EVALUATION OF GROWTH TRAITS AND SEMEN QUALITY USING BIOCHEMICAL AND IGF-1 AS A MOLECULAR MARKER IN THREE GENOTYPES OF NIGERIAN INDIGENOUS CHICKENS

BY

Anthony Kayode, OLUTUNMOGUN

DEPARTMENT OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

JANUARY, 2015.

EVALUATION OF GROWTH TRAITS AND SEMEN QUALITY USING BIOCHEMICAL AND IGF-1 AS MOLECULAR MARKER IN THREE GENOTYPES OF NIGERIAN INDIGENOUS CHICKENS

BY

Anthony Kayode, OLUTUNMOGUN
B.Agric ABU, (2011)
M.Sc /Agric/11787/2011-2012

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE AWARD OF THE DEGREE OFMASTERS OF SCIENCE IN ANIMAL SCIENCE.

DEPARTMENT OF ANIMAL SCIENCE,
FACULTY OF AGRICULTURE,
AHMADU BELLO UNIVERSITY, ZARIA,
NIGERIA.

JANUARY, 2015.

DECLARATION

I hereby declare that the work in the thesis entitled **"EVALUATION OF GROWTH TRAITS AND SEMEN QUALITY USING BIOCHEMICAL AND IGF-1 AS MOLECULAR MARKER IN THREE GENOTYPES OF NIGERIAN INDIGENOUS CHICKENS"** was carried out by me in the Department of Animal Science under the supervision of Dr. (Mrs.) M. Orunmuyi and Dr. M. Kabir. The information derived from literatures has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at any university.

Anthony Kayode OLUTUNMOGUN	………………..	………………
Name of student	Signature	Date

CERTIFICATION

This thesis titled **"EVALUATION OF GROWTH TRAITS AND SEMEN QUALITY USING BIOCHEMICAL AND IGF-1 AS MOLECULAR MARKER IN THREE GENOTYPES OF NIGERIAN INDIGENOUS CHICKENS"** by Anthony Kayode, OLUTUNMOGUN meets the regulations governing the award of the degree of Master of Science in Animal Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

………………………………… …………………………
Dr. (Mrs.) M. Orunmuyi Date
Chairman, Supervisory Committee

………………………………… …………………………
Dr. M. Kabir Date
Member, Supervisory Committee

………………………………… …………………………
Dr. S. Duru Date
Head, Department of Animal Science
Ahmadu Bello University, Zaria.

………………………………… …………………………
Prof. A.Z. Hassan Date
Dean, Postgraduate School
Ahmadu Bello University, Zaria

DEDICATION

I dedicate this work to the I am that I am (GOD), The Lamb of God (Jesus Christ) and to the Comforter (Holy Spirit) for making this work achievable; to my parents Sir and Lady (Mrs.) J.T. Olutunmogun and to my ever caring and supporting siblings Toyin, Babajide, Yemisi and Adeshola.

ACKNOWLEDGEMENT

I am grateful to my supervisors, Dr. (Mrs.) M. Orunmuyi and Dr. M. Kabir who with their tireless and endless commitments and with their great academic wealth of knowledge directed and guided me through the actualization of this M.Sc research work.

I am most grateful to the management and staff of the Department of Animal Science Teaching and Research Farm, ABU, Zaria for their encouragement and support during the period of the research.

I express thanks to the management and staff of the Artificial Insemination (AI) Unit of National Animal Production Research Institute (NAPRI), Shika. Similarly, I thank the Director of Centre for Biotechnology Research and Training in person of Dr. J. Kabir of the Department of Public Health, Faculty of Veterinary Medicine, ABU, Zaria and Staff of the Centre most especially Dr. (Mrs.) Afolabi-Balogun Bola, Mr. J.J. Elisha, Mallam Samir, Mal. Y.Y. Palla, Miss Chachet and Mrs. James for their contributions and advice during the extraction and quantification of the IGF-1 gene.

I sincerely appreciate Mal. Bukar Muhammed, Mairo Ahmad and Sunday Andrew of the Parasitology Laboratory of the Faculty of Veterinary Medicine, ABU, Zaria, Mal. Muhammed Ibrahim of the Chemical Pathology Laboratory of ABUTH, Shika, Zaria and the management of S and T Hatchery, Mando, Kaduna State.

I appreciate the contributions of Prof. I.A. Adeyinka, Prof. G.N. Akpa, Prof. C.I. Alawa, Prof. G.S. Bawa, Prof. J.J. Omage, Dr. S. Duru, Dr. T.S. Olugbemi, Dr. (Mrs.) O.O. Olusola, Dr. S.B. Abdu, Dr. (Mrs.) R. Daudu, Dr. P.A. Onimisi, Dr. M.R. Hassan, Dr. S.M. Yashim, Dr. I.I. Adedibu and other lecturers as well as all the staff in the Department of Animal Science. I also wish to thank Dr. Iyiola-Tunji, A.O and Dr. (Miss) Ojo, O.A of National Agricultural Extension and Research Liaison Service (NAERLS) for their assistance.

I sincerely appreciate my uncle Prof. S.A. Adewuyi, for his fatherly role, academic and spiritual contribution, his wife Mrs. R.K. Adewuyi, children Emmanuel, Joshua, Michael, Grace and Jummai for their care and love. I also thank my cousins Jide Fatai, Joseph and Samson Adewuyi for their support. I sincerely appreciate the moral and spiritual contributions of my siblings (Toyin, Babajide, Yemisi and Adeshola).

I equally thank my friend, Kemi Ramson, for her support. My appreciation will not be complete if I do not appreciate my colleagues Abdulraheem Arome Musa and Mal. Hassan Muhammed for their help, support and tireless effort to see to the completion of this work. Special thanks to all my 2011-2012 M.Sc class mates in the Department of Animal Science and my fellow colleagues from Biochemistry, Biological Science and Microbiology as well as other dear friends and loved ones too numerous to mention.

Finally I thank my Heavenly Father, God Almighty for being in control of everything.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURE

ABSTRACT

A study was conducted using 231 chicks (101 Normal feather, 56 frizzle and 74 Naked neck) to evaluate growth traits and semen quality characteristics using biochemical and IGF-1 as molecular marker on three genotypes (normal, frizzle and naked neck) of Nigeria indigenous chickens. Each of the genotypes comprised of twenty five hens and five roosters mated in the ratio of 1 rooster: 5 hens (1:5). Body weight (BW) and measurements of breast girth (BG), shank length (SL), body length (BL), wattle length (WL), wattle width (WW), comb length (CL), comb height (CH), wing span (WS) and shank diameter (SD) were taken at an interval of 4 weeks starting from 8 weeks of age. Semen quality characteristics (volume, colour, pH, concentration and live and dead ratio) and morphological defects (acrosoma swelling, detached head, mid piece drop, bent tail, curled tail and full tail) were evaluated thrice at 24, 28 and 32 weeks of age, respectively. Blood samples for the haematological, biochemical and molecular studies (IGF-1) were collected at 20 weeks of age. The data collected were subjected to General Linear Model procedure of SAS. The result showed that mean BW between 8 and 20 weeks ranged from 310.46g to 1117.80g and between 1096.80g and 1285.80g from 24 to 32 weeks with significant ($P<0.05$) differences with the normal feathered birds having the superior BW between 20 to 32 weeks of age. However, apart from BW and BL which were significant at all ages, other body linear measurements were not significantly ($P>0.05$) different at all the ages (8 to 32 weeks). The semen quality characteristics for the parameters were significantly ($P<0.05$) different except for semen colour and semen pH which were not significant ($P>0.05$) affected by the age of the birds. The morphological

defects showed that most of them were significantly ($P<0.05$) different for all the genotypes, but the mid piece was not significantly ($P>0.05$) different at 32 weeks. The results of the haematological and biochemical profile show that only Chloride in the biochemical parameter was significantly ($P<0.05$) different. The phenotypic correlation coefficients between traits show that some traits had positive correlation but not statistically significant ($P>0.05$) while others traits showed between moderate and high correlations amongst themselves. Two alleles; A and B and three genotypes: AA, AB and BB were observed with the A allele (0.72) being predominant and conforms to Hardy-Weinberg equilibrium. The relationship among IGF-1 marker, BW and semen quality was significant ($P<0.05$). The AA genotype was superior in body weight over the AB and BB genotypes at 28 and 32 weeks. Therefore, it is concluded that the normal feather genotype did better in terms of BW and body linear measurements (BLM). The semen characteristics revealed that as age increased the quality improved up to 28 weeks, while the IGF-1 gene had a positive relationship with BW and semen characteristics making it a good genetic marker for selection for growth traits (BW) and semen characteristics in the Nigerian indigenous chickens.

CHAPTER ONE

1.0 INTRODUCTION

The poultry industry is a versatile business in Nigeria but the development has been to the detriment of the indigenous birds which have been neglected and not properly managed (Adebambo *et al.,* 1999). The indigenous chickens (*Gallus domesticus)* have been kept in Africa for many generations in subsistence systems.

The Nigerian indigenous chicken constitutes between 80 and 90 percent of the local population of chickens in Nigeria. Three other major genes have been identified in these local chickens apart from the normal feathered type. These are: Frizzle, naked neck and sex-linked dwarfism. Each of these genes plays a significant role in the productive adaptability of the Nigerian local chicken (Ozoje and Ikeobi, 1995). Despite being in Nigeria for long, the indigenous chickens consist of various unimproved sub-populations of heterogeneous characteristics, not yet classified into breeds and varieties since they do not breed true to type and have no clear plumage colours (Obioha, 1992; Ibe, 2001).

The indigenous chicken, also known as the local chicken is the general term given to those chickens kept on an extensive system, scavenging free-range, having no identified description, and being dual-purpose and unimproved (Horst,1989; Pedersen, 2002). They appear to be the cheapest and sustainable means of producing high quality animal protein for the expanding populations of developing countries like Nigeria. They are self reliant and hardy birds with the capacity to withstand harsh weather conditions and adaptation to adverse environments. They are valued for their ability to scavenge, disease tolerance, meat quality and general hardiness (Ssewayana *et al.*, 2003); they also have the ability to hatch and brood. This is clear evidence of the positive attributes of the indigenous chickens. Their products are preferred by the majority of Nigerians because of the pigmentation, taste, leanness and suitability for special dishes (Horst, 1989). However, the Nigerian indigenous chickens are inferior in growth and egg production characteristics compared to the exotic chickens (Ajayi, 2010). Despite these drawbacks, the indigenous chicken plays a vital role in the socio-economic life of those who keep them. Apart from being a source of meat and egg, they are also a source of income for many resource-poor people particularly those living in the rural areas (Ssewayana *et al.*, 2003).

Growth is affected by genetic and non genetic factors (Singh and Singh, 1983; Gupta *et al.*, 1988). Growth is a fundamental property of biological systems and it can be defined as an increase in the number of cells in the body size per unit of time (Schulze *et al.*, 2002; Lawrence and Fowler, 2002). Growth of fowl is analogous to growth of mammalian; consisting of three or four cycles (Grossman, 1988), two of these cycles, however, occur after hatching.

Body weight and body conformations are two important parameters for measuring growth in the domestic chicken (Udeh and Ogbu, 2011). The price of chicken largely depends on body weight. The indigenous chicken, like the improved breed, has a sigmoid growth pattern with differences in growth rate and feed efficiency (Nwosu, 1979), although the indigenous fowl seemed to complete the rapid growth phase earlier than improved breeds (Oluyemi, 1980). However, there is generally no weighing machine or scale available in poultry markets in villages. It is important that the farmer monitors the growth performance of his birds on regular basis to know when they have attained the desired market weight. In places where scales are not available, as is the case in most rural African communities (Nesamvuni *et al*., 2000), linear body measurements such as shank length, drum stick length and wing length can be used in a predictive equation to predict body weight in broilers (Akanno *et al*., 2007). For instance, the reports of Kabir *et al.* (2006) showed that there was a positive interrelationship between body weight and shank length in Rhode Island chickens and similar observations were reported by Malik *et al.* (1997).

Reproductive performance is critical for efficient production in poultry and suitable selection criteria for males based on semen characteristics in roosters (McDaniel *et al*., 1998). The male chicken is known to be responsible for fertilizing the eggs of a number of females and most studies concerning the reproductive efficiency of breeder birds have centered on the cocks and artificial insemination (AI) of large breeder hens for the production of hatching eggs. The artificial insemination is one method showing promise in improving reproductive efficiency of cocks (McDaniel, 1994; McCartney, 1996).

Semen quality of cocks determines the fertility of the male chicken while the female contributes the eggs (Liu *et al.,* 2008). Body weight, length of shank, comb and wattle lengths have been reported to be good predictors of semen attributes in chicken (Galal, 2007). For good results in the artificial insemination of chickens, the quality of semen should be ensured (Tabatabaei *et al.,* 2009). The importance of semen evaluation in poultry breeding for selecting breeding males or for routine monitoring their reproductive performance is well recognized (Cheng *et al.,* 2002). The assessment of semen quality characteristics of poultry gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters *et al*., 2004). Also, sperm morphology is used as one of the most important criteria in the evaluation of semen quality (Den Daas, 1992).

Semen of the domestic fowl, according to Hafez (1990), varies from dense opaque suspension to a watery fluid with a relative high density. The author further stated that the differences in volumes and sperm concentration of the domestic fowl depends largely on the relative contribution of the various reproductive glands, the number of spermatozoa that could be obtained from a breed/strain and the extent to which the genetic potentials can be exploited.

The use of blood examination as a way of assessing the health status of animals has been documented (Owoyele *et al.,* 2003; Muhammad *et al.,* 2004). This is because it plays a vital role in physiological, nutritional and pathological status of organisms (Muhammad *et al.,* 2000; Muhammad *et al.,* 2004) and it also acts in regulatory, protective and homeostatic tissue of an organism (Nasyrova *et al.,* 2006; Eze *et al.,* 2010).

Haematological parameters provide valuable information on the immune status of animals (Kral and Suchy, 2000). This information ranges from giving the level of the blood to detecting ailment or disorders through them. Such information, apart from being useful for diagnostic and management purposes, could equally be incorporated into breeding programmes for the genetic improvement of indigenous chickens.

Glucose, cholesterol, calcium, total protein, alkaline phosphatase, uric acid, sodium, potassium and chloride levels are diagnostic values for diabetes mellitus, liver disease, hypoparathyroidism, chronic hepatopathy and liver disease, gout, kidney disease, chronic diarrhea and dehydration (Simaraks *et al.,* 2004). Besides, haematological values are important to clinicopathological diagnosis such as traumatic injury, parasitism, organic disease, bacterial septicemia and nutritional deficiencies. Moreover, managing abnormalities in birds requires an understanding of how diseases change the biochemical function and electrolyte of the bodies. Because the clinical signs of illness in birds are frequently subtle, clinical chemistry is necessary to evaluate cellular changes (Ritchie *et al*., 1994).

The insulin-like growth factors (IGFs) gene is a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou *et al.,* 2005). Insulin-like growth factors (IGFs) belong to the family of polypeptide hormones; they are structural homologues of insulin and also have a similar function (Li *et al.*, 2003). The IGF-1 and IGF-2 stimulate the proliferation, differentiation, and metabolism of myogenic cell lines from different species (Florini *et al.,* 1996 and Siddle *et al.,* 2001). The IGFs have been shown to regulate body and muscle growth in chickens (Duclos *et al.,* 1999). Similarly, the metabolic effects of avian

IGFs include increase in amino acid and glucose uptake and the regulation of DNA and protein synthesis (McMurtry, 1998). This molecular characterization of the chicken IGF-1 and IGF-2 has provided valuable clues for understanding how they are regulated and expressed.

Most of the functions of the growth hormone in chickens are mediated by insulin-like growth factors (IGF) (Lei *et al.,* 2005) which stimulate amino acid uptake, glucose metabolism, DNA synthesis (McMurtry 1998), protein synthesis, proliferation of different cell types and regulation of growth (McMurtry *et al.*, 1997). Growth rate stimulation by IGF is known in many species of animals but studies have shown that there is no direct relationship between the levels of growth hormone (GH) and the growth rate in chickens. It might be useful therefore to study insulin-like growth factors (Beccavin *et al.*, 2001), as mediators of the functions of the growth hormone (Lei *et al.*, 2005). Surprisingly, higher GH levels in chickens are consistently associated with low growth rates, whether in unrelated genotypes (such as broiler vs. layer) (Goddard *et al.*, 1988) or in divergently selected genotypes (Nir *et al.*, 1987). This suggests that GH levels do not directly explain growth rate in the chicken and implies the need for studies on the factors acting downstream of GH, namely the insulin-like growth factors (IGFs).

1.1 JUSTIFICATION

Great genetic resources embedded in the indigenous chicken await full exploitation that will provide basis for genetic improvement and diversification to produce breeds that are adapted to local conditions for the benefit of farmers in developing countries (Sonaiya *et al*., 1999). Basic morphometric data, sperm reserve and quality on the three genotypes (frizzle, naked-neck and normal feathered chicken) of indigenous chickens are limited (Oke and Ihemeson 2010).

This study will help in improving breeding practices, since a baseline assessment of the growth and reproductive system in relation to the sperm quantity and quality of the genotypes will be established. The biochemical testing being investigated will help to ascertain the blood profile in the three genotypes which will enhance proper management, feeding, breeding, prevention and treatment of disease since it is desirable to know the normal physiological information and the biochemical values which can be obtained under local conditions of rearing since valuable information in literatures towards this line of research is limited.

The inclusion of the IGF-1 molecular marker will check out the level of relationship among this genetic marker, IGF-1 gene polymorphism, body weight and semen quality in the cockerels in respect to fertility and chronological age since this gene of interest does not only have influence on growth and body weight, but it is also critical for reproductive neuroendocrine (Wang *et al.,* 2011) and reproduction in chicken (Bian *et al.,* 2008). Therefore, this experiment was designed to evaluate growth traits, semen ejaculate quality, and biochemical and IGF-1 molecular markers in three genotypes (normal feathered, naked neck and frizzle) of the Nigerian indigenous cockerels.

1.2 HYPOTHESES

H_0: There is no difference in growth, morphometric traits and semen quality characteristics of the F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

H_A: There is difference in growth, morphometric traits and semen quality characteristics of the F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

H_0: There is no difference in some haematological and serum biochemical parameters of F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

H_A: There is difference in some haematological and serum biochemical parameters of F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

H_0: There is no relationship among the IGF-1 polymorphism, body weight and semen quality characteristics of the F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

H_A: There is relationship among the IGF-1 polymorphism, body weight and semen quality characteristics of the F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

1.3 OBJECTIVES

The followings are the objectives of the study:

1. To evaluate the growth, morphometric trait and semen quality characteristics of F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

2. To evaluate the haematological and serum biochemical parameters of the F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

3. To determine the relationship of IGF-1 polymorphism with body weight and semen quality characteristics of the F1 offspring produced from the three genotypes of the Nigerian indigenous cockerels.

4. To evaluate the correlation among body weight, morphometric traits and semen quality characteristics at 24, 28, and 32 weeks of age in the F1 offspring of the indigenous cockerels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORICAL ORIGIN OF THE INDIGENOUS CHICKENS

The domestic folworiginated from South-Western Asia as a descendent of the Red Jungle Fowl and was first introduced into China in about 1400 before the common era (BC). Chickens are also depicted in Babylonian carvings in about 600 BC and are mentioned by ancient Greek writers, particular Aristophanes in 400 BC (Crawford, 1990).

Following the introduction of the local chickens, Africa became blessed with large number of indigenous chickens. This makes them a focal point for researchers as 90% of the 150 million chickens in Nigeria are local varieties which contribute 90% and 72% of the egg and meat consumption respectively (Nwanta *et al.,* 2006). These chickens probably went through several genetic changes leading to their sizes being reduced (in areas of food scarcity) or increased (in areas where food was plenty). The indigenous chickens are resistant to most of the endemic poultry diseases and pests ravaging the exotic stocks (Sil *et al.,* 2002).

2.2 GROWTH PERFORMANCE OF THE INDIGENOUS CHICKENS

Growth is one of the main attributes of living things and is such an obvious process that it hardly seems to justify any particular formal definition. In biology, growth can be defined as the changes throughout life accompanied by the utilization of materials, leading to an increase in volume, size or shape of an organism (Aggrey, 2002). The study of growth traits helps in judging the efficiency of management and in determining the optimum managerial practices to maintain the gain at optimum level with least cost, since feed cost decreases with increasing growth rate (Aboul-Seoud, 2008).

Growth and body weight gain are affected by genetic and non-genetic factors such as genotype, year and season of production, sex, nutrition, adaptability, climatic conditions and management. The indigenous chicken breeds in the tropics have been described as having slow growth rate. Studies conducted on large Beladi type chicken of the Sudan revealed that the birds can hardly attain 2kg at adult stage (Yousif and Osman, 1994). Similar study showed that prior to the age of sexual maturity, these indigenous chickens also lack the ability to attain appreciable weight of 2kg (Yousif *et al.,* 2006).

2.2.1 Body Weight and Body Linear Measurements

The estimated body weight at hatch (BW0) is limited due to its small magnitude and the fragility of the neonates. In respect of insufficient records on neonates, Dana *et al.* (2011) revealed that mean hatch weight (BW0) of Horro chicken was about 25 g which increased to 621 g at16 weeks of age. The report further showed that the male Horro chicken had a mean body weight at hatch (BW0) to be about 25g and at 16 weeks the body weight was about 701g and for the female chicken it was 25g at hatch and 573g at 16 weeks of age. The mean body weights of Horro chicken were generally within the ranges reported for unselected indigenous populations in northwestern Ethiopia (Halima *et al.,* 2007) and many other countries of Africa (Gueye, 1998). Also, a similar report of 28g was reported by Yousif *et al.* (2006) on Sudanese indigenous chicken and was similar to that of Adedeji *et al.* (2008) who reported body weight at hatch of between 33.57 and 41.56g and 421.40 to 1471.22g at twelve weeks in pure and crossbred chickens in savannah environment of Nigeria. Also, Oke (2011) reported a range of 22.33 to 30.90g in the three genotypes of the Nigerian indigenous chickens and their crosses. A similar report was made by Ndofor-Foleng *et al.* (2010) who recorded 21.82 to 28.06g for light and heavy ecotype chicken. Yousif *et al.* (2006) reported an average body weight of 926.3±125.5g at 18 weeks of age in the male Sudanese indigenous chickens' prior to sexual maturity and are in accordance with those reported by Yousif and Osman (1994) and Safloah (1997). Also, Abd El Ghany *et al.* (2011) recorded a general linear increment with increasing age in two strains (El- Salam and Mandarah cockerels) with significance during the early period (12-18 weeks) for body weight, length of both keel and shank and comb length and width in the two strains.

Mature body weights of indigenous chickens in Africa under scavenging system ranged from 1170g to 1500g and from 1354g to 1652g under intensive managements; while it was between 1140g and 2000g and 1125g to 2000g respectively in their Asian counterparts (Huque, 1999 as cited in Sonaiya and Swan, 2004). The differences, which are observed in both body weight and body linear measurements as affected by sex within breeds and genotypes at different age is linked to sexual dimorphism. Adedeji *et al.* (2008) reported that sexual dimorphism appears in some growing animals characterized by the manifestation of visible differences between males and female (species). The author reported that males were superior in body weight and body linear measurements compared with their female counterpart. Similar reports were made by Adedeji (2004) and Adedeji *et al.* (2008) that reported sexual dimorphism is in favour of male birds which could be attributed to differences in hormonal profile, aggressiveness and dominance of males when feeding, especially when the sexes are reared together (Ibe, 1998).

2.3 THE TESTIS

2.3.1 The Male Anatomy

Males of all avian species have testis that are internally located in the body cavity. In domestic birds, they are located just anterior to the kidneys and are attached to the dorsal body wall. In many species of birds, there is a bilateral asymmetry, with the left gonad larger than the right: whether the mechanisms that are responsible for testicular asymmetry are the same as those that cause the marked ovarian asymmetry, in birds, are unknown.

The colour of the testis varies from white to creamy white with the testis being soft and lack the connective-tissue septa commonly found in mammals (Parker *et al.,* 1940) as cited in Ruth, 2002. The weight of the testis in chickens constitutes about 1% of the total body weight, or about 9-30 g per testis at sexual maturity depending on the breed (Sturkie and Opel, 1976, as cited by Ruth, 2002).

2.3.2 Spermatogenesis

Sperm development can be separated into three processes: spermatocytogenesis, spermatogenesis and spermiation. The first stage of spermatogenesis occurs in the periphery of the seminiferous tubules lined with spermatogonia. Spermatogonia are diploid dividing mitotically, to retain a constant population of stem cells for spermatogenesis and to produce the spermatocytes (Lake, 1956 as reviewed by Ruth 2002). Through a total of 10 stages, spermatogonia are transformed from primary spermatocytes into two secondary spermatocytes, four spermatids and spermatozoa. At each stage of spermatogenesis, the cell is transported closer to the lumen of the tubule, where it is finally released as a complete spermatozoon.

The final phase of sperm formation, spermatogenesis, consists of the elongation of the spermatid nucleus to form the head and the shedding of most of the cytoplasm (Lake, 1956 as cited by Ruth, 2002). Since each region of the seminiferous tubule contains spermatozoa in different stages of differentiation, the production of spermatozoa by the testes is continuous, although spermatogenesis by each section of the seminiferous tubule is phasic (Aire *et al.*, 1980 as cited by Ruth, 2002).

2.3.3 Production of Semen in Cocks

The paired testes of the male fowl are situated within the abdomen and are suspended from the dorsal body wall on either side of the midline adjacent to the anterior parts of the kidney and just posterior to the lungs (Lake and Stewart, 1978 as cited by Kabir, 2006). Semen consists of spermatozoa and fluid produced within the testes and the duct portion of the genital tract called the ducti deferentes. Semen is stored in the ducti deferentes which end in two papillae projecting into the cloaca (Lake, 1966 as cited by Kabir, 2006).

The male fowl has no penis, but an erectile phallus which is believed to make contact with the everted vagina (cloaca) during copulation (Kamar and Hafez, 1975 as cited by Kabir, 2006). Erection of the phallus is caused by its becoming engorged with blood as are the related cloacal structures (lympholds). Semen is the complete generative fluid emitted by the male. It is a suspension of living cells (the spermatozoa) in a colloidal or semi-gelatinous medium known as seminal plasma.

As in mammals, chicken spermatozoa mature in the epididymis. However, there are no seminal vesicles or Cowper's glands. The testes are without septa and lobules and consist of seminiferous tubules, rete tubules and vas differentia. There is also relatively less seminal plasma in chicken semen than in mammalian semen (Kamar and Hafez, 1975).

2.3.4 Semen Composition

In the male bird, semen is composed of sperm and seminal plasma secreted by the epididymis and vas deferens with the sperm quite variable (Thatohatsi, 2009). The sperm cells are usually mixed with secretary fluids from the engorged phallic apparatus and with digestive and urinary tract wastes. The sperm cells are produced in the testes and in the case of avian species; the seminal fluid is also produced in the testes. All these secretions in the testes are controlled by the endocrine hormones carried to them in the blood stream. The pituitary FSH (follicle stimulating hormone) and LH (luteinizing hormone) regulates the testes which in turn produce testosterone which controls the testicular development and secretions (Thatohatsi, 2009). The seminal plasma consists of protein, fructose, sorbitol, citric acid, inositol, glyceryl phosphoryl choline, ergothioneine, sodium, potassium, calcium, magnesium, and chloride (Lake, 1971 as cited in Ruth, 2002). Semen that is viscous and white has a high spermatozoa concentration, while semen that has a watery appearance is low in spermatozoa concentration and therefore reveals that semen varies in appearance and leading to differences in concentration (Ruth, 2002).

2.4 SEMEN QUALITY CHARACTERISTICS

The ultimate goal of semen collection is to harvest the maximum number of spermatozoa of the highest possible quality per ejaculation. Quality of semen can be lowered by poor collection procedures, feeding regime and health status of the cocks. Therefore, semen quality like other phenotypic expressions, no doubt consists of a genetic component, an environmental component and a variety of interactions between the two. The major contributor to variation in semen quality is the environment. This environmental effect could be temporary or permanent. The permanent effects occur during prenatal and prepubertal periods (in animals and not birds) and temporary or permanent factors acting after spermatogenesis is initiated can alter semen quality. Semen quality improves during the first few months after puberty and declines in old age both in animals and in poultry species which is due to age advancement and reduction in the reproductive potential of the reproductive gland. Therefore knowing semen quality characteristics is necessary.

2.4.1 Semen Volume and Concentration

The cockerel produces between 0.1 to 1.5ml per ejaculate, with 0.6ml being the average ejaculate volume (Anderson, 2001). Also, different cockerels of the same species often produce different volume of semen at different times (Anderson, 2001). The reports of Orunmuyi *et al.* (2013) on Hubbard broiler showed mean semen volume to be 0.55 ml, 0.34 to 0.59 ml by Bilcik *et al.* (2005) on broiler cocks and 0.40 to 0.73 ml by Peters *et al.* (2008) on seven different strains of indigenous chickens in Nigeria. Machebe and Ezekwe (2006) reported values of 0.27ml, 0.24ml and 0.22ml for naked neck, frizzle and normal feather roosters respectively and while Bah *et al.* (2001) in Sahel regional local breeding cocks reported average of 0.28ml. However, Ajayi *et al.* (2011) reported much higher values of 0.83ml and 1.10ml respectively for normal feather and frizzle and a lower value of 0.18ml for naked neck. Tuncer *et al*. (2006) also reported 0.7mls on Denizli cocks.

Average concentration of spermatozoa is 3.5 million per ml of semen (Sturkie and Opel, 1976 as cited in Ruth, 2002). Reports have also shown an average of 7 billion and a maximum of 8.2 billion per spermatozoa ejaculate in Brown Leghorn roosters (Ruth, 2002). The findings of Orunmuyi *et al.* (2013) in Hubbard broilers indicated concentrations of 3.91 $x10^9$ while 6.6 billion sperm/ml was reported by Tabatabaei *et al.* (2009) in Ross broiler breeders. However a lower value of 2.17 to 3.14 $x10^9$ was reported by Tabatabaei *et al.* (2010) in Iranian indigenous broiler breeder chickens. A low value of 2.26 x 10^9 was also reported by Bah *et al.* (2001) in Nigerian local breeder cocks. In a similar report on broiler breeder roosters, Gumulka and Kapkowska (2005) obtained a range of 4.68 to 5.53 x 10^9 when the birds were 29 to 60 weeks of age.

2.4.2 Semen Motility, pH and Colour

The report of Selvan (2007) in Rock fowls indicated that sperm motility ranged from 78.28 to 85.31% based on the influence of age and dietary protein, calcium and vitamin E. Similar result was observed by Orunmuyi *et al.* (2013) who found that the semen motility of Hubbard broilers was 83.50%. Also, Peters *et al.* (2008) reported a range of 62.55% to 87.35% on seven strains of Nigeria chickens which also fell in the range of 74.5 to 85.67% reported by Tabatabaei *et al.* (2010) on Iranian broiler breeder chickens. However Bah *et al.* (2001) reported a lower value of 73.9% in Nigerian local breeder cocks when compared to similar work of Peters *et al.* (2008) who used similar strains in the humid tropics of Nigeria.

Semen pH varies slightly between different breeds and bird species. The optimum semen pH ranged between 7.0 and 7.4 and sperm motility is generally high between these slightly alkaline ranges and also increases the fertilizing ability. A pH of 6.4 (acidic) is not suitable for semen preservation, as it may cause damage to the plasma membrane of the sperm cell (Latif *et al*., 2005). Contrarily, Donoghue and Wishart (2000) and Siudzinska and Lukaszewicz (2008) reported several trials that indicated chicken sperm to tolerate a pH range of 6.0 to 8.0. Peters *et al.* (2008) also found the semen pH of the cockerel to be slightly alkaline, with a mean of 7.01 ± 0.01, while Tuncer *et al.* (2008) and Bah *et al.* (2001) recorded a semen pH ranging between 7.54 ± 0.04 to 7.80 ± 0.03. Similarly, Orunmuyi *et al.* (2013) recorded a pH of 7.4 in Hubbard broilers. This variation in semen pH may be due to many factors. The pH, especially that of ejaculated semen is dependent on several secretions involved. Poor quality semen generally contains large amounts of fluid from the accessory glands, which increases the semen pH. The pH of semen is likely to decrease as the time between collection and measurement increases, and the semen collection tubes are narrow in shape causing sperm to break down fructose in the semen to lactic acid under anaerobic conditions. Semen samples that contain many dead sperm cells may evolve ammonia, which will also increase the pH (Salisbury *et al.*, 1978 as cited in Hunderra, 2004).

The colour of semen is generally an indication of the density of the ejaculate (Thatohatsi, 2009) which depends on the species of bird used, but generally semen should be creamy which indicates a high sperm concentration. The semen of the domestic fowl varies from a dense opaque suspension to a watery fluid secreted by various reproductive glands, from a relative high sperm density or degrees of clarity to milky white, with declining sperm numbers (Peters *et al*., 2008). Certain degrees of colour reflect certain numbers of sperm cell. Watery semen contains few sperm cells and is almost without fertilizing ability. Sperm samples with a pearly- white colour and a thick consistency usually have a high concentration of spermatozoa and are preferred under practical conditions. Colour could also serve as an indication of contamination for example by faeces or urine and thus become brown or green in colour (Lake, 1983 as cited in Thatohatsi, 2009). Sometimes flakes of blood may be present, which may be a result of excessive force being used during the collection process, injury or infection. Semen samples that are contaminated by faeces do not have to be discarded, but diluted with antibiotics such as penicillin and dihydro-streptomycin or neomycin to reduce the loss of sperm. This is recommended as the antibiotics can also increase fertility when used as diluents in semen (Bearden *et al*., 2004).

2.4.3 Semen Quantity and Testis Size

Traditionally, the only way to determine testicular size in birds was upon post mortem necropsy because of their internal location. However, with new ultrasound technology, it is possible to identify the size and if present, the pathological state of internal organs (Emily, 2003). From literature, it is observed that there is a close association between testis size and the amount of semen produced and once the testis reaches the minimum critical size (7 g), additional size increases have little effect on semen concentration. Only males with testes weighing less than 9 g at the time of necropsy yield semen irregularly throughout production (Fontana *et al.,* 1990). Testes weight has been positively correlated to body weight, semen volume, concentration and total number of sperm (Lee *et al.,* 1999). However, combined testes weight was not correlated to sperm mobility (Froman and Feltmann, 1998).

2.5 MORPHOLOGICAL DEFECTS ASSOCIATED WITH SEMEN QUALITY

Usually, sperm abnormalities or morphological deviation from the normal structure in the sperm cells indicate disturbances of spermatogenesis and this could be attributed to age, nutrition and pollution (Bah *et al*., 2001). Ejaculated semen can contain some morphologically abnormal spermatozoa which are associated with lowered fertility until the proportion of abnormal spermatozoa exceeds 20% (Hafez, 1993). An accurate morphological examination of spermatozoa thus enables the elimination of males with potentially low fertility prior to the preservation of their semen (Rodriguez-Martinez and Barth, 2007). Ajayi *et al.* (2011) stated that improper handling of ejaculates during processing for microscopy has been identified as a major cause for sperm abnormality. The authors also indicated that the low percentage of sperm abnormality of less than 10% observed in the semen of cocks may be insignificant as to cause fertility worries in the local strains examined. The morphological defects as recorded by Ajayi *et al.* (2011) showed no breed or strain differences in the indigenous strains examined but percentage defect was more in the tail region than in other parts of the spermatozoa (2.36±0.06, 3.71±0.60 and 9.01±2.31%), respectively, for head, mid-piece and tail. Tabatabaei *et al*. (2009) reported a range of 1.64±0.07% and 3.32±0.05% for Ross and indigenous cocks at 30 weeks of age. Higher percentages of tail defect were also reported by Tabatabaei *et al*. (2009) in exotic Ross-308 (41.04±10.19%) and indigenous chicken in Iran

(44.1±0.26%). The authors also reported mean morphological defects of spermatozoa on the same breeder rooster samples to be 10.13±0.40 for Ross and 7±0.53% for indigenous Iranian cocks. In a similar study, morphological defect rate in Leghorn and New Hampshire roosters were 5.44±0.95 and 6.76±0.95% respectively and abnormalities in acrosome, head, mid piece and tail region were 6.81, 60.2, 26.98 and 6.01% in Leghorn roosters and 0.37, 4.98, 1.69 and 3.17% in New Hampshire cocks respectively (Alkan *et al.*, 2002). In another study conducted by Selvan (2007), abnormal spermatozoa were 3.09 to 5.7% dependent on influence of age, dietary protein, vitamin E and calcium. Alkan *et al.* (2002) observed much higher morphological defects 17±0.06% in spermatozoa sample of American Bronze male turkeys.

2.6 CORRELATION OF BIOCHEMICAL AND HAEMATOLOGICAL WITH SEMEN CHARACTERISTICS IN THE NIGERIAN INDIGENOUS COCKERELS

Reports have revealed that biochemical parameters and semen characteristics correlate. For instance, blood glucose concentration and sperm motility in three laying lines of cockerels have been reported to correlate Jarinkovicova *et al.* (2012). They also stated relationship between ejaculate volume and concentration with glucose level and a non-relationship between creatinine and ejaculate volume. Sperm motility was also documented to be positively correlated with concentration in cockerels of three different egg lines by the same authors.

In Haematological parameters such as AST negative correlation with most quantitative traits of semen in boar Jacyno *et al.* (2009). Similar reports in bulls have revealed negative correlation between sperm concentration and trace elements such as Na, K, Cl and Ca (Cevik *et al.,* 2007). This may indicate that these trace elements rarely perform roles related with quantitative characteristics but rather work at a physiological level. Na, Cl and K have been associated with proper spermatozoa functioning through modulation of a variety of ion channels (like Cl) of spermatozoa. This characteristic event is associated with capacitating and acrosome reaction of mammalian spermatozoa with K being a natural metabolic inhibitor and a higher K concentration in seminal plasma decreases the metabolic activity of spermatozoa. Therefore, there is generally a negative correlation between K and sperm motility (Meizel, 1997; Purohit *et al*., 1999; Gur and Demirci, 2000; Machal *et al*., 2002; Massanyi *et al*., 2003 and Mosaferi *et al.,* 2005).

2.7 PHENOTYPIC CORRELATION AMONG BODY WEIGHT, BODY LINEAR MEASUREMENTS AND SEMEN CHARACTERISTICS

Simple reliable and indirect method for in vivo estimation of semen quality based on correlation between body parameters and semen traits is needed by poultry farmers. Understanding the interrelationships between body weight and body linear measurements in these birds will help the researchers predict their body weight at various ages especially in the rural areas where scales may not be available Udeh *et al.* (2011). Galal (2007) reported that body weight, length of shank, comb, and wattle were good predictors of semen attributes in chicken. Similarly, Udeh *et al.* (2011) reported that lengths of comb, shank and wing could be used to predict semen traits of cocks. Body weight was reported by Gabriel *et al*. (2009) to have significant effect on concentration and motility but not on semen volume and pH in Norfa chickens while Abd El Ghany *et al.* (2011) reported that body weight influence on volume, concentration and motility but not pH in two local strains of chickens. These authors reported that heavy weight had the advantage of high concentration but motility was to the advantage of the light weight group.

2.8 SELECTION FOR SEMEN QUALITY

In the poultry industry, breeding soundness, examination and evaluation of quality semen in the breeder cocks is important so as to identify and select sires of superior genetic worth (Nwagu *et al.*, 1996). Many male birds exhibit dramatic and conspicuous sexual signals that likely provide information to females about male quality because these traits are condition dependent. At hen-house placement, roosters are selected based on physical characteristics associated with a reproductively matured male. Such characteristics include comb and wattle size and colour as well as overall body size and shank length (Wilson *et al.*, 1979 as cited in Kabir, 2006).

However, (Wilson *et al.* 1979 as cited in (Kabir, 2006) showed that physical characteristics are not strongly predictive of male fertility. According to Donoghue (1999), semen evaluation can be a valuable tool in the management of roosters or toms but that semen quality tests currently available to the poultry industry are time consuming, labour intensive and unreliable predictors of fertility and semen quality. If a quick and accurate semen quality test was available to predict fertility, males could be chosen for hen-house placement based on semen quality and not physical characteristics alone. By testing roosters for semen quality, fertility and ultimately hatchability could be improved. One method of semen analysis that is quick and has been correlated to sperm concentration, viability, and motility is the semen quality index (SQI) in rooster (McDaniel *et al.,* 1998) and mammalian semen (Bartoov *et al*., 1991; Matilsky *et al*., 1993; Johnston *et al*., 1995; Zavos *et al*., 1996). The SQI is generated by the OptiBreed sperm quality analyser (SQA) in 20seconds. The analyser has a photocell that monitors the number of times sperm movement disrupts path of light. In a laboratory trial using artificial insemination, Parker *et al*. (2000) selected males based on the SQI. These authors demonstrated that by removing males whose semen quality ranked in the lowest 22% of the SQI population, fertility was increased by as much as 4% over an unselected population. In a different artificial insemination study, they also reported using semen from males in the lowest 25% of the SQI population result in fertility that is approximately 20% lower when compared to semen from males in the upper 75% of the SQI population. As a result of previous mammalian and avian research using the SQI, it was reasoned that this method of semen evaluation could be used to select roosters in the poultry industry for improved reproductive performance.

2.9 SEMEN QUALITY

Saacke, 1983 as cited by Thatohatsi, 2009 stated historically, that semen quality traits can be classified according to the sperm viability or morphology. The morphology of sperm is also considered to reflect the physiological status of the male for sperm production and reflects the viability of storage in the gonadal ducts. Viability, as other semen traits, can reflect the fertility status, but it also measures the human's interaction with semen as it is collected, processed and inseminated. Semen traits related to fertility e.g. sperm motility, velocity, acrosome morphology etc., may affect the penetration of the cervical mucus and are thus important for semen preservation and ultimately fertilizing capacity. Fertility and hatchability, according to Tabatabaei *et al*. (2009) depend on genetic, physiological, social and environmental factors. These are interrelated heritable traits, which may vary between breeds. Sperm number, type of hens (broiler or layer) and age may affect the in vivo storage of sperm, and subsequently the fertility of the eggs. The conditions which cause low sperm numbers or single sperm activity at the site of fertilization can cause a reduction in the actual number of chicks being hatched. Low fertility and early embryonic mortality may be the result of low sperm activity or single activity. This condition is normally associated with older breeder hens or any flock experiencing infrequent mating activity (Bramwell, 2002).

2.10 FACTORS AFFECTING SEMEN QUALITY

2.10.1 Age and Strain

Age has an adverse effect on the reproductive success of birds. A male fowl is capable of producing small amount of semen at 16 to 20 weeks old (Lake and Stewart, 1978 as cited in Kabir, 2006). However, maximum volume of good quality semen may not be obtained until 24 to 32 weeks of age. Omeje and Marire (1990) as cited by Machebe and Ezekwe (2006) reported that genotypic differences affected body size and semen characteristics of cocks, while age difference significantly affected variation in body size, pH and semen volume. According to Lee *et al.* (1999), birds aged 21 to 30 weeks which were not earlier trained to ejaculate showed less sensitivity to massage thereby proving more difficult in emitting semen. They further concluded that semen at this age had low concentration. However, the factors that influence the age-related decline in avian reproduction are still poorly understood. In hens, reduction in fertility occurs by the middle of reproduction period and is characterized among other things, by a decrease in duration of fertility (DF) (Gumulka and Kapkowska, 2005).

Studies on turkey showed that the age of the toms affected the sperm quality of both the fresh and stored semen. Ageing was accompanied by a reduction in the number of spermatozoa in the ejaculate and in semen volume and by a decrease in motility, viability and membrane integrity of spermatozoa. Consequently, these changes led to a progressive decline in the fertilizing ability of turkey semen and may also affect its preservability during storage (Iaffaldano *et al.*, 2007).

In a study by Tabatabaei *et al.* (2010), it was reported that the difference in spermatozoa concentration between roosters aged 26 - 34 weeks (3.41±0.23 against 3.28±0.48M/L) was not significant; but, its concentration reduced significantly in 45 weeks old roosters (2.17±0.76 x10^9 M/L). Kotlowska *et al.* (2005) and Rosato *et al*. (2006) reported that with ageing of toms, spermatozoa concentration reduced.

Age effect was also reported by Tabatabaei *et al.* (2010) showing sperm motility (85.67±0.69, 80.10±0.46 and 74.5±0.27%) for 26, 34 and 45 weeks, respectively and the sperm viability (90.64±1.47, 82.30±1.62 and 74.11±35%) for 26, 34 and 45 weeks, respectively. Similar findings by Rosato *et al*. (2006) in turkey showed negative correlations between age of toms and motility and viability of spermatozoa. Morphological defect rates of spermatozoa have been shown increase with ageing of indigenous roosters (7.12±0.25, 10.54±0.83 and 15.10±50%) for 26, 34 and 45, respectively Tabatabaei *et al.* (2010).

In studies conducted by Hanafy (2006), El-Sheikh and Hanafy (2006) and El-Tantawy *et al*. (2007), semen volume, sperm concentration, sperm motility and live sperm of Inshas and Matrouh cocks increased linearly with increasing age, while, abnormal sperm decreased linearly with increasing age. In contrast, Ali *et al*. (2006) and Shahein *et al*. (2007) reported no significant effect of age on semen volume, sperm motility, sperm concentration and live sperm. Peters *et al*. (2008) reported that strain differences significantly affected semen ejaculate volume, sperm concentration and sperm motility but not semen pH. The differences in most semen physical characteristics between strains were found to be insignificant (Aly and El- Sahn, 2006; El-Fiky and Soliman, 2006; El-Sahn, 2007 and Habeb, 2007). Morphological defects of spermatozoa in indigenous roosters at 28 weeks ages were identified as Acrosome swelling, Acrosome detachment, Coma shaped acrosome, 90º bent head, 180º bent head, Head detachment, Knotted head, Large head, Small head, Mid-piece sweeling, Knotting at head mid-piece-border, 90º bent tail, 180º bent tail, Tail knotting, Tail detachment and Curled tail (Tabatabaei *et al.*, 2009).

2.10.2 Feed Nutrition

Feed has been implicated by some researchers (Hocking and Bernard, 1997; Omeje and Ude, 1998) as one of the factors known to affect semen production and quality in the male bird. With intensive production and improved feeding, Kabir *et al.* (2007) reported a remarkable improvement in the reproductive traits of Rhode Island Cocks. This therefore shows that adequate nutritional environment is essential to maintaining the breeding flock in good reproductive condition. Pana *et al.* (2000) reported that Cornish broiler cocks whose daily feed consumption was limited to 130g produced ejaculates whose concentration did not differ significantly from their full-fed counterparts. According to Ezekwe *et al.* (2003) the effects of underfeeding on semen quality traits appeared to be more severe on the physical rather than biochemical characteristics, which imply that the spermatogenic functions of the testis are more responsive to underfeeding than the secretary activities of the reproductive tract. Dietary crude protein and feed intake were reported to affect semen production of broiler breeder males (Hocking and Bernard, 1997). Moderate underfeeding was reported to affect semen production and semen quality attributes of local cocks whereas volume, motility and concentration were significantly depressed by severe underfeeding (Ezekwe *et al.*, 2003). In a similar study by Kabir *et al.* (2007), semen quality traits including semen volume, motility and concentration began to fall as the level of underfeeding decreased from 70 to 50% in Rhode Island cocks. This connotes that the spermatogenic functions of the testis can be said to be more responsive to underfeeding than the secretory activities of the reproductive tract.

2.10.3 Ambient Temperature

A decrease in ambient temperature after collection of semen decreases the activity (motility) of the sperm and semen should also not be exposed to the sun (Anderson, 2001). An elevated body temperature of 41.8ºC does not affect spermatogenesis in chicken (McDaniel *et al.*, 1995), but when the ambient temperature goes up to 43ºC, infertility due to heat stress is not uncommon. The sperm membrane is susceptible to changes in temperature, and this may affect the movement of the sperm, causing deterioration in quality and fertilizing capacity. Therefore care must be taken to maintain the required temperatures (Senger, 2003). The ambient temperature is never constant and higher temperatures can increase the metabolism of the sperm cell, while cooler temperatures are less of a problem as it reduces the metabolic rate and slows down the sperm movement (Hafez and Hafez, 2000). On the other hand, Perek and Snapir, 1963 as cited by Kabir, 2006) reported that changes in environmental temperatures during an 11 month experimental period had no significant effect on semen volume and sperm concentration in egg-type cocks.

2.10.4 Photoperiod

Photoperiod affects the semen quality both at the pre and post ejaculation storage and light of adequate intensity and duration is necessary to maintain good semen production. Lake and Stewart, 1978 as cited by Kabir, 2006) reported that the light supplied to encourage production in the female is sufficient to allow optimum semen production in the male. It also shows that no significant effect of photoperiod on semen volume and total sperm per ejaculate in the effect of photoperiodicity on semen characteristics of poultry. Despite the necessity of photoperiod/light, Bearden *et al.* (2004) reported that lighting in the laboratory can suppress the metabolic rate, motility, and fertilizing capacity of the sperm. A greater effect was observed when semen was in contact with oxygen. The enzyme catalase will prevent the harmful effect of light or photoperiod demonstrating that light causes a photo-chemical reaction in the semen that result in the production of hydrogen peroxide, which is detrimental to the sperm cell.

2.10.5 Semen pH

The accessory fluids are of blood origin and contain aldose (presumably blood glucose). Cockerel semen lacks aldose and has a lower pH than the seminal fluid. If a large amount of seminal fluid is ejaculated, it will increase the semen volume, pH and glucose content and cause a decrease in the density (concentration) of the semen. The pH of semen (pH 6.8) is slightly below neutral (pH 7) immediately after ejaculation in most poultry species. The loss in carbondioxide (CO_2) may result in an increase of the pH, which may again be neutralized by the production of lactic acid during sperm metabolism (Cole and Cupps, 1977 as cited by Thatohatsi, 2009). This semen extenders used must provide the necessary buffers to prevent changes in pH, produced by the sperm metabolism.

2.11 BLOOD CHEMISTRY

Blood chemistry refers to the chemical composition of blood and is important for detecting any blood disease of the animals as well as birds. Many researchers have focused their genetic studies on improving only economic traits. A broader perspective of improved and efficient genetic study has emerged because selection, breeding and genetic improvement have a marked effect on characteristics of the blood biochemistry; as researchers utilize the biochemical parameters of the blood as markers in livestock species to enhance productivity and reproductive performance (Nguyen and Tran, 2003; Emmerson, 2003).

This blood chemistry test includes normal haematological, biochemical and serological tests. In haematological studies, such common parameters as the haemoglobin percentage, lymphocytes and monocytes are usually estimated. In biochemical studies, common parameters like calcium (Ca), glucose, and cholesterol are assayed. For serological tests, however, albumen, globulin and total protein are usually estimated.

2.12 HAEMATOLOGICAL PARAMETERS

Analysis of normal haematological parameters of chickens is essential in diagnosing the various pathological and metabolic disorders. It can be used as a diagnostic tool in order to assess the health status of an individual and/or a flock. Haematological parameters and values are routinely used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors. These parameters in birds have been identified to be influenced by various factors such as age, sex, season and nutrition (Elagib *et al.*, 2012). Other factors reported are breed, climate, geographical location, day length, time of day, life habit of species, present status of individual and other physiological factors that can influence the value of the parameters. These parameters includes; Packed Cell Volume (PCV), Haemoglobin (Hb), Total Protein (TP), white blood cell (WBC), Eosinophiles (Eosi.), Hetrophiles (Het) pH, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC).

2.12.1 Packed Cell Volume (PCV)

The packed cell volume is also known as the haematocrit index or volume of packed red cells (PCV). PCV is the fraction or proportion of blood occupied by the red blood cells. It does not include the white blood cells or platelets. Generally, findings have shown that low values of PCV indicates anemia and high values indicates dehydration. The report of

Jain (1993) showed a range of PCV in *Gallus gallus domesticus* as 22.0 to 35%. Isidahomen and Njidda (2012) reported values of 38.46, 42.60 and 46.40% for normal, naked neck and frizzle chickens. Similar results were reported by Ladokun *et al.* (2008) who obtained values of 35.90 and 41.00% for normal feathered and naked neck chickens. Islam *et al.* (2004) recorded an increase in PCV of between 26.56 to 34.60% in the three breeds reared in Bangladesh. The authors detected a low PCV (26.56 to 28.12%) at early age (1month) which gradually increased with advancement in age (3 to 12 months). The lowest PCV were observed at 1month of age (26.56, 28.12 and 27.73%) and the highest was at 12months (30.08, 32.25 and 34.60%) in the three breeds (Fayoumi, Assil and Local chickens) in Bangladesh. The results from Islam *et al.* (2004) on PCV of Fayoumi and local chickens were slightly lower than the result reported by Islam *et al.* (1999). This variation in PCV values was reported by Islam *et al.* (2004) which could be as a result of age, sex, breed and the status of the birds.

2.12.2 Haemoglobin (Hb)

The main function of the red blood cell is to carry oxygen to the tissue and to remove carbon-dioxide. Oxygen is efficiently transported in a haemoglohin ion complex. Haemoglobin is a complex of the protein globulin linked with four pigments or haem-radicals. The globulin molecule is a complex of peptide chains composed of certain amino acids arranged in a definite sequential and spatial pattern. Normal blood haemoglobin values in animals are between 13 and 15g per 100ml of blood. However, Jain (1993) reported that haemoglobin values in *Gallus gallus domesticus* could range from 7.0 to 13.0g/dl with an average of 9.0g/dl. Isidahomen and Njidda (2012) reported values of 13.09, 15.96 and 14.29g/dl for normal, frizzle and naked neck chicken genotypes. Also, Ladokun *et al.* (2008) reported values of 11.60 and 13.68g/dl for normal and naked neck birds. From the findings of Islam *et al.* (2004), it was evident that haemoglobin concentration increased with the advancement of age being lowest at 1month (7.06, 8.23, and 7.73g/dl) and highest at 12months (7.94, 9.54 and 9.37g/dl) in the three different chicken breeds of Fayoumi, Assil and Local chickens. Similar works in ducks was reported by Datta *et al.* (1994) gave slightly lower values.

2.12.3 Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)

The MCV is a measure of the average size of Red Blood Cells while the MCH is a calculation of the average amount of oxygen-carrying haemoglobin inside a red blood cell and the MCHC is a calculation of the average percentage of haemoglobin inside a red cell. These erythrocyte indices have shown to play vital roles in both the health and in the immunological status of birds. Talebi *et al.* (2005) reported that MCV and MCH decreased during breeding period in the four broiler strains with regards to increasing age and MCHC fluctuated in the study. Similar fluctuation of MCHC, which was observed for all the broiler strains during the study, had also been reported (Kral and Suchy, 2000, Islam *et al.*, 2004). In a report by Ladokun *et al.* (2008) on MCV, the authors reported values of 89.10fl and 81.60fl for normal and naked neck genotypes and values of 28.90pg and 27.20pg on the same genotypes for MCH and values of 32.41% and 33.37% for MCHC. In similar findings for MCV, Isidahomen and Njidda (2012), reported values of 134.02fl, 141.19fl and 134.91fl for normal, frizzle and naked neck genotypes. The authors also reported values of 44.53pg, 48.57pg and 45.32pg for MCH and 33.42%, 34.37% and 33.52% for MCHC.

2.13 BIOCHEMICAL PARAMETERS

Blood biochemical analysis is important in the diagnosis and clinical monitoring of diseases (Karesh *et al*., 1997). Recent studies have shown that there were no differences in serum parameters among juvenile males and females (Schmidt *et al.,* 2007) and serum calcium concentrations were found to decrease during peak egg production of the chickens (Peebles *et al.,* 2009). In a similar report, Isidahomen *et al.* (2011) reported no significant genetic effect of calcium and phosphorus but reported inconsistency for cholesterol.

Glucose and cholesterol produce energy. Ibrahim *et al.* (2012) who reported on cholesterol in Local Saudi chicken of different ages (adult and young) and between the two sexes (male and female) revealed that at both ages the male chicken had higher values (82.8mg d/L and 75.2mg d/L) when compared to the female (71.8mg d/L and 63.4mg d/L). Glucose value obtained was high in Local Saudi chickens than reference values recorded in other birds. Ritchie *et al.* (1994) observed that the glucose level in young chickens was lower than those of matured chickens and that glucose level of females are lower than that of the male chickens. Abdi-Hachesoo *et al.* (2013) obtained value of 3.87g/dl in male adult indigenous chickens which was similar to that reported by Elagib *et al.* (2012) who did not find significant differences in total protein values when comparing male and female indigenous Sudan chickens. However, Meluzzi *et al.* (1992) demonstrated that total protein in females was higher than males' broiler chickens. Similarly, the total protein in female Thai indigenous bird was higher than in males (Simaraks *et al.*, 2004).

Kaneko (2008) reported that the values of Aspartate transaminase (ALT) and Alanine aminotransferase (AST) in chickens were 5-15 U/L and 174.8 U/L, respectively. In the report of Abdi-Hachesoo (2013), ALT and AST values were 7.8 U/L and 191 U/L in males and 7.2 U/L and 125.2 U/L in females, respectively with ALT and AST values being higher in males.

Plasma proteins are formed by lymphocytic tissues and the liver. The plasma protein concentration is usually lowest at birth in animals being commonly less than 5.0g/dl. In chicken (*Gallus gallus domesticus*), the plasma protein as reported by Jain (1993) ranged from 4.0 to 5.5g/dl with an average of 4.5g/dl. Abdi-Hachesoo *et al.* (2013) reported a value of 3.87g/dl in male adult indigenous chickens similar to the findings of Elagib *et al.* (2012) who found no significant differences in total protein values between male and female indigenous chickens of Sudan. In a similar work, Orunmuyi *et al.* (2011) obtained higher values in females when comparing plasma protein values in the Nigeria indigenous chickens of different sex, age and genotypes. This was attributed to increase concentration of the plasma protein concentration just before egg production since the proteins produced in the liver (vitellogenin and lipoproteins) are transported in the blood and incorporated into the oocytes of the ovary (Lumeij, 1997).

2.14 INSULIN GROWTH FACTOR

2.14.1 The IGF ligands

Insulin growth-like factors (IGFs), including IGF-1 and IGF-2, are small polypeptide hormones with an approximate molecular weight of 7 kDa (Daughaday and Rotwein, 1989). These growth factors and their receptors are essential players in the biological process of the proliferation, regeneration, differentiation, and metabolism of myogenic cell lines from different species (Florini *et al.,* 1996). IGFs are the circulating growth factors/polypeptides which belong to a family of soluble peptides known as somatomedins (mediators of growth) which makes them one of the most important factors required for growth (Marquardt *et al.*, 1981). IGF-1 and IGF-2 are produced by a variety of cells such as liver, fat, muscle, kidney, brain, skin, gonads, heart (Han *et al.*, 1987). Chicken insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) are polypeptidic hormones that exert their function by binding to specific type 1 receptors (Zhou *et al*., 1995). The metabolic effects of avian IGFs include an increased amino acid and glucose uptake and the up regulation of DNA and protein synthesis (McMurtry, 1998) and it has potent ability to prevent programmed cell death (apoptosis) during normal development and also during stress or disease Vincent and Feldman (2002). The synthesis of IGF-1, IGF-2 and insulin is regulated by distinct mechanisms (Jones and Clemmons, 1995). Unlike insulin and other peptide hormones, IGFs are not synthesized and stored within specialized cells in a given tissue, but are ubiquitously produced and released by virtually every tissue. Insulin concentration *in vivo* is primarily gauged by blood glucose fluctuation.

2.14.2 Insulin Growth-like Factor-1 (IGF-1)

The IGF-1 gene play important roles in growth of multiple tissues, including muscle cells (myocyte differentiation cell multiplication), cartilage (chondrocyte colony formation, alkaline phosphatase activity) and bone (osteoblast division and proliferation) (Zapf and Froesch, 1999) and its synthesis is regulated by a variety of factors, including pituitary growth hormone (GH). Several studies have shown that circulating IGF-1 affects growth rate in poultry (Goddard *et al.,* 1988; Scanes *et al.,* 1989; Ballard *et al.,* 1990). Insulin-like growth factor 1 (IGF-1) is important for regulating growth, development and metabolism in humans and animals. In humans, Yang *et al.* (2008) identified critical effects of IGF-1 on adult height variation in Caucasians. For dogs, a single IGF-1 allele is the major determinant of body size (Sutter *et al.,* 2007; Boyko *et al.,* 2010). It was confirmed by Reyna *et al*. (2010) that a single nucleotide polymorphism in the promoter region of bovine IGF-1 is associated with three specific growth traits in the Charolais breed while the administration of IGF-1 to rats increased protein synthesis and body growth (Tomas *et al*., 1998). In case of poultry, Tang *et al.* (2010) found that a single nucleotide polymorphism (SNP) in the IGF-1 gene was significantly associated with body weight in chickens, and Bian *et al*. (2008) also reported that haplotypes based on three IGF-1 SNPs were associated with body weight traits in chicken.

2.14.3 Allele Frequency and Genotypic Frequency in Different Chickens Populations

Hossein and Mohsen (2011) reported A and B allele frequencies of 0.51and 0.49 in Mazandaran native chicken of the IGF-1 gene respectively and stated the presence of polymorphisms in IGF-1 segment, as previously reported by Abbasi and Kazemi (2011) and Li *et al*. (2010). The genotypic frequencies within 100 chickens examined was 25.88 for AA, 23.89 for BB and 50.23 for AB with gene frequencies for A and B alleles of 0.51 and 0.49 and the chi square (χ^2) test of 2.71 which corresponded with the principle of Hardy-Weinberg equilibrium for the population considered. The observed and expected heterozygosis in the report was 0.42 and 0.50, respectively. In another study by Shah *et al.* (2012), it was reported that the genotypic frequencies in the samples were 0.066, 0.166 and 0.766 and allelic frequencies were 0.15 and 0.85 respectively on broiler chickens.

Amills *et al*. (2003) reported similar result in Black Penedesenca chicken strain for genotypic and allelic frequencies and observed high χ^2value in the sample which showed that the population was not in Hard Weinberg equilibrium for both IGF-1 and 2 genes.

2.14.4 Associations of IGF-1 Genes in Chicken with Growth Traits and Reproduction

Growth is a complex process that involves the regulated coordination of a wide diversity of neuro-endocrine pathways. There are obvious physiological connections between body weight homeostasis and the reproductive axis in both sexes. The rate of sexual maturation is much more closely associated with body growth or weight than with chronological age (King, 2000). There are ample evidences suggesting that IGFs like IGF-1 might influence growth rate, body composition, and lipid metabolism in poultry (McMurtry, 1998; Tomas *et al*., 1998; Beccavin *et al.*, 2001). In the report of Li *et al.* (2006), it was observed in IGF Binding protein 2 (IGFBp2) that from week 1 to week 12, birds with the BB genotype had higher weight followed by the AB genotype and the AA when growth, skeletal and body composition traits were considered. Similarly, Wang *et al.* (2004) reported that Ningdu yellow chicken and Wanzhai yellow chicken that the BB genotype had heavier body weight at four months of age followed by the AB and the AA genotype. In another report by Yan *et al*. (2002), association between phenotypic variation and several growth and carcass traits with one polymorphism at exon 2 of chicken when the IGF-2 gene was considered. Also in a similar report, Zhou *et al.* (2005) revealed that the allele A had positive effect than allele B on economical traits.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION OF THE STUDY AREA

The study was conducted at the Poultry Unit of the Department of Animal Science Research Farm, Faculty of Agriculture, Ahmadu Bello University, Zaria. The geographical location of Zaria is in the Northern Guinea Savannah of Nigeria which lies between latitude 11º 09’ 06” N and longitude 7º 38’ 35” E (Ovimaps, 2012). The annual rainfall of 1100mm starts from late April and early May and ends in mid October. The peak of the rainy season is between June and September, which is followed by the hamattan period of cool and dry weather which lasts from October to January. This is then followed by hot-dry weather from February to April. The maximal temperature varies from 26ºC to 35ºC depending on the seasons, while the mean relative humidity during the hamattan period, hot season and the wet season are 21%, 37% and 77% respectively.

3.2 EXPERIMENTAL BIRDS

3.2.1 Base Population (Foundation Stock)

Ninety growing birds were sourced as base population from different markets in Kaduna (Zaria, Shika), Katsina (Giwa), and Kogi (Anyigba and Okene) States. Each of the three genotypes (Normal feather, Frizzle and Naked Neck) comprised of twenty five hens and five roosters.

3.2.2 Housing and Management

The birds were raised on the deep litter with each genotype in a separate pen and were fed grower mash with crude protein content of 16.08% and metabolizable energy of 2400 Kcal/Kg DM until when the hens started laying (sexual maturity) before they were given breeders mash with crude protein content of 18.05% and metabolizable energy of 2520Kcal/Kg DM (Table 3.2). Feed and water were given *ad-libitum.*

3.2.3 Vaccination and de-worming

The birds obtained as foundation flock were vaccinated against New Castle disease and treated against coccidiosis, de-wormed from endo-parasites and de-loused against ecto-parasites since they were obtained from different village markets. Other routine management practices were adhered to strictly and with those related to health care.

3.2.4 Mating and Egg Collection

Mating was done in the ratio of 1 rooster: 5 hens within each of the genotypes. Eggs laid in each pen were labeled based on the sire genotype (so as to have a pedigree record) and put into egg trays with the broad part of the egg pointing upwards and to keep it safe and avoid mechanical damage (cracking) and stored at room temperature of between 20 to 25ºC for 7days during the egg collection period between November and February before setting to obtain the F1 generation. Two hundred and seventy chicks were generated from the mating within the three genotypes of indigenous chicken. The mating are explained in the table 3.1

Table 3.1: Mating Scheme of the Foundation Stock for the Production of F1 Generation

Male	Female	Progeny
Normal feathered	Normal feathered	N
Frizzle feathered	Frizzle feathered	F
Naked neck	Naked neck	Na

N=Normal Feather, F=Frizzle, Na=Naked neck.

3.2.5 Incubation Process

Eggs laid were labeled with sire identification numbers and collected at an interval of one week and then taken to the hatchery. Settable eggs were sorted out sire-wise and within genotypes before setting. The eggs were set into trays after which they were fumigated with potassium permanganate (K_2Mno4) and Hydrogen peroxide (H_2O_2) in the fumigation room for about 30 minutes and later set into the incubator at a temperature of about 37ºC. The incubator used was an automated type (GQF incubator model 1520 Sportman) with capacity of 1488 for quails, 288 for chickens and 198 for geese.

Candling of the eggs to select the fertile ones was done at the 18th day after which the fertile eggs were transferred into the hatcher. Hatching trays were partitioned into a number of compartments according to sire genotype. Eggs belonging to the same genotype were put into the same compartment. After 3 days in the hatcher, the chicks were hatched.

3.2.6 Brooding and Rearing of the Progeny

Immediately after hatching, the chicks were wing tagged and pedigreed according to sire genotype. The chicks were then vaccinated against Mareks and Newcastle diseases. The birds were subjected to standard poultry practices of brooding, vaccination, provision of feed (Table 3.2) and water *ad lib.*

3.2.7 Management of F1Generation

The chicks were reared on the deep litter with each genotype in a separate pen and with electric bulb, kerosene stove and charcoal pots to generate heat for the chicks. The birds were fed with chick mash during 0 – 8weeks, grower mash between 8 – 20 weeks of age and breeder mash (Table 3.2) as from 20 weeks on wards. Both feed and water were offered *ad-libitum* through out the thirty two weeks period of the experiment.

Table 3. 2: Composition of the Experimental Diets

Ingredients	Chick Mash	Grower Mash	Breeder Mash
Maize %	45.00	34.45	43.45
Maize Offal %	10.00	10.00	10.00
Groundnut Cake %	13.05	10.00	13.50
Soya bean cake %	20.90	10.50	13.00
Wheat offal %	6.00	15.00	9.00
Rice offal %	0.00	15.00	0.00
Bone meal %	3.00	3.00	3.00
Limestone %	1.50	1.50	7.50
salt %	0.30	0.30	0.30
Premix %	0.25	0.25	0.25
Total	100	100	100
Calculated analysis			
ME(Kcal/ kg DM)	2652.45	2400.18	2520.08
CP %	20.99	16.08	18.05
CF	5.97	10.82	5.74
EE	3.40	3.81	3.50
Ca	1.42	1.72	3.56
P	0.55	0.56	0.60
Lysine	0.90	0.70	0.80
Methionine	0.36	0.24	0.35

Metabolizable Energy (ME) Crude protein (CP), Crude Fiber (CF), Ether Extra (EE), Calcium (Ca), Phosphorus (P)

3.3 BODY WEIGHT AND LINEAR BODY MEASUREMENTS

The birds were sexed at 8 weeks of age using the vent sexing method to separate males from female birds. The body weight of each of the cockerels was measured using a sensitive top loading weighing balance (Citizen Electronic Balance, Goldair) with maximum weight of 3kg sensitive at 0.1g monthly from 8 weeks to 32 weeks of age and morphometric traits were measured using ruler, flexible tape rule and venire caliper all in centimeters from 8 to 32 weeks of age. The traits measured included: Breast girth (BG), Body length (BL), Wing span (WS), Shank length (SL), Shank thickness (ST), Comb length (CL), Comb height (CH), Wattle width (WW) and Wattle lenght (WL). The linear body measurements and the anatomical reference points were measured and records taken as described by Gueye *et al*. (1998), Fayeye *et al*. (2006) and Teguia *et al*. (2008).

The measurements were taken as follows:

Linear body traits:

Body length: Distance from the tip of the beak through the body trunk to the toe.

Breast girth: Circumference round the chest from under the wings.

Wing span: Length of the wings from the scapula joints to the last digit of the wing.

Shank length: Distance between posterior aspects of the hock joint to the foot pad.

Shank thickness: Diameter of the tarso-metatarsus just below the spur.

Morphometric traits:

Comb length: The width of the comb along the head.

Comb height: Distance between the point of attachment of the comb to the head and its highest point.

Wattle width: The width of the wattle along the wattle at the base of the head.

Wattle length: Distance between the points of attachment of the wattle to the highest point.

Table 3. 3: The Vaccination Schedule used for the F1 Generation

Age of bird	Vaccination/medication/Route
Day 1	NDV(i/o) + Mareks vaccine (SC)
Day 1 – 15	Anti stress (Oral in water)
Day 10	IBDV – 1^{st} dose (Intra muscular)
Day 24	IBDV – 2^{nd} dose (Intra muscular)
Week 3 (for 5 days)	Coccidiostat (Oral in water)
Week 4	NDV (L)
Week 6 (for 5 days)	Coccidiostat
Week 7	NDV (K) 1^{st} dose + FPV
Week 8	Dewormer
Week 9 (for 5 days)	Coccidiostat
Week 12 (for 5 days)	Coccidiostat
Week 16	NDV (K) 1^{st} dose
Week 17 (for 1 day)	Dewormer

NDV (i/o) – Newcastle Disease Vaccine (intra occular); NDV (L) - Newcastle Disease Vaccine (Lasota); NDV (K) - Newcastle Disease Vaccine (Kumarov); IBDV – Infectious Bursal Disease Vaccine (Gumboro); FPV – Fowl Pox Vaccine.

3.4 SEMEN COLLECTION AND EVALUATION

Prior to the semen collection, twenty five cockerels from each of the genotype were randomly given preliminary training for 2 weeks starting from twenty one weeks of age (to get them acquainted to ejaculation) by the massage technique so as to ejaculate. During the training, cockerels that persistently defecated and those that do not emit semen or refused to erect their phallus were removed. The semen collections were done on twenty cockerels from each genotype which commenced early in the morning between the hours of 8:00am – 12:00am with an interval of four weeks apart. While in a sitting position, each bird was held between the legs then the abdomen was massaged with one hand and simultaneously stroking the lower back and tail feathers with the other hand by the collector. This action caused an erection of the copulatory appendages in the cloaca (phallus) with subsequent expulsion of semen. The semen was collected in a clean, plastic calibrated test tube. The samples were taken to the laboratory in cooler containing warm water at 37 - 40^oC to maintain the sperm cell in a viable state for immediate commencement of evaluation. Semen evaluation was carried out thrice from each cockerel at 24, 28 and 32 weeks of age.

3.4.1 Ejaculate Volume

The massage technique as described by Kalamah *et al*. (2002) was adopted to obtain adequate volume of semen from each cockerel. The volume of the ejaculate was read (in milliliters) directly from the calibrated tube held at eye level and was recorded against the tag number of each cockerel.

3.4.2 Ejaculate Colour and Consistency

Further visual assessment was done on each samples to determine the colour of semen which should be a homogenous creamy, milky or colourless (watery) devoid of pus or blood with a score of 1, 2 and 3, respectively.

3.4.3 Sperm Motility

The motility of the sperm cells (wave pattern of semen) of each sample collected was observed through a light microscope. A drop of the ejaculate was put on a glass slide and covered with a cover slip. This was then observed under the light microscope at x100 magnification. The result was recorded as described in Table 3.4 on the evaluation of semen for microscopic wave pattern.

Table 3. 4: Evaluation of Semen for Microscopic Wave Pattern

Motility	Appearance of pattern
0-20%	Spermatozoa are immotile. No wave pattern
20-40%	Stationary bunting or weak rotary movements are exhibited by spermatozoa. No wave pattern
40-50%	Oscillatory or rotary movements and fewer than 50% of the spermatozoa are in motion. Barely distinguishable wave or eddies.
50-80%	Progressive rapid movement of spermatozoa with slowly moving waves and eddies. Usually 50-80% of the spermatozoa must be progressively motile to produce wave and eddies.
80-90%	Vigorous, progressive movement with rapid and abruptly forming waves and eddies; indicating about 90% motile spermatozoa. Dark, distinct waves.
90-100%	Very vigorous forward motion. Extremely rapid waves and eddies indicating about 100% actively motile spermatozoa.

Source: Artificial Insemination (A.I) Unit, NAPRI, Shika, Zaria.

3.4.4 Sperm Concentration

Sperm concentration was taken as the number of spermatozoa contained in 1ml of ejaculate, and it was determined using the Neubauer Haemocytometer (Lake and Stewart, 1978). The haemocytometer contains grids of 25 large squares with each containing 16 smaller squares. The total number of smaller squares on the haemocytometer is 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot *et al.*, 1997). The freshly collected semen was diluted with buffered normal saline solution. Some other common solutions include 5% Triphenyl-tetrazolum chloride in physiological saline, 5% chlorozene in physiological saline, 5g $NaHCo_3$ and 1 ml of 35% concentrated formaldehyde made up to 100ml in Physiological Saline. Using an automatic micro pipette (aunopet), semen was drawn to the mark at the top of the bubble chamber. The preparations were thoroughly mixed by shaking and allowed to settle for 15–30 minutes. This incubation period increases the accuracy of the count so that all floating sperm cells must have settled down (Lake and Stewart, 1978). As a rule, during counting only those sperm cells that appear within the five-diagonal squares of the chamber were counted using the light microscope at x400 magnification. Those falling outside the chamber were ignored and not counted. A basic formula (Lake and Stewart, 1978) was then used to estimate the sperm concentration as given below;

Sperm Conc/ml = Total No. of sperm cells counted x multiplication factor ($x10^9$).

To obtain sperm concentration per ejaculate, the concentration per ml was multiplied by the volume of the ejaculate.

3.4.5 Semen pH

The hydrogen ion concentration (pH) of individual sample collected was determined using Chemo Craft pH paper. This is a pH paper calibrated from1 to 14 with different colours. One inch of the paper was inserted into each sample of the semen while in the tube for about 5seconds. It was then removed and checked for the corresponding colour match on the pH paper chart. The corresponding pH number for the colour on the chart was recorded as the pH of the sample.

3.4.6 Live and Dead Ratio

The live and dead ratio was estimated by the preparation of a smear of individual semen sample using Eosin Nigrosin stain on the farm and read in the laboratory. A drop of semen was diluted and placed on a clean glass slide using automatic pipette. A drop of Eosin Nigrosin solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the semen and the Eosin Nigrosin solution. A quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide. This was allowed to dry and thereafter labeled. This was done for each sample and they were later mounted on the microscope for counting the live and dead sperm cells. The principle is that the dead sperm cells take up the stain and appear purple in colour while the live sperm cells reject the stain and remain unstained (Hancock, 1951).

3.4.7 Sperm Morphological defects

The same slides prepared for live and dead ratios were used for the morphology determination. Live and abnormal spermatozoa percentages were counted using hand counter (tally). A total of fifty spermatozoa were examined for each sample to see the various defects under a light microscope at x100 magnification. The total number of abnormal cells were counted and recorded. Types of abnormalities observed were: detached mid- piece and tail, detached head, mid piece droplet, coiled and bent tail, and acrosomal abnormalities. Acrosomal abnormalities were determined by using smears made from the raw semen and stained by Giemsa stain according to Watson (1975).

3.5 BLOOD COLLECTION AND EVALUATION

Blood samples of about 5ml were collected from the wing vein of the cockerels using syringe and needle and poured into a plain test tube which contains anticoagulant (Ethylene Diamine Tetra Acetic Acid) for the evaluation of haematological parameters and for the determination of the insulin-like growth factor-1 (IGF- 1) DNA polymorphism while for the biochemical parameters, plain test tubes were used which had no anticoagulant. The collection was done between the hours of 09:00 to 10:00 to minimize any variation in blood chemicals caused by the circadian rhythms. The haematological analysis was carried out in the Parasitological Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The biochemical analysis was similarly carried out in the Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria and the Insulin-like Growth Factor-1 (IGF-1) DNA polymorphism was carried out at the Centre for Biotechnology Research and Training (CBRT) Laboratory, Ahmadu Bello University, Zaria.

3.5.1 Evaluation of Haematological Parameters

The following haematological parameters were analyzed and calculated using the following methods; haemoglobin (Hb), white blood cells (WBC), pH, percentage neutrophils, lymphocytes and eosinophils using the cyanomethamoglobin method (Schalm *et al.*, 1975). The packed cell volume (PCV) was analyzed using the microhaematocrit method. Total protein (TP), was determined using the haemcytometer. Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were estimated.

3.5.2 Evaluation of Biochemical Parameters

Biochemical parameters were evaluated for glucose, creatinine, cholesterol, electrolyte profile (sodium, phosphorus, calcium and chloride) and liver function test (Aspartate aminotransferase, Alanine amino transferase and Alkaline phosphatase) for each of the samples collected from the three genotypes. Serum was separated from the blood plasma after centrifugation at 3,000 rpm for 15 minutes and stored in the freezer at -10ºC until assay. Alkaline phosphatase, aspartate aminotransferase (AST) enzyme activity level was determined using the Reitman-Frankel AST method, Alanine amino transferase, total cholesterol were all carried out as described by Cheesbrough (1991). Serum sodium and potassium ions were determined by flame emission spectrometry as described by Cheesbrough (1991), and chloride level was analylsed by the method of Schales and Schales (1941).

3.5.3 Insulin-Like Growth Factor-1 (IGF-1) DNA Isolation

Genomic DNA was isolated by using Thermo Scientific GeneJET Genomic DNA Purification kit. The protocol used is as described for DNA purification from nucleated blood. The DNA purification procedure was as follows; 10 µL of nucleated blood was taken for each blood samples collected and the volume was adjusted to 200 µL with the addition of PBS (phosphate buffer solution). 20 µL of Proteinase K Solution was added to 200 µL of whole blood and PBS and mixed by vortexing automatically.

Then 400 µL of Lysis solution was added and thoroughly mixed by vortex to obtain a uniform suspension. The mixture was incubated at 56ºC for 10 minutes while vortexing occasionally until the cells were completely lysed.

Then 200 µL of ethanol (96-100%) stored under room temperature was added and mixed by pipetting. The prepared mixture was transferred to the spin column and was centrifuged for 1 minute at 6,000 × g (~8,000 rpm). The collection tube containing the flow-through solution was discarded and the column was transferred into a new 2 ml collection tube and 500 µL of Wash Buffer 1 (with ethanol added) was added. The mixture was centrifuged for 1 minute at 8,000 × g (~10,000 rpm) and the flow-through was discarded and column was placed back into the collection tube.

Then 500 µL of Wash Buffer 2 (with ethanol added) was added to the column and centrifuged for 3 minutes at maximum speed 8,000 × g (~10,000 rpm). The collection tube containing the flow-through solution was then discarded and the column was transferred to a sterile 1.5 mL microcentrifuge tube.

200 μL of Elution Buffer was added to the centre of the column membrane to elute genomic DNA. It was incubated for 2 minutes at room temperature and centrifuged for 1 minute at 8,000 × g (~10,000 rpm). The purification column was discarded and eluted DNA was stored at -20ºC.

A Spectrophotometer was used for investigating quality and quantity of DNA. The purity and concentration of DNA samples was estimated using UV-visible range spectrophotometer. DNA was also examined by loading samples on 0.75% agarose gel and visualizing the band under gel documentation system.

Polymerase chain reaction (PCR) and restricted Fragment length Polymorphism (RFLP)

The Insulin- like growth factor- 1 gene was ascertained and the level of concentration of the gene located from the digested PCR. The PCR-RFLP of the IGF-1 genes was amplified to understand the polymorphic forms of the genes by using the PCR product which was digested using EcoR1 restriction enzymes that has cutting site on the fragment. This is so that the different fragment lengths are shown.

The PCR primers (forward: 5′-AGCTGTTCGAATGATGGTGTTTT-3′; reverse: 5′-GCCCCAGCATTCTCTTTCCTT-3′) for chicken IGF-1 gene were used. According to Nie *et al.* (2005), the sequences of the candidate genes of the somatotropic axis were from Genbank. The primers had been designed using the GENETOOL program. Primers were synthesised through a commercial service (BioNEER Corp., USA). As reported by Amills *et al*. (2003).

The PCR was performed in a total volume of 50 μL in each PCR tube, containing 25 μL of 2 x PCR master mix, 1 μL each of the forward and reverse primers, 1 μL of genomic DNA and 22 μL of nuclease free water. The PCR tube was put in FTGENE5D thermocycler (by TECHNE Cambridge) and the PCR condition was set at 94ºC for 5 minutes for initial denaturing, followed by 35 cycles at 94ºC for 30 seconds for denaturing, 52ºC for 45 seconds for annealing, and 72ºC for 1.30 minutes extension, and a final extension step at 72ºC for 5 minutes.

Agarose gel was prepared by weighing 0.75% of agarose on a sensitive weighing scale and it was diluted with 1 x TAE buffer in side a conical flask. The agarose was solubulized by heating in a microwave oven for two minutes. The solution was then allowed to cool to 60ºC before 5µl of GR Green stainer was added which inflorescence the gel. The solution was then poured into the electrophoresis tank with sixteen combs to create the wells.

Following the preparation of the agarose gel, the PCR products were removed from the thermocycler, 10 μL of DNA PCR ladder was loaded in the first well and 10 μL of each PCR product in each well. Then the electrophoresis tank was filled with distilled water and connected to electrical source to run for 40 minutes at 75-100 volts. The gel was removed and viewed under UV light to observe the band. The restriction patterns were visualized by 0.75% agarose gel electrophoresis; gels were stained with GR Green DNA Stainer and were visualized and photographed using a gel documentation system (Uvipro Silver, Uvitec).

3.6 STATISTICAL ANALYSIS

Model for investigating the effect of genotype and age on growth and semen quality characteristics is as shown:

$$Y_{ijk} = \mu + G_i + A_j + e_{ijk}$$

Where;

Y_{ijk} = observation on the i^{th} genotype at the j^{th} age.

μ = overall population mean.

G_i = Fixed effect of genotype (i=1, 2, 3).

A_j = Fixed effect of age (j=24, 28, 32).

e_{ijk} = Random error.

Model for the Haematological and Biochemical Studies:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where;

Y_{ij} = observation on the i^{th} genotype.

μ = overall population mean

G_i = effect of the i^{th} genotype (i=3: Normal feather, Frizzle, Naked neck)

e_{ij} = random error

Genotype and allele frequencies of the IGF-1 gene calculation:

The gene frequencies were calculated according to Hardy-Weinberg's equation as follows:

$$p = 2(AA) + (AB) / 2N \quad \text{and} \quad q = 2(BB) + (AB) / 2N$$

Where;

p = the gene frequency of allele A;

q = the gene frequency of allele B and

N = the total number of birds tested.

A chi-squared test for goodness-of-fit was performed to verify if genotype frequencies agree with Hardy-Weinberg's equilibrium (HWE) expectations using the following formula:

$$\chi^2 = \frac{\sum (O - E)^2}{E}$$

Where;

χ^2= Chi square

$\sum$ =Summation

O= Observed frequency

E= Expected frequency

Relationship between chicken IGF-1 marker, body weight and semen quality at various ages:

The following model was used:

$$Y_{ijk} = \mu + I_i + G_j + e_{ijk}$$

Where;

Y_{ijk} = observation on the i^{th} IGF-1 marker on the j^{th} genotype.

μ = overall population mean

I_i = effect of the i^{th} IGF-I marker

G_j = effect of the j^{th} genotype

e_{ijk} = random error

All data for growth, semen quality characteristics were subjected to the GLM procedure of SAS (2002). Duncan's Multiple Range Tests of SAS (2002) was used to separate the significant means. Correlations between the growth and body linear measurements and semen quality were evaluated using the correlation procedure of the same SAS software. For the haematological and biochemical analyses, the values were evaluated and mean separated by means of multiple comparisons using Tukey- Honestly Significant Difference (HSD) test of the SAS 9.0 package. Also, the differences among genotypic and allelic frequencies of IGF-I were analyzed using a χ^2 test, which was performed using the same SAS 9.0 package (SAS, 2002).

The phenotypic correlations between traits were estimated using formulae:

$$\sigma_{xy} = \frac{Cov_{xy}}{\sqrt{var_x \, var_y}}$$

Where;

Cov_{xy} = Covariance of traits x and y

Var_x = Variance of trait x

Var_y = Variance of trait y

CHAPTER FOUR

4.0 RESULTS

4.1 GROWTH PERFORMANCE AND SEMEN QUALITY CHARACTERISTICS OF THE THREE GENOTYPES

4.1.1 Body Weight and Growth Traits

Least Square Means and standard errors for the growth traits studied are presented in Tables 4.1 and 4.2 for the three genotypes from 8 to 32 weeks. The results showed that at 8 weeks of age, there were significant (P<0.05) effect of genotype on BW, BG, BL and WL while other traits (SL, WW, CL, CH, WS and SD) showed no significant (P>0.05) difference. At 12 weeks, BW, SL, BL, WS and WL showed significant (P<0.05) difference with the normal feather being the heaviest having an average BW of 600.0±19.66g while the other traits showed no significant (P>0.05) difference. At 16 weeks of age, significant (P<0.05) difference were obtained for BW, BG, BL, WL and WS with frizzle and naked neck having heavier BW when compared to the normal feathered birds while other traits were not significant. BL and WL were lower in the naked neck while WS was highest in the frizzle. Similarly, the result at 20 weeks of age showed that significant (P<0.05) differences were obtained for BW, BG, SL, BL and WS. BW was highest in the normal feathered birds while naked neck recorded the best BG and frizzle recorded the best BL but WL was not significant in the three genotypes. However, the frizzle recorded the best WS.

At 24 weeks, the result (Table 4.2) showed that apart from WW, CL, CH and SD other traits were significantly (P<0.05) different. However, at 28 and 32 weeks all the traits showed significant ($P<0.05$) difference with the exception of SD. The normal feather genotype had better performance for BW and other observed traits from 24 to 32 weeks (Table 4.2).

Table 4.1: Least Square Means (±SE) of Body Weight (g) and Body Linear Measurement (cm) in the Three Nigerian Genotype Cockerels from 8 to 20 weeks of Age

Age(weeks)	Traits	Genotype Normal	Frizzle	Naked neck	LOS
8	BW	333.37 ± 9.86^{ab}	348.7 ± 13.95^{a}	310.46 ± 10.99^{b}	*
	BG	12.69 ± 0.16^{b}	12.56 ± 0.22^{b}	13.26 ± 0.18^{a}	*
	SL	4.63±0.07	4.67±0.09	4.76±0.08	NS
	BL	27.27 ± 0.3^{a}	27.65 ± 0.42^{a}	26.61 ± 0.33^{b}	*
	WL	0.90 ± 0.05^{a}	0.90 ± 0.07^{a}	0.73 ± 0.05^{b}	*
	WW	0.22±0.09	0.21±0.04	0.21±0.03	NS
	CL	0.88±0.04	0.86±0.05	0.77±0.04	NS
	CH	2.39±0.06	2.45±0.09	2.26±0.07	NS
	WS	35.85±0.4	36.58±0.56	35.34±0.45	NS
	SD	0.78±0.01	0.78±0.02	0.75±0.02	NS
12	BW	600.0 ± 19.66^{a}	555.65 ± 27.81^{b}	578.27 ± 29.92^{ab}	*
	BG	17.5±0.22	17.1±0.31	17.8±0.24	NS
	SL	7.04 ± 0.12^{ab}	6.71 ± 0.16^{b}	7.21 ± 0.13^{a}	*
	BL	32.27 ± 0.29^{ab}	32.65 ± 0.41^{a}	30.61 ± 0.33^{b}	*
	WL	1.00 ± 0.04^{a}	1.00 ± 0.06^{a}	0.83 ± 0.05^{b}	*
	WW	0.82±0.03	0.81±0.04	0.81±0.03	NS
	CL	1.39±0.04	1.36±0.06	1.27±0.04	NS
	CH	2.39±0.06	2.45±0.08	2.26±0.06	NS
	WS	38.47 ± 0.43^{b}	39.38 ± 0.61^{a}	38.14 ± 0.48^{b}	*
	SD	0.88±0.01	0.89±0.02	0.86±0.02	NS
16	BW	695.63 ± 23.82^{b}	743.91 ± 33.69^{a}	733.11 ± 26.56^{a}	*
	BG	20.43 ± 0.37^{b}	22.17 ± 0.53^{a}	23.28 ± 0.42^{a}	*
	SL	8.34±0.15	8.39±0.21	8.69±0.16	NS
	BL	35.17 ± 0.29^{ab}	35.55 ± 0.41^{a}	34.51 ± 0.33^{b}	*
	WL	1.2 ± 0.04^{a}	1.2 ± 0.06^{a}	1.03 ± 0.05^{b}	*
	WW	0.91±0.03	0.91±0.04	0.91±0.03	NS
	CL	1.58±0.04	1.56±0.06	1.47±0.04	NS
	CH	2.89±0.06	2.95±0.08	2.76±0.06	NS
	WS	44.37 ± 0.49^{b}	45.68 ± 0.7^{a}	44.44 ± 0.55^{b}	*
	SD	1.03±0.01	1.03±0.02	1.00±0.02	NS
20	BW	1117.8 ± 32.1^{a}	1046.7 ± 45.39^{b}	1061.89 ± 35.79^{b}	*
	BG	23.05 ± 0.28^{b}	20.67 ± 0.39^{c}	24.39 ± 0.31^{a}	*
	SL	9.28 ± 0.15^{a}	9.08 ± 0.21^{b}	9.64 ± 0.16^{a}	*
	BL	39.57 ± 0.29^{ab}	39.95 ± 0.41^{a}	38.91 ± 0.33^{b}	*
	WL	2.00±0.04	2.00±0.06	1.83±0.05	NS
	WW	2.31±0.03	2.31±0.04	2.31±0.03	NS
	CL	2.89±0.04	2.86±0.06	2.77±0.04	NS
	CH	4.99±0.06	5.05±0.08	4.86±0.06	NS
	WS	48.87 ± 0.49^{b}	50.18 ± 0.7^{a}	48.93 ± 0.55^{b}	*
	SD	1.16±0.01	1.16±0.02	1.13±0.02	NS

^{abc} =Means having different superscript along rows for a given trait differ significantly (P<0.05), BW=Body weight, SL=Shank length, BL=Body length, WL=Wattle length, WH=Wattle width, CL=Comb length, CH=Comb height, WS=Wing span, SD=Shank diameter, LOS = Level of significance.

Table 4.2: Least Square Means (±SE) of Body Weight (g) and Body Linear Measurement (cm) in the Three Nigerian Genotype Cockerels from 24 to 32 weeks of Age

Age(weeks)	Genotype				
	Traits	**Normal**	**Frizzle**	**Naked neck**	**LOS**
24	BW	1167.80 ± 32.1^{a}	1096.74 ± 45.39^{b}	1111.89 ± 35.79^{b}	*
	BG	25.15 ± 0.28^{b}	22.76 ± 0.39^{c}	26.48 ± 0.31^{a}	*
	SL	9.78 ± 0.15^{ab}	9.57 ± 0.21^{b}	10.13 ± 0.16^{a}	*
	BL	44.96 ± 0.29^{ab}	45.00 ± 0.41^{a}	43.96 ± 0.33^{b}	*
	WL	2.53 ± 0.04^{a}	2.53 ± 0.06^{a}	2.36 ± 0.05^{b}	*
	WW	2.56±0.03	2.56±0.04	2.56±0.04	NS
	CL	3.72±0.04	3.69±0.06	3.60±0.04	NS
	CH	6.21±0.06	6.27±0.08	6.08±0.06	NS
	WS	53.87 ± 0.49^{b}	55.18 ± 0.7^{a}	53.87 ± 0.55^{b}	*
	SD	1.24±0.01	1.24±0.02	1.21±0.02	NS
28	BW	1227.80 ± 32.1^{a}	1146.74 ± 45.39^{b}	1164.89 ± 35.79^{b}	*
	BG	25.65 ± 0.28^{b}	23.16 ± 0.39^{c}	26.93 ± 0.31^{a}	*
	SL	10.03 ± 0.15^{ab}	9.78 ± 0.21^{b}	10.34 ± 0.16^{a}	*
	BL	47.82 ± 0.29^{ab}	48.00 ± 0.41^{a}	47.06 ± 0.33^{b}	*
	WL	2.93 ± 0.04^{a}	2.83 ± 0.06^{a}	2.66 ± 0.05^{b}	*
	WW	5.76 ± 0.03^{a}	5.56 ± 0.04^{b}	5.66 ± 0.04^{b}	*
	CL	6.11 ± 0.04^{a}	6.11 ± 0.06^{a}	5.90 ± 0.04^{ab}	*
	CH	9.51 ± 0.06^{a}	9.47 ± 0.08^{ab}	9.28 ± 0.06^{b}	*
	WS	55.16 ± 0.49^{b}	56.38 ± 0.7^{a}	55.13 ± 0.55^{b}	*
	SD	1.26±0.01	1.26±0.02	1.23±0.02	NS
32	BW	1285.8 ± 32.1^{a}	1206.74 ± 45.39^{b}	1222.89 ± 35.79^{ab}	*
	BG	26.44 ± 0.28^{b}	23.96 ± 0.39^{c}	27.73 ± 0.31^{a}	*
	SL	10.33 ± 0.15^{ab}	10.03 ± 0.21^{b}	10.64 ± 0.16^{a}	*
	BL	51.82 ± 0.15^{ab}	52.00 ± 0.41^{a}	51.06 ± 0.33^{b}	*
	WL	3.13 ± 0.04^{a}	3.03 ± 0.06^{a}	2.86 ± 0.05^{b}	*
	WW	6.27 ± 0.03^{a}	6.06 ± 0.04^{b}	6.16 ± 0.04^{b}	*
	CL	6.72 ± 0.04^{a}	6.59 ± 0.06^{ab}	6.50 ± 0.04^{b}	*
	CH	10.31 ± 0.06^{a}	10.26 ± 0.08^{ab}	10.09 ± 0.06^{b}	*
	WS	57.17 ± 0.49^{b}	58.38 ± 0.02^{a}	57.14 ± 0.55^{b}	*
	SD	1.31±0.01	1.31±0.02	1.28±0.02	NS

^{abc} = Means having different superscript along rows for a given trait differ significantly ($P<0.05$), BW=Body weight, SL=Shank length, BL=Body length, WL=Wattle length, WW=Wattle width, CL=Comb length, CH=Comb height, WS=Wing span, SD=Shank diameter, LOS = Level of significance.

4.1.2 Semen Characteristics of the Three Genotypes

Table 4.3 shows the least square mean (±SE) for semen characteristics of the three genotypes studied. The mean semen volume (ml) obtained in this study showed that normal feathered genotype had semen volume range of between 0.12±0.03 and 0.32±0.05, the frizzle genotype had 0.08±0.04 and 0.24±0.03 and the naked neck genotype had between 0.12±0.03 and 0.22±0.04. The normal feather and naked neck had significantly lower semen volume than the frizzle at 24 weeks but the trend was reversed at 28 and 32 weeks. Semen colour which was creamy showed no significant (P>0.05) difference at week 24 and between the three genotype but there was significant (P<0.05) difference at week 28 and 32 with creamy and milky colour respectively.

Semen motility was significantly (P<0.05) affected by genotype and ranged between 74.60 and 91.8% for the normal feathered genotype and between 62.60 and 90.0% for the frizzle genotype and between 79.88 and 90.0% for the naked neck genotype. The normal feathered and naked neck genotype had higher semen motility than the frizzle genotype. The pH also had significant (P<0.05) genotype effect at 24 and 32 weeks of age although no significant (P>0.05) genotype effect was observed at 28 weeks of age. The values obtained for the three genotypes were between the neutral and alkaline pH range however the naked neck genotype had an acidic pH value (6.88) at 24 weeks of while the normal and frizzle genotype also had the same acidic pH values (6.60) at 32 week of age. The concentration and live cells also had a significant (P<0.05) genotype effect at the various ages with the normal and frizzle having better semen concentration over the naked neck while for the live cells, the naked neck genotype had better live cells.

4.1.3 Morphological Defects

Table 4.4 shows the morphological defects related to the cockerel spermatozoa. There were significant ($P<0.05$) differences in all the parameters considered with reference to age and genotypes. The Acrosome swelling and the detached head increased from 24 to 32 weeks of age for Normal and Frizzle genotypes but the values decreased for the two parameters at 32 weeks of age for the naked neck. The mid piece increased between 24 and 28 weeks of age for the three genotypes. This increase was maintained up to 32 weeks of age for Normal and Frizzle genotypes while naked neck dropped significantly from 4.00 to 1.82%. The bent tail was increasing as the ages of the cockerel in the three genotypes increased. The curled tail for the Normal feathered roosters took a similar trend to that of bent tail. However, for the Frizzle and Naked necks, values increased as the age increased from 24 to 28 weeks of age but reduced at 32 weeks of age. The full tail also had an increasing value as age advanced but the increasing trend was maintained for only Frizzle genotype while Normal and Naked neck had a reduction in their defects.

The overall abnormality shows that the naked neck genotype had the highest abnormal sperm cells of 22.50% at 28 weeks of age while the normal and the frizzle genotype had 21.61 and 21.67% respectively.

4.1.4 Interaction between age and genotype on the semen quality characteristics

Table 4.5 shows the interaction between age and genotype on the semen quality characteristics between the three genotypes of the Nigerian indigenous cockerels. The interaction significantly affected only semen volume ($P<0.05$). Concentration and live cells decreased as age increased while motility increased from 24 to 28 weeks of age.

Table 4.3: Least Square Means (± SE) for Semen Characteristics in the Three Nigerian Genotype Cockerels at 24, 28 and 32 Weeks

Age	Genotype				
24	**Traits**	**Normal**	**Frizzle**	**Naked neck**	**LOS**
	Volume(ml)	$0.12±0.03^{b}$	$0.24±0.03^{a}$	$0.12±0.03^{b}$	*
	Colour	1.14±0.17	1.40±0.15	1.25±0.17	NS
	Motility (%)	$85.71±9.54^{ab}$	$90.0±7.98^{a}$	$79.88±8.93^{b}$	*
	pH	$7.43±0.2^{a}$	$7.20±0.17^{a}$	$6.88±0.2^{b}$	*
	Conc($x10^{9}$/m)	$6.42±11.38^{b}$	$7.02±9.52^{a}$	$5.73±10.65^{c}$	*
	Live (%)	$54.3±11.11^{c}$	$75.0±9.2^{b}$	$85.0±10.39^{a}$	*
28	Volume(ml)	$0.32±0.05^{a}$	$0.17±0.0^{b}$	$0.18±0.05^{b}$	*
	Colour	$1.20±0.35^{a}$	$1.67±0.4^{b}$	$1.80±0.35^{b}$	*
	Motility (%)	$91.8±5.75^{a}$	$83.3±7.4^{b}$	$81.0±5.75^{b}$	*
	pH	7.20±0.18	7.00±0.23	7.20±0.18	NS
	Conc($x10^{9}$/ml)	$5.27±12.26^{a}$	$4.13±15.8^{b}$	$4.30±12.26^{b}$	*
	Live (%)	$78.0±10.47^{a}$	$60.0±13.52^{b}$	$80.0±10.47^{a}$	*
32	Volume (ml)	$0.18±0.04^{a}$	$0.08±0.04^{b}$	$0.22±0.04^{a}$	*
	Colour	$1.20±0.22^{a}$	$1.00±0.22^{a}$	$2.00±0.25^{b}$	*
	Motility (%)	$74.60±5.20^{b}$	$62.60±5.82^{c}$	$90.00±5.20^{a}$	*
	pH	$6.60±0.28^{b}$	$6.60±0.28^{b}$	$7.00±0.32^{a}$	*
	Conc ($x10^{9}$/ml)	$4.31±12.24^{a}$	$4.31±12.24^{a}$	$3.80±13.68^{b}$	*
	Live (%)	$73.0±16.75^{a}$	$57.0±16.75^{c}$	$67.50±18.73^{b}$	*

[abc] = Means with different superscript within the same row for a trait differ significantly ($P<0.05$), Conc. =Concentration, LOS = Level of significance.

Table 4.4: Morphological Defects in the Semen of the Three Nigerian Genotype Cockerels at Various Ages

			Genotype		
Age (Weeks)	**Sperm segment**	**Defects (%)**	**Normal**	**Frizzle**	**Naked neck**
24	Acrosome	Acro. Swelling	1.39 ± 0.77^{b}	0.89 ± 0.64^{c}	1.77 ± 0.72^{a}
	Head	DH	0.50 ± 0.56^{b}	0.84 ± 0.46^{a}	0.92 ± 0.52^{a}
	MPD	MPD	0.18 ± 0.15^{b}	0 ± 00^{c}	0.44 ± 0.15^{a}
	Tail	BT	1.40 ± 0.65^{a}	1.68 ± 0.54^{a}	0.06 ± 0.60^{b}
		CT	0.50 ± 0.37^{ab}	0.1 ± 0.31^{b}	1.3 ± 0.35^{a}
		FT	0.69 ± 0.48^{ab}	0.86 ± 0.40^{a}	0.59 ± 0.45^{b}
Overall (%)			**4.66±2.98**	**4.37±2.35**	**5.08±2.79**
28	Acrosome	Acro. Swelling	3.61 ± 0.54^{ab}	3.24 ± 0.7^{b}	4.00 ± 0.54^{a}
	Head	DH	4.08 ± 0.60^{c}	4.48 ± 0.77^{b}	5.50 ± 0.60^{a}
	MPD	Mpd	1.72 ± 0.99^{b}	1.60 ± 1.29^{b}	4.00 ± 0.99^{a}
	Tail	BT	3.59 ± 0.49^{b}	4.06 ± 0.64^{a}	3.00 ± 0.49^{c}
		CT	3.59 ± 0.64^{a}	3.67 ± 0.82^{a}	3.25 ± 0.64^{b}
		FT	3.09 ± 0.78^{ab}	3.26 ± 0.78^{a}	2.75 ± 0.78^{b}
Overall (%)			**19.68±4.04**	**20.31±5.0**	**22.50±4.04**
32	Acrosome	Acro. Swelling	3.52 ± 0.66^{a}	1.95 ± 0.13^{b}	3.28 ± 0.66^{a}
	Head	DH	4.18 ± 1.12^{b}	5.35 ± 1.12^{a}	4.22 ± 1.26^{b}
	MPD	Mpd	1.73±0.60	1.71±0.60	1.82±0.67
	Tail	BT	5.56 ± 1.17^{b}	6.56 ± 1.17^{a}	4.17 ± 1.31^{c}
		CT	3.74 ± 0.76^{a}	2.47 ± 0.76^{b}	2.98 ± 0.85^{ab}
		FT	2.88 ± 0.99^{b}	3.63 ± 0.99^{a}	2.42 ± 1.11^{c}
Overall (%)			**21.61±5.3**	**21.67±4.77**	**18.89±5.86**

^{abc}= Means with different superscript within the same row for a trait differ significantly ($P<0.05$), Acro. Swelling= Acrosome swelling, DH = Detached head, MPD= Mid piece drop, BT= Bent tail, CT= Curl tail, FT= Full tail.

Table 4.5: Interaction between Age and Genotypes on Semen Quality Characteristic of the Nigerian Indigenous Cockerels at Different Ages

Parameters	**Genotypes**	**Age (weeks)**			**SEM**	**LOS**	**P-value**
		24	28	32			
Vol.(ml)	NF	0.12^{b}	0.32^{a}	0.18^{b}	0.01	*	0.03
	F	0.24^{b}	0.17^{b}	0.08^{b}			
	Na	0.12^{b}	0.18^{b}	0.22^{b}			
Colour	NF	1.14	1.20	1.20	0.06	NS	0.29
	F	1.40	1.67	1.00			
	Na	1.25	1.80	2.00			
Mot. (%)	NF	85.71	91.80	74.60	2.93	NS	0.41
	F	90.00	83.33	62.60			
	Na	79.88	81.00	91.80			
pH	NF	7.43	7.20	6.60	0.06	NS	0.23
	F	7.20	7.00	6.60			
	Na	6.88	7.20	7.00			
Conc($x10^{9}$)	NF	6.42	5.27	4.31	2.47	NS	0.90
	F	7.02	7.00	4.31			
	Na	5.73	7.20	3.80			
Live	NF	54.3	78.0	73.0	3.06	NS	0.28
	F	75.0	60.0	57.0			
	Na	85.0	80.0	67.0			

^{ab}=Means with different superscript within the same row for a trait differ significantly (P<0.05), NF=Normal feather, F=Frizzle, Na=Naked neck, Vol.=Volume, Mot.=Motility, Conc.=Concentration, SEM=, LOS= Level of significance, NS=Not significant.

4.2 LEAST SQUARE MEANS (±SE) FOR BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS OF THE COCKERELS

Table 4.6 shows the least square means of biochemical parameters of cockerels in the three genotypes of the Nigeria indigenous chickens. There were no significant ($P>0.05$) differences in the parameters considered except chloride which was significantly ($P<0.05$) higher in the normal feather cockerels than the other two genotypes.

Table 4.7 shows the least square means of the haematological parameters of cockerels in the three different genotypes of the Nigerian indigenous chickens. Genotype had no significant ($P>0.05$) effect on all the parameters considered.

Table 4.6: Least Square Means (± SE) of Biochemical Parameters in the Three Nigerian genotype cockerels

	Genotypes		
Parameters	**Normal**	**Frizzle**	**Naked neck**
Na (mmol/l)	138.0±2.33	136.8±2.33	134.8±2.33
K (mmol/l)	4.16±0.33	4.02±0.33	3.84±0.33
Cl (mmol/l)	106.6±2.72^{a}	100.2±2.72^{b}	97.4±2.72^{b}
Ca (ml/dl)	8.8±0.08	8.72±0.08	9.21±0.08
AST (iu/l)	176.94±2.38	85.14±2.38	122.54±2.38
Cholesterol (mg/dl)	149.31±4.96	143.71±4.96	147.21±4.96
ALT (iu/l)	4.60±1.48	4.40±1.48	6.20±1.48
ALP	141.02±4.07	117.02±4.07	102.02±4.07
Cre (µmol/l)	77.60±34.83	80.00±34.83	70.60±34.83
Glu (mg/dl)	179.05±1.12	190.55±1.12	153.55±1.12

^{ab}=Means with different superscript within the same row for a trait differ significantly ($P<0.05$), Na = Sodium, K = Phosphorus, Cl = Chloride, Ca = Calcium, AST = Aspartate transaminase, ALT = Alanine aminotransferase, ALP =Alkaline phosphatase, Cre. = Cretinine, Glu. = Glucose.

Table 4. 7: Least Square Means (± SE) of Haematological Parameters in the Three Nigerian Indigenous Genotype Cockerels

	Genotypes		
Parameters	**Normal**	**Frizzle**	**Naked neck**
PCV (%)	25.8±1.9	25.8±1.9	25.0±2.1
Hb (g/dl)	8.56±0.64	8.56±1.9	8.30±0.71
WBC(10^3ml)	2.02±0.45	1.64±0.45	1.98±0.45
TP (g/dl)	3.28±0.23	3.28±0.23	3.70±0.26
Hetrophiles (%)	9.20±1.26	10.0±1.26	9.50±1.41
Lymphocytes(%)	90.20±1.15	88.80±1.15	90.50±1.28
pH	7.86±0.1	8.02±0.1	7.70±0.1
MCV (fl)	60.24±0.21	60.03±0.21	60.24±0.23
MCH (pg)	28.11±8.30	27.87±8.30	30.00±9.29
MCHC (%)	33.17±0.06	33.18±0.06	33.20±0.07
Eosinophils (%)	3.00±1.41	3.00±1.41	na

PCV=Packed cell volume, Hb=Heamoglobin, WBC=White blood cell, TP=Total protein, MCV= Mean corpuscular volume, MCH= Mean Corpuscular Hemoglobin, (MCHC) =Mean Corpuscular Hemoglobin Concentration, na=non estimable

4.3 CORRELATION BETWEEN BIOCHEMICAL, HEAMATOLOGICAL PARAMETERS AND SEMEN QUALITY OF THE NIGERIA INDIGENOUS COCKERELS

Table 4.8 shows the correlation (r) between the pooled biochemical data and the semen quality regardless of age and genotype. The result showed that most of the correlation coefficients were not significant ($P>0.05$) and had no relationship with semen quality. However, there was very high significant ($P<0.001$) and positive correlation between sodium and phosphorus (0.74), sodium and chloride (0.79) and motility and concentration (0.82). Also, there was a highly significant ($P<0.01$) correlation between Alkaline phosphatase and total protein (0.63), Alanine aminotransferase and total protein (0.65), volume and motility (0.64), motility and live (0.60) and a negative but significant ($P<0.01$) correlation between motility and pH (-0.61). A significant ($P<0.05$) positive correlation existed between phosphorus and chloride (0.54), cretinine and total protein (0.52), glucose and motility (0.55), total protein and colour (0.55) and volume and concentration (0.55).

Table 4.9 shows the correlation (r) between the pooled haematological data and the semen quality regardless of age and genotype. The result showed that most of the correlation coefficients were not significant ($P>0.05$) and had no relationship with semen quality. However, there was very high significant ($P<0.001$) and positive correlation between WBC and lymphocytes (0.91), haemoglobin and PCV (0.99), concentration and motility (0.82). Lymphocytes and hetrophiles (-0.87) and WBC and hetrophiles (-0.76) also had very highly significant ($P<0.001$) but negative correlations. There was also highly significant ($P<0.01$) and positive correlation between pH and WBC (0.66), motility and volume (0.64) and live cells and motility (0.60) while pH and motility (-0.61) had a negative correlation. Similarly, significant ($P<0.05$) positive correlation exited between colour and MCHC (0.55), pH and PCV (0.58), pH and Hb (0.58), pH and lymphocytes (0.51) and between concentration and volume (0.55) while MCHC and MCV (-0.50) had a significant but negative correlation.

Table 4. 8: Correlation Coefficients on the Pooled Data between Biochemical Parameters and Semen Characteristics

Traits	Na	K	Cl	ALP	CHO	AST	ALT	Cre	Ca	Glu	TP	Vol.	Col.	Mot.	pH	Conc.	Live
Na																	
K	0.74***																
Cl	0.79***	0.54*															
ALP	-0.05	-0.13	0.03														
CHO	0.001	0.06	0.09	0.49													
AST	0.20	0.32	0.29	-0.27	-0.04												
ALT	-0.42	-0.24	-0.22	0.36	0.20	0.34											
Cre	-0.04	0.13	0.25	0.34	0.21	-0.02	0.29										
Ca	0.38	0.45	0.06	-0.07	0.13	0.32	-0.18	-0.16									
Glu	-0.20	-0.32	-0.05	0.29	0.13	0.02	0.25	0.32	-0.16								
TP	-0.31	-0.05	-0.07	0.63**	0.32	0.29	0.65**	0.52*	-0.03	0.25							
Vol.	-0.11	-0.07	0.17	-0.02	0.13	0.19	0.37	0.02	-0.28	0.07	-0.12						
Col.	-0.16	-0.16	-0.22	0.32	0.44	0.07	0.23	0.36	0.21	0.18	0.55*	-0.48					
Mot.	-0.04	-0.04	0.10	0.06	0.03	0.38	0.42	0.14	-0.01	0.55*	0.03	0.64**	0.29				
pH	0.05	-0.10	0.15	-0.16	0.01	0.08	-0.06	0.01	-0.04	-0.18	0.10	-0.22	0.44	-0.61**			
Conc.	-0.26	-0.13	-0.003	-0.18	-0.15	0.49	0.27	0.04	-0.02	0.47	0.09	0.55*	-0.3	0.82***	-0.37		
Live	0.12	0.12	0.11	0.47	0.27	0.16	0.37	0.40	0.45	0.39	0.27	0.34	0.13	0.60**	0.29	0.34	

*Significant (P<0.05) **Significant (P< 0.01) *** Highly significant (P<0.001) Na=Sodium, K= Phosphorus, Cl=Chloride, ALP= Alkaline phosphatase, CHO=Cholesterol, AST = Aspartate transaminase, ALT = Alanine aminotransferase, Cre= Cretinine, Ca= Calcium, Glu= Glucose, TP=Total Protein, Vol. =Semen volume, Col.= Colour, Mot.=Motility, Conc.=Concentration.

Table 4. 9: Correlation Coefficients on the Pooled Data between Haematological Parameters and Semen Characteristics

Traits	MCV	MCH	MCHC	PCV	Hb	Het	Lymp	pH	WBC	Vol.	Col.	Mot.	pH	Conc.	Live
MCV															
MCH	-0.04														
MCHC	-0.50*	0.32													
PCV	0.07	-0.06	0.27												
Hb	0.06	-0.05	0.29	0.99***											
Het	0.17	-0.09	-0.38	-0.11	-0.12										
Lymp	-0.10	0.08	0.29	0.14	0.14	-0.87***									
pH	-0.32	0.01	0.14	0.20	0.20	-0.27	0.07								
WBC	-0.07	-0.03	0.31	0.19	0.20	-0.76***	0.91***	-0.05							
Vol.	-0.06	0.06	0.25	-0.06	-0.05	0.29	-0.11	-0.15	-0.03						
Col.	-0.36	0.24	0.55*	0.28	0.29	-0.44	0.23	0.17	0.18	-0.48					
Mot.	-0.11	0.26	0.27	-0.38	-0.37	-0.001	-0.09	-0.17	-0.10	0.64**	-0.29				
pH	0.01	-0.19	0.19	0.58*	0.58*	-0.39	0.51*	-0.01	0.66**	-0.22	0.44	-0.61**			
Conc.	0.18	0.42	0.34	-0.16	-0.16	-0.10	0.01	-0.23	-0.004	0.55*	-0.26	0.82***	-0.36		
Live	-0.30	0.34	0.39	-0.39	-0.37	-0.09	0.09	0.33	0.02	0.34	0.13	0.60**	0.29	0.34	

*Significant (P<0.05) **Significant (P< 0.01) *** Highly significant (P<0.001) MCV= Mean corpuscular volume, MCH= Mean Corpuscular Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration, PCV=Packed cell volume, Hb=Heamoglobin, Het. = Hetrophiles, Lymp. =Lympocytes, WBC=White blood cell, Vol. =Semen volume, Col.=Colour, Mot.=Motility, Conc.=Concentration.

4.4 PHENOTYPIC CORRELATION BETWEEN GROWTH TRAITS AND SEMEN QUALITY IN THE NIGERIA INDIGENOUS COCKERELS AT 24, 28 AND 32 WEEKS OF AGE

Tables 4.10, 4.11 and 4.12 show the phenotypic correlations between body weights, body linear measurements and semen quality at different ages in the three indigenous cockerel genotypes. Correlation coefficients varied in magnitude and direction with the age and genotypes considered. Relationships between body weight and most of the body measurements and semen quality were positive and non significant (P>0.05) at the three age groups of the roosters. However, significant (P<0.05) and positive correlations were recorded for wattle length and comb height (0.54), wing span and shank diameter (0.56), motility and concentration (0.61) and wattle length and comb length (0.52) at (P>0.01) and very highly significant (P<0.001) positive correlation for comb length and comb height (0.85), comb length and wing span (0.86) as well comb height and wing span (0.69) at 24 weeks of age.

At 28 weeks of age, significant (P<0.05) positive correlations were obtained between pH and colour (0.55), body weight and breast girth (0.51) and at (P<0.01) for breast girth and shank length (0.68) and a very highly significant (P<0.001) positive correlation between body length and wattle length (0.79), comb length and body length (0.76), comb height and wattle length (0.94), wattle length and wattle width (0.79), shank length and body weight (0.75), wing span and body length (0.96) and shank diameter and wing span (0.79).

At 32weeks of age, significant ($P<0.05$) positive correlations were obtained between comb height and comb length (0.53), body weight and breast girth (0.58), body weight and shank length (0.61) and very highly significant ($P<0.001$) positive correlation were obtained for body length and comb height, shank diameter and body length (0.74).

Table 4.10: Phenotypic Correlation for Body Weight, Body Linear Measurement and Semen Quality at 24 Weeks of Age

Traits	BW	BG	SL	BL	WW	WL	CL	CH	WS	SD	Vol.	Col.	Mot.	pH	Conc.	Live
BW																
BG	0.34															
SL	0.21	-0.02														
BL	0.18	-0.19	0.22													
WW	-0.13	0.11	-0.09	0.02												
WL	0.35	-0.03	-0.09	0.04	0.34											
CL	0.22	-0.21	-0.08	0.36	0.47	0.52**										
CH	0.3	-0.28	-0.03	0.43	0.33	0.54*	0.85***									
WS	0.33	-0.16	-0.02	0.43	0.31	0.36	0.86***	0.69***								
SD	0.34	-0.23	0.29	0.14	-0.04	0.45	0.59**	0.46	0.56*							
Vol.	0.05	-0.17	-0.34	-0.07	-0.04	0.17	0.03	0.1	0.29	-0.05						
Col.	-0.29	-0.65	0.005	0.06	-0.09	0.24	0.27	0.35	0.18	0.24	0.03					
Mot.	0.09	-0.13	-0.29	0.11	0.24	0.43	0.01	0.04	-0.04	0.009	0.27	-0.06				
pH	0.11	-0.36	0.11	0.04	-0.15	0.16	0.15	0.18	-0.03	0.14	-0.29	0.31	-0.24			
Conc.	0.04	0.19	-0.15	0.06	0.18	0.39	0.1	0.03	-0.07	0.07	0.008	-0.06	0.61*	-0.13		
Live	-0.23	0.24	-0.21	-0.21	0.04	-0.28	-0.23	-0.33	-0.32	-0.11	-0.29	0.23	0.16	-0.29	0.18	

*Significant (P<0.05) **Significant (P< 0.01) *** Highly significant (P<0.001) BW=Body weight, SL=Shank length, BL=Body length, WW=Wattle width, WL=Wattle length, CL=Comb length, CH=Comb height, WS=Wing span, SD=Shank diameter, Vol. =Semen volume, Col.=Colour, Mot.=Motility, Conc.=Concentration.

Table 4. 11: Phenotypic Correlation for Body Weight, Body Linear Measurement and Semen Quality at 28 Weeks of Age

Traits	BW	BG	SL	BL	WW	WL	CL	CH	WS	SD	Vol.	Col.	Mot.	pH	Conc.
BW															
BG	0.51*														
SL	0.75***	0.68*													
WW	-0.13	-0.21	-0.19	0.76***											
WL	-0.08	-0.37	-0.24	0.79***	0.79***										
CL	-0.18	-0.41	-0.12	0.76***	0.64*	0.69**									
CH	-0.14	-0.36	-0.27	0.86***	0.81***	0.94***	0.81***								
WS	-0.15	-0.09	-0.43	0.96***	0.62*	0.48	0.43	0.58*							
SD	0.003	-0.2	-0.4	0.62*	0.46	0.51*	0.29	0.52*	0.79***						
Col.	0.16	0.51*	0.28	-0.78	-0.66	-0.82	-0.65	-0.79	-0.36	0.39	-0.44				
Mot.	-0.08	-0.35	0.03	0.16	-0.08	0.35	0.12	0.24	-0.28	-0.12	0.69*	-0.42			
pH	-0.08	0.28	0.04	-0.17	0.08	-0.18	-0.06	-0.11	0.003	-0.14	-0.27	0.55*	-0.63		
Conc.	0.25	-0.17	0.36	0.06	0.005	0.33	-0.14	0.2	-0.38	-0.34	0.57*	-0.35	0.83***	-0.43	
Live	0.16	0.26	0.21	-0.18	-0.23	0.11	-0.33	-0.03	-0.26	-0.05	0.41	0.04	0.59*	-0.28	0.35

*Significant ($P<0.05$) **Significant ($P< 0.01$) *** Highly significant ($P<0.001$) BW=Body weight, SL=Shank length, BL=Body length, WW=Wattle width, WL=Wattle length, CL=Comb length, CH=Comb height, WS=Wing span, SD=Shank diameter, Vol.=Semen volume, Col.=Colour, Mot.=Motility, Conc.=Concentration.

Table 4.12: Phenotypic Correlation for Body Weight, Body Linear Measurement and Semen Quality at 32 Weeks of Age

Traits	BW	BG	SL	BL	WW	WL	CL	CH	WS	SD	Vol.	Col.	Mot.	pH	Conc.	Live
BW																
BG	0.58^{*}															
SL	0.61^{*}	0.34														
BL	0.004	-0.33	0.17													
WW	-0.09	0.27	-0.17	0.46												
WL	0.12	0.04	0.0009	0.61^{*}	0.68^{*}											
CL	0.001	-0.12	0.01	0.47	0.09	0.14										
CH	-0.14	-0.34	0.09	0.87^{***}	0.22	0.33	0.53^{*}									
WS	0.09	-0.07	0.34	0.35	0.18	0.199	-0.47	0.22								
SD	0.34	-0.15	0.41	0.74^{***}	0.27	0.39	0.51^{*}	0.55^{*}	0.28							
Vol.	-0.11	0.50^{*}	-0.08	-0.17	0.35	-0.18	0.27	-0.15	-0.26	-0.25						
Col.	-0.11	0.32	0.34	-0.49	-0.31	-0.46	-0.27	-0.36	-0.16	-0.56	0.32					
Mot.	0.09	0.45	-0.06	-0.17	0.16	-0.21	0.21	-0.21	-0.05	-0.04	0.65^{**}	0.23				
pH	0.07	0.36	0.03	-0.11	0.07	-0.16	0.19	-0.12	0.04	-0.02	0.53^{*}	0.28	0.95^{***}			
Conc.	0.1	-0.35	0.09	0.51^{*}	0.23	0.47	0.25	0.29	0.39	0.71^{**}	-0.42	-0.74	-0.26	-0.23		
Live	0.13	0.42	-0.19	-0.08	0.29	0.05	0.2	-0.19	-0.05	0.07	0.47	-0.01	0.92^{***}	0.87^{***}	-0.1	

*Significant (P<0.05) **Significant (P< 0.01) *** Highly significant (P<0.001) BW=Body weight, SL=Shank length, BL=Body length, WW=Wattle width, WL=Wattle length, CL=Comb length, CH=Comb height, WS=Wing span, SD=Shank diameter, Vol. =Semen volume, Col.=Colour, Mot.=Motility, Conc.=Concentration.

4.5 INSULIN GROWTH FACTOR-1 (IGF-1) POLYMORPHISM AND ASSOCIATION WITH BODY WEIGHT AND SEMEN QUALITY

4.5.1 Insulin-like growth factor-1 (IGF-1) polymorphic forms

Plate 4.1 shows the different polymorphic forms of the IGF-1 gene. The amplification showed that three pattern fragments exist at; 235bp (AA), 235/500bp (AB) and 500bp (BB). The genotypic distribution and allele frequency of IGF-1 genes in the three genotypes of Nigeria indigenous cockerels was studied. The genotypic frequencies of the different alleles observed in the samples were AA=56.25, AB=31.25 and BB=12.50 and the allelic frequencies were 0.72 (A) and 0.28 (B) respectively with a chi-square value of 0.82.

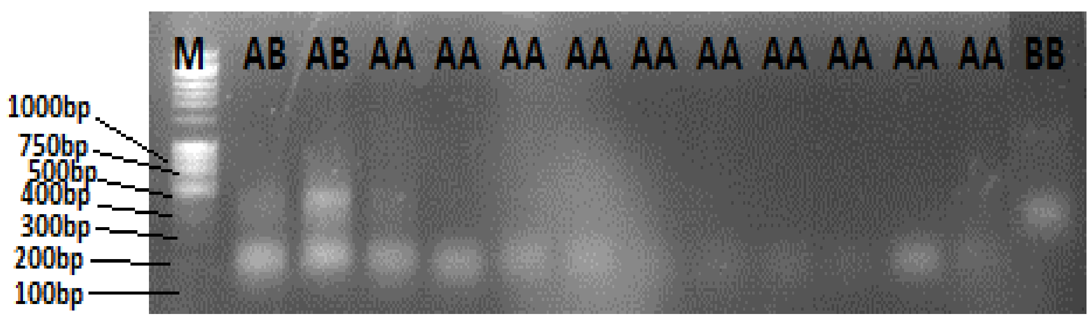

Plate1: Electropherogram showing genotyping profiles of IGF-1 gene detected by PCR method.

Table 4.13: Genotypic distribution and allele frequencies of IGF-1 genes in three Genotypes of Nigeria indigenous cockerels

	Genotypic frequencies			Allele frequencies		χ^2
Genotype	AA	AB	BB	A	B	
IGF-1	56.25	31.25	12.5	0.72	0.28	0.82

IGF = Insulin growth factor, χ^2 = Chi-square

4.5.2: Association of the different alleles on IGF-1 gene, body weight and semen characteristics of the indigenous cockerels

Table 4.13 shows the association of IGF-1 polymorphism with body weight and semen characteristics of the Nigerian indigenous cockerels. Body weight was significantly ($P<0.05$) different with the AA genotype being the heaviest at all ages while AB was significantly ($P<0.05$) higher than BB at 24 and 28 weeks of age. However, the BB genotype had heavier body weight than the AB genotype at week 32. Semen volume was significantly ($P<0.05$) different at week 24 and 32 but was not significantly ($P>0.05$) different at week 28. However, the BB genotype had a very low volume at week 32 when compared to that of AA and AB genotypes. Semen colour, motility, pH, concentration and live cells were significantly ($P<0.05$) different at the different polymorphic forms as the age of the cockerels increased.

Table 4.14: Association between IGF-1 Marker and body weight and semen characteristics in the three Nigeria indigenous cockerels

Genotype (No)	Wt (g)	Vol.(ml)	Colour	Mot. (%)	pH	Conc. (10^9)	Live (%)
				Week 24			
AA (9)	1107.38±118.02^{a}	0.29±0.09^{a}	0.81±0.35^{c}	88.89±16.55^{b}	7.11±0.47^{b}	3.32±17.08^{c}	70.88±18.02^{b}
AB (5)	1071.29±77.47^{a}	0.13±0.04^{c}	1.37±0.23^{b}	76.02±10.86^{c}	7.64±0.31^{a}	6.37±11.21^{b}	53.69±11.83^{c}
BB (2)	976.43±58.53^{b}	0.18±0.03^{b}	1.68±0.17^{a}	91.61±8.21^{a}	7.20±0.24^{b}	7.94±8.47^{a}	77.19±8.90^{a}
SEM	41.64	0.02	0.12	5.84	0.17	0.60	6.36
LOS	*	*	*	*	*	*	*
				Week 28			
AA (9)	1230.27±68.31^{a}	0.19±0.04	2.63±0.3^{a}	93.51±3.60^{a}	7.01±0.26^{b}	5.16±6.04^{a}	92.41±13.19^{a}
AB (5)	1155.27±44.84^{b}	0.20±0.03	1.17±0.19^{b}	80.42±2.36^{c}	7.35±0.17^{a}	4.27±3.96^{b}	72.81±8.66^{c}
BB (2)	1039.27±33.87^{c}	0.20±0.02	1.11±0.15^{b}	87.36±1.78^{b}	7.02±0.13^{b}	4.19±2.99^{b}	77.16±6.54^{b}
SEM	24.1	0.02	0.11	1.27	0.09	0.21	4.66
LOS	*	NS	*	*	*	*	*
				Week 32			
AA (9)	1386.07±73.07^{a}	0.20±0.04^{a}	0.98±0.30^{b}	94.29±18.11^{a}	6.74±0.54^{b}	5.05±5.34^{a}	88.58±18.19^{a}
AB (5)	1249.72±47.96^{c}	0.17±0.03^{a}	0.92±0.20^{b}	90.96±11.90^{b}	7.20±0.35^{a}	5.16±3.5^{a}	87.85±11.94^{a}
BB (2)	1280.28±36.23^{b}	0.09±0.02^{b}	1.42±0.15^{a}	76.68±8.99^{c}	7.06±0.26^{a}	4.36±2.64^{b}	79.02±9.02^{b}
SEM	25.78	0.02	0.11	7.43	0.19	0.19	6.42
LOS	*	*	*	*	*	*	*

Means within the same column with different superscript are significantly ($P<0.05$) different, NS= Not Significant, Wt= Weight, vol. = volume Mot. = Motility, Conc. = Concentration, No= Number, SEM= Standard error of mean, LOS= Level of significance.

CHAPTER FIVE

5.0 DISCUSSION

5.1 MEAN PERFORMANCE

5.1.1 Body weight and growth traits

The mean body weight of the roosters at eight weeks (310.46±10.99g) was within the range of 306-312g reported by Fayeye *et al.* (2005) on Nigerian local chickens. The result at 20 weeks (1167.80±32.1g) was close to the range of values (1017.63 – 1121.78g) reported by Egahi *et al.* (2013) at 20 weeks but slightly higher than the 926.3±125.5g reported by Yousif *et al.* (2006) at 18 weeks of age in the male Sudanese indigenous chickens' prior to sexual maturity. The progressive increase in weight was in accordance to the report of Abd El Ghany *et al.* (2011) that a general linear increment existed with increasing age in the two strains (El- Salam and Mandarah cocks) with significance during the early period (12-18weeks) for body weight, length of both keel, shank and comb length and width in the two strains. The values for body weight and morphometric traits in this study were lower than the values reported by Gabriel *et al.* (2009) in Norfa cocks and El-Sahn, (2007) in Bandarah cocks. Despite the great potentials in the indigenous chickens, the relatively low performance is due to lack of adequate selection of the indigenous stocks.

5.1.2 Semen characteristics of the three genotypes

Semen characteristics and assessment are important indicators of the reproductive potential of breeding cockerels. The result of this study on semen volume (0.32ml, 0.24ml and 0.22ml) for normal feather, frizzle and naked neck respectively was similar to those of Machebe and Ezekwe (2006) who recorded values of 0.27ml, 0.24ml and 0.22ml for naked neck, frizzle and normal feather roosters respectively. The result also agrees with that of Bah *et al.* (2001) in Sahel regional local breeding cocks with semen volume average of 0.28 ml but not in agreement with the report of Ajayi *et al.* (2011) who recorded a much higher values for normal feather (0.83ml) and frizzle feather (1.10 ml) and a lower value for naked neck (0.18ml). Tuncer *et al*. (2006) who worked with Denizli cocks reported volume of 0.7 ml and Peters *et al*. (2008) reported values of 0.37 to 0.73 ml and 0.76 ml for Nigerian indigenous breeds. Differences in the volume of semen observed can probably be attributed to breed, age and environmental factors (Ezekwe and Machebe 2004).

The colour of semen of the cockerels did not differ significantly being creamy white in almost all the ejaculates collected except at 32 weeks for the naked neck which was milky. The creamy white colour of semen collected in this study, did not concur with the observed milky colour of exotic Rhode Island Red and White breeder cocks reported by Nwagu *et al.* (1996) and Kabir (2006). It has been observed that variations in semen colour may arise partly due to the presence of contaminants and the level of sperm concentration (Etches, 1998). However, the creamy white colour observed in this study in the indigenous cockerels of different genotypes could be as a result of their own genetic attribute.

The semen motility (91.80%), pH (7.43), semen concentration (7.02 x10^9/ml) as well as live cell (85.0%) recorded in this study were higher than those reported by Ajayi *et al.*

(2011) and Machebe and Ezekwe (2006). However, the values obtained for sperm motility ranged between 3.80 to 7.43 x10^9/ml which is similar to the 4.3 billion sperm/ml obtained by Moya *et al.* (1996) in broiler cocks and 6.6 billion sperm/ml reported by Tabatabaei *et al.* (2009) in Ross broiler breeders. The result of Orunmuyi *et al.* (2013) revealed that the semen motility of Hubbard broilers was 83.50% which was within the range obtained by Peters *et al.* (2008) who obtained a range of 70% to 87.35% and are in agreement with the result in this study. Also, Tabatabaei *et al.* (2009) reported sperm concentration of 6.6x10^9 ml in Ross broiler breeder cocks which is in agreement with the result of this study. Similarly, the report of Tabatabaei *et al*. (2010) on Iranian broiler breeder chickens was within the range of 74.5 to 85.67% which agrees with this study. However, the report by Bah *et al.* (2001) in local breeder cocks with the value of 73.9% was not in agreement with this study. Similarly, the results of this work was in the range and in agreement with that of Peters *et al.* (2008) who reported semen pH of cockerel to be slightly alkaline, with a mean of 7.01 ± 0.01, while Tuncer *et al.* (2008) and Bah *et al.* (2001) recorded semen pH ranging between 7.54 ± 0.04 and 7.80 ± 0.03. Similar reports were given by Orunmuyi *et al.* (2013) who recorded a pH of 7.40 in Hubbard broilers. The variations arising from the mean semen motility, pH, semen concentration as well as live cell in the three genotypes having high and better semen concentration, motility and a normal pH of these genotypes at the ages considered could be attributed to their different genetic background and their natural tendencies.

5.1.3 Morphological defects related to cockerels spermatozoa

The acrosome swelling in this result at 24 weeks of age was between 0.89±0.64% to 1.77±0.72% which was in the range reported by Tabatabaei *et al.* (2009) for Ross cocks.

Also, the result obtained at 24 and 32 weeks fell within the range of values in indigenous cocks at 30 weeks of age in the report by Tabatabaei *et al.* (2009). The percentage of detached head (0.50±0.56% - 5.50±0.60) in this study was high and did not conform with the report by Tabatabaei *et al.* (2009) and with those of Ajayi *et al.* (2011) at all ages.

The percentage of mid piece detachment obtained in this study was similar to those of Tabatabaei *et al.* (2009) for indigenous cocks at 30 weeks. However, this result was not in agreement with the report of Ajayi *et al.* (2011) who reported a higher value using similar type of genotypes but at an age range of 50 – 86 weeks. These variations could thus be as a result of age and strain differences. The implication of the values obtained for the morphological defects of this study is that the sperm cells have negligible numbers of abnormal cell and is good enough for breeding purpose.

5.1.4 Interaction between age and genotypes of the Nigerian indigenous cockerels

The reports of Hanafy (2006), El-Sheikh and Hanafy (2006) and El-Tantawy *et al*. (2007), on Inshas and Matrouh cocks regarding semen volume, sperm concentration, sperm motility and live sperm had linear increase with age which is not in agreement with the same parameters considered in this work. However, motility was the only parameter in agreement with a linear increase. Also, the report of this work with the non-significant interactions apart from semen volume obtained in this study agreed with the report of Ali *et al*. (2006) and Shahein *et al*. (2007) who reported non significant effect of age on semen volume, sperm motility, sperm concentration and live sperm of Inshas and Matrouh strains.

5.2 BIOCHEMICAL PARAMETERS OF THE THREE GENOTYPES

The non- significant effect of genotype on biochemical parameters obtained in this report is consistent with the values obtained by Ladokun *et al.* (2008) who observed no significant genetic effect in his study. They are also in agreement with the report of Isidahomen *et al.* (2011) on calcium and potassium but varies for cholesterol. The value of sodium (Na) in this report was in agreement with the range of 130-155 mmol/l for serum Na in mature birds (Clinical Diagnostic Division, 1990).The serum Chloride level in this study was below the range of 108-124mmol/L in birds. However, the differences across the three groups for serum Chloride agreed with the reports of Ibrahim *et al*. (2012).

AST, Glucose, Cholesterol, Potassium and Calcium values were within the range of values reported by Abdi-Hachesoo *et al.* (2013) for male domestic fowls, Meluzzi *et al.* (1992), Simaraks *et al.* (2004) and Elagib *et al.* (2012) who reported that there was no significant difference in Ca between the sexes of indigenous chickens. However, the glucose value was not consistent with the findings of Ibrahim *et al.* (2012) who obtained very high range of values in Local Saudi chickens and Ladokun *et al.* (2008) who reported a range of 42.1 – 48.90 mg/dl in Nigerian indigenous chickens. However, the result of glucose in this study was consistent and was within the reported range of 167-305 mg/dl by Aengwanich *et al*. (2004).

Cholesterol level obtained in this study fell within the range of 129 -297mg/dl reported by the Clinical Diagnostic Division (1990) but higher than the report of Ibrahim *et al.* (2012) on two sexes of local Saudi chicken of different ages (adult and young). AST values were consistent with the findings of Abdi-Hachesoo *et al.* (2011) on indigenous and Ross-308 broiler breeders.

The result on total protein (3.28±0.23 - 3.70±0.26 g/dl) in this study fell within the normal range of value for chickens and was in agreement with the works of Abdi-Hachesoo *et al.* (2013) who reported 3.87g/dl in male adult indigenous chickens. Also, the result is in agreement with the report of Elagib *et al.* (2012) who did not find significant differences in total protein values when male and female indigenous chickens in Sudan were compared. The values obtained were also within the range of 3.0-4.9 mg/dl as reported by Clinical Diagnostic Division, (1990). The result is also in agreement with the findings of Aengwanich *et al*. (2004) who reported 4.0–5.5g/dl and similar to the 4.5g/dl reported by Jain (1993). The result of the study is not in agreement with the findings of Orunmuyi *et al.*, (2011) where values obtained were higher when compared to sex, age and genotypes of Nigeria indigenous chickens and those of Meluzzi *et al.* (1992) who demonstrated that total protein of female was higher than males in broiler chicken. These differences may be attributed to environment, season or the condition of the experiment (Ritchie *et al.*, 1994).

Values of ALP in this study were within the range of values reported by Clinical Diagnostic Division, (1990) and were in line with the differences in activities across genotypes and strain reported the report by Orunmuyi (2006).

5.3 HAEMATOLOGICAL PARAMETERS OF THE COCKERELS

The mean values for haematological parameters obtained in this study though with no significant difference between genotypes fell within the normal physiological range and agreed with the reports of Ikhimioya *et al.* (2000), Islam, *et al.* (2004) and Simaraks *et al.* (2004). The result is also consistent with those of Oke *et al.* (2007) where no significant effect of genotype was observed. However, the result is in contrast with the findings of Isidahomen *et al.* (2011) who reported significant difference in the various haematological parameters amongst the different genotypes. Similarly, the non significant differences obtained by this work agree with the report of Njidda *et al.* (2006) who obtained a range of 24 to 27% for PCV. However the results on PCV and Hb were similar to those of Islam *et al.* (2004) who also reported lower values in adult native chickens reared in Bangladesh.

Heterophil and Lymphocyte are leukocyte found in the blood. Heterophils however, are the most abundant leukocyte in the peripheral blood of most species of birds in most studies, whereas in some avian species lymphocytes are the predominant cell type in the differential count (Fudge, 2000, Latimer and Bienzle, 2000 as cited in Abdi-Hachesoo *et al.* (2013); Schmidt *et al.,* 2009). In this study, the lymphocytes had the most abundant leukocyte in the peripheral blood which is consistent with the reports of Abdi-Hachesoo *et al.* (2013). A similar result which is in agreement with this study was reported by Wakenell (2010) who observed that lymphocytes were the predominant leukocyte in the peripheral blood of chickens and turkeys. However, the percentage heterophils had a much lower value as reported in this study which does not agree with the reports of Abdi-Hachesoo *et al.* (2013) who reported higher values of 28.30% for adult cocks.

The values of MCV in this study was lower and is not in agreement with the reports by Islam *et al.* (2004), Isidahomen *et al.* (2011) and Elagib *et al.* (2012) for different chicken types. Similarly, the report of Ladokun *et al.* (2008) on MCV gave values of 89.10fl and 81.60fl for normal and naked neck genotypes which were higher than the result obtained in this study. The authors also reported values of 28.90fl and 27.20fl on the same genotypes for MCH and values of 32.41pg and 33.37pg for MCHC which were in agreement with the findings of this study. Similarly, for MCHC the values obtained were all consistent with the findings of Isidahomen *et al.* (2011) in relation to different genotypes in sub humid environment. The result on MCH was lower in this result than those reported by Isidahomen *et al.* (2011) on males of Nigeria indigenous chickens. Talebi *et al.* (2005) reported that MCV and MCH decreased during breeding periods in the four broiler strains of both sexes that were compared while MCHC fluctuated in the study which agreed with the present result. Kral and Suchy, (2000) and Islam *et al.* (2004) had similar reports in MCHC.

5.4 CORRELATION BETWEEN BIOCHEMICAL, HEAMATOLOGICAL PARAMETERS WITH SEMEN CHARACTERISTICS OF THE NIGERIAN INDIGENOUS COCKERELS

The significant and positive correlations between blood glucose concentration and sperm motility obtained in this work were in contrast with the observation of Jarinkovicova *et al.* (2012) who reported negative though significant correlations between these variables among cockerels of three laying lines. However, relationship between ejaculate volume and concentration with glucose level were positive in this study and those of Jarinkovicova *et al.* (2012). The non-significant relationship between creatinine and ejaculate volume (0.02), creatinine and motility (0.14) and creatinine and concentration (0.04) were not in consonance with the observations of Jarinkovicova *et al.* (2012) who reported -0.03, 0.08 and -0.20, respectively from the same correlations. Ejaculate volume was observed to be significant and positively correlated with motility and concentration. This was at variance with the negative correlation reported by Jarinkovicova *et al,* (2012). Sperm motility's positive relationship with concentration also contradicted what was obtained by these authors in cockerels of three different egg lines. The relationships obtained between traits correlated for can enhance identification for quality semen for breeding purposes.

Jacyno *et al.* (2009) reported significant and negative correlation between AST and most quantitative traits of semen in boar. This was at variance with what was obtained in this study relationship were mainly positive but not significant. However, the observation of non-significant but negative correlation between sperm concentration and trace elements such as Na, K, Cl and Ca obtained was in agreement with the findings of Cevik *et al*. (2007) in bulls. This may indicate that these trace elements rarely perform roles related to quantitative characteristics but rather work at a physiological level. Since Na, Cl and K have been associated with proper spermatozoa functioning through modulation of a variety of ion channels like Cl. The K in the spermatozoa as a characteristic event associated with capacitation and acrosome reaction of mammalian spermatozoa. K has being a natural metabolic inhibitor and a higher K concentration in seminal plasma decreases the metabolic activity of spermatozoa. Therefore, there is generally a negative correlation between K and sperm motility (Meizel, 1997; Purohit *et al*., 1999; Gur and Demirci, 2000; Machal *et al*., 2002; Massanyi *et al*., 2003 and Mosaferi *et al.*, 2005).

5.5 PHENOTYPIC CORRELATION BETWEEN BODY WEIGHT, LINEAR BODY TRAITS AND SEMEN QUALITY OF THE NIGERIAN INDIGENOUS COCKRELS AT VARIOUS AGES

The positive relationship between body weight and most of the body measurements showed that bodyweight can be predicted from body linear measurements and agrees with the observation of Ajayi *et al.* (2008). Among the body weight and body linear measurements, the highest correlation with reference to age was found between wing span and comb length (0.86) at 24 weeks, wattle length and comb height (0.94) at 28 weeks and body length and comb height (0.87) at 32 weeks indicating very strong association between the variables. This is in agreement with the reports of Udeh, (2012) and Yakubu *et al.* (2009) who reported similar results with other body linear traits.

Abd El Ghary *et al.* (2011) reported that body weight had influence on semen quality trait like volume, concentration and motility but not pH in two local strains of chickens and that the heavier weight bird had advantage of high concentration but the motility was to the advantage of the lighter weight group which was similar to report of this study. Similarly, positive and high correlation coefficients were obtained between semen colour and breast girth (0.51) at 28 weeks and between semen volume and breast girth (0.50), concentration and body length (0.51) at 32 weeks. A high correlation of (0.71) between concentration and shank diameter suggest high predictability between these variables were also obtained at 32 weeks of age. A similar observation was reported by Udeh *et al*. (2011) and Udeh, (2012) on similar body traits and semen quality characteristics of layer type cocks. The correlation coefficients between motility, pH and live cells and body linear measurements were mostly negative and non significant at all ages which was similar to the findings of Udeh, (2012).

5.6 INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) POLYMORPHISM

The result of this study on polymorphism clearly revealed different forms of alleles and is in agreement with the findings of Abbasi and Kazemi (2011) and with those of Li *et al.* (2010) who showed the presence of polymorphisms in IGF-1 segment in chickens. Hossein and Mohsen (2011) also stated the presence of polymorphisms in IGF-1 segment in Mazandaran native chickens. The low and non significant Chi- square (0.82) value in the sample showed that the population considered was in Hardy-Weinberg's equilibrium and this agreed with those of Abbasi and Kazemi (2011) but was not in agreement with the reports of Amills *et al.* (2003) who studied the Black Penedesenca chicken which showed different polymorphic forms.

5.7 VARIATION IN BODY WEIGHT AND SEMEN QUALITY WITH IGF-1 POLYMORPHIC FORMS AT DIFFERENT AGES

The significant variations observed in body weight and semen quality with IGF-1 was in agreement with King (2006) who reported that there was a physiological connection between body weight and growth with reproductive traits in males and the rate of sexual maturation was much more closely associated with body weight than with chronological age. However, Wang *et al.* (2004) on Ningdu yellow chicken and Wanzhai yellow chicken reported that the BB genotype had heavier body weight at 4 month of age followed by the AB before the AA genotypes. However in this study the AA genotype had heavier body weight at all ages while BB had the least body weight.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

A study was carried out to evaluate the effect of three major genotypes in the Nigerian indigenous chickens on growth traits and semen quality characteristics using blood chemistry (heamatological and biochemical) and IGF-1 as a molecular marker. Using 90 sexually matured local chickens randomly purchased and used as foundation stock. Twenty five (25) hens and five (5) cocks each of the three genotypes (normal feathered (N), frizzle feathered (F) and naked neck (Na)) were mated using a ratio of 1 cock: 5 hens to obtain the progeny used for the study.

The effect of the main genes on growth traits, semen quality, blood chemistry and IGF-1 were obtained from the F1 offspring's produced. Results obtained for body weight of the cockerels showed significant difference between the three different genotypes and ranged between 310.46g to 1117.80g at 8 to 20 weeks and 1096.80g to 1285.8g at 24 to 32 weeks of age. The results obtained from this study also showed that breast girth differed significantly ($P<0.05$) between the three genotypes with the naked neck been superior. Shank length, wattle width, comb length, comb height and shank diameter were not significantly ($P>0.05$) different at weeks 8, 16 and 20. However, body length and wattle length were significantly ($P<0.05$) different across the ages. Wing span differences was not significant ($P>0.05$) at 8 week but as from week 12 it was significantly ($P<0.05$) higher in the frizzle genotype.

Results obtained for semen quality characteristics showed that semen volume for normal feathered genotype had a range of 0.12±0.03ml to 0.32±0.05ml, the frizzle genotype had a range of 0.08±0.04ml to 0.24±0.03ml and the naked neck genotype had 0.12±0.03ml to 0.20±0.04ml. There was significant ($P<0.05$) effect on semen volume (ml), motility (%), concentration (%) and live cell (%) across the genotypes at each age interval. However, semen colour and pH, had no significant ($P>0.05$) difference at week 24 and 28 respectively.

Semen morphological defects were significantly ($P<0.05$) affected by genotype and down the ages with the exception of the mid piece at 32 weeks.

Blood chemistry, was not significantly ($P>0.05$) different in all the parameters evaluated except Chloride. The values obtained for the blood chemistry reflected that the birds were all in a healthy condition.

The insulin like growth-like factor-1 (IGF-1) revealed the presence of polymorphic forms of AA, AB and BB. The low and non significant ($P>0.05$) Chi - square (0.82) value in the sampled population showed that it was in Hardy – Weinberg's equilibrium. The association of the IGF-1 with body weight and semen quality hence showed that heavy body weight is related to the semen quality output with advancement of the chronological age of the cockerels.

6.2 CONCLUSIONS

1. The normal feather had the heaviest body weight and body linear measurements when compared to the frizzle and naked neck genotypes.

2. The result on semen quality revealed that the cockerels may be used for breeding when they attain 28 weeks of age since the traits measured at this age reflected quality semen for good fertility.

3. The IGF-1 allele identified shows which polymorphic form had the better association for body weight which translated to production of high quality semen.

6.3 RECOMMENDATIONS

1. For higher body weight the normal feathered is recommended for the northern guinea environment.

2. The cockerels may be used for mating as from 24 – 28 weeks of age to get a good fertility rate since the semen quality characteristics at those ages are good enough for breeding purposes.

3. Variations could be obtained if the three genotypes are further studied in a different geographical location, management system and at a different season and at advanced ages different from this study.

REFERENCES

Abbasi, H.A. and Kazemi, M. (2011). Detection of polymorphism of the insulin-like growth factor-1 (IGF-1) gene in Mazandaran native chicken using PCR-RFLP method. *African Journal of Biotechnology*, 10(61): 13351-13354. ISSN 1684-5315.

Abd El Ghany, F.A., Alm El Dein, A.K., Soliman, M.M., Rezaa, A.M. and El Sodany, S.M. (2011). Relationship between some body measurements and fertility in males of two local strains of chicken. *Egypt Poultry Science,* 31(2): 331-349.

Abdi-Hachesoo, B., Talebi, A. and Asri-Rezaei, S. (2011). Comparative study on blood profile of indigenous and Ross-broiler breeders. *Global Veterinary,* 7(3): 238-241.

Abdi-Hachesoo, B., Talebi, A., Asri-Rezaei, S. and Basaki, M. (2013). Sex related differences in biochemical and hematological parameters of adult Indigenous chickens in Northwest of Iran. *Journal Animal Science Advance*, 3(10): 512-516.

Aboul-Seoud, D.I.M. (2008). Divergent selection for growth and egg production traits in Japanese quail. Ph.D Thesis, Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Egypt.

Adebambo, O.A., Ikeobi, C.O.N., Ozoje, M.O and Adenowo, J.A. (1999). Colour variations and performance characteristics of the indigenous chickens of Southwest Nigeria. *Nigeria Journal of Animal Production*, 26: 15-22.

Adedeji, T.A., Ojedapo, L.O., Ige, A.O., Ameen, S.A., Akinwumi, A.O and Amao, S.N. (2008). Genetic evaluation of growth performance of pure and crossbred chicken progenies in a derived savannah environment. *Proceedings of the 13thAnnual Conference of the Animal Science Association of Nigeria, (ASAN). Ahmadu Bello* University, Zaria. 15th – 19th September, 2008. Page 8-12.

Adedeji, T.A. (2004). Effect of sire strain of chickens on growth and laying performance of crossbred progenies. An MSc. Agriculture Dissertation, Department of Animal Breeding and Genetics, University of Agriculture, Abeokuta, Nigeria. Page 112.

Aengwanich, S., Simaraks, S. and Chinrasi, O. (2004). Haematological electrolyte and serum biochemical values of the Thai indigenous chickens (Gallus domesticus) in Northeastern Thailand. Songklanakarim. *Journal of Science and Technology*, 26: 425-430.

Aggrey, S.E. (2002). Comparison of three nonlinear and spline regression models for describing chicken growth curves. *Poultry Science,* 81: 1782-1788.

Aire, T.A., Olowo-Okorun, M.O and Ayeni, J.S. (1980). The seminiferous epithelium in the Guinea Fowl (*Numidia meleagris*). *Cell Tissue Resources,* 205: 319-329.

Ajayi, F.O., Ejiofor, O. and Ironkwe, M.O. (2008). Estimation of body weight from body measurements in two commercial meat type chickens. *Global Journal of Agricultural Science,* 7(1): 57 – 59.

Ajayi, F.O. (2010). Nigeria indigenous chicken: A valuable genetic resources for meat and egg production. *Asian Journal Poultry,* 4: 164-172.

Ajayi, F.O., Agaviezor, B.O. and Ajuogu, P.K. (2011). Semen characteristics of three strains of local cocks in the humid tropical environment of Nigeria. *International Journal of Animal and Veterinary Advances,* 3(3): 125-127.

Akanno, E.C., Ole, P.K., Okoli, I.C. and Ogundu, U.E. (2007). Performance characteristics and prediction of body weight of broiler strains using linear body measurements. *Proceeding 22nd Annual Conference Nigeria Society for Animal Production* Calabar, page 162-164.

Ali, M.N., Qota, E.M.A., Hassan, R.A. and Abou-Elmagd, M.K. (2006). Novel methods of detoxification of aflatoxin B1 in contaminated local laying hen diets. *Egyptian Poultry Science*, 26 (111): 911-940.

Alkan, S., Baran, A., Ozdas, O.B and Evecen, M. (2002). Morphological defects in turkey semem. *Turkey Journal Veterinary Animal Science*, 26:1087-1092. http://journals tubitak.gov.tr/veterinary/issues/vet 02-26-5/vet-26-5-16-0107-40pdf.

Aly, O.M. and El-Sahn, A. (2006). Effect of crossing on the performance of three local strains. Semen quality, electrophoretic pattern of seminal plasma proteins, fertility and hatchability in Bandara, Gimmizah and their reciprocal crosses. *Egyptian Poultry Science*, 26: 123-136.

Amills, M.N., Jimenez, D., Villalba, M., Tor, E., Molina, D., Cubilo, C., Marcos, Francesch, A., Sanchez, A. and Estany, J. (2003). Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. *Poultry Science,* 82: 1485-1493.

Anderson, J. (2001). The semen of animals and its use for artificial insemination. Green World Publishers India.

Bah, G.S., Chaudhari, S.U.R., and Al-Amin, J.D. (2001). Semen characteristics of local breeder cocks in the Sahel region of Nigeria. *Revue Elev. Medical Veterinary Pays tropical,* 54(2):153-158.

Ballard, F.J., Johnson, R.J., Owens, P.C., Francis, G.L., Upton, F.M., McMurtry, J.P. and Wallace, J.C. (1990). Chicken insulin-like growth factor-I: Amino acid sequence, radioimmunoassay, and plasma levels between strains and during growth. *General Comprehensive Endocrinology,* 79: 459-468.

Bartoov, B., Ben-Barak, J., Mayevsky, A., Sneider, M., Yogev, L., and Lightman, A. (1991). Sperm motility index: A new parameter for human sperm evaluation. *Fertility Sterility,* 56:108-112

Bearden, H.J., Fuquay, J.W. and Willard, S.T. (2004). Applied Animal Reproduction. Pearson Education, Inc. New Jersey. 6th edition.

Beccavin, C., Chevalier, B., Cogburn, L.A., Simon, J. and Duclos, M.J (2001). Insulin-liken growth factors and body growth in chickens divergently selected for high or low growth rate. *Journal Endocrinology,* 168: 297–306.

Bian, L.H., Wang, S.Z., Wang, Q.G., Zhang, S., Wang, Y.X. and Li, H. (2008). Variation at the insulin-like growth factor-1 gene and its association with body weight traits in the chicken. *Journal of Animal Breeding Genetics,* 125: 265–270.

Bilcik, B., Estevez, I., and Russek-Cohen, E. (2005). Reproductive success of broiler breeders in natural mating systems: The effect of male-male competition, sperm quality and morphological characteristics. *Poultry Science, 84*: 1453-1462.

Boyko, A.R., Quignon, P., Li, L., Schoenebeck, J.J., Degenhardt, J.D., Lohmueller, K.E., Zhao, K., Brisbin, A., Parker, H.G. and vonHoldt, B.M. (2010). A simple genetic architecture underlies morphological variation in dogs. PLoS Biol 8

Bramwell, K.R. (2002). Fertility and embryonic mortality in breeders. Avian advice of University of Arkansas. Summer, 4, http://poultryscience.uark.edu

Cevik, M., Tuncer, P.B., Tasdemir, U and Ozgurtafis, T. (2007). Comparison of Spermatological Characteristics and Biochemical Seminal Plasma Parameters of Normozoospermic and Oligoasthenozoospermic Bulls of Two Breeds. *Turkish Journal of Veterinary Animal Science,* 31(6): 381-387.

Cheesbrough, M. (1991). Medical Laboratory Manual for tropical countries. *2nd edition, ELSB Cambridge,* 11: 508 – 511.

Cheng, F.P., Guo, T.J., Wu, J.T., Lin, T.E., Ursem, P.J.F., Colenbrander B and Fung, H.P (2002). Annual variation in semen characteristics of pigeons (Columba livia). *Poultry Science,* 81: 1050-1056. PMID: 12162344.

Clinical Diagnostic Division. (1990). Veterinary Reference Guide, Eastman Kodak Company, Rochester, New York.

Cole, H.H. and Cupps, P.T. (1977). Reproduction in domestic animals. 3rd edition New York Academic press.

Crawford, R.D. (1990). Origin and history of poultry species. Chapter 1. In*: Poultry Breeding and Genetics, Edited* by R.D Crawford. Elsevier, Amsterdam.

Dana, N., vander Waaij, E.H. and van Arendonk, J.A.M. (2011). Genetic and phenotypic parameter estimates for body weights and egg production in Horro chicken of Ethiopia. *Tropical Animal Health Production*, 43: 21–28.

Datta, D.J., Mahanta, J.D. and Dutta, A. (1994). Haematological values of local duck of Assam in relation to sex. *Indian Veterinary Journal,* 71: 1048-1082.

Daughaday, W.H. and Rotwein, P. (1989). IGF-1 and 2, Peptide, messenger RNA and gene structures, Serum and tissue concentrations. *Endocrinology Review*, 10:68-91.

Den Daas, N. (1992). Labouratory measurement of semen characteristics. *Animal Reproductive Science,* 28: 87-94.

Donoghue, A.M. and Wishart, G.J. (2000). Storage of poultry semen. *Animal Reproduction Science,* 62: 213-232.

Donoghue, A.M. (1999). Prospective approaches to avoid flock fertility. In: Predictive assessment of sperm function traits in poultry. *Poultry Science*, 78: 437- 443.

Duclos, M.J., Beccavin, C. and Simon, J. (1999). Genetic models for the study of insulin-like growth factors (IGF) and muscle development in birds compared to mammals. *Domestic Animal Endocrinology,* 17: 231–243.

Elagib, H.A.A., Elamin, K.M., Ahmed, A.D.A., Malik, H.E.E. (2012). Blood Biochemical Profile of Males and Females of Three Indigenous Chicken Ecotypes in Sudan. *Journal Veterinary Advance,* 2(12): 568-572.

Egahi, J.O., Dim, N.I. and Momoh, O.M. (2013). Crossbreeding and reciprocal effect on egg weight, hatch weight and Growth Pattern and the Interrelationships between these Traits in Three Genetic Groups of Native Chickens of Nigeria. *Asian Journal of Biological Sciences*, 6: 187-191. DOI**:** 10.3923/ajbs.2013.187.191

El-Fiky, A.A. and Soliman, M.M. (2006). Effect of blood groups on some physiological parameters of chickens. *Egyptian Poultry Science,* 26: 267 – 279.

El-Sahn, A. A. (2007). Use of phenotypic traits to predict cocks fertility. The secondary sexual traits and the skeletal conformation. *Egyptian Poultry Science,* 27: 521 – 536.

El-Sheikh, A.M.H. and Hanafy, M.M. (2006). Physiological effect of gibberellic acid (GA3) on some productive and reproductive traits of Bandarah local chicken strain. *Egyptian Poultry Science,* 26: 1071-1087.

El-Tantawy, S.M.T., Atta, A.M., Bakir, A.A.M., EI-Manawey, M.A. and Habeeb, A.R.H. (2007). Evaluation of some productive and reproductive performance of Inshas and Matrouh chickens. *Egyptian Journal of Applied Science,* 22: 466-479.

Emily, R.B. (2003). Sperm mobility in broiler breeders. An MSc Thesis Submitted to the Graduate Faculty of the University of Georgia, Athens, Georgia.

Emmerson, D.A. (2003). Breeding objectives and selection strategies for broiler production. In: Poultry Genetics, Breeding and Biotechnology. W.M. Muir and S.E Aggrey, eds. CABI Publishing, Wallingford, UK, Pages 113-126.

Etches, R.J. (1998). *Reproductions in Poultry*. CAB International Wallingford.

Eze, J.I., Onunkwo, J.I., Shoyinka, S.V.O., Chah, F.K., Ngene, A.A., Okolinta, N., Nwanta, J.A and Onyenwe, I.W. (2010). Haematological profile of pigs raised under intensive management system in South Eastern Nigeria. *Nigerian Veterinary Journal,* 31(2): 115-123.

Ezekwe A.G. and Machebe N.S. (2004). Ejaculate characteristics of two strains of local cocks (Naked-Neck and Frizzle) in Nigeria. In: *Proceedings 29th Annual Conference of the Nigeria Society for Animal Production* 29: 92–95.

Ezekwe A.G., Udozor I.J. and Osita C.O (2003). Effect of quantitative feed restriction on the semen quality of Nigerian Local Cocks. *Nigerian Journal of Animal Production,* 30 (1): 127–132.

Fayeye, T.R., Ayorinde, K.L., Ojo, V. and Adesina, O.M. (2005). Frequency and influence of some major genes on body weight and body size parameters of Nigeria local chickens. *Livestock Research for Rural Development,* Volume 18.

Florini, J.R., Ewton, D.Z. and Coolican, S. A. (1996). Growth hormone and the insulin-like growth factor system in myogenesis. *Endocrinology Review,* 17: 481–517.

Fontana, E.A., Weaver Jr. W.D. and Van Krey, H.P. (1990). Effects of various feeding regimens on reproduction in broiler males. *Poultry Science,* 69: 209-216.

Froman, D.P. and Feltmann, A.J. (1998). Sperm mobility: A quantitative trait of the domestic fowl (Gallus domesticus). *Biology Reproduction,* 58: 379-384

Fudge, A.M. (2000). Laboratory Medicine-Avian and Exotic Pets. W.B. Saunders Company, Philadelphia, Page 486.

Galal, A. (2007). Predicting semen attributes of naked neck and normally feathered male chickens from live performance traits. *International Journal of Poultry Science* 6: 36-42.

Gabriel, G.M., Kalamah, M.A., El-Fiky, A.A. and Ali, A.F.A. (2009). Some factors affecting semen quality traits in Norfa cocks. *Egypt Poultry Science,* 29 (2): 677-693.

Goddard, C, Wilkie, R.S and Dunn, I.C. (1988). The relationship between insulin-like growth factor-1, growth hormone, thyroid hormones and insulin in chickens selected for growth. *Animal Endocrinology,* 5: 165–176.

Grossman, R.D. (1988). Multi-phasic analysis of growth curves in chicken. *Poultry Science,* 67: 33-42.

Gueye, E.F., Ndiaye, A. and Branckaert, R.D.S. (1998). Prediction of body weight on the basis of body measurements in mature indigenous chickens in Senegal. LRRD 10:http://www.cipav.org.co/lrrd10/3/sene103.htm.

Gueye, E.F. (1998). Village egg and fowl meat production in Africa. *World's Poultry Science Journal,* 54: 73–86.

Gumulka, M. and Kapkowska, E. (2005). Age effect of broilers on fertility and sperm penetration of the perivitelline layer of the ovum. *Animal Reproduction Science,* 90: 135-147. PMID ; 16257603.

Gupta, R.D., Joshi, S.K., Alkare, S.S. and Bagheel, K.K. (1988). Influence of divergent egg weight on performance of progenies from reciprocal cross of dwarf X normal population. *Indian Journal of Animal Science,* 58: 130-132.

Gur, S. and Demirci, E. (2000). Holfltayn bogaların seminal plazmasındaki kalsiyum, magnezyum, sodium ve potasyum duzeylerinin spermatolojik özellikler üzerine etkisi. *Turkish Journal of Veterinary Animal Science,* 24: 275-281.

Habeb, A.R.H. (2007). A comparative study of meat and egg production and some physiological aspects in some local strains of chickens. MSc. Thesis, Faculty of Agriculture Cairo University Egypt.

Hafez, E.S.E. (1990). Artificial insemination. In: Hafez, E.S.E (Ed); Reproduction in Farm Animals. *5th Edition, Lea and Febieger, Philadelphia,* 451- 497.

Hafez, E.S.E. (1993). Reproduction in Farm Animals. 6th ed. Philadelphia: Lea and Febiger, page 405-439.

Hafez, B. and Hafez, E.S.E. (2000). Reproduction in Farm Animals. *7th Edition. New York*. Lippincott Williams and Wilkins, USA.

Halima, H., Neser, F.W.C., van Marle-Koster, E. and de Kock, A. (2007). Phenotypic variation of indigenous chicken populations in northwest Ethiopia. *Tropical Animal Health and Production*, 39: 507–513.

Han, V.K., Hill, D.J., Strain, A.J., Towle, A.C., Lauder, J.M., Underwood, L.E. and D'Ercole, A. J. (1987). Identification of somatomedin / IGF immunoreactive cells in the human fetus. *Pediatric Residency,* 22:245-249.

Hanafy, M.M. (2006). Effect of essential phospholipids (EPL) injection on total lipids and cholesterol contents of Gimmizah laying hens. *Egyptian Poultry Science,* 26: 281-295.

Hancock, J.I. (1951). A staining technique for the study of temperature shock in semen. *Nature*, London 197: 323-343.

Hocking P.M. and Bernard R. (1997). Effect of dietary crude protein content and food intake on production of semen in two lines of broiler breeder males. *British Poultry Science,* 38:199–202.

Horst, P. (1989). Native fowl as reservoir for genomes and major genes with direct and indirect effects on adaptability and their potential for tropical oriented breeding plans. Archiv Fur Guflugelkunde, 53(3): 93-101.

Hossein, A.A. and Mohsen, K. (2011). Detection of polymorphism at the insulin like growth factor-1 gene in Mazandaran native chicken using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Method. *American Journal of Animal and Veterinary Sciences,* 6 (2): 80-83.

Hunderra, S.A. (2004). Evaluation of semen parameters in Ethiopian indigenous bulls kept at Kaliti Artificial insemination Center, Addis Abba. A MSc. Thesis, Faculty of Veterinary Medicine of Addis Ababa University in Tropical Veterinary Medicine Page 16-18.

Huque, Q.M.E. (1999). Nutritional status of family poultry in Bangladesh. *Livestock Research for Rural Development,* 11: 3. http://www.lrrd.org/lrrd11/3/huq113

Iaffaldano, N., Manchisi, A., and Rosato, M.P. (2007). The preservability of turkey semen quality during liquid storage in relation to strain and age of males. *Journal Animal Reproduction Science*, 11: 024. DOI: 101016

Ibrahim, A., Thnaian, A. and El-Bahr, S.M. (2012). Investigation of selected biochemical parameters of local chickens with different age and sex in Al-ahsa, Saudi Arabia. *Pakistan Journal of Biological Sciences*, 15 (17): 827-832.

Ibe, S.N (2001). Problems and proposal of developing Nigerian poultry breeds. *Journal of Science, Agriculture, Food Technology and Environment,* 1: 1-3.

Ibe, S.N (1998). Improving productive adaptability of the Nigerian local chicken. In: proceedings of the silver anniversary conference of the Nigerian Society for Animal Production held at Gateway Hotel, Abeokuta, Nigeria 21-26th March, 1998. Page 331-335.

Ikhimioya, I.A., Arijeniwa, I.T., Oteku, I.T. and Ahmed, A. (2000). Preliminary investigation on the haematology of Nigerian indigenous chickens. *Proceedings of the 5th Animal Conference Animal Science Association Nigeria*, 19th-22nd September, Port-Harcourt, Nigeria page 10-12.

Isidahomen, C.E., Ozoje, M.O. and Njidda, A.A. (2011). Haematology and serum biochemical indices of local and exotic chickens in a sub-humid tropical environment. *European Journal of Biological Science,* 3(1): 16-21. ISSN 2079-2085.

Isidahomen, C.E. and Njidda, A.A. (2012). Haematology and carcass characteristics of Naked Neck, Frizzled and Normal Feathered local chickens in Southern Nigeria. *Savannah Journal of Agriculture*, 7 (2): 12-19.

Islam, M.K., Ahmed, N., Islam, M.A., Sha, S. and Roy, P.K. (1999). Effect of cecectomy on body weight and certain haematological parameters in chickens. *Bangladesh Veterinary Journal*, 33: 55-59.

Islam, M.S., Lucky, N.S., Islam, M.R., Ahad, A., Das, B.R., Rahman, M.M. and Siddiui, M.S.I. (2004). Haematological Parameters of Fayoumi, Assil and Local Chickens Reared in Sylhet Region in Bangladesh. *International Journal of Poultry Science,* 3 (2): 144-147.

Jacyno, E., Kołodziej, A.,Kawęcka, M., Pietruszka, A., Matysiak, B. and Kamyczek, M. (2009). The relationship between blood serum and seminal plasma cholesterol content in young boars and their semen qualitative traits and testes size. *ArchivTierzucht*, 52 (2): 161-168, ISSN 0003-9438.

Jain, N.C. (1993). Essential of Veterinary Haematology. Published by Willians and Wikin,U.S.A and edited by George, H. Mundorff. page 417

Jarinkovičová, L., Máchal, L., Máchal, J., Filipčík, R., Tůmová, E. and Horský, R. (2012). Relationship of ejaculate quality and selected biochemical parameters of blood in cockerels of three laying lines. *Czech Journal of Animal Science,* 8: 370–376.

Johnston, R.C., Clarke, G.N., Liu, D.Y., and Barker, H.W.G. (1995). Assessment of the sperm quality analyzer. *Fertility Sterility,* 63:1071-1076.

Jones, J.I., and Clemmons, D.R. (1995). Insulin-like growth factors and their binding proteins: Biological actions. *Endocrinology Review,* 16:3-34.

Kabir, M. (2006). Estimation of Genetic parameters for semen characteristics in Rhode Island Red and White Chickens. An MSc Thesis, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria.

Kabir, M., Oni O.O., Akpa, G.N. and Adeyinka, I.A. (2006). Heritability estimates and the inter relationships of body weight and shank length in Rhode Island Red and White breeder chickens. *Pakistan Journal of Biological Science,* 9: 2892-2896.

Kabir, M., Oni, O.O., Akpa, G.N., Adeyinka, I.A. and Rekwot, P.I. (2007). Effect of underfeeding on semen quality of Rhode Island cocks. *Pakistan Journal of Biological Science,* 11(6): 986-988.

Kalamah, M.A.A. El-Nady, M.M., Abdou F.H and Esa E.K. (2002). Effect of heat stress and Vitamin C on some productive traits and physiological aspects in chickens. *Minufiya Agricultural Resources,* 27: 57–74.

Kamar, G.A.R. and Hafez, E.S.E. (1975). Sperm maturation and fertility in poultry. Animal Breeding. Abstract Volume 43 No 4.

Kaneko, J.J. (2008). Clinical biochemistry of domestic animals. Academic press Inc, San Diego. 6th Edition, page 897-898.

Karesh, W.B., Campo, A.D., Braselton, E., Puche, H. and Cook, R.A. (1997). Health evaluation of free ranging and hand reared macaws (*Ara* spp.) in Peru. *Journal of Zoo and Wild Life Medicine,* 28: 368-377.

King, J.C. (2000). Physiology of pregnancy and nutrient metabolism. *American Journal of Clinical Nutrition,* 71: 1218-1225.

Kotlowska, M., Glogowski, J., Dietrich, G.J., Kozlowski, K., Frruga, A., Jankowski, J. and Ciereszko, A. (2005). Biochemical characteristics and sperm production of turkey semen in relation to strain and age of the males. *Poultry Science,* 84:1763-1768.

Kral, I. and Suchy, P. (2000). Haematological studies in adolescent breeding cocks. Acta Vet. Brno. 69:189-194.

Ladokun, A.O., Yakubu, A., Otite, J.R., Omeje, J.N., Sokunbi, O.A., Onyeji, E. (2008). Haemtological and serum biochemical indices of naked neck and normally feathered Nigerian indigenous chickens in a sub humid tropical environment. *International Journal of Poultry Science,* 7: 55-58.

Lake, P.E. (1956). The structure of the germinal epithelium of the fowl testis with special reference to the presence of multinuclear cell. *Q.J. Microscopical Science*, 97:487-497.

Lake, P E. (1966). Physiology and biochemistry of poultry semen. In: Advances in Reproductive Physiology. Volume 1 America McLaren edition. Academic press, New York 93-123.

Lake, P.E. (1971). The male in reproduction. In: Physiology and Biochemistry of the Domestic Fowl, Volume III. (D.J. Bell and B.M. Freeman, editions.). London and New York. Academic Press, Chapter 60.

Lake, P.E. and Stewart, J.M. (1978). Artificial insemination in poultry: Biochemistry of the domestic fowl. Academic Press London. New York. 3:1411-1425.

Lake, P.E. (1983). Factors affecting the fertility level in poultry with special reference to artificial insemination. Agricultural Research Council. U.K.

Latif, A., Ijaz, A., Aleem, M. and Mahmud, A. (2005). Effect of osmotic pressure and pH on the short-term storage and fertility of broiler breeder sperm. *Pakistan Veterinary Journal*, 25: 179-183.

Lawrence, T.L.J and Fowler, V.R. (2002). Growth of Farm Animals. 2nd Edition CABI Publishing, Oxon UK. Page 347.

Lee, Y.P., Lee, S.L., Ho, Y.J. and Chen, T.L. (1999). Behavioural responses of cockerels to semen collection and their influence on semen characteristics. *British Poultry Science,* 40: 317-322.

Lei, M.M., Nie, Q.H., Peng, X., Zhang, D.X. and Zhang, Q. (2005). Single nucleotide polymorphism of the chicken insulin-like factor binding protein 2 gene associated with chicken growth and carcass traits. *Poultry Science,* 84: 1191–1198.

Li, H., Zhu, W., Chen, K., Song, W., Shu, J. and Han, W. (2010). Effect of the polymorphism of GHR gene and IGF-1 gene on egg quality in Wanchang chicken. *Medwell Journal,* 3: 19-22. DOI: 10.1093/molbev/ 211

Li, Z.H., Li, H., Zhang, S.Z., Wang, Q.G. and Wang, Y.X. (2006). Identification of a single nucleotide polymorphism of the Insulin Growth Factor binding protein

2 gene and its association with growth and body composition traits in chicken. *Journal of Animal Science,* 84: 2902-2906.

Li, H., Deeb, N., Zhou, H., Mitchell, A.D., Ashwell, C.M. and Lamont, S.J. (2003). Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-beta genes. *Poultry Science*, 82:347–356.

Liu, S.J., Zheng, J.X. and Yang, N. (2008). Semen quality factor as an indicator for fertilizing ability for Geese. *Poultry Science,* 87: 155-159.

Lumeij, J.T. (1997). Avian Clinical Biochemistry. In: Kaneko, J.J., Harvey, J.W and Brass, M.L. (Eds). *Clinical Biochemistry of domestic animals.* Academic Press, San Diego. Page 857-883.

Machal, L., Chladek, G. and Strakova, E. (2002).Copper, phosphorus and calcium in bovine blood and seminal plasma in relation to semen quality. *Animal Feed Science*, 11: 425-436.

Machebe, N.S. and Ezekwe, A.G. (2006). Ejaculate characteristics of three genotypes of local cocks in the humid tropics. *Journal of Agriculture, Food, Environment and Extension*, 3(2): 33-37.

Malik, B.N., Mishra, P.K. and Mishra, S.C (1997). Inheritance of 6 week body weight breast angle, shank length and keel length in a broiler chicken. *Indonesia Journal Poultry Science*, 32: 249-252.

Marquardt, H., Todaro, G., Henderson, L.E. and Oroszalan, S. (1981). Purification and primary structure of a polypeptide with multiplication stimulating activity from rat liver cell cultures. *Journal Biology Chemistry,* 256: 6859-6863.

Massányi, P., Trandzik, J., Nad, P., Toman, R., Skalická, M. and Koréneková, B. (2003). Seminal concentrations of trace elements in various animals and their correlations. *Asian Journal of Andrology,* 5:101-104.

Matilsky, M., Ben-Ami, M., Eyali, V., Geslevieh, Y. and Shalev, E. (1993). Correlation between sperm motility index as measured by the sperm quality analyzer and the outcome of intrauterine inseminations. *Human Reproduction* 8 (Suppl. 1):98. (Absract).

McCartney, M.G. (1996). The effect of semen dosage and insemination frequency on the fertility of broiler breeder hens. *Poultry Sci*ence, 75: 669-671.

McDaniel, C.D., Bramwell R.K., Wilson J.L. and Howarth B. Jr. (1995). Fertility of male and female broiler breeders following exposure to elevated ambient temperature. *Poultry Science,* 74:1029–1038.

McDaniel, C.D., Hannah, J.L., Parker, H.M., Smith, T.W., Schultz, C.D and Zumwalt.C.D. (1998). Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. *Poultry Science,* 77: 888-893.

McDaniel, G.R. (1994). The production of broiler hatching eggs in cages. *Poultry Science,* 73: 19-54.

McMurtry, J.P., Francis, G.L. and Upton, Z. (1997). Insulin like growth factors in poultry. *Domestic Animal Endocrinology,* 14:199–229.

McMurtry, J.P (1998). Nutritional and developmental roles of insulin-like growth factors in poultry. *Journal of Nutrition,* 128: 302-305.

Meizel, S. (1997). Amino acid neurotransmitter receptor/chloride channels of mammalian sperm and the acrosome reaction. *Biology Reproduction,* 56: 569-574.

Meluzzi, A., Primiceri, G., Giordani, R. and Fabris, G. (1992). Determination of blood Constituents' reference values in broilers. *Poultry Science,* 71: 337-345.

Mosaferi, S., Niasari-Naslaji, A., Abarghani, A., Gharahdaghi, A.A. and Gerami, A. (2005). Biophysical and biochemical characteristics of Bactrian camel semen collected by artificial vagina. *Theriogenology,* 63: 92-101.

Moya, A, Gonzalez, A., and Capote, M. (1996). Selection of White Leghorn cocks for artificial insemination 1: sire semen evaluation. *Poultry Science,* 75: 1112-1119.

Muhammad, N.O., Adeyina, A.O. and Peters, O.M. (2000). Nutritional evaluation of fungi treated cocoa bean shell. *Nigerian Journal of Pure and Apply Science,* 5: 1059-1064.

Muhammad, N.O., Oloyede, O.B. Owoyele, B.V and Olajide, J.E. (2004). Deleterious effect of defatted *Terminalia catappa* seed meal-based diet on haematological and urinary parameters of albino rats. *Nigerian Society of Experimental Biology Journal,* 4(2): 51-57.

Nasyrova, D.I., Sapronova, A., Nigmatullina, R.R and Ugrumov, M.V. (2006). Changes in blood plasma volume in rats during Ontogenesis. *Russian Journal of Developmental Biology,* 27: 1062-3604.

Ndofor-Foleng, H.M., Ngongeh, L.A., Uberu, C.P.N, and Nwosu, C.C. (2010). Evaluation of the performance of two indigenous chicken ecotypes and the main cross reared in Nsukka, Enugu State, Nigeria. *International Journal of Science and Nature,* 1(2): 179-182.

Nesamvuni, A.E., Malandzii, J., Zamanyimi, N.D and Taylor, G.J. (2000). Estimates of body weight in Nguru type cattle under commercial management conditions. *South Africa Journal of Animal Science,* Volume 30.

Nguyen, Q.C. and Tran, P.T.T. (2003). Study on some Biological Characteristics of Local Chicken Breeds. Ri, Ac, Ho and Dongtao, *Special Scientific Papers, Vietnam, Nien-Vietnam,* 1:24-34.

Nie, Q., Lei, M., Ouyang, J., Zeng, H., Yang, G. and Zhang, X. (2005). Identification and characterization of single nucleotide polymorphisms in 12 chicken growth correlated genes by denaturing high performance liquid chromatography. *Genetics Selection Evolution*, 37:339-360.

Nir, I., Harvey, S., Cherry, J.A., Dunnington, E.A., Klandorf, H., and Siegel, P.B. (1987). Growth-associated traits in parental and F1 populations of chickens under different feeding programs for growth and thyroid hormones. *Poultry Science*, 66: 32–37.

Njidda, A.A., Igwebuike, J.U., Ngoshe, A.A and Tijjani, A.O. (2006). Effect of substituting maize with graded levels of cane molasses on the performance of broiler finisher birds in the semi arid region of Nigeria. *Journal of Sustainable Agriculture and Environment,* 8(1): 1-13

Nwagu B.I., Rekwot P.I., Abubakar B.Y., Oni O.O., Adeyinka I.A. and Egbuedu U.C. (1996). Semen characteristics of Rhode Island Red and White breeder cocks. *Nigerian Journal of Animal Production*, 23 (1):1–4.

Nwanta, J.A., Umoh, J.U., Abdu, P.A., Agogi I. and Alibalogun .J.K. (2006). Management of losses and New Castle disease in rural poultry in Kaduna State. *Nigerian Journal of Animal Production*, 33(2): 274-285.

Nwosu, C.C. (1979). Characterization of the local chicken of Nigeria and its potential for egg and meat production. Poultry Production in Nigeria. In*: proceedings of the 1st National Seminar on Poultry Production in Nigeria*, December 11th-13th, 1979, Ahmadu Bello University, Zaria, Nigeria page 87-210.

Obioha, F.C. (1992). A Guide to Poultry Production in the Tropics. Enugu Atena Publishers 1st edition.

Oke, U.K., Herbert, U., Ebuzoeme, C.O. and Nwachukwu, E.N. (2007). Effect of genotype on the haematology of Nigerian local chickens in a humid tropical environment. In: proceeding *32nd Animal Conference of NSAP,* Calabar, Nigeria, 18th -21st page 123-125.

Oke, U.K and Ihemeson, C. (2010). Effect of genotype on the morphometric differentiation of the reproductive organs and sperm reserves in the Nigeria local chicken. *Livestock Research for Rural Development*, 22(3): 2010.

Oke, U.K. (2011). Influence of some major genes on growth traits of indigenous pullets in humid tropical environment. *Agriculture and Biology Journal of North America*, 2151-7525. DOI: 10.5251/abjna.2011.2.4.570.576 ©2011, ScienceHuβ.

Oluyemi, J.A. (1980). A comparison of five commercial laying strains of fowl in tropical environment. *Nigerian Journal of Animal Production,* 7: 91- 96.

Omeje, S.I and Marire, B.N. (1990). Evaluation of the semen characteristics of adult cocks of different genetic background. *Theriogenology* 34(6): 1111-1118.

Omeje, S.I. and Ude, I. (1998). Effect of feed restriction on body weight and semen characteristics of native (broiler) cocks. *Journal of Applied Animal Resources,* 14:81–86.

Orunmuyi, M., Akanwa, C.L. and Nwagu B.I. (2013). Semen quality characteristics and effect of mating ratio on reproductive performance of Hubbard broiler breeders. *Journal of Agricultural Science,* 5 (1): 154-159.

Orunmuyi, M., Olutunmogun, A.K., Daudu, O.M., Akinsola, O.M., Akinfenwa, F.A and Olugbemi, T.S. (2011). Effect of sex, age and genotypes on some haematological parameters of the Nigerian indigenous chickens reared intensively. *Proceedings of the 36th Annual Conference of the Nigerian Society for Animal Production.* University of Abuja, 13th – 16th March, 2011. Page 90-92.

Orunmuyi, M. (2006). Genetic evaluation of plasma alkaline phosphatase activity in two strains of Rhode Island chickens. A PhD Dissertation, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria, Page 51-53.

Ovimaps, (2012). Ovilocation map; ovi earth imaginery. Dated July, 2012.

Owoyele, B.V., Adebayo, J.O., Muhammad, N.O and Ebunlomo, A.O. (2003). Effect of different processing techniques of tunbran meal on some haematological indices in rats. *Nigerian Journal of Pure and Applied Science,* 18: 1340-1345.

Ozoje, M.O. and Ikeobi, C.O.N. (1995). Productive performance and occurrence of major genes in the Nigeria local chicken. *Nigerian Journal of Genetics,* 10: 67-77.

Pana C.I., Stoica D., Dregotoiu E.P., Midosanu E.P. and Stanescu, M. (2000). Influence of quantitative and qualitative feed restriction on sperm quality and fecundity of broiler breeder males. *Abstract 14th International Congress of Animal Reproduction.* Stockholm Sweden, Volume 1:89.

Parker, J.E., McKenzie, F.F. and Kempster, H.L (1940). Observations on the sexual behavior of New Hampshire males. *Poultry Science,* 19: 191-197.

Parker, H.M., Yeatman, J.B., Schultz, C.D., Zumwalt, C.D., and McDaniel, C.D. (2000). Use of sperm analyzer for evaluating broiler males. Selection of young broiler breeders for semen quality index increases fertile egg production. *Poultry Science,* 79: 771-777.

Pedersen, C.V. (2002). Production of semi-scavenging chicken in Zimbabwe. PhD Thesis, Royal Veterinary and Agricultural University, Copenhengen, Denmark.

Peebles, E.D., Park, S.W., Burnham, M.R., Branton, S.L., Gerard, P.D. and Womack, S.K. (2009). Influence of supplemental dietary poultry fat on the blood characteristics of commercial layers inoculated before or at the onset of lay with F-Strain *Mycoplasma gallisepticum*. *International Journal of Poultry Science,* 8 (9): 824-828.

Perek, M. and Snapir, N. (1963). Seasonal variations in semen production of different breeds of cocks and the effect of Vitamin C feed supplementation upon the semen of White Rocks. *Poultry Science,* 42:19–26.

Peters, S.O., Omidiji, E.A. Ikeobi, C.O.N. Ebozoje, M.O. and Adebambo. O.A. (2004). Effect of naked neck and frizzle genes on egg traits, fertility and hatchability in local chickens. In: self sufficiency of Animal protein in Nigeria. *Proceedings of the 9th Annual Conference of Animal Science Association of Nigeria*. Ebonyi State University, Abakaliki, Nigeria. September 13th-16th, page: 262-264.

Peters, S.O., Shoyebo, O.D., llori, B.M., Ebozoje, M.O., Ikeobi, C.O.N., and Adebambo, O.A. (2008). Semen quality traits of seven strain of chickens raised in the humid tropics. *International Journal of Poultry Science, 7*(10): 949-953.

Purohit, S.B., Laloraya, M. and Kumar, G.P. (1999).Role of ions and ion channels in capacitation and acrosome reaction of spermatozoa. *Asian Journal of Andrology,* 1: 95-107.

Rekwot, P.I., Oyedipe, E.O., Dawuda, P.M. and Sekoni, V.O. (1997). Age and hourly related changes of serum testosterone and spermiogram of pre-puberty bulls fed two levels of nutrition. *The Veterinary Journal,* 153: 341-347.

Reyna, X.F., Montoya, H.M., Castrellon, V.V., Rincon, A.M., Bracamonte, M.P. and Vera, W.A. (2010). Polymorphisms in the IGF-1 gene and their effect on growth traits in Mexican beef cattle. *Genetics Molecular Resources*, 9: 875–883.

Ritchie, B.W., Harrison, J.G. and Harrison, R.L. (1994). Avian Medicine, Winger's publishing Inc. Florida.

Rodriguez-Martinez, H. and Barth, A.D. (2007). In vitro evaluation of sperm quality related to in vivo function and fertility. In: Juengel, J.I., Murray, J.F. (Ed.). *Reproduction in Domestic Animal VI*. Nottingham, UK: Nottingham University Press. Page: 39-54.

Rosato, M.P., Cinone, M., Meluzzi, A., Manchisi, A., and Iaffaldano, N. (2006). Changes in the quality characteristics of stored semen during reproductive period of Hybrid toms. *World Poultry Science Journal,* 62:538.

Ruth, H.M. (2002). Reproduction in male broiler breeders. PhD Dissertation, Faculty of Agriculture, Forest and Home Economics, University of Georgia, Athens.

Saacke, R.G. (1983)**.** Semen quality in relation to semen preservation. *Department of Dairy Science,* 66: 2635-2644.

SAS. (2002). Statistical Analysis System, Computer Software, Version 9: Statistics SAS Institute Inc. Cary, Northern Carolina 27513, USA.

Safloah, A.C. (1997). Characteristics of indigenous chickens of Malawi. *Animal Genetic Resources Information,* 22: 61-69.

Salisbury, G.W., Van Demark, N.L. and Lodge, J. R. (1978). Physiology of reproduction and artificial insemination of Cattle. 2nd edition. San Francisco: W. H. Free man, page 385-479.

Scanes, C.G., Dunnington, E.A., Buonomo, F.C., Donoghue, D.J. and Siegel, P.B. (1989). Plasma concentrations of insulin-like growth factors 1 (IGF-1) and IGF-2 in dwarf and normal chickens of high and low weight selected lines. *Growth Development Aging,* 53: 151–157.

Schales, O. and Schales, S.S. (1941). A aimple and accurate method for determination of chloride in biology fluid. *Journal of Biology and Chemistry,* 140(5): 879-882.

Schalm, O.W., Jain, N.C and Carrol, E.J. (1975). Veterinary Haematology, Lea and Febiger Philadelphia 3rd (ed) page 15-218.

Schmidt, E.M.S., Paulillo, A.C., Martins, G.R.V., Lapera, I.M., Testi, A.G.P., Junior, L.N., Denadai, J. and Fagliari, J.J. (2009). Haematology of the Bronze Turkey (Meleagris gallopavo): Variations with Age and Gender. *International Journal of Poultry Science*, 8(8): 752-754.

Schmidt, E.M.S., Paulillo, A.C., Santin, E., Dittrich, R.L. and Oliveira, E.G. (2007). Hematological and serum chemistry values for the ring-necked pheasant (*Phasianus colchicus*): Variation with sex and age. *International Journal of Poultry Sci*ence, 6(2): 137-139.

Schulze, V., Rohe, R., Looft, H and Kalm, E. (2002). Genetics analysis of the course of individual growth and feed intake of group-penned performance tested boar (In German Language). Arch. Tievz. Dummerstorf, 44: 139-156.

Selvan, S.T. (2007). Influence of dietary protein, calcium and vitamin E on the semen quality in broiler breeder males. *Tamilnadu Journal of Veterinary Animal Science*, 3(2): 60-64.

Senger, P.L. (2003). Path ways to pregnancy and Parturition. (99164-6332) 2nd Edition Pullman, Washington, USA.

Shah, T., Deshpande, S., Dasgupta, U., Singh, K. M. and Joshi, G. C. (2012). Genotypic and allelic frequencies of IGF-1 and IGF-2 genes in Broilers analyzed by using PCR-RFLP technique. *Iranian Journal of Applied Animal Science*, 2(4): 357-360.

Shahein, E.H.A., El-Sahn, A.A. and Rizk, R.E. (2007). Hatching traits and embryonic development as influenced by flock age in local chicken. *Egyptian Poultry Science*, 27(1): 309-319.

Siddle, K., Urso, B., Niesler, C.A., Cope, D.L., Molina, L., Surinya, K.H. and Soos, M.A. (2001). Specificity in ligland binding and intracellular signaling by insulin and insulin-like growth factor receptors. *Biochemistry Society of Transgenetics,* 29: 513-525.

Sil, G.C., Das, P.M., Islam, M.R. and Rahman, M.M. (2002). Management and disease problems of cockerels in some farms of Mymensingh, Bangladesh. *International Journal Poultry Science*, 1: 102-105.

Simaraks, S., Chinrasri, O. and Aengwanich, W. (2004). Hematological, electrolyte and serum biochemical value of the Thai indigenous chickens (*Gallus domesticus*) in northeastern of Thailand. *Songklanakarin Journal of Science Technology,* 26: 425-430.

Singh, H.N. and Singh, B.P. (1983). Inheritance of eight week body weight in pure and cross breed population of two broiler strains. *Indian Veterinary Journal,* 60: 560-563.

Siudzinska, A. and Lukaszewicz, E. (2008). Effect of semen extenders and storage time on sperm morphology of four chicken breeds. *Applied Poultry Resource,* 17: 101-108.

Sonaiya, E.B. and Swan, S.E.J. (2004). Small-scale poultry production, technical guide manual. FAO Animal Production and Health 1. Food and Agriculture Organization of the United Nations (FAO), Rome.

Sonaiya, E.B., Branckaert R.D.S. and Gueye, E.F. (1999). Research and development options for family poultry. First International Network for Family Poultry Development/FAO. *Electronic Conference on Family Poultry*. www.Infpd.net/cms/sc

Ssewayana, E., Onyait, A.O., Ogwal, J., Mukasa, B., Nsamba, P., and Musaba, J. (2003). Characteristics of rural chickens in Apac and Knmi districts of Uganda. *Uganda Journal of Agriculture Science,* 8: 159-164.

Sturkie, P. D. and Opel, H. (1976). Reproduction in the male, fertilization, and early embryonic development. In: Avian Reproduction (3rd ed.) (P. D. Sturkie, Ed.). New York: *Springer- Verlag,* Chapter 17.

Sutter, N.B., Bustamante, C.D., Chase, K., Gray, M.M., Zhao, K., Zhu, L., Padhukasahasram, B., Karlins, E., Davis, S. and Jones, P.G. (2007). A single IGF-1 allele is a major determinant of small size in dogs. *Science,* 316:112–115.

Tabatabaei, S., Chaji, M., and Mohammadabadi, T. (2010). Correlation between age of rooster and semen quality in Iranian indigenous broiler breeder chickens. *Journal of Animal and Veterinary Advances,* 9(1): 195-198.

Tabatabaei, S., Batavani R.A. and Talebi, A.R. (2009). Comparison of semen quality in Indigenous and Ross broiler breeders. *Journal of Animal Veterinary Advances,* 8(1): 90-93. http://medwelljournals.com/fulltext/java/2009/90-93.pdf.

Talebi, A., Asri-Rezaei, S., Rozeh-Chai, R. and Sahraei, R. (2005). Comparative studies on haematological values of broiler strains. (Ross, Cobb, Arbor-acres and Arian) *International Journal of Poultry Science,* 4 (8): 573-579.

Tang, S., Sun, D., Ou, J., Zhang, Y. and Xu, G. (2010). Evaluation of the IGFs (IGF-1 and IGF-2) genes as candidates for growth, body measurement, carcass, and reproduction traits in Beijing You and Silkie chickens. *Animal Biotechnology*, 21: 104–113.

Teguia, A., Ngandjou, H.M., Defang H. and Tchoumboue. J. (2008). Study of the live body weight and body characteristics of the African Muscovy Duck (*Cairina moschata*). *Tropical Animal Health and Production,* 40: 5- 10.

Thatohatsi, M.B.M. (2009). Characterization and Cryopreservation of semen of four South African chicken breeds. A MSc. Thesis, Department of Animal, Wildlife and Grassland Science, University of the Free State, Bloemfontein.

Tomas, F.M., Pym, R.A., McMurtry, J.P. and Francis, G.L. (1998). Insulin-like growth factor IGF-1 but not IGF-2 promotes lean growth and feed efficiency in broiler chickens. *General Comparative Endocrinology*, 110: 262–275.

Tuncer, P.B., Kinet, H., Ozdogan, N. and Demiral, O.O. (2006). Evaluation of some spermatological characteristics in Denizli cocks. *Journal Faculty of Veterinary Medicine University Erciyes,* 3(1): 37-42.

Udeh, I. (2012). Use of factor scores for determining the relationship between body measurements and semen traits of cocks. *Open Journal of Animal Sciences*, 2(1): 41-44.

Udeh, I., Ugwu, S.O.C and Ogagifo, N.L. (2011). Predicting semen traits of local and exotic cocks using linear body measurements. *Asian Journal of Animal Science,* 5: 268-276. DOI:10.3923/ajas.2011.268.276

Udeh, I. and Ogbu, C.C. (2011). Principal component analysis of body measurements in three strains of broiler chicken. *Science World Journal*, 6(2): 11-14.

Vincent, A.M., Feldman, E.L. (2002). Control of cell survival by IGF signaling pathways. *Growth Hormone and IGF Research*, 12: 193-197.

Wang, P., Tan, Y., Zhang, B., Chu, M., Deng, L., Fan, Q. and Liu, C. (2011). DNA Polymorphisms of 5´-flanking region of insulin-like growth factor-1 gene and their association with reproduction traits in Goats. *Agricultural Sciences in China,* 10(10): 1609-1617.

Wang, W., Kehui O., Jianhua O., Haihua L., Shumao., L. and Han Sun. (2004). Polymorphism of insulin-like growth factor-1 gene in six chicken breeds and its relationship with growth traits. *Asian-Australia Journal Animal Science*, 17(3): 301-304.

Watson, P.P. (1975). Use of Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Veterinary Research,* 97: 12-15.

Wakenell, P.S (2010). Haematology of Chickens and Turkeys. In: Weiss, D.J., Wardrop, K.J., Schalm's Veterinary and hematology Sixth Edition. *Blackwell Publishing Ltd*. page 958-967.

Wilson, H.R., Piesco, N.P., Miller, E.R. and Nesbeth, W.G. (1979). Prediction of the fertility potential of broiler breeder males. *World's Poultry Science Journal,* 35: 95-118.

Yan, B.X., Li, N., Deng, X.M., Hu, X.X., Liu, Z.L., Zhao, X.B., Lian, Z.X. and Wu, C.X. (2002). Single nucleotide polymorphism analysis in chicken insulin-like growth factor-2 and its associations with growth and carcass traits. *Yi Chuan Xue Bao,* 29: 30–33.

Yakubu, A., Kuje, D. and Okpeku, M. (2009). Principal components as measures of size and shape in Nigerian indigenous chickens. *Thai Journal of Agricultural Science,* 42(3): 167-176.

Yang, T.L., Xiong, D.H., Guo, Y., Recker, R.R. and Deng, H.W. (2008). Comprehensive association analyses of IGF-1, ESR2, and CYP17 genes with adult height in Caucasians. *European Journal of Human Genetics,* 16: 1380–1387.

Yousif, I.A., Ismail, A.M., Mohamed, A.A. and Suleiman, M.F. (2006). Growth curves and factors affecting body weight in indigenous chickens prior to age at sexual maturity. *University of Khartoum Journal of Agricultural Science,* 14(3): 450-461.

Yousif, I.A. and Osman, A. (1994). Production traits and parameters of the chicken of Sudan. *Sudan Journal of Veterinary Science and Animal Husbandry,* 33: 90-95.

Zapf, J. and Froesch, E.R. (1999). Insulin-like growth factor-1 actions on somatic growth. In: Handbook of Physiology. J.L. Kostyo, ed. Oxford University Press, New York.

Zavos, P.M., Correa, J.R. and Zarmakoupis-Zavos, P.N. (1996). Measurement of the sperm motility index via the sperm quality analyzer and its relationship to other qualitative sperm parameters. *Theriogenology,* 46: 421-427.

Zhou, M., Ma, Z. and Sly, W.S. (1995). Cloning and expression of the cDNA of chicken cation independent mannose-6-phosphate receptor. *Proceeding National Academics Science,* 92: 9762-9766.

Zhou, H., Mitchell, A.D., McMurtry, J.P., Ashwell, C.M., Lamont, S.J. (2005): Insulin-like growth factor-1 Gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. *Poultry Science*, 84: 212‒219.