Sudan University of Science andTechnology

Collegeof Graduate Studies

Phenotypic and Genotypic Characterization of Sudanese Indigenous Bare Neck Chicken

التوصيف المظهري والوراثي للدجاج البلدي السوداني عاري الرقبة

By

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Dedication

This piece of work is dedicated to the souls of my deceased parents

Awan

and

Veronica Nyathow Amolker

To my family

Dear Wife Dak Othownh Dak

and

Beloved Daughter Mujojwok

For their patient and sacrifices they made during my absence

And

for the difficult days from May 2013 to January 2015 they have undergone in Malakal

Ojang

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Appreviations

AD: After Death

AFLP: Amplified Fragment Length Polymorphism

AP-PCR: arbitrarily primed

Camp: cycle adenosine monophosphate

BC: Before Christ

EBV: Estimated Breeding Value

ECD: Extracellular domain

e.g.: For example

CAPs: Cleaved Amplified Polymorphic Sequence

DAD-IS: Domestic Animal Diversity Information System

DAF: DNA Amplified Fingerprinting Technique

DNA: Deoxy ribonucleic acid

PCR- RFLP: Polymerase Chain Reaction- Restriction fragment Length Polymorphism

FAO: Food and Agriculture Organization

INRA: International Nuclear Regulation Association

G.gallus: Gallus gallus

G. Sonnerattii: Gallus Sonneratii

USA: United State of America

UV: Ultraviolet

QTL: Quantitative traits loci

GH: Growth hormone

IGF: Insulin-like Growth factor

GHRH: Growth releasing hormone

GLP1: glucagon-like peptide

VIP: Vasoactive intestinal

SST: somatostatin

PACAP: Pituitary adeaylate cyclase-peptide

SSR: Simple Sequence Repeats

STS: Sequence-Tagged sites

1-SSR: Inter-simple Sequence Repeats

SCAR: Sequence Characterized Amplified Regions

SAMPL: Selective Amplified Microsattelite Locus Sequence

S- SAP: Sequence Amplification Polymorphism

SNP: Single Nucleotide Polymorphism DAP: DNA amplified finger printing technique

UV: Ultraviolet

GH: growth hormone

IGF: Insulin-like Growth Factor

GHRH: growth hormone releasing hormone

GLPI: glucagon-like peptide 1

PACAP: pituitary adenylate cycle-activating peptide

A (PKA): activate protein kinase

TRH: Thyrotropin releasing hormone

GHRPs: growth hormone releasing peptides

GC: glucocorticoid

PIT1: pituitary-specific transcription factor!

TR: Thyroid hormone receptor

TATA box: Teleosts, amphibians, birds, primate and non- primate mammals

CORT: corticosterone

ER: estrogen receptor

TM: transmembrane domain

1CD: intracellular domain

ECD: Extracellular domain

ADG: Average daily again

PACAP: Pituitary adenylate cycle-activating peptide

GLP 1: glucagon-like peptide 1

PKA: protein kinase A

TRH: Thyrotropin releasing hormone

GHRPs: growth hormone releasing peptides

CRH: corticotrophin releasing hormone

GC: glucocorticoid

CORT: corticosterone

FACT: Farm Animal Conservation Trust

SSCP: Single Stranded Conformation Polymorphism

ASO: Allele Specific Oligonucleotides

DASH: Dynamic Allele-specific Hybridization

ARMS: amplification refractory mutation system

TM: Taqman

MALDITOF: Matrix-assisted laser desorption/ionization time of flight mass spectrometry

ET: East Lansing

GHRH: growth hormone-releasing hormone

SST: somatostatin

TR: Thyroid hormone receptor

cAMP: cyclic adonsine mono phosphate

Mg: milligram

ME/CAL/KG: Metabolizable energy/calory/ kilogram

EDTA: Ethylene Diarnine Tetra acetic Acid 4 micro liter

AOAC: American Organization for Analytic Chemistry Min: minute

EL: elution

Ca: capacity?

GC: Binding buffer

: Washing buffer 1 W2: Washing buffer 2 EL: Elution buffer

CRI: chicken repeat element

Na: Naked neck gene Na Na: homozygous Na na: heterozygous

SLDA: single locus and diallelic

MOET: multiple ovulation and embryo transfer

DAF: DNA amplification fingerprinting technique

SSR's: simple sequence repeats

STR's: short tandem repeats

VNTR: variable number length repeats

STMs: sequence tagged microsatellite

SNPS: single nucleotide polymorphisms

SSCP: single stranded conformation polymorphism

ASO: allele specific oligonucleotides

DASH: Dynamic Allelie-Specific Hybridization

ARMS test: Allele Specific PCR (amplification refractory mutation system

MALDITOF: matrix-assisted laser desorption/ionization time of flight mass spectrometry EL: east lansing

GHRH: growth hormone releasing hormone

SST: somatostatin

GR: glucocorticoid receptor

TR: thyroid hormone receptor

Camp: cyclic adenosine monophosphate

ADG: average daily gain

VIP: vasoactive intestinal peptide

PACAP: pituitary adenylate cyclase-activating peptide

GLP1: glucagon-like peptide 1

PKA: protein kinase A

TRH; thyrotropin releasing hormone

GHRPs: growth hormone- releasing peptide

PKC: protein kinase C

NPY: neuropeptide

GC: glucocorticoid

TS1-113: 13-subunit of thyroid-stimulating hormone

CORT: corticosterone

T3: triiodothyronine

PIT 1: pituitary-specific transcription factor 1

TSS: transcription start site

EGF: epidermal growth factor

ECD: extra cellular domain

TM: transmembrane domain

ICD: intracellular domain

Abstract

The indigenous chickens are known of low productivity and adaptability to their local environment which made them to be resistant to some extend to some diseases prevalent in their localities more than exotic breeds.

In this study two experiments were conducted. The trial was concerning phenotypic trial of indigenous Bare neck chicken. The second experiment was handling three genes\in order to show polymorphism in the three genes GH, PIT1 and PRLR.

In the first trial phonetic performance of the Sudanese indigenous bare neck was determined which include body weight, body weight gain, feed consumption, feed conversion ratio, age at first egg, egg quality characteristics, inedible and edible carcass cuts and the mean values of chemical analysis and sensory evaluation percentages are as below:

The mean weights of body weight observed were 27.37±2.12g, 131.89±11.25, 339.24±72.42, 525.43±97.17g and 739.33±147.13g during rearing period of 0, 4, 8, 12, 16 weeks of age. The initial and final weights of males and at (0, 16) weeks were (28.79, 816.43g) for males and (26.96g, 699.63g) for females. The body weight gains were 104.24g, 207.36g, 187.18g, and 213.09g at 4, 8, 12 and 16 weeks of age. Feed consumption was 401g, 702, 920g and 932.06g at 4, 8, 12 and 16 weeks of age. Feed conversion ratios were 3.84, 3.39, 4, 98 and 5.47 at 4, 8, 12 and 16 weeks of age.

The age at first egg was 148.44 days with mean egg weight and body weight of 33.50 g and 1006.7g respectively.

The egg quality characteristics showed mean values of external and internal egg quality. The external egg quality of egg weight, egg length, egg diameter, shell weight, shell thickness and egg shape index mean vales were 31.43±0.85g, 46.11±0.42mm, 34.93±0.40mm, 4.30±0.08g, 0.38±0.04 mm and 0.75.87+1.17. The internal quality characteristics of albumen weight, albumen height, yolk weight, yolk height, yolk diameter, yolk index and Haugh unit mean values were 18.01±0.65g, 4.31±0.39 mm, 9.05±0.28g, 13.54±0.29mm, 32.55±0.33mm, 0.42±0.01 and 75.2012.34.

The measurement of carcass cuts consists of inedible and edible carcass cuts weights. The inedible carcass cuts weights were composed of blood weight, feather weight, head weight, neck weights, legs weights, intestine weights, abdominal fat weights, wings weights,

shank weights, shank length and back weights. The mean weights of these traits at 8, 12 and 16 weeks of age were (15.00±6.55g, 26.56±11.06g and 30.00±9.78g) blood weights, (15.65±8.84g, 17.81±5.76g and 37.19±19.58g) feather weights, (19.87±3.38g, 23.38±2.80g and 29.06±12.44g) head weights, (13.67±3.96g,, 17.63±3.20g and 29.69±5.87g) neck weights, (23.33±6.70g, 25.13±4.38g and 27.62±6.70g) legs weights, (27.47±4.9g, 37.94±7.75g and 47.94±7.51 g) intestine weights, (0, 2.50±1.67 and 3.19±2.12g) abdominal fat weights, (24.93±4.13g, 42.81±9.00g and 57.62±12.76g) wings weights, (5.87±2.17g, 6.13±2.42g and 6.8711.93g) shank weight, (4.53+0.74 cm, 5.31+0.48 cm and 5.94±0.77cm) shank length and (41.33±10.07 g, 87.13±24.29 g and 138.76±26.30 g) back weights.

The edible carcass cuts weights of giblets, (gizzard weights, heart weights and liver weights) and commercial carcass cuts weights (breast, thigh and drumstick) mean at 8, 12 and 16 weeks of age were (12.73±2.84g, 15.88±2.06g and 17.25±3.62g) gizzard weights, (3.00±1.36g, 3.44±0.63g and 4.50±1.71g) heart weights and (14.60±3.80g, 20.81±4.02g and 21.94±6.31g) liver weights. The commercial carcass cuts weights of breast, thigh and drumstick at the same period above were (58.27+39.41 g, 71.25±14.14g and 115.38±30.74g) breast weight, (40.93±25.06g, 48.00±11.08g and 73.88±16.18g) thigh weight and (37.33±22.50g, 39.88±6.98g and 60.63±12.94g) drumstick weights. The meat and bone mean weights of breast, thigh and drumstick at the same period were (45.07+36.32g, 56.50±14.76 and 95.87±25.44g) breast meat weights and corresponding breast bone weights were (12.27±4.33g, 15.75±5.36g and 19.38±8.66g), (31.53±21.68g, 37.88±9.65g and 59.25±14.25g) thigh meat weights and (9.33±8.06g, 10.00±2.63g and 14.63±4.66g) corresponding thigh bone weights and (25.87±18.49g, 29.63±6.21g and 46.88±11.31g) drumstick meat weights and (9.0014.52g, 10.50±3.46g and 13.87±4.76g).corresponding drumstick bone.

The chemical analysis percentages of moisture, protein, fat and ash were 77.06±0.70% 19.60±0.59% 2.07±0.01 % and 1.04±0.01% respectively.

The sensory evaluation traits of breast, thigh and drumstick attributes as judged by the panelists showed the scores on attributes of tenderness of breast, thigh and drumstick scored 35.52%, 33.73% and 30.75% respectively. The flavor of breast, drumstick and thigh scored 36.39%, 32.95% and 30.66%. The colour of breast, thigh and drumstick scored 34.66%, 34.66% and 30.68%. The juiciness of breast, thigh and drumstick scored 37.08%,

34.19% and 28.75% respectively.

The second experiment was based on polymorphism of three genes, namely PIT1; GH and PRLR which were genotyped by using PCR-RFLPs technology in six Sudanese indigenous chicken lines and they are: Bare neck (1; 2); Large Baladi (1; 2); Frizzle and Betwil. For each line, twenty blood samples were collected from wing vein; DNA was extracted, PCRs were performed using specific primers for the genes understudy, the PCR products were characterized after cutting the products with previously reported restriction enzymes. The results showed the absence of polymorphism for the PRLR gene which shows only one allele or genotype (B; BB) and the other two genes were polymorphic. The GH gene showed two alleles (A; B) with three genotypes (AA; AB; BB), while the PIT1 gene showed also two alleles (A; B) with only two genotypes (AA; AB), frequencies of alleles and genotypes were varying according to the line. In conclusion the GH and P1T1 genes can be used in genetic improvement of Sudanese chickens while PRLR gene cannot be used due to the lack of polymorphism in it.

ملخص الدراسة

إن الدجاج البلدي في السودان معروف بقلة إنتاجها وملائمتها للبيئة المحلية مما تجعلها تقاوم بعض الامراض المستوطنة في مناطق تواجدها أكثر من السلالات المستوردة.

في هذا البحث تم تناول تجربتين اللأولى خاصة بأداء الصفات الظاهرية للدجاج السوداني العاري الرقبة والتجربة الثانية تناولت ثلاثة جينات هي GH, PIT1 و PRLR وذلك لمعرفة تعدد أشكالها. في التجربة الأولى تم تناول أداء الصفات الظاهرية للدجاج البلدي العاري الرقبة وتتمثل هذه الصفات في وزن الجسم والزيادة في وزن الجسم، استهلاك العلف ونسبة التحويل الغذائي، العمر عند أول بيضة، الصفات النوعية للبيضة، وزن الذبيحة المأكول والغير مأكول ومتوسط القيم والنسب المئوية للتحليل الكيميائي وتقييم التذوق، وكذلك تم رصد متوسط القيم كما يلي:

(2.129±27.37,11.25±131.89,72.42±339.2,979±525.43, 147.13±739.33) جرام في فترة النمو (16,12,8,4,0) أسبوع. بينما كان الوزن الابتدائي والنهائي للدجاج الذكور والإناث خلال فترة النمو (16,0) هي (816.43,28.79) جرام للذكور، (699.63,26.69) جرام للإناث. أما زيادة وزن الجسم فقد كانت (213.09,187.18,207.36,104.24) جرام للفترة (16,12,8,4) أسبوع. وبالنسبة لاستهلاك العلف فقد كانت (932.06,9209,702,401) جرام خلال الفترة (16,12,8,4) أسبوع. ونسبة التحويل الغذائي كانت (5,47,4.98,3.39,3.84) في الفترة (16,12,8,4) أسبوع.

إن العمر عند أول بيضة فقد كان 148.44 يوم بمتوسط قيم وزن البيضة ووزن الجسم هي (33.50 و 1006.7) جرام على التوالي.

كما تم تناول الصفات النوعية للبيضة الخارجية والداخلية حيث أن الصفات النوعية للبيضة الخارجية هي وزن البيضة، طول البيضة، عرض البيضة، وزن القشرة، سمك القشرة، معامل شكل البيضة فقد كانت متوسط القيم هي (75.87±1.17,0.38±0.04mm,4.30,31.43±0.85g±0.08g,34.93±0.40mm,46.11±0.42mm) بينما كان متوسط القيم الداخلية للبيضة من وزن البياض، طول البياض، وزن الصفار، طول الصفار، عرض الصفار، معامل الصفار ووحدة هوف هي (13.54±0.29mm,9.05±0.28mm, 4.31±0.39mm, 18.01±0.65g, 75.20±2.34,0.42±0.01,32.55±0.33mm). وبالنسبة لقياس قطع الذبيحة فيتكون من متوسط قيم المأكول والغير المأكول. قطع الغير مأكول تتكون من وزن الدم، وزن الريش، الرأس، الرقبة، الأحشاء، دهن البطن، الأجنحة ووزن الساق وطول الساق والظهر بمتوسط قيم

(30.00±9.78,26.56±11.06,15.00±6.55) جرام للدم

(37.19±19.58,17.81±5.76,15.65±8.84) جرام للريش

(29.06±12.44,23.38±2.80,19.87±3.38) جرام للرأس

(29.69±5.87,17.63±3.20,13.67±3.38.69) جرام للرقبة

(27.62±6.70,25.13±4.38,23.33±6.70) جرام للأرجل

(47.94±7.51,37.94±7.75,27.47±4.9) جرام للأحشاء

(3.19±2.12,2.50±1.67,0) جرام لدهن البطن

(57.62±12.76,42.81±9.00,24.93±4.13) جرام للأجنحة

(5.94±0.77,5.31±0.48,4.53±0.74) سم لطول الساق

(138.76±26.0,87.13±24.29,41.33±10.07) جرام للظهر

في الفترة 12.16.8 أسبوع

وقطع الذبيحة المأكولمن القوانص التي تتمثل في القانص، القلب، الكبد، وقطع الذبيحة التجاري التي تتكون من الصدر، الفخذ، المضرب في الفترة (16,12,8) أسبوع متوسط القيم هي

(17.25±3.62,15.88±2.06,12.73±2.84) جرام للقانص

(4.50±1.71,3.44±0.63,3.00±1.36) جرام للقلب

(20.81±4.02,14.60±3.80 21.94±6.31) جرام للكبد

بينما متوسط قيم قطع الذبيحة للصدر، الفخذ والمضرب خلال الفترة أعلاه هي

(115.38±30.74,71.25±14.14,58.27±39.41) جرام

(73.88±16.18,48.00±11.08,40.93±25.06) جرام

(60.63±12.94,39.88±6.98,37.33±22.50) جرام، على التوالي

متوسط قيم اللحم والعظم للصدر، الفخذ والمضرب فكانت

(19.39±8.66,15.75±5.36,12.27±4.33) جرام للحم والصدر

(95.87±25.44,56.50±14.76,45.07±36.32) جرام لعظم الصدر

(14.63±4.66,10.00±2.63,9.33±8.06,59) جرام للحم الفخذ

(13.87±4.76,10.50±3.46,9.00±4.52) جرام للحم المضرب

(46.88±11.31,29.63±6.21,25.87±18.49) جرام لعظم المضرب

التحليل الكيميائي أوضحت متوسط قيم ونسب مئوية للرطوبة، البروتين والدهن والرماد فكانت على النحو التالي:

77.06±0.709%, 19.60±0.59%, 2.07±0.01%, 1.04±0.01% على التوالي.

إن قيم قياس التذوق التي تتكون من الصدر، الفخذ والمضرب بعد التحكيم فقد أوضحت طراوة الصد، الفخذ والمشرب على النسب التالية (35.52%, 33.73%, 30.75%).

نكهة الصدر، الفخذ والمضرب أعطت النسب (36.39%, 32.95%, 30.66%). بينما أعطى لون الصدر، الفخذ،المضرب النسب (36.39%, 32.95%, 30.66%) فيحين كانت عصيرية الصدر، الفخذ والمضرب النسب(37.08%,34.19%,28.75%).

أما التجربة الثانية فكانت مبنية على تعدد أشكال (polymorphism) ثلاثة جينات GH, PIT1 و PRLR والتي تم تنميطها الجيني باستعمال تكنولوجي PCR- RFLPs لستة دجاج بلدي (محلي) سوداني (BN2, BN1) الدجاج العاري الرقبة البلدي الكبيرة (LB2, LB1)، (F) النافوش و

البتول BT حيث تم أخذ 20 عينة دم من كل نوع والتي تم جمعها بواسطة وريد الجناح. وتم استخلاص الحامض النووي (DNA). وقد تم استخدام البلمرة المتسلسل (PCR) مستخدمين بادئات معينة خاص بالجينات قيد الدراسة. نواتج تفاعل البلمرة المتسلسل تم توصيفها بعد قطع النواتج بواسطة أنزيمات التقييد التي تم استعراضها سابقا. أوضحت النتائج غياب تعدد الأشكال (polymorphism) للجين PRLR والتي أوضحت اليل واحد أو النمط الجيني (B; BB) بينما الجينين الآخرين أوضحا تعدد الأشكال (polymorphism)، أن جين النمو أوضح اليلين (A; B) بثلاثة أنماط جينية (AA; AB; BB) بينما PIT1 جين أوضح اليلين (A, B) بنمطين جين (AA; AB)، وبتكرارات اليلات وأنماط جين تختلف باختاف نوع الدجاج.

وفي الختام فإن جين هرمون النمو (GH) و PIT1 جين يمكن استغلالهما في تحسين الدجاج البلدي السوداني بينما جين PRLR لا يمكن استغلاله لعدم وجود تعدد الأشكال فيها.

CHAPTER ONE

INTRODUCTION

Indigenous chickens are widely distributed in the rural areas of tropical and sub-tropical countries where they are kept by the majority of rural poor. Whereas in Africa they are in general hardy, adaptive to rural environments, survive on little or no inputs and adjust to fluctuation in feed availability. Chickens largely dominate flock composition and make up about 98% of the total poultry numbers (chicken, ducks and turkeys) in Africa (Gueye, 2003).

harsh weather condition and adaptation to adverse environment. They are known to possess qualities such as the ability to hatch on their own, brood and scavenge for major parts of their food and possess appreciated immunity for endemic diseases.

The indigenous poultry species represents valuable resources for livestock development because of their extensive genetic diversity allows for rearing of poultry under varied environmental conditions, providing a range of products and functions. Thus, great genetic resources embedded in indigenous poultry await full exploitation that will provide a basis for genetic improvement and diversification to produce breeds that are adapted to local conditions for the benefit of farmers in developing countries (Horst, 1989; Sonaiya *et al*., 1999).

The local chicken breeds contain genes and alleles pertinent to their adaptation to a particular environments and local breeding goals (Romanov *et al*., 1996). Indigenous chickens are becoming seriously endangered owing to the high rate of genetic erosion resulting from diseases and predation. Attempts must be made so that these adaptive

features of the local stocks will not be eroded before they are characterized and conserved. Molecular markers have played a leading role in characterization of diversity which provides relatively rapid and cheap assay in the absence of quality phenotypic measures (Toro *et al.,* 2006). As a result the classification of genetic resources based on geographical location needs to be supported by molecular data to provide unbiased estimates of genetic diversity (Pimm and Lawton, 1998) for the purpose of genetic resource conservation and utilization. Characterization includes a clear definition of the genetic attributes of an animal species or breed, which has a unique identity and the environment to which the species or breed population are adapted. The genetic distinctiveness of animal forms the basis for distinguishing it among different animal genetic resources and for assessing the available diversity (FAO, 1984).

Many studies have been conducted with regard to phenotypic performance of Sudanese indigenous chickens. Therefore, in this study the concern was mainly on Bare neck chickens. On the other hand, there were few studies done pertaining genetic study of Sudanese indigenous chickens. This was the reason which led to selecting the six lines of Sudanese indigenous chickens in order to find out their performances by using RELP as a marker which gave positive.

The identification and utilization of potential candidate genes with significant effects on economically important traits have become increasingly important in poultry breeding programmes. They could be consider as marker for quantitative trait loci (QTL) and can be used for genetic improvement through the genetic assisted selection (GAS) (Zhang *et al.,* 2012) The candidate genes used in poultry breeding are many among them are pituitary-specific transcription factor 1 (PIT1), growth hormone (GH) and prolactin

receptor (PRLR) are considered the most important candidate genes which can influence the performance of production traits

The objective of this study is to characterize the Sudanese indigenous bare neck chickens phenotypically and genotypically with the aim to improve the productive traits for the rural populations who rely on this stock in their livelihood and to conserve them as well because these stocks are confined in rural areas.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conservation of native chicken populations

Extinction of endangered farm animal breeds leads to an irreversible loss of genetic diversity. The need to conserve genetic diversity is widely accepted for biological, economic and cultural reasons (Oldenbroek, 1999). A main reason is that an abundant source of genetic diversity with each livestock species is the prerequisite of copying with putative changes in livestock farming conditions (Bennewitz *et al*., 2006). The maintenance of high levels of genetic variability and low levels of inbreeding are major objectives in conservation programme. Genetic variation is a prerequisite for populations to be able to face future environmental changes and ensure long-term response to selection, either natural or artificial, for traits of economic or cultural interest (Frankham *et al*., 2003). Also, inbreeding levels should be kept as low as possible in order to avoid deleterious effects on fitness related traits which could compromise the variability of the population (Fernandez *et al*., 2005).

Three factors are considered as being largely responsible for declining genetic diversity of livestock (1) Destruction of native habitats of livestock breeds (2) The development of genetically uniform livestock breeds (3) Farmer and/or consumer preference for certain varieties and breeds (changes in these consumer preferences over time) (Gholizadeh *et al.,* 2008). The genetic characterization of populations, breeds and species allows evaluation of genetic variability, of fundamental element in working out breeding strategies and genetic conservation.

Molecular markers have been comprehensively exploited to access this variability as they contribute information on every region of the genome regardless of the level of gene expression (Pandey *et al*., 2006). Lynch *et al.,* (1995) showed that small populations might decline in fitness due to the accumulation of detrimental mutations. Hedrick *et al*., (1996) suggested that low genetic variation in a species might be indicative of recent population bottleneck and such a bottleneck did potentially indicate vulnerability to

extinction. In small populations genetic drift tends to reduce genetic variation, leading eventually to homozygosity and loss of evolutionary adaptability to environmental changes (Lande, 1988). Low genetic variation in a species may be an indication of recent population bottleneck and such bottleneck could result in inbreeding depression. Species with low genetic variation may be more vulnerable to environmental change and consequently is vulnerable to extinction (Zhang *et al*., 2002).

When a population goes through a bottleneck rare alleles tend to be lost and the average number of alleles per locus, allelic diversity, is reduced. Heterozygosity, however, is not reduced proportionally because rare alleles contribute little heterozygosity. The difference between allelic diversity and heterozygosity is used as the basis of statistical detects presence of recent genetic bottleneck (Piry *et al*., 1999).

Throughout Africa most research efforts towards the conservation and utilization of indigenous livestock have been focused on cattle and sheep and to lesser extent on goats (Weigend and Romanov, 2002). Although the production potential of local birds is poor in comparison with commercial lines, the native breeds evolved the crucial ability to adapt and survive in often challenging environmental and ecological conditions associated with their geographical origins. Generally, however, exact research data on the genetic potential towards disease resistance and adaptation mechanisms of indigenous livestock and poultry are limited (Anderson, 2003).

In lieu of the above arguments there has been a renewed interest over the past two decades in conservation and utilization of indigenous livestock and this has resulted in the establishment of a programme for Global Management of Farm Animal Genetic Resource by the Food and Agriculture Organization (FAO). The main objective of this programme is to stimulate conservation activities and create an awareness of possible losses of genetic resources on an international scale (Scherf, 1995; Shrestha, 2005). The information system, Domestic Animal Diversity Information System (DAD- IS), forms one of the main components of the programme and provides an inventory of all existing breeds. The biodiversity of 52 chicken breeds from Europe was assessed in a European Union Project and a chicken DNA bank and poultry biodiversity database have been established at INRA Jouy-en-Josas (Weigend, 2000).

In South Africa, the Farm Animal Conservation Trust (FACT) was established in 1994 to facilitate and promotes conservation of native animal genetic resources. Three South Africa native chicken lines (Naked Neck, Ovambo and Lebowa-Venda chickens) and one locally developed breed (Potchefstroom Koekoek) have been listed by FACT) (Ramsey *et al*., 2000).

There are numerous reasons for conserving genetic variation in indigenous farm animals including fowl. From the view point of the conservationist, the native fowl should be conserved as a genetic resource against future disasters, commercial chicken stocks are always in danger of severe potential erosion by infectious diseases. Secondly, native fowls could be a source of unique alleles and contribute to the search for genes associated with health quality traits (Mendelsohn, 2003). Native fowl types throughout Africa contribute significantly to household food security and are resource with considerable socio-economic value (Marle-Köster *et al*., 2008).

2.2 Origin and domestication of domestic fowl

The genus of Gallus is composed of four species, Gallus gallus (Red Jungle Fowl), Gallus Sonneratii (Grey Jungle Fowl), Gallus Lafayettei (Ceylon Jungle Fowl) and Gallus Varius (Green Jungle Fowl). The red jungle fowl inhabits South East Asia including North East India covering Bhutan and Nepal and some part of Tibet and China. The grey jungle fowl is found in India, while the Ceylon jungle fowl, originally inhabited Sri Lanka. The green jungle fowl also called Java jungle fowl is a native of Java Island in Indonesia (Stevens, 1986).

With regard to the origin of domestic fowl, there was a debate on whether the domestic fowl is monophyletic or polyphyletic origin (Crawford,1990a).Supporters of monophyletic theory take their cue from Darwin 1868 as reported by Hutt (1949), who considered Gallus gallus the sole ancestor of domestic fowl for the following reasons: 1) domestic fowls mated freely with G. gallus and rarely with others 2) progeny from this cross were fertile 3) the voice of G. sonneratii and its hybrids differs from that of the domestic fowl 4) black- breasted Red Game resemble G. gallus in clour and in other

respect 5) Darwin's own experiments crosses of various breeds occasionally produced fowls resembling G. gallus.

Whereas the supporters of polyphyletic origin may choose between two possibilities either 1) all fowls are descended from two or more species of the four existing wild species of Gallus or 2) the Mediterranean breeds may have had such an origin, but some other ancestor now extinct gave rise to the Asiatic breeds (Hutt,1949).

Many research studies indicated that the red jungle fowl is the direct ancestor of the domestic chicken (Gallus gallus domesticus used in commercial production for meat and eggs) (Moreng and Avens, 1985; Crawford, 1990a; Siegel *et al*., 1992; Hillel *et al*. , 2003 ; Vaisaen *et al.* , 2005). Fumihito *et al*., (1994) reported that G. g. gallus of the red jungle fowl is the origin of all domestic breeds. Collias and Collias (1996) also reported that the red jungle fowl is the principal and perhaps the sole ancestor of the domestic fowl. Moreover, the use of molecular genetics and microsatellite techniques provided evidence that the origin of domestic fowl is monophyletic. Hillel *et al*. (2003) evaluated the gene pool of 52 chicken populations from wide range of origins using the microsatellite markers technique. They found that the red jungle fowl is the main progenitor of domestic chickens.

Opinions among scholars are divided concerning when the species arrived and the routes by which chickens entered and dispersed across the Africa continent (Gifford-Gonzalez and Hanotte 2011). The initial trigger for the adaptation of domestic chickens by African communities could have been socio-cultural and/or recreational (e.g., cockfighting) rather than as a new source of food. Textual records indicated that chickens were already present in Egypt by the time of the Third Dynasty of Ur (C, 2113–2006 BC), and the oldest recognizable occurrence of the bird in art dated from the second half of the fourteen Century BC (Smith, 1965). Houlihan and Goodman (1986) further suggested that chickens were probably brought to Egypt as creatures of curiosity and admiration because of their unusualness and that they did not become a regular feature of the Egyptian farm yard until the Ptolemaic period (304–330 BC). Domestic chickens could have been introduced to African continent several times fulfilling different societal needs at different periods.

In North Africa, domestic chickens were depicted in Egypt from the second millennium BC (Houlihan and Goodman 1986). In Sub-Saharan Africa, the earliest widening accepted evidence of domestic chickens dated to the mid-first millennium AD in Nubian and the late first millennium AD in East and South Africa (e.g., Horton and Mudida 1993; Plug 1996 a, b). In West Africa several sites attest to the presence of chickens in the late first millennium AD. Chickens were first discovered in Egypt, where they were reared as foreign pets and game cocks. However, in the year 650C they became common and economically important. They then spread from there to sub- Saharan Africa during the first AD (Dalby, 2003).

New evidence from Kirikongo (Burkina Faso) and Daboya indicated that chickens were already a significant component of the Iron Age economy in West Africa by six Century AD at the latest and probably several Centuries earlier Duephen (2011).

Undoubtedly, the complex and ancient interactions resulting from the Indian Ocean trading networks must have facilitated maritime and terrestrial intercontinental translocations of several domestic and nondomestic plant and animal species among Africa, the Middle East and South, South East Asia (Boivin and Fuller 2009; Fuller and Boivin 2009; Fuller *et al*., 2011).

The arrival of European settlers as part of the development of terrestrial and maritime empires of several European nations across Africa and Asia (Portugal, Britain, etc.) from the fifteen Century AD onward provided further opportunities for the arrival and movement of chickens within Africa. Also, since the second half of the twentieth century, the African continent as other parts of the world has witnessed the arrival of exotic chicken breeds developed for higher productivity (Kitalyi 1997), including crossbreeding purposes with local flocks to improve meat and egg production, these developments might have had an impact on the genetic make-up of some African village chicken populations.

Historically chickens were kept for cockfighting until mid-1800 when cockfighting became illegal, thereafter poultry breeders in USA and Europe started to breed chickens for exhibition (Moreng and Avens 1985; Crawford, 1990b).The chickens

were selected for uniformity and cloured plumage patterns. These efforts were important in the development of the standard breeds and varieties of chickens that were used in the development of commercial chickens of today.

The strange looking Bare neck chickens have been known by many other names such as Transylvanian Naked Necks, Hackle less and even Rubber Necks. They are also called "Turk–hens" or "chickens"- products of cross between chickens and turkeys. Formerly the condition was attributed by some writers to be a dermatitis supposed to have been induced either by fighting or by depluming mites and subsequently inherited (Hutt, 1949). However, a careful study of the case by (Greenwood, 1927 as reported by Hutt, 1949) showed that it is quite unnecessary in order to account for the naked neck to invoke the theory that an acquired character has been inherited. Whereas the origin of the strange looking naked neck chickens is disputed according to archaeologists these birds originated in Malaysia; from there they spread all over the world while others think that the naked neck apparently were originated in the Transylvanian region in the current Romania as a result of a risky mutation. Today they are dispersed in the entire world and they are very appreciated by their productive performance, especially in warm climates for their resistance to epidemic diseases and for the best flavor of their meat (http:// www.Sideral.com.ve / naked_neck.htm). Record in South Africa revealed that the naked neck chickens have been found in areas as far apart as Central Europe to Africa and are thought to have originated in Malaysia and are now found mainly in native huts of the local populations in South Africa (http:// www.arc.agric.za/ home. Asp? pid: 2706). While the origin of the frizzle feathered chicken was first described by Western explorers in Fiji during the seventeenth century (FAO, 2000).

2.3 Types and characterization of indigenous chickens

In most of developing countries indigenous chicken populations are the result of uncontrolled crossbreeding programmes between various lines of local and exotic breeds (Dare, 1977). Distinct indigenous chicken ecotypes have been identified and named in Cameroon, Egypt, Kenya, Morocco and Sudan. The names used to describe the common phenotype in Kenya are frizzle feathered naked neck, barred feathered, feathered shanks, bearded, dwarf size etc. (Nyaga, 2007). The local types of chickens vary in body size,

conformation, plumage color and performance. Some of the different indigenous breeds and strains that are abundant in coastal Kenya and Sub-Sahara Africa in general are Dwarf, Frizzle, and Naked neck and normal feathered.

In Sudan indigenous chickens with their various types are collectively called Baladi as they were early described and classified by Desai, (1962). These birds are commonly known as Large Baladi, Bare neck and Betwil. Large Baladi the most common type and are distributed all over the country. The birds are of medium size, with adult body weight of 1.350-1.362 kg and small crushed combs and plenty of plumage of various colours with egg production of 78 eggs/bird/year. The Bared neck is smaller than large baladi, it is common in south Sudan and mainly characterized by featherless neck which is a dominant character and characterized by various colours, very active and comparatively more resistant to diseases than the other two types and egg production 106 eggs/bird/year. The Betwil is found in Nuba Mountain in southern Kordofan. It is a dwarf chicken with small compact body, adult body weight ranged from 0.681-0.908 kg with egg production of 86 eggs/bird/year.

The indigenous domestic fowl of Bangladesh are varied and non-descript with poor egg production which is 50-55 eggs/bird/year (Yoshimura *et al*.,1997) compared to 35-40 eggs/bird/year of fully feathered indigenous fowl (Sazzad *et al*., 1990) and mature body weight of 1.0-1.2 kg (Panda,1989; Okada *et al*., 1987; Barua and Howlider, 1990).

Several authors have reported the production performance of indigenous chickens to be below that of standard commercial layers and broilers with smaller bodies (Barua *et al*., 1998; Ebangi and Ibe 1994 and Safaloah, 2001). Mature body weight of 1.02kg in males and 1.00 kg in females at five months of age (Barua and Toshimura, 1997).

The indigenous chickens are characterized as dual purpose birds due to their ability to supply both meat and eggs for human consumption. They are broody, able to take care of their own chicks (Horst, 1989). When compared with exotic chickens they lay fewer eggs which weighed about 43 grammes (Ramlah, 1996) with a maximum of four clutches per hen per year composed of up to ten eggs per clutch (Awuni, 1989 and Akinola *et al*., 2011).

2.4 Indigenous chickens use as food and other functions

There is ample evidence to support the impact of indigenous chickens in the livelihood of rural populations in terms of nutrition, health status, and income and socio-

cultural aspects. Therefore, indigenous chickens are valuable in the rural communities because they fulfill major functions and benefits in the livelihood of rural communities. Minh (2005) described indigenous chickens as efficient converters of left over grains as well as insects into valuable protein, for instance, eggs and meat. They are also seen as controllers of insects and weeds and suppliers of manure as well for vegetable gardens.

Ganabadi *et al*., (2009) reported that indigenous chickens are always thought to be better in terms of carcass composition than commercial broilers due to its low fat content. Ramlah, (1996) stated that the indigenous chickens have low fat and muscle weight compared to broilers because they need to use energy to find food.

2.5 The importance of indigenous chickens in religious and socio-cultural aspect of African rural communities

Indigenous chickens are important for other activities for example colours of the plumages are reported to be very important in various religious and social-cultural lives of many Africans. For instance, Sonaiya *et al.,* (1999), reported that red cock is used for sacrifice to ask for rain and good harvest, a white cock is used for thanks giving and black cock is for protection from evils e.g. diseases, wars or quarrel. According to Ojo (2003) a lot of social and traditional values are placed on naked neck and frizzled chickens due to their roles in ritual and sacrifices.

Mopate and Lony (1999) on the other hand reported some taboos associated with consumption of chickens, especially in circumcised women and first borne children moreover, in some cultures children under the age of three and girls in general are traditionally forbidden from eating eggs.

2.6 Major genes identified in indigenous chickens in the tropics

Horst (1989) described nine major genes of indigenous chicken that can be used in genetic improvement programmes in the tropic and sub-tropics. The same author indicated that there is little information on the genetic make-up of the indigenous chickens of Africa. The major genes identified in indigenous chickens in the tropics are as in the Table (2.1) below:

Table 2. 1. Major genes in local fowl populations with important effects on tropical orientated breeding

Genes	Mode of inheritance	Direct effects	Indirect effects
Dw: dwarf	-Recessive -Sex-linked -Multiple allele	Reduction of body size between 30 and 10% from normal size	-Reduction metabolism -Improved fitness -Disease tolerance
Na: naked neck	Incomplete dominant	-Loss of neck feathers -Reduction of pterylae width -Reduction of secondary feathers	-Improved ability for convection -Reduced embryonic liveability (hatchability) -Improved adult fitness
F: frizzle	Incomplete dominant	-Curling of feathers -Reduced feathering	-Decreased fitness under temperate conditions -Improved ability for convection
h: silky	Recessive	-Lack of hamuli on the barbules -Delicate shafts -Long barbs at contour feathers	Improved ability for convection from shank and skin
K: slow feathering	-Sex-linked -Dominant -Multiple allelic	Delay of feathering	-Reduced protein requirement -Reduced fat deposition during juvenile life -Increased heat loss during early growth -Reduced adult viability
Id: non-inhibitor	-Recessive -Sex-linked -Multiple allelic	-Dermal-melanin deposition in The skin and shanks	Improved ability for radiation from shanks and skin
Fm: fibro-melanosis	Dominant with multi-factorial modifiers	-Melanin deposition all over the body -Sheath of muscles and nerves, tendons, esenterium -Blood vessel walls	-Protection of skin against UV-radiation -Increased pack cell volume and plasma protein
P: peacomb	Dominant	-Change of skin structure -Compact comb size -Reduction of pterylae width -Development of breast ridges	-Improved ability for convection, increased frequency of breast blister -Sex limited (O) -Improvement of late juvenile growth
O: blue shell	Dominant, sex limited (E)	Deposition of blue pigment (bilverdinIX) into egg shell	Improved egg shell stability

Source: Horst, 1989

2.6.1 Major Genes that Affect Heat Tolerance

2.6.1.1Naked neck gene

The naked neck (Na) gene reduces feather mass by 20% and 40% (relative to body weight) in the heterozygous (Na/ na) and homozygous (Na/Na) birds respectively compared with fully feathered counter parts, especially with regard to high temperature (Merat, 1986). The advantageous effect of naked neck gene in hot environment has been recognized since 1980s (Hanzl and Somes, 1983). The lower feather mass increases the effective surface of heat dissipation and increases the sensible heat loss from the neck (Yahav *et al*., 1998). Currently, resistance to heat dissipation is decreased because the reduced plumage is associated with lower skin mass due to reduced fat deposition within it (Cahaner *et al*., 1993; Raju *et al*., 2004). The high growth rate of the Na genotype at high temperature may be related to the relative high T_3 (triiodothyronine) concentration (Decuypere *et al*., 1991).

2.6.1.2 Frizzle gene

The frizzle (F) gene may reduce the heat insulation of feather by curling the feathers and reducing their size. The beneficial effect of the F gene on broiler growth at high temperature is less than that of Na allele and the effect is only significant in slow growing line. However, there is an additive effect in the double heterozygous (Na/ na F/ f) broilers (Yunis and Cahaner, 1999).

2.6.1.3 Dwarf (dw) gene

The gene results in reduction of 30 – 40% of adult body size and leads to speculation about the inherent heat tolerance of dwarf broiler breeders. However, the inherent heat tolerance of dw genotype in laying hens seems uncertain (Decuypere *et al*., 1991). In fast growing broiler chickens the dw gene has been proven to have no practical value for improving tolerance to chronic heat stress (Deeb and Cahaner, 2001b).

2.6.1.4 Slow feathering (K) gene

The slow feathering (K) gene has been widely used to 'auto-sex' strain and breed crosses. At hatching the primary and secondary feathers of the recessive birds (Kw or Kk) project well beyond the wing coverts while those of slow-feathered chicks (Kk or Kw) do not. There are two other alleles in series, both dominant to the wild- type or K gene which is not used commercially (Somes, 1990). Horst (1989) also credited the K gene with the indirect effects:(i) reduced protein requirement (ii) reduced fat deposit during juvenile life, and (iii) increased heat loss during early growth all of which may assist the bird in resisting heat stress.

2.6.1.5 Other Genes (h, p, id)

It has been suggested by (Host 1988, Host 1989) that several other genes may be useful in making fowl tolerant to tropical conditions. The recessive gene for silky (h), which affects the barbules on the feathers, may improve the ability to dissipate heat. The dominant gene for pea comb (P) reduced feather tract width, reduced comb size and changed skin structure. These may improve the ability to dissipate heat. The recessive sex – linked, multiple – allelic locus for dermal melanin (id) may improve radiation from the skin.

2.6.1.6 Interactions of major genes

There is evidence that the Na and F genes can interact to improve the performance of egg stocks under heat stress (Host 1988, Host 1989). In a brief report (Mathur and Horst 1992) claimed that the three genes Na, F, and dw interact so that the combined effects of one or two genes are lower than the sum of their individual and additive effects but still higher than the individual gene effects. The cross of the Dahlem Red naked-neck strain with the Dahlem white frizzle strain (both developed at Berlin University) has competed successfully in Singapore random sample test (Mukherjee, 1992). Suggesting that the interaction of the genes Na and F had a positive effect on performance.

In broiler stocks there is one report that 6-week-old broilers with the Ff Na na genotype had fewer feathers than the ff Na na comparable stock, which had fewer

feathers than the ff Na na stock. The effects, which are not fully additive, indicated that it might be advantageous to introduce both genes (F and Na) into a broiler sire line (Deeb *et al*., 1993). Birds from strains selected for slow feathering (S) within the k genotype and carrying the Na gene had lower feather cover than S birds not carrying Na indicating that both Na and selecting for slow feathering within k genotype were improving performance in hot warm environment (Lou *et al*., 1992).

Selecting for quantitative genes for slower feather growth in a broiler line breeding true for k genes (Edriss *et al*., 1988; Ajang *et al*., 1993) reduced feathers at 48 days, reduced carcass fat. Increased carcass protein, carcass meat, and increased growth and feed efficiency. Although these results were obtained at a moderate test temperature of 20^{o} C, selection for slow feather growth in birds with k gene should increase their heat tolerance by enhancing their ability to dissipate heat.

2.7 Feathers and heat stress

Commercial broilers are more susceptible to acute/chronic heat stress and which in turn results in poor performance due to increased mortality and decreased growth rate in tropical climate (Singh *et al*., 1996). Efforts to alleviate heat stress problems through genetic selection, management practices and dietary adjustments have been unsuccessful. Therefore, a genetic approach of naked neck gene (Na) incorporation in modern broiler strains was considered to mitigate heat stress.

Selection under normal climate stress can solve the problem of stimulating a complex environment. Singh *et al,* (1998) stated that a broiler stock selected in India for 10 generations exhibited better adaptation to local environment as compared to counterpart in USA.

The naked neck gene (Na) has been associated with increased heat resistance and different effects of this gene on poultry have received considerable attention in recent years with particular reference to high ambient temperature and commercial broiler production (Merat 1986; Cahaner and Leenstra 1992; Eberhart and Washburn 1993a, Cahaner 1996; Singh *et al*., 1996).

Heat dissipation is hindered due to the insulation provided by feather coverage. This insulation is advantageous in slow growing chickens or when broilers are reared at low ambient temperature, but under high ambient temperature the feather coverage has a negative effect on thermoregulation (Yaron *et al*., 2004). This negative effect can be encountered by costly cooling and ventilation system. A genetic alternative would be to eliminate the feather coverage, as a way to maximize inherent heat tolerance in fast-growing broilers under hot condition.

It was demonstrated by (Richard, 1976) that poorly feathered birds burn up to 40-45% more energy than the well feathered birds. The poorly feathered birds had a small advantage in very hot conditions. At 35oC their body temperatures were lower and they produced 4% less metabolic heat than the normally feathered birds.

Feathered broilers under heat waves showed poor liveability and growth performance under heat stress a steep alleviation in body temperature occurred in feathered broilers, followed by any apparent reduction in feed intake and a substantial decrease in daily weight gain (Yaron *et al*., 2004). Whereas no elevation in weight gain were exhibited by featherless broilers. Moreover, the elevated body temperature in the feathered broilers resulted in over 33% mortality due to heat stress. These results clearly indicate that being featherless improves the welfare and livability of broilers under hot conditions.

Reduction of feather coverage was found advantageous in the case of naked neck broilers (Deeb and Cahaner, 2001). Yaron *et al*., (2004) observed that body temperatures were significantly (<0.001) lower in featherless broilers regardless of sex at all ages. The difference in body temperature between genotypes is greater during heat wave period.

Heat stress negatively affects broilers because their feather coverage hinders the dissipation of internal heat, leading to alleviated body temperatures (Singh *et al*. 1998). To avoid a dangerous increase in body temperature, chickens minimize endogenous heat production by reducing feed intake and consequently exhibit decreased growth and meat yield in broilers. Decreasing the feather coverage should enhance heat dissipation and

consequently alleviate the heat stress on chickens reared in hot climate. Further reduction in feathers spares valuable protein which is turned into meat tissues (Ajang *et al*., 1993).

Furthermore, comb, wattle and shank length are considered as means to heat dissipation or heat release. Naked neck genotype had longer shank than the normal feathered counterpart. This higher length in leg enhanced the releasing of additional heat along the main pathways through comb and wattles. Therefore, the naked neck birds had more bare area and this assist to tolerance of more heat stress than fully feathered sib Galal (2007). McGary *et al*., (2003) also highlighted that the higher length in leg enhanced the releasing of additional heat a long with the main pathways through comb and wattles.

2.8 Effect of (Na) gene on indigenous Chicken

2.8.1 Effect of (Na) gene on growth rate

Growth rate is a complex physiological trait and there appears to be a major difference in its mode of inheritance (Hurry and Nordskog, 1953). In the case of growth rate no single gene effects have been yet isolated. So growth rate may thus be considered a typical multifactorial or polygenenic trait which is highly influenced by the environment. Hanzl and Somes (1983) conducted an experiment at 38^{o}C and they stated that naked neck homozygotes showed a marked advantage for body weight which was less for heterozygotes. Mahrous *et al*., (2008) stated that the presence of Na gene in a single state or combined with F gene significantly increased body weight of chicks at hatch compared with na na ff genotype.

Cahaner *et al*., (1993) reported that heterozygous naked neck broilers gain about 3% more weight than their normally feathered siblings under commercial conditions during the spring and summer months and this advantage is almost tripled at a constant high temperature of about 32^{o}C. The advantage of these heterozygous naked neck broilers at the high temperature was greater than had been recorded earlier. This could be attributed to the higher growth rate of the stock used in more recent study. Males from broiler lines with the highest inherited growth rate show the largest reduction in weight gain under a high constant temperature of 32^{o}C (Cahaner and Leenstra, 1992).

Cahaner et al., (1993) in a study on the effect of (Na) gene on fast growing broilers at high 32^{o}C and normal 23^{o}C temperatures concluded that reduced plumage was associated with lower skin mass resulting from reduced fat deposition. The advantage associated with reduced plumage increased with broiler size and ambient temperature.

Yunis and Cahaner (1996) reported that the heterozygous naked neck broilers were superior to conventional broilers at normal temperatures and demonstrated even greater superiority at high temperatures. Further reductions in plumage as seen in the (NaNa) bird increases this advantage.

2.8.2 Effect of the (Na) gene on feed consumption, feed conversion ratio and feed efficiency

The excess of feed consumption of (NaNa) and to lesser than that of (Nana) hens in comparison with the (nana) genotype may contribute to this increase in egg weight by the supply of additional material, protein and so on for egg formation. The naked neck birds consume more food at 15^{o}C low ambient temperature (Yahav *et al*., 1998).

The greater food intake of the naked neck chickens at 15^{o}C was associated with significantly higher packed cell volume, haemoglobin concentration, heart and liver size. These appear to involve both higher heat production and haemodynamic changes to accommodate the higher oxygen demands of the naked neck chickens at lower ambient temperature.

Yalcin *et al*., (1999) indicated that the dietary protein requirements of the naked neck birds were similar to those for normally feathered birds. Among the pure breeds feed intake was highest in naked neck desi followed by Rhode Island Red followed by Fayoumi and White Leghorn. Over all feed intake differences appeared to be arising for differences in later growth period (6-17weeks). The feed intake differences also resulted in variable feed conversion in the later growth period (6-17weeks).

Jianxia (2002) reported that under the high ambient temperature (340C) male broilers with frizzle and naked neck genes increased feed intake by 6.0% on an average when compared with normally feathered broilers.

Under high ambient temperature, Galal and Fathi (2001) concluded that the naked neck gene was associated with higher feed consumption compared with fully feathered one. They also found that the Na allele had better effect on feed conversion ratio, whereas the Nana genotype had significantly low feed conversion ratio as compared with nana one.

The presence of Na gene in a single state or interacted with F gene significantly improved feed conversion ratio compared with nanaff sibs (Mahrous *et al*., 2008).

The feed efficiency of naked neck chickens deteriorated in comparison with normally feathered chick as might be expected from the larger extension of unfeathered areas, increasing the heat loss of the former. Feed efficiency seemed slightly better although not significant for the naked neck genotypes, in addition the data suggested the advantage is more marked when a ration with lower protein level is given. Feed efficiency was slightly, but not significantly better for naked neck chicks at 31oC and 38^{o}C respectively (Hanzl and Somes, 1983).

According to Eberhart and Washburn (1993a, 1993b), naked neck birds had equal or better feed efficiency at a temperature of 21^{o}C than normally feathered birds; this was attributed to their superior growth. Leenstra and Cahaner (1992) and Leenstra (1993) reported that broiler line selected over 10 generations for feed efficiency was more tolerance to heat stress and heat failure than a counterpart selected for growth rate.

Feed efficiency of the (Nana) birds was lower by about 4% but in the summer climates, feed efficiency of the (Nana) birds was about 9% higher than that of their (nana) counterpart (Yalcin *et al*., 1997). The improved feed efficiency of the naked neck broilers in the chronic heat stress environment has been reported by Singh *et al*., (1996), the naked neck birds had a slight advantage but not significant on feed efficiency utilization at temperatures of 31^{o}C or 38^{o}C. Merat (1986) reported that the (NaNa) hens were the least efficient in the control experiment, easily explained by their excessive heat dissipation. This handicap is not found in the high temperature group. Among dwarf hens the feed efficiency was found always better for (Nana) genotypes compared to (nana) genotype whatever the environmental temperature is.

2.8.3 Age at first egg of bare neck chicken

Age at sexual maturity is generally determined by the age at first egg laid and is an important factor determining the overall profitability of commercial flock. Yousif and Eltayeb., (2011) reported age at sexual maturity was significantly (P<0.05) lower in Betwil than in Bare neck (163.9 vs184.9 days). Whereas Elfaki, (2000) found the age at first egg for Sudanese Large Baladi range 136-148.25 days. While Elamin *et al*., (2004) stated that age at sexual maturity for the Large Baladi bird was 180.52±18.85 days. Ershad (2005) also reported the age at first egg in Bangladesh native chicken was 175 days.

2.8.4 Effect of Na on egg quality and characteristics

Shell quality, particularly shell thickness, is an important trait that primarily breeders of egg laying flock incorporate into their breeding programme to reduced egg-shell breakage (Mekki *et al*., 2005). The same authors found significant differences (P<0.05) in average egg-shell thickness among Sudanese local types. The average egg-shell thickness of Betwil (36.2±4.2u) and of Bare neck (36.2±4.0u) was similar and both were significantly thicker than that of Large Baladi (34.3±3.6u).

Yousif and Eltayeb, (2011) reported significant differences (P<0.05) between the two ecotypes (Betwil and Bare neck) for other egg traits with the results being lower in Betwil than in Bare neck. Moreover, albumen weight, yolk weight and shell weight represented approximately 53.77g, 33.08g and 13.15g of the total egg weight respectively in Bare neck, while the corresponding results in Betwil were 52.79, 33.88 and13.15 percent. On the other hand, the yolk-albumen ratio was not significantly different (P<0.05) between the two ecotypes (0.649 and 0.625) respectively. The general trend exhibited high and positive correlations between egg weight and other egg characteristics including albumen weight, yolk weight and shell thickness. The highest correlation was between egg weight and albumen weight, whereas the lowest correlation was between egg weight and shell strength. Elamin *et al*., (2004) reported that the albumen height, shell thickness and Haugh unit for the Sudanese Large Baladi type under relatively improved environmental conditions were 8.35mm, 3.68 micrometer and 99.02 percent.

Fayeye *et al*., (2005) investigated egg characteristics of Fulani chicken and reported 20.33g for albumen weight, 4.92mm for height, and 73.43 for Haugh unit, 13.03g for yolk weight, 14.27mm for yolk height, 24.68mm for yolk diameter, and 5.04g for shell weight, 0.58mm for shell thickness, 35.24mm for egg length and 23.59mm for egg diameter respectively. Similarly Parmar (2006) reported egg characteristics (egg weight, shape index, albumen index, Hugh unit, yolk index, yolk weight and albumen height) of indigenous Kadaknath in India were 41.99g, 74.35%, 8.52%, 80.24%, 37.53%, 15.18g and 21.27g respectively.

Less feather cover associated with the naked neck gene can allow more access to solar radiation in birds in open-sided houses or outdoors, which may facilitate greater vitamin D3 synthesis and in turn contribute to better shell quality (Barua *et al*., 1998). The Na gene improves shell thickness and breaking strength by a factor of 9-10 respectively (Galal and Fathi, 2002). In addition El-Safty (2006) concluded that laying hens carrying Na gene had superior shell thickness and strength compared with normally feathered ones. Sharifi and Simianer (2007) reported that under heat stress conditions the presence of the Na gene led to significant improvements in weight of egg shell, breaking strength, thickness and egg elasticity as assessed by deformation. On the other hand, Abdel- Rahaman (1990) and El-Safty (2006) stated that normally feathered genotype gave significantly thicker egg shells compared to heterozygous birds (Nana) raised under low ambient temperatures with respect to breaking strength, the nana had a slightly stronger egg shell than Nana. Furthermore, eggs produced from nana hens had higher area comparatively to Nana when kept under winter conditions in Egypt (El-Safty, 2006).

2.8.5 Effect of Na gene on meat and carcass yield

The differences in meat yield traits between genotypes do not seem to depend on the environmental conditions (e.g. temperature) Singh *et al*., (2001). Zein-el-Dein *et al*., (1984b) reported that the meat yield from the (Nana) genotype was superior to that from the (nana) genotype. El Attar and Merat (1985) indicated this difference is probably a result of greater amounts of breast muscle in the former and added that the advantage of the (NaNa) chicken in terms of meat yield can be available.

Cahaner *et al*., (1987) indicated also that the higher meat yield in naked neck genotypes probably results from the greater yield of breast muscle. Ajang *et al*., (1993) reported that birds of a slow growing line had lower feather and skin weights, higher body weights and beast meat yield than those of a fast growing line. Weights of the breast and skin (relative to body weight) were similar in fully feathered fast and slow growing birds to those found in the naked neck (Nana) counterparts. It therefore, seems that the meat production of chickens can be improved by reducing their plumage cover either by the (Na) gene or by selection for quantitative loci controlling the rate of feather development.

Several mechanisms appear to be responsible for higher meat production in chickens with reduced plumage, which was summarized by Merat (1986) as follows: (i) less feather production leaves more protein for the synthesis of other tissues, mainly muscle (meat); (ii) the more rapid dissipation of heat results in less appetite depression and consequently better growth at high ambient temperatures; and (iii) lower carcass fat content resulting from a higher proportion of lipid being used for thermoregulation.

Cahaner *et al*., (1987) pointed out that, because there is less water in feathers than in muscles a reduction of 1.0g of feathers may increase body weight gain by 1.5g. Higher yield of meat can be achieved from broilers by reducing their plumage. At normal temperatures this effect results mainly from reduced skin weight and higher breast meat yield. The naked neck broilers were found to have lower skin weight and this was attributed mostly to a reduction in the amount of subcutaneous fat (Cahaner *et al*., 1993).

Haque *et al*., (1999) showed that (Na) genotypes superiority in dressed yield, breast meat and edible portion than in full-feathered (nana) pure breeds with few exceptions. The higher breast meat in genotypes appeared to be major advantage. As skin weight contains variable amount of subcutaneous fat, lower skin weight obtained in (Na) genotypes may be an indication of leanness of their carcass, but little differences were found in abdominal fat between (Na) and full-feathered genotype.

Fraga and Febles, (2001) observed that the naked neck (Nana) genotype had lower neck weight that may be due to decreased fat deposition. These broilers had a

higher relative proportion of prime cuts since their weights of wings; neck and back are lower in the (Nana) genotype. Thus these birds are leaner and have more muscle. (Nana) broiler carcasses are characterized by a better distribution of prime cuts and lower neck proportion. Dressing percentage was significantly higher in two naked neck genotypes than their normal siblings, which has been attributed to higher body weights and less losses due to feathering in naked neck birds and meat yield due to the presence of the Na gene (El-Attar and Fathi, 1995; Deeb and Cahaner, 2001; Yalcin *et al*., 1999; Fathi and Galal, 2001 and Fathi *et al*., 2008).

Zein El-Dein *et al*., (1981) under hot ambient conditions in Egypt showed that the introduction of the Na gene in broilers improved not only the dressing percentage but also the meat/bone ratio. Zein El-Dein *et al*., (1984) reported a relatively higher percentage of muscular tissue with Nana compared to nana males at 63 days old and naked neck males had a significantly higher eviscerated carcass percentage than their normally feathered counter parts. When naked neck and normally feathered indigenous fowls were kept in free range system (Barua and Howlider, 1990) found that the carcass dressing averaged 57.1% and 53.6% respectively, while total meat yield (as percentage of live weight) was 37.2% and 29.9% respectively but breast meat averaged 10.7% and 10.9% and thigh meat was 10.8% and 8.7% respectively.

In a study on Nana and nana chickens fed a diet containing 20, 18 and 16% protein Perez *et al*., (1993) found that the final live body weight was 1571g and 1548g respectively. Carcass yield percentage was 66.8% and 65.3% and thigh plus breast as a percentage of live weight was 37.0% and 36.2%. A genotype diet interaction was found for weight of thigh muscle; with the superiority of this traits noted in Nana chickens fed low protein diet. The high plus breast index in absolute and relative terms was greater in Nana chickens regardless of total protein levels. Under Egyptian environmental conditions the naked neck birds had a higher dressing percentage, more breast muscle, giblets and meat yield feathered birds (Fathi *et al*., 1993) they concluded that the naked neck gene exhibited some advantage in meat production particularly where fowls were kept in hotter environmental conditions. Yunis and Cahaner (1999) concluded that the naked neck gene had more pronounced effects on carcass traits through reducing

feathering, skin coverage and total body fat (skin plus abdominal fat) as well as increasing meat yield and edible parts. The Na gene improved carcass quality measurements, especially breast, thigh and giblets (Abdel-Rahman 1990).

The Na allele can increase breast meat especially at high temperatures (Yunis and Cahaner, 1999; Deeb and Cahaner 2001). The advantageous effect of the Na genotype is more pronounced in broiler chickens with higher growth rates and breast meat yields and increases with broiler size and ambient temperature (Deeb and Cahaner, 2001). El-Attar and Fathi (1995) found differences between Nana and nana genotypes for certain carcass composition measurements. Heterozygous naked neck males exhibit higher breast meat yields when compared with normally feathered sibs.

Fathi and Galal (2001) reported that the Na gene significantly improved dressing percentage and breast meat muscle compared to normally feathered genotype. Under high ambient temperature in South Africa, Van Marle-Köster and Webb (2000) found the Na gene increased breast muscle compared to normally feathered sibs. Regardless of ambient temperature Mahrous et al., (2003) observed that the Na allele improved dressing percentage and breast muscle compared with fully feathered birds.

Rajkumar et al., (2009) reported that the giblets proportion was not significantly varied among genotypes. However, liver and heart weights were heavier in naked neck genotypes while gizzard was heavier in normal birds. The larger liver and heart in naked neck genotypes may be correlated with high metabolic rate and more blood pumping from the heart to meet these metabolic needs. The significantly higher blood proportions in NaNa and Nana birds confirmed the association between heart and liver in naked neck chickens. Mahrous et al., (2008) reported that the presence of the Na gene significantly increased blood percentage compared to normally feathered genotypes. The higher blood content in naked neck genotypes was probably due to greater haemoglobin concentration and packed cell volumes associated with the naked neck gene (Luger et al., 1998; Raju et al., 2004 and Galal et al., 2007), as a consequence of greater oxygen demand. This also may be due to higher blood supply to organs and muscles.

2.8.6 Effect of (Na) gene on abdominal fat and fat deposition

The main biological purpose of fat deposition in poultry is to store energy in times of abundant feed supply against times when the feed supply is limited. In broiler chickens fat constitute 15-20% of the total body weight (Leenstra, 1987). Evans (1977) stated that over 85% of total body fat is stored in adipose tissue (subcutaneous, inter-muscular and abdominal fat) as an energy reserve. A maximum of 2 to 2.5% of the total body weight is fat which is present in the blood and other tissues as physiologically necessary fat. Yoshida and Morimoto (1970) stated that at least 0.9% of total fat weight should be for normal body function.

In broilers, fat is the most variable component (Lohman, 1973). The amount of fat deposit in the abdominal cavity is far more variable than that deposited inter-muscularly (Becker et al., 1979; Leenstra, 1982). The coefficient of variation of abdominal fat is 25 to 30% while the coefficient variation of total fat (abdominal fat included) varies between 15 and 20% (Leenstra, 1984). The coefficient of variation of total fat content is large compared to the coefficients of water content (approximately2%) and protein content (approximately8%) (Leenstra, 1982).

The abdominal fat percentage in relation to live weight was significantly lower in both NaNa and Nana genotypes compared to the normal birds. The significant effect of the Na gene in reducing subcutaneous and intramuscular fat deposition is well documented by (Cahaner et al., 1993; Raju et al., 2004 and Fathi et al., 2008). Body fat is negatively correlated with heat tolerance (Macleod and Hocking, 1993) which substantiates the present findings of less fat in naked neck birds (Fathi et al., 2013). The decrease in abdominal fat in naked neck chickens may be due to combined insulation effects of less plumage cover and utilization of a higher proportion of energy for thermoregulation (Merat, 1990). The Nana chickens had more skin protein and less fat in breast muscle and skin than their normal sib.

Although body fatness can be affected by diet, conventional wisdom is that genetic factors are more important determinants of the body composition than are dietary factors (Donaldson, 1985).

Bordas et al., (1979) reported that the abdominal fat in male chickens raised at 31oC was not significantly affected by the genotypes at (na) locus. There was a tendency for the naked neck birds to have a significantly higher value, possibly reflecting their higher growth rate at this temperature. While abdominal fat does not differ according to genotype, the total percentage of subcutaneous and inter-muscular fat is significantly lower in naked neck heterozygous.

Zein-et-Dein et al., (1984b) found the trend was the same, although not significant in the results obtained by El-Attar and Merat (1985). This may be related to the analysis of total lipids in the carcass made by Hanzl and Somes (1983) showed a significantly lower value for the naked neck genotype than for wholly feathered birds. An increase in the rate of heat loss by the naked neck birds as the ambient temperature decrease should increase fat deposition Leenstra and Cahaner (1991).

2.9 Chemical composition

There are two major aspects of meat quality; "nutrition quality, which is objective and eating quality," as perceived by the consumer, which is highly subjective. Meat from poultry contains several important classes of nutrients and it is low in calories. The fat contains essential fatty acids; proteins are good sources of essential amino acids (Mounteny and Parkhurst, 1995; Van Heerden *et al*., 2002; Wattanachant *et al*., 2004) and also excellent sources of water-soluble vitamins and minerals, such as iron and zinc (Van Heerden *et al.*, 2002; Boccia *et al*., 2005). Chicken contains about 16.44-23.31% protein, 0.37-7.20% fat, 0.19-6.52% ash and 72.8-80.82% moisture content (Smith *et al*., 1993; Xiong *et al*., 1999; Abeni and Bergoglio, 2001; Van Heerden, *et al*., 2002; Wattanachant *et al.,* 2004; Chuaynukool, *et al*., 2007). The chemical composition of poultry meat has been shown to be related to species, breed, muscle type, sex, age and method of processing of carcass (Ngoka, *et al*., 1982; Smith and Smith 1993, Ding *et al*., 1999; Abeni and Bergoglio, 2001; Van Heerden, *et al*., 2002; Wattanachant *et al.,* 2004; Boccia *et al.,* 2005; Chuaynukool *et al.,* 2007; Wattanachant and Wattanachant, 2007). Ding *et al*., (1999) showed significant differences in fat contents between broiler and local chickens. Wattanschant *et al.,* (2002) found that Thai indigenous chicken muscle contained higher protein content but lower fat and ash content compared to broiler

muscles, much of their value of protein food is based on its amino acids content whereby the high nutritional value is related to presence of essential amino acids. Amino acids present over 90% of the crude protein in the body of poultry (Hunton, 1995).

2.10 Sensory evaluation (Panel taste)

Five main characteristics which contribute to the overall eating quality of meat are taste, texture, juiciness, appearance and odor. Among these characteristics, texture is probably considered to be the most important attribute by the average consumer (Dransfield, 1994; Chrystall, 1994). Mechanical factors (tenderness) and juiciness (succulence) contribute to different meat textures. The tenderness of meat is the sum total of the mechanical strength of the skeletal muscle tissue and it's weakening during the post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animals and fowls (Takahashi, 1996). Meat tenderness originates in structural and biochemical properties of skeletal muscle fibers, especially myofibrils and intermediate filaments and of the intramuscular connective tissue, the endomysium and perimysium which are composed of collagen fibrils and fibers. Attractive appearance to consumer of indigenous chicken meat is performed by its carcass conformation, skin or meat color which might be related to chicken genotypes, feeds, rearing system or even processing condition. There are many intrinsic and extrinsic factors including genotype or breed, age, rearing system, feeds, chemical composition, structure and properties of muscle and processing condition which can have influence on different quality characteristics of chicken meat (Wattanachant, 2008).

Naked neck chicken breast and thigh muscles had slightly higher fat content when compared to those of the Southern Thai native chicken. The difference in muscle color profile between both chicken breeds contributed to significantly lower sensory scores on color preference of naked neck chicken (Adulyathan *et al.*, 2006).

The high shear value relating to Thai collagen content of Thai chicken meat results in lower sensory score on tenderness and juiciness of cooked meat compared to broiler (Adulyatham, *et al*., 2006). The tenderness of chicken meat decreased during muscle growth (Nakamura *et al*., 1975; Wattanachant and Wattenchort, 2007). Probably

because of the structural changes of collagen (Nishimura *et al.*, 1996; Fang *et al*., 1999; Nakamura *et al*., 2004). Wattanachant and Wattanachant (2007) stated that the appropriate age for indigenous chickens to possess economical live weight and high quality was in 16-18 weeks of age.

2.11 Molecular Genetic markers

Recent advances in molecular biology provide novel tools for addressing evolutionary, ecological and taxonomic research questions. Variation in DNA sequence can be observed with a level of accuracy and throughput that was previously impossible. The bulk of variation at the nucleotide level often not visible at phenotypic level. This DNA variation frequently is exploited in molecular genetic marker systems and the application of molecular markers to advance research and commercial activities is now well established (Gupta *et al*., 2001). DNA based markers have many advantages over phenotypic markers in that they are highly heritable, relatively easy to assay and are not affected by the environment (Duran *et al*., 2009). These are opportunities for using molecular genetics to identify genes that are involved in variety of traits. Armed with this information it would be possible to select improved livestock on the basis of their genetic makeup. If applied with care, the use of molecular information in selection programmes has the potential to increase productivity, enhance environmental adaptation and maintain genetic diversity (Naqvi 2007).The use of molecular genetics technologies potentially offer a way to select breeding animal at an early age (even embryos) to select for a wide range of traits and to enhance reliability in predicting the mature phenotype of individual. To date, most genetic progress for quantitative traits in livestock has been made by selection on phenotype or on Estimated Breeding Values (EBV) derived from phenotype, without knowledge of the number of genes that affect the trait or the effects of each gene (Naqvi 2007). In this quantitative genetic approach to genetic improvement, the genetic architecture of traits has essentially been treated as a 'black box'. Genetic progress may be enhanced if we could gain insight into black box of quantitative traits. Molecular genetics allows studying the genetic make-up of individuals at the DNA level.

The main reasons why molecular genetic information can result in greater genetic gain than phenotypic information are:

1) Assuming no genotyping errors, molecular genetic information is not affected by environmental effects and therefore, has heritability equal to 1.
2) Molecular genetic information can be available at an early age, in principle at the embryo stage thereby allowing early selection age, and reduction of generation intervals.
3) Molecular genetic information can be obtained on all selection candidates, which is especially beneficial for sex-limited traits, traits that are expensive or difficult to record, or traits that require slaughter of the animal (carcass traits) (Naqvi, 2007)

Definition of molecular marker

Living organisms are known to be made up of cells that are programmed by genetic material called DNA. Only a small fraction of the DNA sequence typically makes up genes coding for proteins, while the vast majority of remaining DNA represents non-coding sequences. The genetic material is organized into sets of chromosomes and the entire set is called the genome. In a diploid individual (i.e. where chromosomes are organized in pairs), there are two alleles of every gene-one from each parent. Molecular markers can be considered as constant landmarks in the genome, as a matter of fact they cannot be thought as a gene because they do not have any known biological function. They are simply identifiable DNA sequences, found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. Moreover, molecular markers rely on DNA assays, in contrast to morphological markers that are based on visible traits and biochemical markers that are based on protein produced by genes.

Although each marker system is associated with some advantaged and disadvantages, the choice of marker system is dictated to a large extent by the intended application convenience and the cost involved. The information provided to the breeder, by the markers varies depending on the type of marker system used. It is crucial the number of differences (polymorphisms) that the molecular marker system is able to discover between two or more individuals, breeds, populations at genome level. A powerful molecular marker is that skilled to identify a high number of polymorphisms.

2.12 Properties of molecular genetic markers

Various types of genetic markers such as morphological, chromosomal, biochemical and molecular markers are used. Morphological (e.g. pigmentation or other features) and chromosomal (e.g. structural or numerical variations) markers usually show low degree of polymorphism and hence, are not very useful for genetic markers. Biochemical markers have been tried out extensively but have not been found encouraging as they are often sex limited, age dependent and are significantly influenced by environment (http://www.ias.ac.in/currsci/oct 25/ articles19.htm). Sometimes, the various genotypic classes are indistinguishable at the phenotypic level owing to dominance effect. Furthermore, these markers reflect variability in their coding sequences that constitute less than 10 per cent of the total genome. The molecular markers capable of detecting the genetic variation at the DNA sequence level have not only removed these limitations but also possess unique genetic properties that make them more useful than other genetic markers. Moreover, they are numerous and distributed ubiquitously throughout the genome. These follow a typical Mendelian inheritance which usually expresses in a co-dominant fashion and are often multiallelic giving mean heterozygosity of more than 70 per cent. They remain un affected by the environmental factors and generally do not have pleiotropic effects on quantitative traits loci (QTL).

For genetic analysis, molecular markers offer several methodological advantages that are both attractive as well as amenable and they are:

i) The DNA samples can not only be isolated very conveniently from blood of live individuals but can also be isolated from tissues like sperms, hair follicle and even from archival preparations. The DNA samples can be stored for longer periods and can be readily exchange between laboratories. The analysis of DNA can be carried out at an early age or even at the embryonic stage irrespective of the sex. the DNA is transferred on to a solid support, such as filter membrane, it can be repeatedly hybridized with different probes and moreover, heterologous probe and in vitro- synthesized oligonucleotide probes can also be used and PCR- based methods can be subjected to automation.

Teneva (2009) summarized the properties of ideal DNA markers as follows:

i) Highly polymorphic nature. ii) Co- dominant inheritance (determination of homozygous and heterozygous status of diploid organism). iii) Frequent occurrence in genome. iv) Selective neutral behavior (the DNA sequences of any organism are neutral to environmental conditions or management practices). Easy access (availability). v) Easy and fast assay. vi) High reproducibility. viii) Easy exchange of data between laboratories.

2.13 Application of molecular genetic markers

Molecular markers can play an important role for livestock improvement through conventional breeding strategies. The various possible applications of molecular markers are short-range or immediate and long- range applications (Naqvi, 2007).

2.13.1 Short- range or immediate applications

Molecular markers have several immediate applications like parentage determination, genetic distance estimation, determination of twin zygosity and free martinism, sexing of pre- implantation embryos and identification of disease carrier and are discussed briefly:

2.13.1.1 Parentage determination

Since the breeding value of an animal is generally estimated using the information available from its relatives, the knowledge of correct parentage is therefore, a prerequisite. Parentage testing using molecular markers yield much higher exclusion probability (>90%) than testing with blood groups (70-90 %) or other biochemical markers (40- 60 %) highly polymorphic DNA fingerprinting markers (Jeffrey *et al.*, 1985) with the advent of PCR- based micro satellite assays. A large number of microsatallite panels have been reported that are useful for parentage testing in different livestock species. In addition, molecular markers also serve as useful tool for animal identification of semen particularly for verification of semen used for artificial insemination.

2.13.1.2 Genetic distance estimation

Genetic distance, a measure of overall evolutionary divergence, i.e. genetic similarities and dissimilarities between two populations (such as between species, breeds and strains) serves as useful tool for authentication of pedigree for characterization of different breeds or strains within a species and for evaluation for the change in variation in species overtime (Naqvi, 2007). In principle genetic distance can be measured on the basis of polymorphic characters occurring at the different levels, viz morphological, biochemical cellular and DNA level. Allelic frequencies of blood groups as well as those of other biochemical loci have been used extensively for estimation of genetic divergence of different livestock species (Baker, 1982). A great amount of genetic variations at protein loci remain undetected since changes in the underlying nucleotide sequences may not necessarily lead to corresponding change in the amino acid sequences owing to degeneracy in the genetic code.

2.13.1.3 Determination of twin zygosity and free martinism

Correct knowledge of zygosity of twins, particularly in monolocus animal is very important. Monozygotic twin provide means for epidemiological as well as for genetical studies and also help in transplant matching. Individual specific DNA fingerprinting techniques have potential applications in determination of twin zygosity and demonstration of spontaneous XX/XY chimerism (Farber *et al*., 1989). Demonstration of XX/YY chimerism in heterosexual bovine twins by PCR- RFLP assay using sex-chromosome -specific primers, has enable the identification of free martin animal.

2.13.1.4 Sex determination

Sexing of pre- implantation embryos can serve as an important tool for improving herd for a desire purpose. A large number of invasive and non invasive methods for sexing embryos are available. However, ideally the technique to be applied should not have any adverse effect on embryo survivability, its conception rate and subsequent development. The molecular markers have potential application in determination of sex of pre- implantation embryo, since the embryos can be sexed using male – specific or Y chromosome- specific DNA sequence as probe. The PCR- based method of sex

determination offers several advantages over all the other methods as stated by (Machaty *et al*., 1993):

i) It can be carried out in less than five hours with almost 100 percent accuracy. i) It is less invasive and requires very small quantities (in nanogrames) of DNA for PCR assay, which can be isolated from two to eight cells from embryos. iii) It can be done at an early stage of embryo e.g. blastocyst stage (6 to 8 days) or even earlier at the 16-32 cell stage. v) The use of multiplex PCR allows simultaneous genotyping for important loci like milk protein, disease carrier etc.

2.13.1.5 Identification of disease carrier

Many of the most serious incurable diseases result not from infections with bacteria or virus but defects in genomes of the host. Certain allelic variations in the host genome lead to susceptibility or resistance to particular disease (Naqvi, 2007). DNA polymorphism occurring within a gene helps to understand the molecular mechanism and genetic control of several genetic and metabolic disorders and allows the identification of heterozygous carrier animals which are otherwise phenotypically indistinguishable from normal individuals.

2.13.2 Long-range application

The foremost long- range application of molecular markers in conventional breeding includes mapping of the QTL by linkage. Such mapping information, if available particularly for those loci which affect the performance traits or disease resistance / susceptibility can be used in breeding programmes by either within- breed manipulations like marker- assisted selection of young sires or between breeds introgression programme.

2.13.2.1 Gene mapping

Molecular markers have three fold applications in gene mapping.

i. A marker allows the direct identification of the gene of interest instead of the gene product and consequently it serves as useful tool for screening somatic cell hybrids.
ii. Use of several DNA probes and easy- to screen techniques, a marker also helps in physical mapping of the genes using in situ hybridization.
iii. The molecular markers provide sufficient markers for construction of genetic maps using linkage analysis.

Genetic maps are constructed on the basis of two classes of molecular markers (O'Brien, 1991). Type 1 marker that represents the evolutionary conserved coding sequences (e.g. classical RFLPs and SSLPs) is useful in comparative mapping strategies where polymorphism is not an essential prerequisite. However, these are mostly single locus and di-allelic (SLDA) and thus are not useful for linkage analysis. On the other hand, the type 11 markers (like microsatellites markers) have higher polymorphism information content than conventional RFLPs and can be generated very easily and rapidly. Therefore, major efforts are being made to produce gene maps based on the type 11 markers. Furthermore, utilization of molecular markers developed from DNA sequences information namely ASO and STMS polymorphic markers are also helpful in rapid progress of gene mapping.

2.13.2.2 Markers assisted selection

The concept of marker assisted selection (MAS) utilizing the information of polymorphic loci as an aid to selection, was introduced as early as in 1900s (Sax, 1923). However, its application in genetic improvement of livestock species has been limited due to lack of suitable genetic markers (Naqvi, 2007). The discovery of DNA- level polymorphism in eighties and their subsequent use in molecular markers has renewed interest in the use of genetic markers in selection of breeding stock. Implementation of MAS essentially involves two steps:

i. Identification of the marker loci that is linked to QTL of economic importance followed by the utilization of linkage association in genetic improvement programme.

ii. Once linkage between a QTL and a marker locus is established, it is possible to recognize the alternative QTL allele inherited by the individual. Such information can then be used for the selection of breeding stock. Inclusion of markers could increase up to 15% the genetic response to selection for growth rate in a population of chickens compared with selection based on family selection. Ruane and Colleau (1996) found an increase of 6 to15% from MAS in response for milk production in cattle nucleus that used multiple ovulation and embryo transfer (MOET) in the first six generations of selection.

2.13. 2.3 DNA- based markers

Classification of molecular markers

The molecular markers can be classified into the following two groups:

i) Southern-blot hybridization, based on DNA markers such as Restriction Fragment Length Polymorphisms (RFLP) and Variable Number of Tandem Repeats (NVTR).

ii) PCR-based DNA marker such as Random Amplified Polymorphism DNA (RAPD), Arbitrarily Primed-Polymerase Chain Reaction (AP-PCR), Simple Sequence Repeats (SSR), Sequence-Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLP), Inter-Simple Sequence Repeats (I-SSR), Sequence Characterized Amplified Regions (SCAR), Cleaved Amplified Polymorphic sequences (CAPS), Selective Amplified Microsatellite Polymorphic Locus Sequences (SAMPL), Sequence-Specific Amplification Polymorphism (S-SAP), Single Nucleotide Polymorphism (SNP). These two main groups of molecular markers can be further subdivided depending on their ability to detect variations at single locus or at multiple locus level. The SCAR, CAPS, SNP, RFLP and VNTR markers belong to the former subgroup, whereas RAPD, AP-PCR, AFLP, SAMPL, S-SAP and I-SSR markers belong to the latter subgroup. For some find molecular markers included in the second group, such as RAPD, AP-PCR, AFLP, no preliminary DNA sequence information is necessary. By contrast, older molecular marker systems, such as RFLP and VNTR, or SNP markers need preliminary DNA sequence information to be investigated.

In the future, due to the necessary development of sequence information for different species and organisms, new DNA marker systems will be set up to meet the new research area needs.

2.13.2.3.1 Restricted Fragment Length Polymorphisms (RFLPs)

Restricted fragment length polymorphisms (RFLPs) are based on the analysis of patterns derived from a DNA sequence digested with known restriction enzyme (Teneva 2009). The technique relies on the amplification of variable regions of the target genome, with one or more amplicon, and then being digested with one or more sequence-specific restriction enzymes. The DNA fragments of different lengths are then subjected to electrophoresis and fragments migrate according to their weights, the smaller fragments faster and the larger fragments more slowly (Nicholas, 1996). Thus, RFLP generally refers to the differences in banding patterns obtained from DNA fragments after sequence-specific cleavage with restriction enzymes. Before the advent of PCR, RFLP analyses typically entailed restriction enzyme (RE) digestion of chromosomal DNA, followed by electrophoretic separation, membrane blotting and hybridization with a labeled probe, usually radioactive. Although reliable, this process is time consuming, cumbersome and expensive. However, the ability to amplify a selected target a million fold through PCR has abolished the need for the problematic blotting and hybridization analyses. Although many enzymes still need to be tested in the initial phase to be able to identify the polymorphism, it is today an easy, reliable and relatively cheap marker to use (Dodgson *et al*., 1997) and PCR-RFLP is therefore, the simplest PCR procedure for comparisons of sequence polymorphism. Restricted enzymes (Bam HI, EcoRI Sacl and Taql) have often been shown to be useful in obtaining RFLP patterns for haplotype identification in individuals (Smith *et al*., 1996; Spike *et al*., 1996). The RFLP marker technique allows the identification of only two alleles per locus and is slow to be used with large genome size in mammals, where about $3x10^9$ individual nucleotides are present in the total DNA component (Brash, 1994; Lewin, 1994; Wilmut *et al*., 1992).

The potential advantages and disadvantages of RFLP markers according to Teneva (2009) are:

Advantages

1) Produces co-dominant (also known as semi-dominant) markers, this allows discrimination of homo-and heterozygotic states in diploid organisms. 2) Stable and reproducible, gives constant results overtime and location. 3) Selective neutrality.

Disadvantages

1**)** Long methodology. 2) Labour intensive.

3) Requires high quality and large quantities of DNA.

4) RFLPs limited identification of the whole genome variation in animals

5) The reduced variability observed in domestic animals by inbreeding makes many RFLPs sites non-informative.

2.13.2.3.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was developed by Mullis in 1983 (Mullis *et al.,* 1986). The PCR is a biochemistry and molecular technique for exponentially amplifying a fragment of DNA, via enzymatic replication without using a living organism (Pavlov *et al*., 2004). This allows to amplification or reproduction in great amounts of particular regions of the DNA. In order to initiate the process of replication of the DNA, two informing sequence codes dominated primers are required which promote the beginning and reversion of the reaction of the polymerase (RNA-pol) at particular locations of the genome. A reproduction or amplification of thousands of copies of a chromosomal region of gene of interest is obtained by repeated cycles of synthesis and denaturalization (chain separation) of the DNA using temperature changes. Since the primers are specific sequences to bond to a determined region of the DNA only the specific amplification of the desired sequence of DNA instead of amplifying the DNA in its totality is obtained (Buratowski, 1994; Koleske and Young, 1995; Stein *et al.,* 1996) PCR is closely patterned after the natural DNA replication. Two oligonucleotide primers flank and define the target sequence to be amplified. These primers hybridize to opposite strands of the DNA to serve as an initiation points

for the synthesis of new DNA strands. A thermostable DNA polymerase, such as a Taq DNA polymerase catalyzes this synthesis. This enzyme needs original template in the 3´-5´ direction and synthesizes a new complementary template in 5´-3´ direction, using free deoxnucleotide triphosphates (dNTP's) as building blocks. A PCR thus consists of a number of cycles of denaturation, annealing and extension. The product synthesized in one cycle serves as a template in the next, so that the original DNA is doubled in every cycle and the target DNA molecules are typically increased by several million folds. This is only in 20 cycles PCR provides million (2^{20}) copies of the target (Mullis *et al*., 1986; Buratowski, 1994; Koleske and Young, 1995; Stein *et al*., 1996). The amplicon or PCR-product can then be visualized on an agarose or polyacrylamide gel (Nicholes, 1996; Buratowski, 1994).

The PCR is a method that efficiently increases the number of DNA molecule in a logarithmic and controlled fashion. PCR is a major scientific development and taq polymerase the enzyme essential to PCR's success. The chemistry involved in the PCR depends on the complimentarily (matching of the nucleotide bases in the double stranded DNA helix). When a molecule of DNA is sufficiently heated, the hydrogen bonds holding together the double helix are disrupted and the nucleotide separates or denature into single strand.

PCR analysis involves the use of DNA polymerase and synthetic primers to replicate DNA in vitro. Starting with just 1 copy of the target sequence, billions of copies can be generated within an hour. The process involves temperature cycle. At high temperature the DNA melts, the temperature is then lowered to one where the primers can base pair (anneal) with the target and DNA polymerase can synthesize the DNA molecule. PCR principles are denaturizing, annealing and elongated.

PCR has the advantage of being a relative fast, sensitive and reliable method. Not only can it amplify very small amounts of DNA, but can also amplify degraded or poor sources of DNA. PCR has become an essential tool in molecular biology and plays a leading role in all techniques that are presently applicable to the analysis and characterization of genomes. PCR is applied to amplify known sequences of a sample of DNA or arbitrary priming of variable regions of genome. The technique is suitable

for the use with various DNA markers such as Variable Number Repeats (VNTR's), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) (Sunnuks, 2001; Freeman *et al*., 2006).

2.13.2.3.3 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA is also known as arbitrarily primed PCR (AP-PCR), or as a DNA amplification fingerprinting technique (DAF). This technique is based on the use of short, arbitrary primers in PCR reaction and can be used to produce relatively detailed and complex DNA profiles for detecting amplified fragment length polymorphisms between organisms. In the simplest format, only one short oligonucleotide usually eight to 10 nucleotides in length is used. However, multiple primers are usually applied and a range of five to 21 nucleotides has proven successful if detection is coupled with polyacryamide gel electrophoresis. Relaxed PCR conditions allow for multiple unspecific priming sites on opposite DNA strands, even if the match is imperfect. A successfully amplified template sequence will however, only span from a priming site sequence to a nearly complementary sequence. Depending on the primer/template combination and ratios, amplified products range from less than 10 to over a 100. In this way, a spectrum of products characteristic for each template and primer combination is typically obtained and these can be adequately resolved and visualized using polyacryamide gel electrophoresis and silver staining. Agarose gel electrophoresis and ethidium bromide staining can also be used but detected only the major fragments Van Marle-Kosster *et al.,* 2003).

Random amplified polymorphic DNA markers have advantages and disadvantages according to (Vignal *et al.,* 2002; Mburu and Hanotte, 2005; Dodgson *et al.,* 1997) as follows:

Advantages

1. Cost effective. 2) Simple and quick. 3) Large number of band is produced. 4) No prior sequence knowledge is necessary. 5) The required samples are very small because DNA will be amplified by PCR technique.

Disadvantages

1) Is limited; reproducibility Detection of polymorphism of results may be inconsistent e.g. low annealing temperature.
2) Some unspecific non reproducible binding primers.
3) Dominant markers (homozygote cannot be discerned from heterozygote so allele frequencies cannot be estimated).
4) The RAPD primers are very sensitive to PCR conditions and this may lead to poor reproducibility.

2.13.2.3.4 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism is a DNA technique that is based on detection of DNA fragments, subjected to restriction enzymes, followed by selective PCR amplification. The DNA is cut with two restriction enzymes and double stranded adapters are then ligated to the ends of the DNA fragments to generate the template for PCR (Van Marle-Kosster *et al.,* 2003). The specific adapter ligated to the DNA fragment, determined the sequence of AFLP primers. These primers are thus in reality semi-arbitrary and the assay determines the distribution of DNA restriction sites throughout the genomes in question by DNA amplification. AFLP procedures can be manipulated to suit specific applications through the selection of the restriction enzyme and the design of the PCR primers. Typically a rare-cutter and frequent-cutter restriction enzyme is combined to ensure the generation of small frequents (frequent-cutter) but to limit the number of fragments (rare-cutter) at the same time. PCR primers can be designed to have no selective bases on the 3' ends if the targeted templates are simple elements such as plasmids or bacterial artificial chromosomes. As in other technique for fingerprinting, fragments are separated and analyzed using gel electrophoresis. The AFLP technique can be performed at a reasonable cost (Van Marle-Kosster *et al.,* 2003).

Compared to RAPDs, higher marker densities are achieved with AFLPs and the stringency of the PCR step is also much higher in the AFLP strategy, although manipulation of band sizes and numbers is still possible. As a result AFLP is more highly reproducible and reliable. Development costs are low but running costs are higher than for RAPD analyses (1.5-2 times the cost of RAPD/sample). Being slightly more technical, the levels of skills required are also higher than those required for RAPD analysis and in addition, DNA preparations must be of particular high quality and purity to ensure restriction enzyme digestion (Van Marle-Kosster *et al.,* 2003).

AFLPs are dominant bi allelic markers (Vos *et al.,* 1995). Variations at many loci can be arrayed simultaneously to detect single nucleotide variations of unknown genomic regions, in which a given mutation may be frequently present in undetermined functional genes (Young, 1999). AFLP provides an effective, rapid and economical tool for detecting a large number of polymorphic genetic markers that are highly reliable and reproducible and are able to be genotyped automatically. AFLP is considered as the "gold standard" for molecular epidemiological studies of the pathogenic microorganisms and it is widely used in forensic sciences. The AFLP has been used extensively to detect polymorphisms, evaluate and characterize breed recourses, assess the relationship between breeds, construct genetic maps and identify genes in the main livestock species (Ajmone-Marson *et al.,* 2002; Marsan *et al*., 2007). AFLP is the ideal molecular approach for population genetics and genome typing except microsatellites.

The advantages and disadvantages of AFLPs as shown by Mburu and Hanotte (2005) as follows:

Advantages

1) Highly sensitive method.2) large number of polymorphisms is generated. 3) Selective neutrality. 3) Highly reproducible. 4) No prior sequence information or probe generation is needed.

Disadvantages

1) Expensive technique. 2) Dominant marker. 3) Technically demanding.

2.13.2.3.5 Minisatellites

Parallel to development of PCR, a new type of polymorphism of DNA also known as hypervariable minisatellites were discovered in the DNA structure. These minisatellites are defined regions of DNA with polymorphisms in the number of repeated nucleotide sequences of around 25 bp in length. These minisatellites can be used for search for marker genes associated and as DNA fingerprints in paternity testing (Smith and Smith, 1993; Stein *et al.,* 1996).

Minisatellites were first described as hypervariable tandem repeats, when found in human genome. This led to the use of the term Variable Number of Tandem Repeats (VNTR) in reference to repetitive units which include mini-and microsatellites. Minisatellites usually range in size from 10 to 100 bases and microsatellites from one to six bases (Nicholas, 1996; Turner *et al.,* 1998). Although both minisatellites and microsatellites occur throughout the eukaryotic genome, the minisatellites tends to be concentrated in the telomere region and in sites associated with a high frequency of recombination (Bruford and Wayne, 1993; Nicholas, 1996). The number of repeats in the satellite DNA is highly hypervariable and differs among individual species. For this reason these repeats may be effectively applied as DNA markers in the study of genetic differences. Minisatellite markers however, have certain limitations for example, they do not uniformly mark the genome, the marker fragment is difficult to clone and they are dominant markers if the repeat is used as probe, which reduces the potential information for genotyping (Dodgson *et al.,* 1997).

2.13.2.3.6 Microsatellites

Microsatellites loci consist of tandem repeats between one to six bp and are also known as simple sequence repeats (SSR's), short tandem repeats (STR's), simple sequence tandem repeats (SSTR), variabl number tendem repeats (VNTR), simple sequence length polymorphisms (SSLP) and sequence tagged microsatellites (STMs). Repeat units may consist of $(A)_n$, $(TG)_n$, $(CA)_n$ or (AAT) n repeats. Microsatellites are highly polymorphic due to the variation in the number of repeats. It is not uncommon to find up to 10 alleles per locus and heterozygosity values of 60% in a relatively small

number of samples (Goldstein and Pollack, 1997). Microsatellites tend to mutate with mutation rates of up to 10-2 per generation (Bruford and Wayne, 1993). A large number of microsatellite markers have been mapped for various species including humans, mice, fruits, flies and farm animals (cattle, sheep, pigs and chickens) (Goldstein and Pollack, 1997; Groenen *et al.*, 2000).These elements are most valuable markers in studies on genetic variability, parentage verifications and genome mapping project. Microsatellites are spread over the eukaryotic genome and have a sufficiently high mutation rate and were suggested in order to overcome the limitations associated with RFLP and RAPD. Micrsatellites are easily amplified using PCR from DNA extracted from a variety of sources including blood, hair, skin or faeces. Polymorphisms can be visualized on a sequencing gel and the availability of automatic DNA sequences allows high-throughput analysis of a large number of samples (Goldstein *et al.*, 1995). Vos *et al*., (1995) stated that the heterozygosity of SSRs is seven to 10 times higher than that of RFLP's.

Many authors including (Mburu and Hanotte, 2005; Erhardt and Weimann, 2007) showed the advantages and disadvantages of microsatellites as follows**:**

Advantages

1) Low quantities of template DNA required (10-100ng).
2) High genomic abundance.
3) Random distribution throughout the genome.
4) Band profiles can be interpreted in terms of loci and alleles.
5) Co-dominant markers
6) Allele sizes can be determined with accuracy.
7) Comparison across different gels possible using size standard.
8) High reproducibility.
9) Different microsatellites may be multiplex in PCR.
10) Wide range of applications.
11) Amenable to automation.

Disadvantages

1) Initial high development costs.

2) Heterozygotes may be misclassified as homozygotes when null-alleles occur due to mutation in the primer annealing sites.
3) Stutter bands may complicate accurate scoring of polymorphisms.
4) Underlying mutation model (infinite alleles model or stepwise mutation model) largely unknown.
5) Homoplasy due to different forward and backward mutations may underestimate genetic divergence.
6) Time-consuming and expensive to develop.
7) Microsatellite markers help to identify neutral biodiversity but do not provide information on functional traits biodiversity.

2.13.2.3.7 Single Nucleotide Polymorphisms (SNP)

Single nucleotide polymorphisms (also referred to as "Snip") are the most recent contribution to studying DNA sequence variation. These markers are abundant and found in both coding and non-coding regions of the genome. They are biallelic markers, indicating a specific polymorphism in two alleles only of a population. SNP in coding regions can be directly associated with the protein function and as the inheritance pattern is more stable, they are more suitable markers for selection (Beuzen *et al.*, 2000). Most of RFLP and AFLP markers are also the result of a SNP in a restriction enzyme recognition site, which confirms the importance of SNP markers. SNP have become the preferred markers in genetic disease studies in humans and they are also being searched for various livestock species. SNPs can be detected using Single Stranded Conformation Polymorphism (SSCP). Allele Specific Oligonucleotides (ASO), single nucleotide polymorphic discrimination by an electronic dot blot assay, (ASO) on semiconductor microchips, reverse dot blot on DNA chips Dynamic Allele-Specific Hybridization (DASH), Allele-Specific PCR (amplification refractory mutation system or ARMS test), mutation detection the ARMS test combination with the Taqman TM 5′ exonuclease assay (exploiting the 5′ > 3′ exonuclease activity of Taq DNA polymerase), minisequencing and analysis of the extension products by PAGE, Minisequencing and analysis of the extension products of DNA chips, Minisequencing and analysis of extension product using matrix-assisted, laser desorption/ionization time of flight mass spectrometry (MALDITOF) (Mburu and Hanotte, 2005) .

There are four major reasons for the increasing interest in the use of SNPs as markers for genetic analysis. Firstly, they are prevalent and provide more potential markers near or in many locus of interest than other types of polymorphism such as microsatellites. Secondly, some SNPs are located in coding regions and directly affect protein function. These SNPs may be directly responsible for some of the variations among individuals in important traits. Thirdly, SNPs are more stably inherited than microsatellites, making them more suitable as long-term selection markers. Finally, SNPs are more suitable than microsatellites for high throughput genetic analysis using DNA microassay technology (Lipshutz *et al*., 1999).

One disadvantage of SNPs markers is the lower information content compared with that of highly polymorphic mirosatellite but it can be compensated by the use of a higher number of markers (Werner *et al.,* 2004).

2.13.2.4 The Chicken Genome and Mapping

The chicken is increasingly becoming of great interest as an intermediate evolutionary model organism, ideally placed between mammals and more distant vertebrates as Pufferfish and Zebrafish. There are a number of different reasons for this increasing interest in the chicken genome. First, the genome size is only one third that of mammals (Tiersch and Wachtel, 1991) mainly because of its low amount of repetitive sequences and reduced intron sizes (Hughes and Hughes, 1995). Furthermore, it has an interesting complex genomic structure with two chromosomal subtypes- macrochromosomes and microchromosomes.

Second, the level of conserved synteny between chicken and humans appears to be very high. Third, the chicken is being studied intensively for affecting polygenic traits (quantitative trait loci or QTL).

The genome-wide comparison between the wild and domestic chicken yielded the discovery of 2.8 million single nucleotide polymorphisms (SNP). The mean nucleotide diversity was estimated to be approximately 5 SNPs/ kb, in comparisons between domestic breeds to red jungle fowl, but also in comparisons between and within breeds of domestic chicken (Wong *et al*., 2004). The high genetic diversity within and between

domestic breeds show that although the chicken has gone through a domestication process, vast nucleotide diversity remains in domestic population. Given the short time scale that elapsed from the start of domestication (7,000-9,000 years ago) to the present, a coalescence time of 1.4 million years would be required to account for observed nucleotide diversity in domestic breeds (Wong *et al.*,2004). Hence, most of the genetic variation observed in the domestic chicken pre-dates to the start of domestication. The estimate of 5 SNP/ kb in chicken is much higher than the observed rate of about 1 SNP/ kb in humans (Schidanandam *et al.,* 2001) and the rate in dog (Lindblad-Toh *et al*., 2005) while similar to different subspecies of mice (Lindblad- Toh *et al.,* 2000). Domestication of the chicken must have involved a large founding population and possibly a large geographical region.

The karyotype (i.e. chromosome organization) in birds differs from that in mammals. The chicken has 38 autosomal chromosome pairs (ten large macrochromosomes and two sex chromosomes (Z and W) (Schmid *et al*., 2005). In contrast to mammalian species, where the male is the heterogametic sex (XY), birds display a reverse order with the female as the heterogametic sex (ZW) and males are homogametic (ZZ). The sex chromosomes in the avian genome are not homologous to the X and Y chromosomes as in mammals and have evolved from a different autosomal chromosome pair (Fridolfsson *et al.,* 1998). Characteristic feature of a typical avian karyotype include a large number of chromosomes and also substantial size variants between chromosomes (Schmid *et al.,* 2005). Macro-and microchromosomes are also found in reptiles and some fish (Burt, 2002 and Stock *and* Mengden., 1975). The macro chromosomes are relatively few, and about the same size as an average-sized mammalian chromosome (i.e. 140 Mb). Micro chromosomes vary in size from 2-15 Mb (Nature 2004). Mammalian chromosomes also show size variation however, the span is small compared to that detailed in birds. There are interesting structural differences between macro- and micro chromosomes, for example, GC content and gene density are higher on the micro chromosomes whilst intronic lengths are shorter. In addition, repetitive sequence elements are generally less common on micro chromosomes (Primmer *et al*., 1997).

The haploid size of the chicken genome is approximately 1x 10^{9} base pair (bp) compared to 3x10^{9} bp in human (Lander *et al*., 2001 and Venter *et al.*, 2001) and 2.7x10^{9} bp in the mouse (Waterston *et al.*, 2002). The smaller genome size of avian species is mainly due to reduction of repetitive sequences in general, less non-coding sequence. The number of genes contained in the avian genome is equivalent to that in the human genome (about 20,000 genes) (Clamp *et al.*, 2007). The streamlined genomes of avian species have been suggested to have evolved as an adaptation to the high metabolic demand associated with flight (Huges and Huges 1995 and Huges 1999). Consistent with this hypothesis, flightless birds have larger genomes compared to flying birds, other mammalian species in the same order (Huges and Huges 1995 and Van den Bussche 1995). The possibility remains that the small genome size of birds could have evolved from the flightless dinosaur ancestor and so merely reflects the neutral evolutionary processes of selfish repetitive DNA sequences (Kazazian 2004). Testing this hypothesis would require a comparative analysis of the genomes from the extinct predecessors of avian species, although to date, an analysis has not been possible. In a recent study by Organ and colleagues, the absence of data from extinct species has been overcome by exploring the positive correlation between genome size and volume (Organ *et al*., 2007). First the authors showed that bone-cell size correlates well with genome size in the extinct vertebrates and then used this relationship to estimate genome size from the bone-cell cavities found in fossilized bone remains. They employed this method to estimate genome sizes from 31 extinct species of dinosaurs together with extinct avian species. The results from their analysis suggested that a small genome size, evolved long before the appearance of the first birds.

Although the first genetic linkage map in chicken was published > 60 years ago (Hutt, 1949), it was not until the development of large numbers of molecular markers in the last decade that the generation of linkage maps in chicken increased. In chicken, three different linkage maps were developed using three different mapping populations. The first genetic maps, based completely on DNA markers (Bumstead and Palyga, 1992). This map, based on the Compton (C) reference population, consisted solely of restriction length polymorphism (RFLP) markers. The second genetic map to be published (Levin *et al.*, 1993, 1994) was based on the East Lansing (ET) reference population and consisted

primarily of RFLPs, random amplified polymorphic DNA (RAPD) markers, (CRI) markers. The third map (Groenen *et al.,* 1998; Herbergs *et al.,* 1999) was based on a large F_2 population and consisted solely of microsatellite and amplified fragment length polymorphism (AFLP) markers. Increasing densities and increased initiatives in physical mapping in chicken have necessitated the need of a single consensus linkage in chicken. Because all three maps have many markers in common, this goal has become feasible for the large and intermediate- size chromosomes.

2.13.2.5. Genetic polymorphism

Genetic polymorphism is a type of variation with sharply distinct qualities co-exists as normal members of a population. The condition is defined as 'the occurrence together in the same habitat of two or more discontinuous forms, or "phases," of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation (Ford, 1940a), it is also defined as the inheritance of a trait controlled by a single genetic locus with two alleles, in which the least common allele has a frequency of about 1% or greater (Leonnard, 1990). In a formal sense there are two forms of DNA polymorphisms, those due to replacement of DNA bases and those due to insertion and deletion of bases, point mutation usually show two alleles at each locus. Those that are due to insertion /deletion events often show more alleles; those that are due to changes in the number of a particular DNA sequence, such as the nucleotides GT repeated many times, may be hyper variable, with more than five alleles at a locus (Fries *et al*., 1990; Moore *et al.,* 1992; Bishop *et al.,* 1994 and Vaiman *et al*., 1994b). In some cases, the DNA sequence that is repeated is not exactly the same every time but there is sufficient similarity to discern the fundamental motif. Depending upon the size of the motif, the element is a microsatellite (repeat unit of 1-5 base pairs), a minisatellite (repeat unit of base pairs) or a satellite. The hypervariable loci tend to be either microsatellite or minisatellite. However, the hypervariable loci are less common than point mutations, with point mutations being approximately two orders of magnitude more common than microsatellites (Georges *et al*., 1987; Steele and Georges, 1991; Steffen *et al.,* 1993; Moore *et al*., 1992 and Vaiman *et al*., 1994b).

The detection of these polymorphisms uses essentially the same technology and it is a rare technique indeed that can be used for only one or the other of these kinds of polymorphism. The techniques either recognize the identity of the sequence or recognize the effect of a change in sequence, and some techniques use a combination of the two. For example, a restriction endonuclease will cut DNA at a specific sequence, and mutations at the sequence will result in DNA of different lengths, corresponding to the presence or absence of the mutation. The lengths can be discriminated once the DNA is separated by size on a gel (Southern, 1975). However, the same system can be used to recognize a polymorphism if there is variation in the length of the fragment, due to repetitive elements, even if there has been no mutation at the restriction endonuclease site (Jeffreys *et al*., 1985b). The restriction endonuclease acts merely to cut the DNA to a manageable size for analysis.

Polymorphism can also be divided into those that are in or extremely near a sequence that is transcribed into RNA. The first kind, often called type I polymorphisms (O'Brien, 1991), are in sequences they are conserved across species and so are useful for studies of evolutionary history and for the practical matter of interpolating, the expected locations of genes between species. The second kind, often called type II polymorphisms (O'Brien, 1991), are in sequences that are usually unique to a species or occur in a narrow range of taxonomic groups. Despite their limitation for some studies, they form the backbone of studies to localize genetic factors to linkage maps, primarily due to their large number, they are often hypervariable.

The methods of detection DNA polymorphisms revolve around three major technologies, not all of which are used in every method. These are: (i) gel electrophoresis to separate DNA molecules by either length or volume; (ii) DNA oligonucleotide hybridization, in which oligonucleotides are hybridized to the target DNA; and (iii) DNA visualization, in which the DNA is either stained by silver, intercalated with ethidium bromide, or labeled with radioactive or fluorescent components with detection by various combinations of photography, ultraviolet (UV) fluorescence, autoradiography and laser excitation, with computerized capture of image. These methods have been revolutionized

by polymerase chain reactions (Mullies *et al*., 1986), which result in the amplification of specific DNA sequences to quantities of any predetermined amount.

Using genetic polymorphism to study evolution and variation in populations: Genes specify proteins, and proteins form the basis of physiology, development, appearance, and behavior of organisms. Genetic variation can be examined at any of these functional levels. Genetic diversity is measurable at any functional level from blueprint to phenotype. Thus, genetic variability within a population can be measured as:

1- The number (and percentage) of genes in the population that are polymorphic. 2- The number of alleles for each polymorphic gene. 3- The number and percentage of genes per individual that are polymorphic (Primack, 1993).

2.13.2.6 Growth Hormone Gene

Growth hormone (GH), a peptide hormone released from the anterior pituitary gland, is essential for embryonic and post hatch growth in domestic chickens. In sex-linked dwarf chickens, a mutation of the GH receptor (GHR) results in a dwarf phenotype due to lack of GH signaling (Huang *et al*., 1993). GH regulates metabolic processes such as lipolysis and muscle accretion. These two processes directly affect the quality of meat in broiler chickens. Post hatch administration of GH has little or no effect on growth rate in chickens. However, in ovo administration of GH at embryonic day 13 (e13) results in improved growth after hatch (Blumenthal *et al.,* 1954), suggesting the establishment of a growth threshold during embryonic development. Hence, understanding the mechanisms that establish growth parameters in the embryo is essential for successful manipulation of this process to maximize agricultural yield. Comparison of the regulation of GH gene expression across vertebrate species has identified several common themes as well as differences.. In the general theme, GH is synthesized and secreted from the somatotrophs (GH producing cells) of the anterior pituitary gland. Signaling pathways and transcription factors orchestrate the development of the pituitary gland and functional maturation of the hormone producing cells. Somatotroph differentiation and GH gene expression are influenced by hypothalamic factors such as growth hormone-releasing hormone (GHRH) and somatostatin (SST) (Romero and Phelps, 1997), as well as hormones from peripheral

glands (adrenal glucocorticoids and thyroid hormones). Several transcription factors, such as PIT1, Sp-1, AP-2 (activator protein-2), glucocorticoid receptor (GR), thyroid hormone receptor (TR) and cAMP (3'-5'-cyclic adenosine monophosphate) response element binding protein (CREB) coordinate to maintain the tissue-specific expression of the GH gene. Absence of one or more key elements of this machinery leads to absence or significant reduction of GH synthesis and secretion.

2.13.2.6.1 Functions of growth hormone

Growth hormone is involved in regulating a number of physiological processes in vertebrates, including long bone growth, lipid, carbohydrate and protein metabolism, immune function (Hooghe-Peters and Hooghe, 1998) and carcinogenesis. The Somatomedin hypothesis, put forward in 1957 (Salmon and Daughaday, 1957), postulated that the effects of GH on its target organs are not direct, and that they involve one or more intermediate factors (termed somatomedins; later identified to be insulin-like growth factors or IGFs) (Van Wyk *et al.*, 1974). Decades of research in this area have proved this hypothesis to be partially true, and the hypothesis has been modified, with its present form stating that some actions of GH are direct while others are indirect (Le Roith *et al.*, 2001). For instance, the GH effect on long bone growth appears to be direct. It is generally seen that the anabolic effects of GH, such as those involving bone, cartilage and skeletal muscle growth are direct and in some of these IGFs act in concert with GH towards the same end, but the action of GH itself is IGF-independent. However, in certain catabolic processes, such as lipid and carbohydrate metabolism (gluconeogenesis and adipogenesis), IGFs and GH have opposite effects, and IGFs are thought to counteract the effects of GH, which would otherwise be harmful (Kaplan and Cohen, 2007). In mammals, GH is the primary regulator of post-natal growth. Transgenic mice over expressing GH grew almost twice as big as control littermates (Palmiter *et al.*, 1983). Exogenous administration of GH in swine results in increased average daily gain (ADG), muscle accretion and lipolysis (Campbell *et al.*, 1989). GH also increases lactation in dairy cattle (Falaki *et al.*, 1997). Apart from its obvious effects on bone growth, GH is necessary for normal female reproductive function, steroid metabolism, tooth development and modulation of gut function.

In fish, apart from regulating growth throughout adult life, GH functions include regulation of metabolism by lipolysis and protein synthesis (Foster *et al.*, 1991; Leatherland and Farbridge, 1992; O'Connor *et al.*, 1996; Fauconneau *et al.*, 2000), reproduction, osmoregulation (Bolton *et al.*, 1987;), and immune development (reviewed in Yada 2007). Osmoregulation by GH is brought about by morphological changes in gill chloride cells and by increasing the number of ion transporters in these cells, and this effect is believed to be IGF-dependent. Other vertebrate classes where GH exerts control over growth are reptiles (Denver and Licht, 1990) and amphibians (Huang and Brown, 2000).

Contrary to all other vertebrates, exogenous GH administration has no effect on growth and body weight in early post hatch broiler chickens (Burke *et al.* 1987; Cogburn *et al.*, 1989; Cravener *et al.*, 1989; Scanes *et al.* 1990). However, pulsatile infusion of GH after the decrease of endogenous levels did have positive effects on bone growth and other metabolic parameters such as weight gain and feed efficiency (Vasilatos-Younken *et al.*, 1988; Leung *et al.*, 1986). Also, unlike in mammals, GH does not promote gluconeogenesis in avian species, and effects on lipid metabolism can be lipogenic or lipolytic depending on the age and mode of administration of GH. While the pleiotropic nature of GH function is evident from the discussion above, regulation of body growth and development appears to be the major function of GH. GH synthesis and secretion is under the control of several hypothalamic and other endocrine factors, and the actions of GH, as mentioned previously, are mediated by IGFs produced either locally or by the liver (carried by blood to the site of action). Hypothalamic regulators, GH, and IGFs constitute the somatotropic axis essential for post-natal growth. Evidence of the essentiality of the components and targets of the somatotropic axis comes mainly from mutant dwarf phenotypes. Classical pituitary ablation/replacement experiments have demonstrated that growth cessation resulting from hypophysectomy can be reversed by exogenous GH in chickens (Thommes *et al.*, 1992; Scanes *et al.*, 1986). In addition, both transgenic mice (Palmiter *et al.*, 1983) and fish (Houdebine and Chourrout, 1991) over expressing the GH gene show marked increases in growth when compared to control animals. Also, it is well known that in humans, GH hyposecretion leads to dwarfism and

hypersecretion leads to gigantism (before puberty) or acromegaly (in the case of adult onset).

2.13.2.6.2 Regulation of GH synthesis and secretion

GH is synthesized and secreted in a ultradian pulsatile manner (Winer *et al.*, 1990) from somatotrophs, one of the five cell types that make up the anterior pituitary. Somatotroph differentiation requires blood-borne signals from outside the pituitary in all species (Nogami *et al.*, 1995; Porter *et al.*, 1995). Somatotrophs are the third pituitary cell type to arise in most species, and they are one of the most numerous cell types in adults. Growth hormone-releasing hormone (GHRH) and somatostatin (SST) are two hypothalamic factors that regulate GH production, with GHRH stimulating the synthesis and secretion of GH and SST inhibiting it. Both SST and GHRH are essential for maintaining the pulsatality of GH secretion (Cella *et al.*, 1990, Katakami *et al.*, 1988,) even though the exact mechanism behind the pulsatality remains unknown. SST, the inhibitor of GH release, probably negatively regulates GHRH to bring about the pulsatility.

GHRH belongs to the family of brain-gut peptides along with vasoactive intestinal peptide (VIP), glucagon, glucagon-like peptide I (GLPI), pituitary adenylate cyclase-activating peptide (PACAP) etc. (Campbell *et al.*, 1991). Functions of GHRH include stimulation of somatotroph differentiation, GH gene transcription, biosynthesis, and secretion (Mayo *et al.*, 1995). GHRH is a peptide hormone produced in the arcuate nucleus of the hypothalamus (Bloch *et al.*, 1983). The actions of GHRH are mediated through its receptor, GHRH-R, a seven transmembrane G-protein coupled receptor linked to a stimulatory G-protein (Gs) (Gaylinn *et al.*,1993, Lin *et al.*,1992, Narayanon *et al.* 1989, Spada *et al.*,1984), which activates adenylate cyclase (Spada *et al.*,1984), leading to an increase in intracellular cAMP and Ca2+ levels (Holl *et al.*,1988). The activated G-protein interacts with ion channels, leading to secretion of GH, whereas the elevated cAMP levels activate the protein kinase A (PKA) pathway that leads to increased transcription of the GH gene, possibly by phosphorylation of transcription factors and activators involved in the process (Holl *et al.*, 1988). Evidence of the essentiality of GHRH signaling in GH regulation comes from the GHRH-R mutant little mouse (*lit/lit*), with a mutation of a conserved aspartic acid residue (Asp 60Gly) in the N-terminal ligand

binding domain above of GH and SST inhibiting it. Both SST and GHRH are essential for maintaining the pulsatality of GH secretion (Cella *et al.*, 1990, Katakami *et al.*, 1985) even though the exact mechanism behind the pulsatality remains unknown. SST, the inhibitor of GH release, probably negatively regulates GHRH to bring about the pulsatility.

SST, the hypothalamic peptide hormone that inhibits GH synthesis (Brazeau *et al.*, 1973), also acts through G-protein couple of receptors, of which there are six subtypes (Patel, 1997). SST function has been conserved in vertebrates. In almost all species, SST does not decrease basal GH production at either RNA or protein levels; neither does it inhibit somatotroph proliferation, but rather it attenuates GHRH-induced GH expression (Billestrup *et al.*,1986, Fukata *et al.*,1985, Simard *et al.*,1986, Tanner *et al.*,1990). In chickens and teleosts, however, SST can affect both basal and GHRH-induced GH expression, while in reptiles and some amphibians, SST acts on thyrotropin releasing hormone (TRH)-stimulated GH expression (Hall and Chadwick, 1984; Jeandel *et al.*, 1998). SST possibly mediates its effect through decreasing intracellular cAMP levels and by affecting voltage-gated ion channels that lead to an increase in intracellular K+ levels and simultaneous decrease in Ca2+ levels, but the precise mechanism is still unclear (Epelbaum, 1992, Patel *et al.*,1995).

Apart from these hypothalamic peptides, numerous growth hormone-releasing peptides, or GHRPs have been identified and characterized. GHRP-6 is the most potent peptide, being more potent than GHRH (Bowers *et al*., 1993; Bowers *et al.*, 1990). The potency of it has been evaluated in monkeys, sheep, pigs, chickens, steers, rats and humans (Bowers *et al.*, 1984, Doscher *et al.*, 1984, Kraft *et al.*, 1984, Malozowski *et al.*, 1991, Bowers *et al.*, 1990, Ilson *et al.*, 1989). GHRP-6 can increase the level of GH mRNA independent of GHRH, and the two together lead to a synergistic activation of *Gh* mRNA (Cheng *et al.*, 1989, Goth *et al.*, 1992). Even though the precise mechanism of action of GHRPs is not known, it is postulated that GHRP binding to GHRP receptor leads to an increase in intracellular Ca2+ (Akman *et al.*, 1993; Sartor *et al.*, 1985), no change in intracellular cAMP (Cheng *et al.*, 1989, Wu *et al.*, 1994) and a possible activation of the protein kinase C (PKC) pathway (Cheng *et al.*, 1991).

Several other hypothalamic peptides participate in the regulation of GH, some of them having dual functions depending on age, pathological state, site of action, etc. For instance, thyrotropin releasing hormone (TRH) is stimulatory in fetal and neonatal stages before the somatotropic axis is established or in pathologies such as acromegaly (Cocchi *et al.*, 1983; Harvey, 1990), whereas it inhibits GH release by its action on the hypothalamus (Cocchi *et al.*, 1983; Müller, 1987). CRH, corticotrophin releasing hormone, and neuropeptide Y (NPY) both have an inhibitory effect (Katakami, 1985), and both are most likely mediated by SST (Katakami, 1985). PACAP, another brain-gut peptide has a pronounced role in GH release in fish (Montero *et al.*, 2000; Wong *et al.*, 2000) and amphibians (Martinez-Fuentes *et al.*, 1994), somewhat of a lesser effect in birds (Peeters *et al.*, 1996), and possibly no effect in mammals (Miyata *et al.*, 1989; Jarry *et al.*, 1992; Chiodera *et al.*, 1996). Among peripheral gland hormones, glucocorticoid (GC) regulation of GH gene expression has been extensively studied in many vertebrate species. A part of the regulation is mediated through GC action on the hypothalamic regulators, GHRH (Seifert *et al.*, 1984; Michel *et al.*, 1984) and SST (Holl *et al.*, 1988;). However, GCs can also directly stimulate *Gh* gene transcription by acting through a ligand-dependent transcription factor (Thakore and Dinan, 1994). These effects of GCs are true for physiologic doses. Physiological concentrations of thyroid hormones are also necessary for the maintenance of GH gene expression (Giustina and Wehrenberg, 1995). Thyroid hormones act in concert with GHRH by enhancing the effect of GHRH on somatotrophs (Martin *et al.*, 1985; Korythko *et al.*, 1997).

Apart from the major regulators of GH mentioned above, a host of other factors influence GH synthesis and secretion in vertebrates. Ghrelin, a stomach peptide hormone, has been shown to act as a stimulator of GH in all species. Neurotransmitters such as serotonin and norepinephrine, metabolic signals such as glucose and leptin, nonesterified fatty acids, nitric oxide, amino acids such as arginine, ornithine, lysine, and tryptophan, have all been shown to have specific roles in GH synthesis, secretion and feedback mechanisms. It suffices to say that GH regulation is a complex process involving numerous players, which act in concert with tissue specificity to maintain normal GH supply and function in complex physiological systems, many aspects of such regulation being still unknown.

2.13.2.6.3 Transcription factor regulation of GH gene expression

The regulatory regions of the GH gene have been identified and characterized in several vertebrate species. In the species studied so far, PIT1 is undoubtedly the single most important transcription factor regulating GH gene expression in the anterior pituitary somatotrophs. PIT1 is essential for terminal differentiation of cells of the PIT1 lineage (thyrotrophs, somatotrophs and lactotrophs) and for gene expression of the hormones secreted by these cells, β-subunit of thyroid-stimulating hormone (TSHβ), growth hormone (GH) and prolactin (Prl), respectively (Ingraham *et al.* 1988). Evidence of this essential role of PIT1 includes pituitary hypoplasia and lack of detectable levels of the hormones in *Pit1* mutant mice (Li *et al.*, 1990). However, although necessary, PIT1 is not sufficient to regulate somatotroph-specific GH gene expression. PIT1 interacts with other transcription factors, both general and specific, as well as with co-activators to bring about this highly cell-type specific gene expression. Several of the cellular factors that bind to and regulate the GH promoter have been identified. Steroid hormone receptors, such as the glucocorticoid receptor (GR) and more extensively the thyroid hormone receptor (TR), binding to the GH promoter have been studied, and the results have shown that the regulation of GH varies considerably across species. Other transcription factors, such as Ets-1 (Yang *et al.*, 2010), Sp-1 (Melamed *et al.*, 1998), NF-1 (Norquay *et al.*, 2003), Ikaros (Ezzat *et al.*, 2005) and Zn finger 15 (Zn-15) (Lipkin *et al.*, 1993) are involved in tissue-specific transcription of GH.

Promoter analysis of the GH gene across species brings out some common features. General transcription regulatory sequences, such as the TATA box, are present in representative species of all classes (teleosts, amphibians, birds, primate and non-primate mammals). In general, the fish species show maximum inter-species variation. Similarly, PIT1 and at least one AP site(s) are present in all species examined so far. The mammalian and chicken promoters have some similarities in terms of the binding sites present, marked by the presence of Sp1 and AP-2 binding sites, cAMP- and vitamin D response elements, and thyroid hormone response elements; however, the copy numbers and relative locations of these sites vary widely (Chuzhanova *et al.*, 2000).

2.13.2.6.4 Somatotroph differentiation and GH gene expression in the chicken

Somatotroph differentiation takes place during the latter half of embryonic/fetal development in chickens and rats. In chickens, growth hormone-secreting cells are first detected on embryonic day 12 (e12) in the caudal portion of the pituitary gland, and they become a significant population around e16 (Porter *et al.*, 1995). Concomitant with this increase in abundance, GH mRNA and protein levels increase and serum GH levels become detectable (Harvey *et al.*, 1979). Somatotroph differentiation is a highly regulated developmental process requiring the participation of signals emanating both from within and outside the pituitary gland. A blood-borne signal is required for somatotroph differentiation. Anterior pituitary cells isolated from e12 to e16 chickens (prior to somatotroph differentiation) fail to differentiate into functional somatotrophs in culture in the absence of serum. Serum from e16 has the highest potency to induce somatotroph differentiation in cultured cells (Porter *et al.*, 1995). Heat-inactivated, but not ether-extracted serum maintains the ability to induce differentiation, suggesting the blood-borne factor is a steroid. This signal was later found to be the adrenal glucocorticoid, corticosterone (CORT), the level of which is known to increase dramatically with the onset of somatotroph differentiation (Morpurgo *et al.*, 1997; Jenkins and Porter, 2004). In both rats and chicks, endogenous thyroid hormone, especially triiodothyronine (T3), is necessary for somatotroph differentiation. However, unlike CORT, thyroid hormone is ineffective in increasing somatotroph abundance by itself and requires the presence of CORT (Liu *et al.*, 2003; Nogami *et al.* 1997). Also, the effect of T3 is age-dependent, as at e20 T3 actually inhibits GH secretion (Liu and Cordes, 2004; Jenkins *et al.*, 2007).

2.13.2.7 Pituitary-specific transcription factor1 (Pit1)

Pituitary-specific transcription factor 1 (Pit1) is a member of POU homeodomain (POU-HD) family or transcription factors (Bastos *et al.*, 2006a; Ingraham *et al.*, 1988). The N terminus of Pit1 is required for transcription, whereas the PUO domain that characterizes this family is involved in DNA binding and is located toward the C terminus and consists of POU-HD and POU-specific domain (Theil *et al.*, 1989; Ingraham *et al.*, 1994). PIT1 gene structure has been conserved across vertebrates.

Mammalian PIT1 genes are comprised of six exons and five introns (Theil *et al.,* 1989; Theil *et al.,* 1992), and /teleost PIT1 genes have seven introns due to the presence of a unique 38-amino acid exon (designated 2 for avian species) located between exon 2 and exon 3 (Van As *et al.,* 2000). Pit1 is essential for differentiation of cells of the Pit1 lineage (Li *et al.,* 1990; Simmons *et al.,* 1990) and for gene expression of the hormone secreted by these cells, GH, prolactin (Prl) and TSHβ (Li *et al.,* 1990; Lefevre *et al.,* 1987; Nelson *et al.,* 1988). Pit 1 also autoregulates its own expression (Chen *et al.,* 1990; Rhodes et al., 1993). Variant forms of Pit1 have been identified in several species that arise by alternative transcription, translation and/or splicing. The first report was of a variant form in rat, where the shorter isoform arose by translation initiation at a down stram inframe AUG codon resulting in loss of the first 27-amino acid residues present in the larger 33- kDa variant. This PIT1 variant did not differ in its ability to activate the PRL promoter (Voss *et al.,* 1991). A second alternative in mammals results from alternative splicing and possesses a short exon encoding for 26 amino acids inserted after exon 1 in the transactivation domain (Morris *et al.,* 1992). This isoform (Pit1β) was unable to activate the rat PRL promoter in CHO cells. This isoform unable to activate PIT 1 and PRL promoter activity, but it retains its ability to activate GH promoter activity (Theil *et al.,* 1992). Later work (Konzak and Moore, 1992) showed this isoform to be a dominant negative repressor of PRL gene expression in pituitary cells. However, Pit 1 β is a repressor of Pit 1 function only in pituitary cell; it potentiates activation of the rat PRL promoter (Diamond *et al*., 1999). Among avian species, three turkeys Pit 1 variants were reported (Kurima *et al*., 1998). Turkey Pit 1β (tPit 1β*) arose by alternative splicing and included a specific exon of 28 amino acids (compared with 26 amino acids in mammals). The identification of tPit1 W* was the first report of a unique avian specific isoform arising by use of a transcription start site (TSS) in intron 1 followed by inclusion of the β-specific exon. The presence of two Pit 1 variants in chicken was first reported by Tanaka *et al*., (Tanaka *et al*., 1999). Later, Van As *et al., (*Van As *et al.,* 2000) reported the presence of three isoforms, Gallus gallus PIT 1 (ggPit 1*), ggPit 1β, and ggPit 1W*. They also adopted the nomenclature Pit 1α for the canonical form, Pit 1β for the derivative of Pit 1 with the β-specific exon, and Pit 1γ for the equivalent of Pit W* (Kurima *et al.,*1998). Even though multiple isoforms are found in many species, the

functions of having multiple isoforms are not clear. Research indicates that the specific amino acid sequence of the β-specific domain mediates the dominant negative repression of PRL gene expression in pituitary cells (Diamond and Gutierrez-Hartmann, 1996). This same sequence makes Pit 1β a more potent protein kinase A-mediated activator of PRL gene expression in non pituitary cells (Diamond *et al*., 1999). Until_now, PIT 1 cDNA has been identified in a variety of species, and previous studies showed that the PIT 1 gene comprised 6 exons in mammals and 7 exons in birds and fishes, seen as differences in precursor length (Tatsumi *et al.,* 1992; Wang *et al*., 1992; Yamada *et al*., 1993). The chicken PIT1cDNA has firstly been isolated and sequenced by Tanaka *et al.,* (1999), and its three isoforms of PIT1*, PIT1β* and PIT1α induced by alternative splicing have also been isolated and found to comprise 335, 363 and 327 amino acids respectively (Van As *et al*., 2000). The alternative splicing of PIT1 gene has also been reported in other species (Morris *et al.,* 1992; Konzak and Moore, 1992). According to the chicken genome sequences released in May of 2006 (MGC/ORFeome Chicken Genome Browser Gateway), the chicken PIT1 gene is located at chromosomes (GGA1) and spans over 14kb in length.

Due to its crucial regulatory function and a variety of bioactivities, PIT1has been regarded as a key candidate gene for production performance. There are indications that variations of PIT1 gene related to growth, carcass traits in cattle (Zhao *et al.,* 2004; Xue *et al.,* 2006). In chickens although a total of 23 single nucleotide polymorphism (SNP) and a 57 bp indel have been lately identified in 2400 bp discrete region of PIT1 gene, their genetic effects on chicken production traits remain unclear (Nie *et al*., 2005). Recently, it has been shown that a non-synonymous SNP at POU domain (A---->T, Asn 229Ile) is significantly associated with body weight at 8 weeks.

2.13.2.7.1 Regulation of target genes by PIT1

The well-studied targets of PIT1 include GH, *Prl*, *TSHβ* and GHRH-*R*. These target genes contain one or more PIT1 binding sites in their promoter regions. The sequence of the PIT1 binding sites has been determined in mammals [(A/T) (A/T) (A/T)TATNCAT] (Nelson *et al.,* 1988) and in avian/teleost species [(A/T)NCTNCAT] (Ohkubo *et al.,* 1996). However, given the redundancy of the PIT1 binding site, it is very

likely that several other pituitary genes are regulated by PIT1 at the transcriptional level. Indeed, results from our lab have shown that Ras-DVA is a novel PIT1 regulated gene (Ellestad, 2010). In addition, PIT1 auto regulates itself (Chen *et al.*, 1990; Rhodes *et al.*, 1993).

Multiple lines of evidence suggest a role of PIT1 in pituitary development and regulation of hormones secreted by cells of the PIT1 lineage. *Pit1* mRNA and protein are expressed in the anterior pituitary before the differentiation of these cell types (Dolle *et al*., 1990; Simmons *et al.*, 1990). Mutations in the *Pit1* gene lead to severe pituitary hypoplasia, absence of the hormone producing cells, and complete lack of hormone gene transcription (Wilson and Wyatt, 1986; Radovick *et al*., 1992). Mice carrying mutations in the *Pit1* gene show dwarfism and severely reduced somatotrophs, lactotrophs and thyrotrophs. The Jackson dwarf mice are characterized by a gross alteration of the *Pit1* gene resulting from an insertion or inversion of a >4kb piece of DNA. The Snell mutant 28 has a point mutation in the POUHD resulting in a substitution of the tryptophan residue at position 261 with cysteine (Li *et al.*, 1990). Mutations in Prop-1, a paired-like homeodomain protein required for *Pit1* gene activation and regulation also lead to dwarfism and absence of the cells of the PIT1 lineage (Sornson *et al.*, 1996). In all the mutants, levels of PIT1 are nearly undetectable (Li *et al.*, 1990; Sornson *et al.*, 1996). Knocking down Pit-1 expression by antisense oligonucleotides blocks GH and Prl transcription along with inhibition of proliferation of somatotroph and lactotroph cell lines (Castrillo *et al.*, 1991). In teleosts, zebrafish *Pit1* mutant lacking 55 amino acid residues due to an internal truncation resulting from alternative splice site-usage shows severe dwarfism and lack of the three pituitary cell types (Nica *et al.*, 2004).

The regulation of cell-type specific expression of target genes by PIT1 has always been a conundrum. PIT1 is expressed in 3 cell types of the anterior pituitary; however, even within the cells of PIT1 lineage, there is specificity of hormone production. This cell type specificity is not just because the chromatin conformation is not conducive to transcription, because both in corticotrophs and in non-pituitary cell lines (Ingraham et al, 1988), where, for instance, the GH promoter should be in closed conformation, transfected *Pit1* brings about expression of GH. However, in lactotrophs, *Gh* gene transcription does not take place even in the presence of PIT1. In the lactotroph-derived

cell line 235-1 (Nelson *et al.*, 1988), transfected GH promoter is not activated by PIT1 (Ingraham *et al.*, 1994), suggesting the presence of factors that either occupy the PIT1 binding site itself or the immediate vicinity, and/or modifies PIT1 in such a way that it is unable to bind GH promoter PIT1 binding sites and/or recruit other essential transcription 29 factors, thus providing a negative regulation. An alternative mode of this regulation could be that factors otherwise ubiquitous are specifically absent from lactotrophs (Crenshaw *et al*., 1989).

Regulation of GHRHR by PIT1 has been studied in mammals, but not in any other vertebrate class. The human and rat GHRHR gene promoters lack a TATA box, and have multiple PIT1 binding sites. Other transcription factor binding sites common between the two species are for estrogen receptor (ER) and CREB (Peterson *et al.*, 1998; Miller *et al.*, 1998; Iguchi *et al.*, 1999; McElvaine *et al.*, 2007). Out of the multiple PIT1 binding sites present, usually a few are functional and even fewer contribute significantly to regulation of transcription. The purpose of having multiple sites is not known. In humans, GHRHR gene transcription is regulated by GCs, even though the 5'flanking region lacks a classical GRE. In rat, two GREs along with a PIT1 binding site and an internal silencer region make up the glucocorticoid response unit. It is hypothesized that PIT1 binding to its element leads to release of an unknown factor from the silencer, allowing GR to bind to the GRE (Nogami *et al.*, 2005).

TSHβ gene expression is regulated positively by TRH (Steinfelder *et al.*, 1992) phorbol esters (Haugen *et al.*, 1993) and forskolin (Kim *et al.*, 1993), and negatively by thyroid hormones and estrogen (Steinfelder *et al.*, 1992, Nagayama *et al.*, 2008). The 5'flanking region of this PIT1 regulated gene has been characterized in mammalian species (human, mouse, rat) (Steinfelder *et al.*, 1992; Haugen *et al.*, 1993 and goldfish (Sohn *et al.*, 1999), but information from other groups of vertebrates is lacking.

In humans, 128bp of the 5'-flanking region is sufficient for the TRH response. This region has multiple functional PIT1 binding sites (Steinfelder *et al.*, 1992). TRH induction leads to increased cAMP, but the effects of cAMP are likely mediated through PIT1, as there is no cAMP response element (CRE) in this region. Also, the effect can be mimicked in non-pituitary cell lines only in the presence of transfected PIT1 (Steinfelder *et al.*, 1992). In all species studied, PIT1 is necessary but not sufficient to facilitate

thyrotroph-specific gene expression of TSHβ. While PIT1 binding to DNA is critical, there was evidence of the involvement of another factor, later identified to be GATA2 (Gordon *et al.*, 1997). Both PIT1 and GATA2 bind to the TSHβ promoter. In mouse, 5'-PIT1 and 3'GATA2 binding sites along with 16bp of spacer make up a composite unit. The spacer contains additional putative overlapping PIT1 and GATA2 binding sites, and the sequence of the spacer is functionally important. When only the spacer is mutated with the flanking PIT1 and GATA2 binding sites left intact, there is markedly reduced binding of both transcription factors and almost no formation of the ternary complex (Gordon *et al.*, 2002). It is hypothesized that binding of one factor causes the DNA to bend and attain a conformation that permits and facilitates the binding of the other factor. Also, PIT1 and GATA2 physically interact with each other; the POU homeodomain of PIT1 interacts with the zinc finger domain of GATA2 (Gordon *et al.*, 2002). PIT1 also interacts with CREB binding protein (CBP) on the TSHβ promoter, but the functional implication of such interaction is not clear (Hashimoto *et al.*, 2000).

Factors that stimulate Prl include TRH, oxytocin, VIP, epidermal growth factor (EGF), estradiol and phorbol esters (Day and Maurer, 1989). Several of these pathways lead to increase cellular cAMP levels in both mammals and birds, and the cAMP effect is thought to be mediated by PIT1 if no CRE are found in the minimal promoter sufficient for activation of the Prl promoter in response to the stimulus (Peers *et al.*, 1991). Also, PIT1 may or may not need to be phosphorylated to bring about the effect, suggesting the involvement of other factor(s) that interact with PIT1 in mediating the effect. The interacting factor(s) of PIT1 in regulating the *Prl* gene are several, including, but not limited to, Ets-1, GR, and Oct-1. A composite PIT1/Ets-1 binding site in the rat Prl promoter is required for mediating the induction of Prl by several stimulatory factors (Howard and Maurer, 1995; Dradford *et al.*, 1997). PIT1 physically interacts with GR to inhibit human Prl transcription (Nalda *et al.*, 1997), while PIT1 heterodimerizes with Oct-1to produce synergistic activation of the rat Prl promoter (Voss *et al.*, 1991; Verrijzer *et al.*, 1992).

PIT1 is the major regulator of GH gene expression by pituitary somatotrophs, but other nuclear factors are essential for the highly cell-type specific expression of GH. The Zn finger transcription factor Zn-15, binds to so-called - boxes in the rat GH promoter

and in other species it regulates GH transcription, and a mutation in the Zn-15 binding site leads to a notable reduction of GH synthesis (Lipkin *et al.*, 1993). GC induction of GH is a well-studied phenomenon, and in most species the effect is mediated through GR binding to GREs in the GH promoter. In species where a canonical GRE is absent, GR is thought to mediate its effect by GR tethering to other protein(s) already present on the *Gh* gene promoter. GHRH induction of GH is known to increase intracellular cAMP levels and activation of the PKA pathway. The exact target of PKA is not known, nor has the mechanism leading to increased GH been elucidated. PIT1 is thought to be involved in the cAMP-induced response, whereby PKA phosphorylates PIT1, leading to an altered conformation and increased binding to the GH promoter. However, evidence available showing the requirement for PIT1 phosphorylation has been conflicting. There are two hypotheses: in one scenario, increase cAMP leads to CREB phosphorylation and activation, CREB binding to CREs present in the promoter and activating transcription. In the absence of functional CREs, however, it is hypothesized that the mechanism is CREB-independent, and according to the alternative hypothesis, activated PKA instead leads to phosphorylation of CREB-binding protein (CBP), which can then act as a co-factor of PIT1 (Cohen *et al.*, 1999). PIT1 dependent negative regulation of GH is mediated by activin by reducing PIT1 binding to GH promoter (Struthers *et al.*, 1992).

2.13.2.7.2 Pit1 and production traits

The PIT1 regulated genes, GH, *Prl* and *TSHβ,* are all involved in metabolic functions such as energy balance and homeostasis, it was tempting to predict a role of PIT1 in production traits. Indeed, several studies have been done in agricultural animals to identify polymorphisms associated with agricultural performances. Single nucleotide polymorphisms of *Pit1* have been identified as genetic markers for reproductive (fertilization) rate in cattle (Khalib *et al.*, 2009). Agricultural traits such as body weight, wither height (Zhang *et al.*, 2009), milk yield and duration of reproductive life (Huang *et al.*, 2009) are a few others that are influenced by PIT1. Similarly, in pigs, birth weight (Song *et al.*, 2007), fat thickness (Franco *et al.*, 2005), growth, meat quality and carcass composition (Brunsch *et al.*, 2002) are correlated with *Pit1* polymorphisms which can be either in the introns or exons. Cashmere wool production in goats is determined by a polymorphism located at the 3'-UTR of *Pit1* (Lar *et al.*, 2009). In chickens, growth traits

such as growth rate, but not carcass composition or fat content are associated with *Pit1* polymorphisms (Jiang *et al.*, 2004; Nie *et al.*, 2005).

2.13.2.8 Prolactin receptor (PRLR)

Prolactin receptor *(PRLR)* is a single transmembrane protein that belongs to class 1 of cytokine receptor super family, which includes growth hormone receptor, interleukins, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, leukemia inhibitory factor, oncostatin M, erythropoietin, thrombopoietin, gp130, ciliary neurotrophic factor, and leptin receptors. By signaling through homodimers of PRLR, prolactin plays a wide variety of physiological roles in vertebrates (reviews by Bole-Feysot *et al.,* 1998; Freeman *et al*., 2000; and Clevenger and Kline 2001). So far, the *PRLR* gene has been cloned in the human (Boutin *et al.*, 1989), mouse (Clarke and Linzer, 1993), rat (Shirota *et al*., 1990), rabbit (Edery *et al.*, 1989), pig (Tomas *et al.,* 2006), cow (Scott *et al*., 1992), frog (Hasunuma *et al*., 2004), chicken (Tanaka *et al*., 1992), duck (Wang *et al.,* 2009), turkey (Zhou *et al.,* 1996), pigeon (Chen and Horseman, 1994), leopard gecko (Kato *et al.,* 2005).

In avian species, a single form of PRLR containing a cytoplasmic domain comparable to that of the longest isoform of mammalian PRLR has been identified by cDNA cloning (Yamamoto *et al*., 2003). The pituitary hormone of PRL is a ligand of PRLR. Chicken PRLR gene was mapped on to the chicken chromosome Zp23-22 (Cheng *et al*., 1995) and disperses over 34 kb on chromosome Z and consists of 5 exons and 14 introns (Leung and Wang, 2005). The membrane-bound PRLR is closely related to growth hormones receptor and is a member of the cytokine receptor family (Bole-Feysot *et al.,* 1998). Consistent with its diverse effects, the PRLR is expressed ubiquitously in chickens and turkeys (Leclerc *et al.,* 2007a,b) and levels of receptor in target tissues are up and down-regulated in response to changes in the concentration of circulating PRL. At around the time of hatch, the secretion of PRL significantly increases in chickens and turkeys in concert with increase in PRLR. Presumably, the increase in circulating PRL is associated with adaptation of the embryo to ex ovo life although in what capacity is not clear (Hiyama *et al.,* 2009). Prolactin and its receptor are involved in the growth and development, control of water and electrolyte balance, reproduction, endocrine signaling

and metabolism. Due to different biological activities attributed to PRL and PRLR, they can be used as major candidate genes in molecular animal breeding programmes.

Prolactin exerts its effects by binding to its membrane-bound cell surface receptor (prolactin receptor, PRLR), which is a member of the cytokine receptor gene super family. These receptors share conserved functional domains, including the extracellular domain (ECD), the transmembrane domain (TM), and the intracellular domain (ICD). The PRLR cDNA have been cloned from several mammalian species (Boutin *et al.*, 1988, 1989; Davis and Linzer, 1989; Edery *et al.,* 1989; Scott *et al*., 1992; Jabbour *et al*., 1996; Bignon *et al.,* 1997; Demmer, 1999; Dalrymple *et al*., 2000; Tomas *et al*., 2006). Two major forms of PRLR that differ in the length of the ICD have been identified in rat, mouse, sheep, cattle, and human (Boutin *et al.,* 1989; Davis and Linzer, 1989; Scott *et al.,* 1992; Moore and Oka, 1993; Bignon *et al.,* 1997; Hu *et al.,* 2001). These 2 forms of PRLR originate from alterative splicing of the mRNA transcribed from a single gene (Ormandy *et al.,* 1998). In avian species such as the chicken, pigeon, and turkey, the PRLR protein contains a cytoplasmic domain comparable to that of mammalian long-form PRLR (Tanaka *et al.,* 1992; Chen and Horseman, 1994; Zhou *et al*., 1996). The avian PRLR are structurally different from mammalian PRLR due to the presence of 2 conserved ligand-binding regions in the ICD. Each repeat contains the essential regions for hormone binding, including 2 pairs of cysteine residues and the WS motif. Prolactin receptors have also been cloned in amphibians (Yamamoto *et al*., 1998; Huang and Brown, 2000) and several teleosts (Sandra *et al*., 1995; Tse *et al*., 2000; Higashimoto *et al*., 2001; Le Rouzic *et al*., 2001; Lee *et al.,* 2006); in these species, only a long-form PRLR was identified that shares the greatest similarity with mammalian long-form PRLR. Until now, the information on gene structure and distribution of PRLR was not reported in ducks. The PRLR in the duck exhibits all of the characteristic features of long forms of PRLR. In common with other species, dPRLR contains a single TM and an ECD and ICD. The ECD comprises 416 amino acids and has 2 repeated units. This is similar to what is observed in the chicken (Tanaka *et al.,* 1992), turkey (Zhou *et al.,* 1996), and pigeon (Chen and Horseman *et al.,* 1994), where each unit of the ECD contains 2 pairs of cysteines and a WSXWS motif, which are conserved in all PRLR sequences characterized to date. In mammals, these 2 pairs of cysteines are believed to

form disulfide linkages to stabilize the ligand-binding domain of the receptor. Mutational studies have demonstrated that these linkages are important for receptor structure and function (Rozakis-Adcock and Kelly, 1991, 1992). In the WS motif of the ECD, the common amino acid sequence is WSXWS. The first 2 amino acids (WS) and the fourth amino acid (W) are conserved in all other PRLR. The third amino acid is more variable among various vertebrates. However, the fifth amino acid of the WS motif in duck is S, which is identical to avian PRLR as well as most other mammalian and teleostean PRLR. The WS motif can be found in all of the members of the class I super family of cytokine receptor with the exception of the growth hormone receptor. The high degree of conservation of the WS motif suggests that it has an important role in receptor function. Mutations in this region are detrimental to receptor affinity. It is generally accepted that tree branches that have more than 70% bootstraps support are likely to be correct with a confidence level of 95% (Rehm, 2001). All tree branches except 2 branches had more than 80% bootstraps, which ensured the accuracy of phylogenetic analysis. In big contrast, the phylogenetic tree of PRLR could be divided into 4 groups: teleost PRLR, amphibian PRLR, avian PRLR, and mammalian PRLR, and there existed much greater homologies among species within each group. These results were supported by Higashimoto *et al.* (2001) and Tse *et al*. (2000). Higashimoto *et al.* (2001) used 17 sequences of PRLR for a phylogenetic analysis, and these 17 sequences could also be divided into 4 groups. The tissue distribution of the PRLR was determined in adult duck by RT-PCR. This shows that PRLR mRNA could be expressed in liver, muscle, kidney, testis, and ovary. The greatest abundance of PRLR mRNA was found in kidney among all tissues detected, and Zhou *et al.* (1996) observed similar phenomena in adult turkey. However, Mao *et al.*, (1999) did not detect PRLR expression in the muscle and liver of chicks using the RNase protection assay method. In addition, the PRLR mRNA was more expressed in testis than in ovary and it may be due to truncated PRLR in testis but not in ovary, which was confirmed by Mao *et al*. (1999) and Tanaka *et al*. (2000) in chicks.

The prolactin receptor (PRLR) has an important role in the PRL signal transduction cascade, which is triggered at the onset of broodiness, and PRL exerts its biological functions by acting through the PRLR (Bole-Feysot *et al.*, 1998). Because the *PRLR* gene is on the Z chromosome (Dunn *et al*., 1998), the hypothesis of sex-linked

inheritance of broodiness may relate to *PRLR* gene via its role in PRL signal transduction.

Although the chicken *PRL* and *PRLR* have been cloned and mapped (Dunn *et al.*, 1998; Ohkubo *et al*., 2000), information on their effects on broody behavior is lacking.

CHAPTER THREE

MATERIALS AND METHODS

3. 1. Phenotypic studies

3.1. 1. Area of research

The experiment was conducted on the farm of Animal production Department (Shambat) Khartoum North, College of Agricultural Studies, Sudan University of Science and Technology. Whereas the trial started on the 25th of September 2011 and ended on the 15th January 2012 which lasted for 16 weeks.

3.1.2. Parent stock

The indigenous bare neck chickens were purchased as parent stock from two different geographical locations Butana area of Gedarif States, Northern Sudan and northern area of Upper Nile State (Maban, Renk, Kaka and Wadakona), Southern Sudan. The stocks purchased were 90 birds. After collection of these birds they were transported to Khartoum and kept out side the department farm for prephylactic treatment. On their arrival They were kept in a pen and immediately treated against external and internal parasites and given multivitamins and vaccinated against Newcastle disease. They were kept in that location for an adaptation period of two weeks. After that when the birds were observed to be healthy they were then shifted to an open house system on the department farm. These birds were then divided into four pens. Each group purchased from one location were divided into two sub-groups and assigned to two separate pens. Then from this stock seventy chickens were collected 10 males and 60 females and were assigned another separate room, and from these stocks the experimental eggs were collected.

3.1.3. Fertility and hatchability

The stored eggs were sorted out by separating dirty, cracked, large and small sizes and miss – shaped eggs were considered bad eggs and therefore were rejected. Then the

eggs were set in plastic trays with large end up and placed in an incubator (Funki model) with a capacity of 1500 eggs which belong to the Department of Animal Production. The temperature through out incubation during the period 1- 18 days was 37.5^{o} C to 38.5^{o} C and humidity of 60%, turning of the eggs was every an hour for each direction until it reached an angle of 45^{o} and turned to the other side. Moisture trays were put at the bottom of the hatchery for humidity balance inside incubator. At the 7th day of incubation eggs were candled for fertility after that the trays were returned in incubator and it continued until the 18th day of incubation was reached. At the 18th day of incubation the trays with fertile eggs were shifted to the hatchery below the incubation unit in the incubator. The eggs stayed for 3 days in the hatchery unit and on the 21 st day of incubation the trays were taken out and the hatched chicks were left in the hatchery for 3 - 4 hours to dry up.

3.1.4. Experimental stock

The chicks were graded and those with deformities were culled. Healthy one hundred chicks of mixed sex were leg banded and were individually weighed and recorded. Thereafter the chicks were transferred to the brooding housed.

3.1.5. Brooding and rearing of the chicks

In the brooding house the chicks were divided into four groups and each group was assigned to a separate pen provided with feeding trays and drinkers, the chicks were fed starter diet. During the brooding period chicks were vaccinated against Gamboro and Newcastle diseases at the second and the third week respectively, the chicks remained in the brooding room for 4 weeks after which they were transferred to an open rearing house with dimensions of 6,20×5,80×3 m made of local materials, the floor was concrete open at the north and south directions and supported with iron bars and covered with wire netting. The east and west directions of the house was closed with bricks plastered with cement to prevent morning and evening direct sun rays and the roof was made of plastic sheets covered with thatch and bamboos on the top. The room in which the birds were housed was fitted with four pens with dimensions of 2×4×3 m. The room

was then burned and disinfected together with feeders and drinkers before the birds were introduced. The pens were prepared for receiving the chicks and covered with saw dust.

3.1.6 Traits meaured

3.1.6.1 Age at first egg

After the end of the first phase of the trial the female birds were kept for the determination of age at first egg. In the early morning the pullets which showed signs of laying were isolated from the rest of the flock and the laid egg was collected and weighed as well as the weight of the bird and the date of lay. Then in the evening the bird was returned to the pen and the same process was done to any bird in the position of lay.

3.1.6.2 Egg quality and characteristics

Eggs collected from experimental stocks were used for determination of egg quality and characteristics. Eggs were collected and weighed and the parameters measured were egg weight, length, width (diameter), albumin height and weight, yolk height, width and weight, weight of shell thickness (middle, narrow and broad end) and yolk colour, these parameters were measured by using digital caliper, electronic sensitive balance for measurement of egg weight and for yolk colour, yolk colour fan was used. Each of the eggs was carefully opened the sharp end, the opened hole was large enough to allow the albumen and yolk to pass through it without being damaged or mixed with one another. The contents (albumen and yolk) were gently poured into a Petri dish, whereby the length of albumin and length of yolk and width were measured. Shell thickness was measured at narrow and broad ends as well as the middle of shell. The color of the yolk was matched with the color of yolk color fan. Haugh Unit Score was calculated using the egg weight and albumen height (Haugh, 1937) as follows:

$$Hu = 100 \log (H + 7.6 – 1.7W)^{0.37}$$

Where:

Hu = Haugh unit, H = observed height of albumin in millimeter, W = weight of egg in grammes.

3.1.6.3 Feed intake and feed consumption

The chicks were provided with water and feed ad libtum to the end of the trial 16 weeks. The feed provided was weighed and checked every day to remove birds' droppings and any other dirt. The feed given was starter and finisher diets with the ingradients composition as calculated in (Table 3.1 and 3. 2).

3.1.6.4 Body weight

Body weight of each individual chick was weighed starting from day old and thereafter every four weeks (week 4, 8, 12 and 16 the end of the experiment).

3.1.6.5 Carcass processing and measurements

At 8 weeks of age selection of individual birds for slaughtering was done. After chicks were individually weighed and their weights recorded in preparation for carcass processing and measurements. From each pen four chicks were randomly selected and slaughtered manually. The neck was cut by severing the right and left carotid and jugular vessels, trachea and esophagus. After bleeding the carcass was weighed to determine the blood weight and the difference between the live weight and dressed carcass was the weight of blood. After bleeding the carcasses were scalded in hot water for a few minutes. This was done for easy removal of feathers which were removed by hand plucking and then the carcasses were washed and any remaining feathers on the carcasses were removed.

The head was removed close to the skull and the feet and shank were cut at the hock joint (head, shank weight and length were measured). Eviscerations were done by posterior ventral cut and complete removal of visceral contents. These contents were then separated and weighed and recorded (gizzard, liver, heart intestines and abdomenal fat). The next day the cold carcasses were weighed and divided into two parts the right and left sides by mid- sawing along the vertebral Column. Then the left side was portioned into wing, thigh, drumstick, breast and back and each cut was weighed and recorded. These cuts were farther deboned and the meat of each part was weighed and recorded and the keel bone was measured. These cuts were then used for chemical analysis.

3.1.6.6 Chemical analysis

The meat which was stored in a deep freezer was thawed and thoroughly mixed for chemical analysis. The moisture, ash, crude protein and crude fat contents of samples were determined according to analytical method of AOAC (1990). Moisture percentage was determined by placing 2g of sample in a clean dried dish. The sample and the dish were weighed and recorded. The dish was then transferred into an air- oven for 24 hours at 100^{o} C. The dish was then removed to cool, after cooling it was weighed. The moisture percentage was determined by the difference between the sample weights before drying and after drying divided by sample weight before drying multiplied by 100. The ash percentage was determined by putting 2g of sample in a clean dry crucible and then placed in a muffle furnace at 550^{o} C for 3 ½ hours and then taken out and allowed to cool and weighed. Ash percentage was determined by calculating weight of ash divided by weight of sample multiplied by 100. Protein percentage was determined by Kjeldahl Method by multiplying Nitrogen% x 6.25. Crude fat percentage was determined by extracting fat from a meat sample for 5 hours using petroleum extract and after the ether extract evaporated in extraction flask, the flask was dried at 100^{0} C for ½ an hour and allowed to cool and then weighed. Crude fat was determined by calculating total fat weight divided by sample weight multiplied by 100.

3.1.6.7 Sensory evaluation (Taste panel)

The remaining right portion of the carcass was used for the taste panel. It was cut into thigh, drumstick and breast and each cut was wrapped up in aluminum foil and roasted in a gas oven at 190^{0} C for 70 minutes. Then the roasted parts were prepared and served. Twenty one semi-trained taste panelists were asked to score colour, flavor, tenderness and juiciness on a scale of 1-8 (Appendix XI). Samples were served randomly to each panelist and water was available for use between samples.

Table 3. 1. Broiler diet formulation

Ingredient % lage (100%)	Starter	Finisher
Dura (Sorghum)	64.29	67.20
Lysine	0.24	0.30
Ground nut cake	12.00	13.00
Sesame cake	17.00	12.00
Methionine	0.11	0.16
Oyster shell	0.49	0.49
Salt	0.25	0.25
Dicalcium phosphate	0.62	0.60
Vegetable oil	-	1.00
Concentrates*	5.00	5.00
Total	100.00	100.00

Concentrates* composition

Crude protein min% 40.00, crude fat min% 03.90, crude fiber max% 1.44, calcium min%10.00, available phosphorus min%6.40, Energy kcal/kg 1950, Methionine min 3.0. Methio + Cysteine min 3.3, Lysine % 10-12, crude mineral%39.30, Sodium min 2.77 Linoleic Acid% 0.24, NaCl(salt)% 6.00,

Vitamins: Vitamin A IU/KG 200 000, Vitamin D3 iu/kg 70 000, Vitamin E mg/kg 400, Vitamin K3 mg/Kg 30, Vitamin B1 Mg/Kg 50, Vitamin B2 mg/kg 120, VitaminB6 mg/kg 50, Vitamin B12 Mcg/kg 180, D antothenic Acid mg/kg 155, Niacine mg/kg 440, FolicAcid mg/kg 8, Choline Chloride mg/kg 5800, Antioxydant(BHT) mg/1000.**Trace minerals:** Manganese mg/kg 1600, Zinc mg/kg 1600, Iron mg/kg 580, Copper mg/kg 450, Iodine mg/kg 55, Selenium mg/kg 8, Cobalt mg/ kg 9, Molybden mg/kg 20.

Table 3.2. Analysis of diet composition

Diet/ %	ME/CAL/KG	CP	Lysine	Methionine	Ca	P	C.fiber
Starter diet	3100	22	1.50	0.60	1.18	0.77	4.01
Finisher diet	3200	21	1.41	0.59	1.08	0.73	2.88

3.1.7 Statistical analysis

The data on phenotypic studies were subjected to the following statistical analysis:

Data on body weight were subjected to descriptive analysis (Excel 2007, SPSS Version 10) and analysis of Variance (Sigma Stat 2.0).

Data on age at first egg, egg quality and characteristic and measurement of carcass cuts (inedible carcass (blood and feather) and edible carcass cuts giblets (gizzard, heart and liver), commercial cuts and (breast, thigh and drumstick) were subjected to descriptive analysis, Correlations and trend (quot.) (SPSS Version 10 + Excel). Chemical analysis was subjected to descriptive analysis and data on sensory evaluation was subjected to Hedonic Scale (Excel).

Mdecular studies:

3.2 Molecular Studies

3.2.1 Chickens

A total of 281 blood samples were collected from six Sudanese indigenous chicken populations including four populations from two breeds namely bare neck (Naked neck) and Large Bladi. Bare neck (BN1, n= 37 from Northern Upper Nile Area); (BN2, n= 38 from Butana Area); Large Baladi (LB1, n= 61 from Butana Area); (LB2, n= 61 from Abu Naama Area), Frizzle (FZ, n= 60 from Lagawa Area) and Betwil (BT, n= 24 from Nuba Mountains Area). These chickens were collected at random and represent mixed sex and different ages.

3.2.2 Blood sample collection

Blood samples were collected by wing vein puncture in a tube containing EDTA as anticoagulant and then blood samples were transferred into an ice box and transported to National Central Laboratory of the Ministry of Science and Technology for DNA extraction.

3.2.3 DNA extraction

DNA extraction was performed by using AccuPrep® Genomic DNA Extraction kits supplied by Bioneer Corporation, Republic of Korea. The extraction was done according to the instructions of the manufacturer as follow:

1. Add 20 µl of Proteinase K to a clean 1.5 ml tube.
2. Apply 200 µl of whole blood to the tube containing proteinase K. If the sample volume is less than 200 µl, make the total volume 200 µl by adding PBS.
3. Add 200 µl of Binding buffer (GC) to the sample and mix immediately by vortex mixer. Completely resuspend the sample to achieve maximum lysis efficiency.
4. Incubate at 60℃ for 10 min.
5. Add 100 µl of Isopropanol and mix well by pipetting. After this step, briefly spin down to get the drops clinging under the lid.
6. Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a 2 ml tube) without wetting the rim.

7. Close the tube and centrifuge at 8,000 rpm for 1 min. If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (>10,000 rpm) until the binding column tube is empty.
8. Open the tube and transfer the Binding column tube to a new 2 ml tube for filtration.
9. Add 500 µl of Washing buffer 1 (W1) without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.
10. Open the tube and pour the solution from the 2 ml tube into a disposal bottle.
11. Carefully add 500 µl of Washing buffer 2 (W2) without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.
12. Centrifuge once more at *ca.* 12,000 rpm for 1 min to completely remove ethanol, and check that there is no droplet clinging to the bottom of Binding column tube
13. Transfer the Binding column tube to a new 1.5 ml tube for elution, add 200 µl of Elution buffer (EL, or nuclease-free water) onto Binding column tube, and wait for at least 1 min at RT (15~25℃) until EL is completely absorbed into the glass fiber of Binding column tube.
14. To increase DNA yield wait for 5 min after adding Elution buffer (EL). The volume of EL added can be adjusted from 50 µl to 100 µl. A smaller volume will result in a more concentrated solution, but total yield may be reduced.
15. Centrifuge at 8,000 rpm for 1 min to elute. About 180 µl ~ 200 µl of eluent can be obtained when using 200 µl of Elution buffer (or nuclease-free water). For an improved yield, elute the sample twice and use after concentration process.
16. For further analysis the DNA was kept at - 20 C.

3.2.4. Quantization and dilution:

DNA concentration was measured after dissolving 5 µl from the stock DNA in 495 µl ddH_2O, the mixture wasre measured in UV spectrophotometer machine (UV-vis, PC 2401, from Shimadzu Company, Japan). The obtained optical density was used in calculation of the concentration using the equation described by Sambrook and Pussel (2000) as follow:

DNA concentration= O.D x 50 x 100/ 1000

Where

O.D = optical density, 50 = constant value for double strand DNA, 100 = inverse of dilution.

The DNA was diluted to 50 ng/µl in order to be used in polymerase chain reactions (PCR).

3.3 Gene amplifications and typing:

The primers sequences for three genes including growth hormone gene (*GH*), *Pit1* and prolactin receptor (*PRLR*) were studied using PCR-RFLPs methodology were presented in Table 3.3. PRLR gene part of Exon 5 was also amplified using a primer set described by (Cui *et al.,* 2006; Jiang *et al.,* 2005).

Table 3.3. Primers sequence for tested genes

Gene	Sequence	Size	AT
GH	F 5'- CTAAAGGACCTGGAAGAAGGG -3' R 5' -AACTTGTCGTAGGTGGGTCTG -3'	1050	60^{o}C
Pit1	F 5'- GGACCCTCTCTAACAGCTCTC- 3' R 5'- GGGAAGAATACAGGGAAAGG-3	599	58^{o} C
PRLR	F 5'-TTGTCTGCTTTGATTCATTTCC-3' 5'-TGCATTTCATTCTTCCCTTTTT-3'	250	59^{o} C

The RFLP-PCR conditions are presented in Table 3.2.

Table 3.4. The standard PCR cocktail was as follows:

Amount	Chemical	Final concentration
1 µl	DNA template	50 ng
1 µl	10X PCR buffer	1 X
0.5 µl	Forward primer	10 pmol
0.5 µl	Reverse primer	10 pmol
1 µl	Deoxynucleotides (dNTPs)	200 mol
0.1 µl	Taq DNA polymerase	0.5 Unit
5.9 µl	Sterile ddH_2O	------
10 µl	Final volume	

The optimized PCR cycle was as follows:

Primary denaturation	94° C /5 min
PCR 35 cycles	94° C for 30 sec 59° C for 1 min 72° C for 1min
Post PCR	72° C for 5 min
	4° C to ∞

The polymerase chain reaction amplifications were performed on Bio-RAD model: T100^{TM} thermal cycler. The following cycles were applied for *GH* gene amplification: initially denatured at 95^{o} C for 4min followed by 35 cycles of denaturation at 95^{o} C for 30 sec, annealing at 60 ^{o}C for 60 sec, and extension at 72 ^{o}C for 60 sec, final extension at 72^{o} C for 7 min. PIT1 amplification: initially denatured at 94^{o} C for 5 min followed by 30 cycles of denaturation at 94^{o} for 30 sec, annealing at 58^{o} C for 30 sec, extension at 72 for 45 sec, and a final extension at 72^{o} C for 5 min. PRLR gene

amplification: initially denatured at 94^{o} C for 5 min followed by 35 cycles of denaturation at 94C for 30 sec, annealing at 60^{o} C for 30sec, extension at 72^{o} C for 30 sec, and final extension at 72^{o} C for 5 min.

After the PCR run finished some of the PCR products were loaded on 2% agarose supported with ethidium bromide for band visualization.

Details of the detection were as follows:

Five µl from the PCR products were mixed with 1µl from PCR loading dye and one was kept for the size marker (50 or 100 bp DNA ladder). The PCR products were run on 2% agarose gel electrophoresis in TAE buffer (mixed of tris, acetic acid and EDTA) for 30 min. at 100 volts, 40mA. The gel was stained for 20 min with Ethidium bromide (5 mg/m/ distilled water). In a dark room, the DNA was visualized using U.V. transilluminator and the photos were captured using Bio-Rad gel documentation system.

After the confirmation of the PCR amplification success, PCR products were subjected to restriction using Sac1, Taq1 and BamH1restriction enzymes which were provided by the manufacturing company. The cutting was done according to the enzyme manufacturing company instructions (Thermo, Germany). PCR product resulting from GHG, PIT1 and PRLR a 5 µl of DNA was treated with 1 µl of buffer, 0.5 µlenzyme Taq and 8.5 µl sterile ddH_2O.1x Fast Digest Green Buffer was used at incubation temperatures of 37^{o} C for 30 min, 65^{o} C for 5min and 37^{o} C for/ min for restriction enzymes Sac1, Taq1 and BamH1, respectively.

After the end of restriction enzyme reaction, the DNA was allowed again to run horizontally on 2% agarose supported with Ethidium bromide and images were captured and analyzed for allele identification.

CHAPTER FOUR

RESULTS

4.1 Body weight

The mean body weights of indigenous bare neck chickens were 27.37±2.12g, 131.89±11.25g, 339.24±72.42g, 525.43± 97.17g and739.33±147.13g during the rearing period of 0, 4, 8, 12 and 16 weeks of age. Whereas the initial and final weights were 27.37±2.12 g and 739.33±147.13g respectively. The result showed that the final body weight at 16 weeks of age was below the market weight of 1000g (Table 4.1). This slow growth is a typical characteristic growth of the indigenous chickens.

The body weight at day and 16 weeks of age of male and female indigenous bare neck chicken were 28.79g, 816.43g respectively for males and 26.96g, 699.63g for females (Table 4.2). The trend of growth pattern clearly showed that males were heavier than females as shown in (Figures 4.1, 4.2, 4.3). There was a statistically significant ($P< 0.001$) difference in body weight between sexes. The difference in mean values at different weeks of age was greater than would be expected by chance. The effect of sex varied with age and there was statistically significant ($P< 0.003$) interaction between sex and age (Table 4.3).

4.2 Body weight gain

The estimates of body weight gains at different ages of 4, 8,12and 16 weeks were 104.24 g, 207.36 g, 187.18 g and 213.09 g respectively. Body weight at the end of the period of 16 weeks was 711.96 g and daily gain was 6.36g Table (4.4 and Figure 4.4).

4.3 Feed consumption

Results on feed consumption in Table (4.4) and Figure (4.4) revealed that feed consumption between ages 0-4, 4-8, 8-12, 12-16 weeks was 401 g, 702.92 g and 932.06 g and 116.74 respectively. The total feed consumption at the end of the period of 16 weeks was 3199.96 g.

4.4 Feed conversion ratio

The results in Table (4.3) and Figure (4.4) represent the feed conversion ratio at different ages of 0-4, 4-8, 8-12 and 12-16 weeks. It was 3.84, 3.39,4.98 and5.47 respectively with the highest feed conversion recorded at 8 weeks of age and the lowest feed conversion efficiency at the age of 16 weeks.

Table 4.1. Body weight during rearing period (0-16) weeks of age

	N	Minimum	Maximum	Mean ±SE		Std.
					Std. Error	
Day-old	100	22	32	27.37	.21	2.12
4 weeks	98	100	165	131.89	1.14	11.25
8 weeks	86	195	525	339.24	7.81	72.42
12 weeks	63	335	780	526.43	12.24	97.17
16 weeks	42	460	1220	739.33	22.98	147.13
Valid N (listwise)	42					

Table 4.2. Body weight of males and females indigenous bare neck chickens

Age (weeks)	Sex	
	Male	Female
0	28.786±21.010	26.963±15.129
4	138.929±21.010	130.556±15.129
8	399.286±21.010	317.037±15.129
12	603.214±21.010	505.926±15.129
16	816.429±21.010	699.630±15.129

Figure 4.1. Male Body Weight (g)

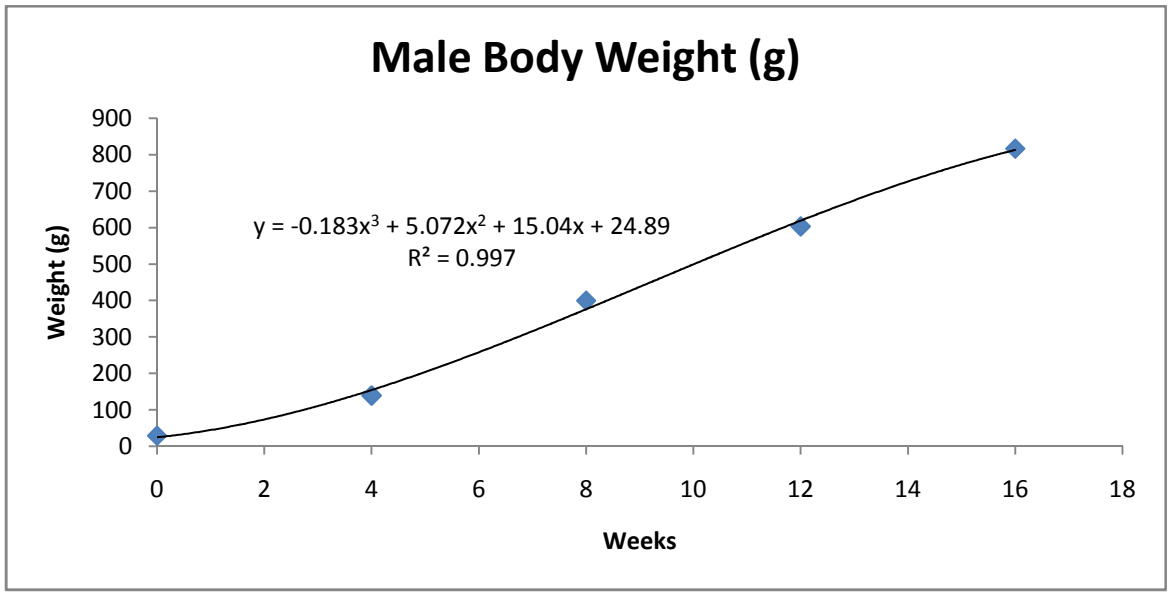


Figure 4. 2. Female Body Weight (g)

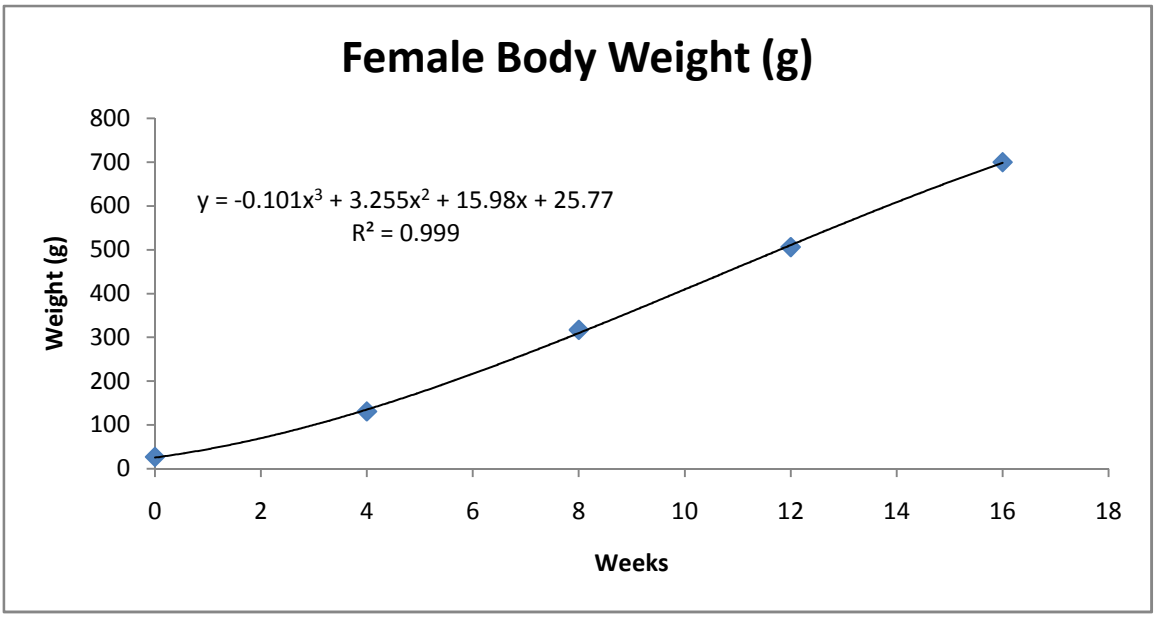


Figure 4. 3. Male and Female Body Weight (g)

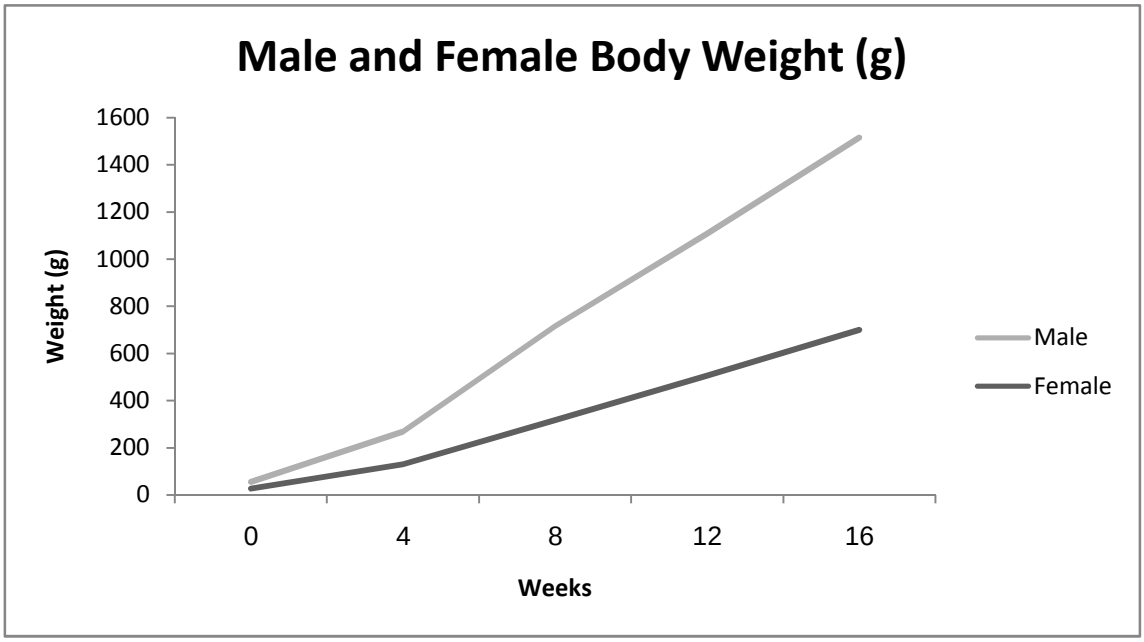


Table 4. 3. ANOVA of body with respect to sex and age (wks) of chickens

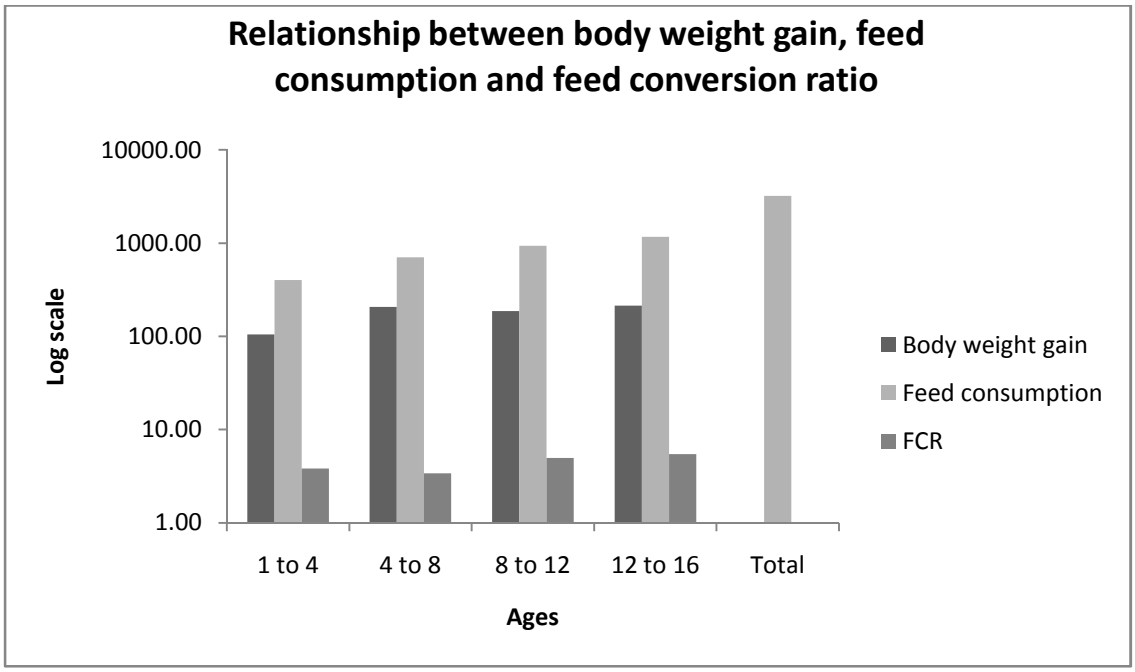
Source of Variation	DF	SS	MS	F	P
Sex	1	173256.229	173256.229	28.036	<0.001
Age	4	13169662.341	3292415.585	532.777	<0.001
Sex x Age	4	102824.761	25706.190	4.160	0.003
Residual	195	1205046.669	6179.727		
Total	204	15344800.605	75219.611		

Age 0-4, 4-8, 8-12 and 12-16 (wks)

Table 4.4. body weight, body weight gain, feed consumption and feed conversion ratio

Weeks	Body weight	Body weight gain	Feed consumption	FCR
0	27.37			
0 to 4	131.89	104.52	401.24	3.84
4 to 8	339.24	207.36	702.92	3.39
8 to 12	526.43	187.18	932.06	4.98
12 to 16	739.33	213.08	1164.74	5.47
Total		711.96	3199.96	

Figure 4.4. Relationship between body weight gain, feed consumption and feed conversion ratio



4.5 Age at first egg

The mean body weight of the birds at the age of first egg (148.44 days) was 1006.76 ±306.78 g (Table 4.5). The mean egg weight at the same age was 33.50± 4.48 g.

The body weight of the birds was positively and significantly (P< 0.01) correlated with egg weight (Table 4.6). It was also negatively but significantly (P< 0.05) correlated with age at first egg. Egg weight on the other hand was positively and significantly (P< 0.01) correlated with age at first egg Table (4.6).

Table 4.5. Mean and standard deviation of age at first egg (days)

	Mean	Std. Deviation	N
BIRD_WT	1006.76	306.78	54
EGG_WT	33.5046	4.4825	54
Age at first egg	148.44	6.81	54

Table 4.6. Correlation of bird weight and egg weight of age at first egg

		BIRD_WT	EGG_WT	AFE
BIRD_WT	Pearson Correlation	1.000	.377**	-.155
	Sig. (2-tailed)	.	.005	.263
	N		54	54
EGG_WT	Pearson Correlation	.	1.000	.385**
	Sig. (2-tailed)	.	.	.004
	N			54
Age at First egg	Pearson Correlation		.	1.000
	Sig. (2-tailed)			.
	N			

**. Correlation is significant at the 0.01 level (2-tailed).

AFE: Age at first egg

4.6 Egg quality characteristics

Egg characteristics parameters showed varingt mean values (Table 4.7). The mean egg weight was 31.43±0.85g, egg length 46.11±0.42mm, egg diameter 34.93±0.40 mm, albumen height 4.31±0.39 mm, albumen weight 18.01±0.65 g, yolk weight 9.05±0.28 g, yolk height 13.54±0.29 mm, yolk diameter 32.55±0.33 mm, shell weight 4.30±0.08 g, shell thickness 0.38±0.04 mm, Haugh Unit 75.20± 2.34 HU, egg shape index 0.75.87± 1.17 and yolk index 0 .42±0.01.

Table (4.8) presents correlations among all egg quality characteristics studied.

Significant (P< 0.01) positive correlations were found between egg weight and each of egg diameter, albumen weight, yolk weight, yolk height, and egg shape index. It was also significantly (P<0.05) and positively correlated with albumen height and negatively with the shell weight. Its correlation with egg length, shell thickness and Haugh unit was not significant.

Egg length was negatively (P<0.05) correlated with egg shape index and negatively correlated with egg diameter, albumen weight, shell weight and Haugh unit Table (4.8).

The correlations between egg length and all other traits were not significant (P>0.05) except for a significant (P< 0.01) negative correlation with egg shape index.

Egg diameter was positively and significantly (P< 0.01) correlated with albumen weight, yolk weight, yolk height and egg shape index. It was positively and significantly (P< 0.05) correlated with yolk index. Correlations with all other traits were not significant (P> 0.05). Albumen height was positively significantly (P< 0.01 and P< 0.05) correlated with Haugh units and yolk weight respectively.

Albumen weight was positively and significantly correlated with yolk weight, yolk height and egg shape index and it was positively and significantly (P< 0.05) correlated with yolk height and significantly (P< 0.05) positively correlated with both egg shape index and yolk index. It was negatively and significantly correlated with shell weight. Yolk height was positively and significantly correlated with yolk index. Shell weight was positively and significantly (P< 0.05) correlated with shell thickness.

Table 4.7. Mean of egg quality and characteristics

	N	Minimum	Maximum	Mean	SE	SD.
Egg weight g	17	25.92	36.96	31.4288	.8493	3.5016
Egg length mm	17	42.95	49.02	46.1106	.4213	1.7370
Egg diameter mm	17	32.64	37.13	34.9324	.4014	1.6548
Albumenheightmm	17	3.17	7.65	4.3053	.3873	1.5968
Albumen weight g	17	13.95	22.15	18.0129	.6509	2.6838
Yolk weight g	17	7.20	12.02	9.0524	.2771	1.1424
Yolk height mm	17	11.49	15.63	13.5394	.2906	1.1982
Yolk diameter mm	17	30.23	35.71	32.5465	.3335	1.3750
Shell weight g	17	3.7	4.8	4.302	0.07614	.314
Shell thickness mm	17	.32	.51	.3776	0.01363	0.05618
Yolk colour	17	1	1	1.00	.00	.00
Haugh unit	17	66.62	95.08	75.1965	2.3431	9.6607
Egg shape index	17	66.78	83.82	75.8747	1.1696	4.8224
Yolk index	17	.35	.49	.4159	0.008955	0.03692
Valid N (listwise)	17					

Table 4.8. Correlations of egg quality and characteristics

		Egg weigh	Egg leng mm	Egg diame mm	Albumen eightmm	Albumen weight g	Yolk weigh	Yolk heig mm	Yolk diame mm	Shell weigh	Shell thickness n	Haugh un	Egg shap inde	Yolk inde
Egg weight g	Pearson Correlation	1.000	.056	.948**	.507*	.977**	.878**	.827**	.358	-.484*	-.381	.330	.661**	.631**
	Sig. (2-tailed)	.	.830	.000	.038	.000	.000	.000	.158	.049	.132	.196	.004	.007
	N	17	17	17	17	17	17	17	17	17	17	17	17	17
Egg length mm	Pearson Correlation		1.000	-.150	-.028	.062	.084	.154	.276	-.117	.214	-.037	-.692**	.036
	Sig. (2-tailed)	.	.	.566	.914	.813	.747	.555	.284	.655	.409	.887	.002	.891
	N			17	17	17	17	17	17	17	17	17	17	17
Egg diameter mm	Pearson Correlation			1.000	.479*	.938**	.792**	.719**	.429	-.401	-.425	.307	.817**	.501*
	Sig. (2-tailed)			.	.052	.000	.000	.001	.086	.110	.089	.230	.000	.041
	N				17	17	17	17	17	17	17	17	17	17
Albumenheightmm	Pearson Correlation	.	.	.	1.000	.465	.574*	.447	.017	-.264	-.304	.980**	.363	.447
	Sig. (2-tailed)	.	.	.	.	.060	.016	.072	.947	.306	.235	.000	.152	.072
	N					17	17	17	17	17	17	17	17	17
Albumen weight g	Pearson Correlation	.	.	.	.	1.000	.769**	.814**	.386	-.478*	-.357	.290	.649**	.605*
	Sig. (2-tailed)	.	.	.	.	.	.000	.000	.126	.052	.160	.259	.005	.010
	N						17	17	17	17	1	17	17	17
Yolk weight g	Pearson Correlation	.	.	.	. *	.	1.000	.701**	.273	-.538*	-.386	.431	.532*	.549*
	Sig. (2-tailed)	.	.	.	.	.	.	.002	.290	.026	.126	.085	.028	.022
	N							17	17	17	1	17	17	17
Yolk height mm	Pearson Correlation	.	.	.	.	.	. *	1.000	.119	-.209	-.141	.320	.440	.901**
	Sig. (2-tailed)	.	.	.	.	.	.	.	.648	.421	.590	.210	.077	.000
	N								17	17	17	17	17	17
Yolk diameter mm	Pearson Correlation	.	.	.	.	.	.	.	1.000	-.039	.139	-.060	.158	-.315
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.883	.594	.819	.545	.218
	N									17	17	17	17	17
Shell weight g	Pearson Correlation	*	.	.	.	.	. *	.	.	1.000	.488*	-.170	-.215	-.161
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.047	.514	.407	.536
	N										17	17	17	17
Shell thickness mm	Pearson Correlation		.	.	.	.	.	.	.	*	1.000	-.224	-.424	-.162
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.387	.090	.535
	N											17	17	17
Haugh unit	Pearson Correlation	.	.	.	.	.	.	.	.	.	.	1.000	.242	.362
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.	.349	.153
	N												17	17
Egg shape index	Pearson Correlation	.	.	.	.	. *	. *	.	.	.	.	.	1.000	.346
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.	.	.174
	N													17
Yolk index	Pearson Correlation	.	.	.	.	. *	. *	. *	.	.	-	.	.	1.000
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.		.	.	.
	N													

*Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

4.7 Measurement of carcass cuts

4.7.1 Inedible carcass cuts

4.7.1.1 Blood and feather

The mean values of blood weight at 8, 12 and 16 weeks of age were 15.00±6.55 g, 26.56± 11.06 g and 30.00±9.78 g respectively. Whereas, the mean values of feather weight at the same ages were 15.65±8.84 g, 17.81±5.76 g and 37.19± 19.58 g (Table 4.9).

Table 4.9. Mean values of live body weight, blood weight and feather weight (g)

	Mean	Std. Deviation	N
LW8	358.67	91.76	15
BW8	15.00	6.55	15
FW8	15.67	8.84	15
LW12	520.31	84.49	16
BW12	26.56	11.06	16
FW12	17.81	5.76	16
LW16	707.19	129.14	16
BW16	30.00	14.61	16
FW16	37.19	19.58	16

Table (4.10) presents the correlations between blood weight, feather weight and live body weight at different ages. Live body weight at 8 weeks was negatively and significantly ($P< 0.05$) correlated with blood weight at 16 weeks. Its correlation with all other traits was not significant ($P> 0.05$).

Live body weight at 12 weeks was positively and significantly ($P< 0.01$) correlated with blood weight at 12 weeks and feather weight at 12 weeks. Blood weight at 12 weeks was positively and significantly ($P< 0.05$) correlated with feather weight at 12 weeks. All other correlations were not significant ($P> 0.05$).

Table 4.10. Correlations of live weight, blood and feathers

		LW8	BW8	FW8	LW12	BW12	FW12	LW16	BW16	FW16
LW8	Pearson Correlation	1.000	.181	.486	.183	-.106	-.196	.009	-.604*	-.065
	Sig. (2-tailed)	.	.518	.066	.515	.708	.483	.974	.017	.819
	N		15	15	15	15	15	15	15	15
BW8	Pearson Correlation	.	1.000	-.062	.031	.120	-.092	-.423	.018	.402
	Sig. (2-tailed)	.	.	.827	.912	.671	.745	.116	.948	.138
	N			15	15	15	15	15	15	15
FW8	Pearson Correlation	.		1.000	.167	-.470	-.070	-.372	-.507	-.014
	Sig. (2-tailed)	.	.	.	.551	.077	.803	.172	.054	.961
	N				15	15	15	15	15	15
LW12	Pearson Correlation	.		.	1.000	.670**	.706**	.041	.092	.099
	Sig. (2-tailed)	.	.	.	.	.005	.002	.879	.735	.714
	N					16	16	16	16	16
BW12	Pearson Correlation	-.	.	-.	.	1.000	.528*	.210	.217	.068
	Sig. (2-tailed)	.	.	.		.	.036	.436	.420	.803
	N						16	16	16	16
FW12	Pearson Correlation	-.			.	*	1.000	.224	.218	-.162
	Sig. (2-tailed)		.		.	.	.	.404	.418	.550
	N							16	16	16
LW16	Pearson Correlation	.			.		.	1.000	.409	-.352
	Sig. (2-tailed)	.	.	.	.	.	.	.	.116	.181
	N			.					16	16
BW16	Pearson Correlation	*			.	.	.	.	1.000	.122
	Sig. (2-tailed)	.	.	.	.	.	.	.	.	.652
	N									16
FW16	Pearson Correlation		.	-.		.	-		.	1.000
	Sig. (2-tailed)	.	.	.	.	.		.	.	.
	N									

*Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

4.7.1.2 Head, neck, legs, intestine, abdominal fat, wings, shank weight, shank length and back

The mean values of inedible carcass cuts at 8, 12 and 16 weeks of age are presented in Table (4.11). The mean values of head weight were 19.87±3.38 g, 23.38±2.80 and 29.06±12.44 g. While the mean values of neck weight were 13.67±3.96 g, 17.63±3.20 and 29.69±5.87 g. The mean values of legs were 23.33±6.70 g, 25.13±4.38 g and 27.62±6.94 g in the three ages, respectively. The mean values of intestines were 27.47±4.9 g, 37.94±7.75 g and 47.94±7.51 g. Abdominal fat means were 0, 2.50±1.67 g and 3.19±2.12 g. The mean values of wings were 24.93±4.13 g, 42.81±9.00 g and 57.62±12.76 g. The mean values of shank weight were 5.87±2.17 g, 6.13±2.42 g and 6.87±1.93 g. Shank length means were 4.53±.74 cm, 5.31±0.48 cm and 5.94±.77 cm. The mean values of back were 41.33±10.07 g, 87.13±24.29 g and 138.76±26.30 g in the three ages, respectively.

Live weight was significantly ($P< 0.05$ and $P< 0.01$) correlated with liver at 8 weeks and at 12 and 16 weeks of age respectively. It was also significantly ($P< 0.05$ and $P< 0.01$) correlated with intestine at 8 weeks and at 12 and 16 weeks of age respectively. It was significantly ($P< 0.05$) correlated with shank weight at 16 weeks and shank length at 8 and 12 weeks of age. It was also significantly ($P< 0.01$) correlated with back weight at 12 and 16 weeks of age.

Liver weight was significantly ($P< 0.05$ and $P< 0.01$) correlated with intestine at 8 and 16 weeks of age respectively. It was also significantly ($P< 0.05$) correlated with abdominal fat weight at 12 weeks of age. It was significantly ($P< 0.01$) correlated with wing weight, shank weight and back weight at 16 weeks of age.

Intestine weight was significantly ($P < 0.05$ and $P< 0.01$) correlated with wing weights at 12 and 16 weeks of age respectively.

Wing weights were significantly ($P< 0.05$) correlated with shanks weight at 16 weeks and shank length at 12 weeks of age.

Shank length was significantly ($P< 0.05$) correlated with back weight at 8 weeks of age.

Table 4.11. Means and standard error of inedible carcass cuts of bare neck chicken (g)

Parameters	8 weeks	12 weeks	16 weeks
LW	358.67±91.76	520.31±84.49	707.19±129.14
DW	343.67±90.80	493.75±77.51	669.06±123.40
DFW	315.00±89.08	475.94±73.63	596.56±123.65
EVW	216.33±58.02	323.75±86.48	449.06±84.07
CW	199.00±51.90	276.56±55.94	435.94±84.09
H	19.87±3.38	23.38±2.80	29.06±12.44
N	13.67±3.96	17.63±3.20	29.62±5.87
L	23.33±6.70	25.13±4.38	27.62±6.94
IN	27.47±4.09	37.94±7.75	46.94±7.51
AF	0.00	2.50±1.67	3.19±2.14
WW	24.93±4.13	42.81±9.00	57.62±12.76
SHW	5.87±2.17	6.13±2.42	6.87±1.93
SHL cm	4.53±.74	5.31±.48	5.94±.77
Back	41.33±10.07	87.13±24.29	138.76±26.30

Abbreviations: LW= Live weight, DW= Dressed weight, DFW= Defeathered weight, EVW= Eviscerated weight, CW= Cold weight, H= Head, N= Neck, L= legs, IN= Intestine, AF= Abdominal fat, WW= wings, SHW= Shank weight, SHL CM= Shank length centimeter,

Table 4.12. Correlation of live weight and carcass cuts (head, neck, legs, gizzard and heart)

Parameter	LW	H	N	LS	GI	HE
LW						
8weeks		.913**	.837**	.200	.717**	.734**
12weeks		.667**	.443	.467	.310	.442
16weeks		.396	.952**	.808**	.408	.680**
H						
8weeks	.		.605*	.311	.659**	.590*
12weeks			.098	-.012	.447	.544**
16weeks			.449	.751**	.412	.324
N						
8weeks	.	.		.023	.722	.609*
12weeks				.369	-.068	.120
16weeks				.831**	.383	.779**
LS						
8weeks	.	.	.		-.235	.016
12weeks					.226	.235
16weeks					.428	.735**
GI						
8weeks	.	.	.			.609*
12weeks						.353
16weeks						.000
HE						
8weeks	.	.	.	.	.	
12weeks						
16weeks						

*correlation is significant at the P< 0.05 level (2-tailed)

**correlation is significant at the P< 0.01 level (2-tailed)

Abbreviations: LW = Live weight, H = Head, N = Neck, L = Legs, GI = Gizzard, HE = Heart

Table 4.13. Correlation of live weight and carcass cuts (liver, intestine, abdominal fat, wings, shank weight, shank length and back)

Parameter	LW	LI	IN	AB	WWS	SHW	SHL cm	Back
LW								
8weeks		.625*	.619*	-	.414	.146	.519*	.587
12weeks		.589*	.709**	.421	.680**	.390	.591*	.556*
16weeks		.733**	.625**	-.242	826**	.573*	.125	.648**
LI								
8weeks		1.000	.555*	-	-.089	.297	.488	.469
12weeks			.368	.510*	.310	-.039	.136	.019
16weeks			.811**	.075	.704**	.564*	.050	.610*
IN								
8weeks	.	.		-	.243	.104	.359	.640*
12weeks				.239	.520*	-.010	.383	.290
16weeks				.088	.672**	.502*	.149	.374
AB								
8weeks					-	-	-	-
12weeks					.246	-.198	-.042	.094
16weeks					-.051	-.059	-.033	.374
WWS								
8weeks	.	-.	.	-		.238	.245	.277
12weeks						.363	.618*	.302
16weeks						.529*	.038	.123
SHW								
8weeks			.		.		.047	-.083
12weeks							.367	.197
16weeks							.398	.507*
SHL cm								
8weeks							1.000	.585*
12weeks								.300
16weeks								.456
Back								
8weeks		.	.					1.000
12weeks								
16weeks								

*correlation is significant at the P< 0.05 level (2-tailed)

**correlation is significant level at the P< 0.01 (2-tailed)

Abbreviations: LW= Live weight, LI= Liver, IN= Intestine, AB= Abdominal fat, WW= Wings, SHW= Shank weight, SHL= Shank length.

4.7.4 Edible carcass cuts

4.7.2.1 Giblets (gizzard, heart and liver)

The mean weight edible carcass (giblets) at 8, 12 and 16 weeks of age are presented in Table (4.14).

The mean of eviscerated carcass weights at the three ages were 216.33±58.02 g, 323.75±86.48g and 449.06±84.07g, respectively.

The mean weights of gizzard were 12.73±2.84 g, 15.88±2.06 g and 17.25±3.62. While the heart weights were 3.00±1.36g, 3.44±.63g and4.50±1.71g. The liver weights were 14.60±2.80 g, 20.81±4.02g and 21.94±6.31 g, respectively.

Table (4.15) represents the correlation estimates between eviscerated weight, gizzard, heart and liver.

The eviscerated weight at 8 weeks was highly positively and significantly ($P< 0.05$) correlated with the liver weight. At 16 weeks it was highly positively ($P< 0.01$) correlated with gizzard, heart and liver weight. Gizzard weight at 8 weeks was positively and significantly ($P< 0.05$) correlated with heart weight. At 16 weeks it was positively and significantly ($P< 0.05$) correlated liver.

Heart weight at 8 weeks and 16 weeks was positively and significantly ($P< 0.05$) correlated with liver weight.

Table 4.14. Mean weights of edible carcass cuts giblets (gizzard, heart and liver) g

Cut/ Traits	8 weeks mean ±SE	12 weeks mean ±SE	16 weeks mean ±SE
EVW	216.33±58.02	323.75±86.48	449.06±84.07
GI	12.73±2.84	15.88±2.06	17.25±3.62
HE	3.00±1.36	3.44±.63	4.50±1.71
LI	14.60±2.80	20.81±4.02	21.94±6.31

Abbreviations: EVW= Eviscerated weight, GI= Gizzard, HE= Heart, LI= Liver

Table 4.15. Correlations of edible of carcass cuts giblets (gizzard, heart and liver)

Parameter	EVW	GI	**HE**	LI
EVW 8weeks 12weeks 16weeks		 .819** .139 .768**	 **.641**** **.482** **.916****	 .556* .166 .738**
GI 8weeks 12weeks 16weeks	 . 	 . 	 **.609*** **.353** **.000**	 .273 .254 .587*
HE 8weeks 12weeks 16weeks				 .656* .351 571*
LI 8weeks 12weeks 16weeks	 . 	 . 		

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed)

Abbreviations: EVW = Eviscerated weight, GI = Gizzard, HE = Heart, LI = Liver

4.7.2.2 Edible carcass commercial cuts

4.7.2.2.1 Commercial cuts (breast, thigh and drumstick weights)

The mean weighs of commercial carcass cuts at 8, 12 and 16 weeks of age are presented in Table (4.16) and Figure 4.5.

The mean weights of breast were 58.27±39.41 g, 71.25±14.14g and 115.38±30.74 g at the same ages respectively. The breast weights showed steady increase during 8, 12 and 16 weeks of age.

The mean weights of thigh were 40.93±25.06 g, 48.00±11.08 g and 73.88±16.12g at the same ages, respectively. This also showed that thigh weights were steadily increasing with up to 16 weeks.

The mean weights of drumstick were 37.33±22.50 g, 39.88±6.98 g and 60.63±12.94 g at the three ages respectively. The drumstick weights were increasing steadily up to 16 weeks.

The results of correlations between the weights of commercial carcass cuts are presented in (Table 4.17).

The eviscerated carcass weight was positively significantly ($P<0.01$) correlated with breast weight, thigh weight and drumstick weight at 8 weeks of age. It was significantly ($P<0.05$ and $P<0.01$) correlated with breast weight and thigh weight respectively at 12 weeks of age. It was positively and significantly ($P<0.01$) correlated with breast weight, thigh weight and drumstick weight at 16 weeks of age.

The breast weight was significantly ($P< 0.01$) correlated with eviscerated carcass weight, thigh weight and drumstick weight at 8 and 16 weeks of age. It was ($P<0.01$) correlated with thigh weight at 12 weeks of age.

The thigh weight at all three ages was significantly ($P< 0.01$) correlated with eviscerated carcass weight, breast weight and drumstick weight.

4.7.2.2.2 Commercial carcass cuts (breast weight, breast meat and breast bone)

The mean weights of breast were 58.27±39.41g, 71.25±14.14g and 115.38±30.74 g at ages 8, 12 and 16 weeks respectively. While the breast meat weights were 45.07±36.32 g, 56.50±14.76 g and 95.87±25.44 g at the same ages, respectively. The breast meat weights increased steadily during the period of 8, 12 and 16 weeks of age. Breast bone weights were 12.27±4.33 g, 15.75±5.36 g and 19.38±8.66g at 8, 12 and 16 weeks of age respectively (Table 4.16 and Figure 4.6).

The results of correlations on commercial cuts breast weight, breast meat and breast bone are presented in (Table 4.18). The breast weight at all ages was significantly ($P< 0.01$) correlated with the breast meat weights. It was significantly ($P< 0.05$) and ($P< 0.01$) correlated with breast bone weights at 12 and 16 weeks, respectively. Breast meat weights was significantly ($P< 0.05$) correlated with breast bone weights.

4.7.2.2.3 Commercial carcass cuts (thigh weight, thigh meat and thigh bone)

The mean values of thigh weights were 40.93±25.06 g, 48.00±11.08 g and 73.88±16.12g at ages 8, 12 and 16 weeks, respectively. Thigh meat weights were 31.53±21.68 g, 37.88±9.65 g and 59.25±14.25 g at the ages respectively. Thigh meat was observed to increase steadily during the period 8 to 16 weeks of age. Thigh bone weights were 9.33±8.06 g, 10.00±2.63 g and 14.63±4.66 g respectively. The thigh bone weight increased gradually from 8 to 16 weeks of age (Table 4.16 and Figure 4.7).

Results of correlations between weights of commercial cuts (thigh weight, thigh meat and thigh bone) are presented in Table (4.19).

Thigh weight at all ages was significantly ($P< 0.01$) correlated with thigh bone at 8 weeks of age. Whereas thigh meat was significantly ($P< 0.05$) correlated with thigh bone at 8 weeks of age.

4.7.2.2.4 Commercial carcass cuts (drumstick weight, drumstick meat and drumstick bone)

The mean values of drumstick weight were 37.33±22.50 g, 39.88±6.98 g and 60.63±12.94 g at 8, 12 and 16 weeks of age, respectively. Whereas drumstick meat weights were 25.87±18.49 g, 29.63±6.21 and 46.88±11.31 g at the same ages respectively. The drumstick meat increased steadily during the period 8 to 16 weeks of age. Drumstick bone weights were 9.00±4.52 g, 10.50±3.46 g and 13.87±4.76 g. at 8,12 and 16 weeks of age respectively (Table 4.16 and Figure 4.8).

The results of correlation between the weight of commercial cuts (drumstick weight, drumstick meat and drumstick bone) and their mean values are presented in (Table 4.20 and Figure 4.8).

Drumstick weight at all ages was significantly ($P< 0.01$) correlated with drumstick meat and was significantly ($P< 0.01$) correlated with drumstick bone at 8 weeks of age. Drumstick meat was significantly ($P< 0.05$) correlated with drumstick bone at 8 weeks of age.

Table 4.16. Commercial carcass cuts (breast, thigh and drumstick) (g)

Parameter	8 weeks	12 weeks	16 weeks
EVW	216.33±58.02	323.75±86.48	449.06±84.07
WB	58.27±39.41	71.25±14.14	115.38±75
BM	45.07±36.32	56.50±14.76	95.87±25.44
BB	12.27±4.33	15.75±5.36	19.38±8.66
WT	40.93±25.06	48.00±11.08	73.88±16.12
TM	31.53±21.68	37.88±9.65	59.25±14.25
TB	9.33±8.06	10.00±2.63	14.63±4.66
WD	37.33±22.50	39.88±6.98	60.63±12.94
DM	25.87±18.49	29.63±6.21	46.88±11.31
DB	9.00±4.52	10.50±3.46	13.87±4.76

Abbreviations: WB = Breast weight, BM = Breast meat, BB = Breast bone, WT= Thigh weight, TM = Thigh meat,

TB = Thigh bone, WD = Drumstick weight, DM = Drumstick meat, DB = Drumstick bone.

Table 4.17. Summary of correlation of commercial cuts (breast weight, thigh weight and drumstick weight)

Parameter	EVW	WB	WT	WD
EVW				
8weeks		.715**	.742**	.765**
12weeks		.567*	.670**	.474
16weeks		.768**	.916**	.738**
WB				
8weeks			.973**	.960**
12weeks			.628**	.393
16weeks			.795**	.788**
WT				
8weeks				.949**
12weeks				.576**
16weeks				.768**
WD				
8weeks		.	.	
12weeks				
16weeks				

*correlation is significant at the P< 0.05 level (2-tailed)

**correlation is significant at the P<0.01 level (2-tailed)

Abbreviations: EVW = Eviscerated weight, WB = Breast weight, WT = Thigh weight, WD = Drumstick weight

Table 4.18. Summary of correlation of commercial cuts (breast weight, breast meat and breast bone)

Parameter	WB	BM	BB
WB			
8weeks		.958**	.324
12weeks		.875**	.553*
16weeks		.971**	.695**
BM			
8weeks			.319
12weeks			.187
16week			.505*
BB			
8weeks			
12weeks			
16weeks			

*correlation is significant at the P< 0.05 level (2-tailed)

**correlation is significant at the P< 0.01 level (2-tailed)

Abbreviations: WB = Breast weight, BM = Breast meat, BB = Beast bone

Table 4.19. Summary of correlation of commercial cuts (thigh weight, thigh meat and thigh bone)

Parameter	WT	TM	TB
WT			
8weeks	1.000	.923**	.820**
12weeks		.978**	.649**
16weeks		.960**	.523*
TM			
8weeks		1.000	.620*
12weeks			.483
16weeks			.264
TB			
8weeks			1.000
12weeks			
16weeks			

*correlation is significant at the P< 0.05 level (2-tailed)

**correlation is significant at the P< 0.01 level (2-tailed)

Abbreviation: WT = Thigh weight, TM = Thigh meat, TB = Thigh bone

Table 4.20. Summary of correlation of commercial cuts (drumstick weight, drumstick meat and drumstick bone)

Parameter	WD	DM	DB
WD			
8weeks	1.000	.913**	.830**
12weeks		.891**	.344
16weeks		.852**	.257
DM			
8weeks		1.000	.591*
12weeks		1.000	-.016
16weeks			.166
DB			
8weeks		.	1.000
12weeks			
16weeks			

*correlation is significant at the P< 0.05 level (2-tailed)

**correlation is significant at the P< 0.01 level (2-tailed)

Abbreviations: WD = Drumstick weight, DM = Drumstick meat, DB = Drumstick bone

Figure 4.5. Commercial Cuts

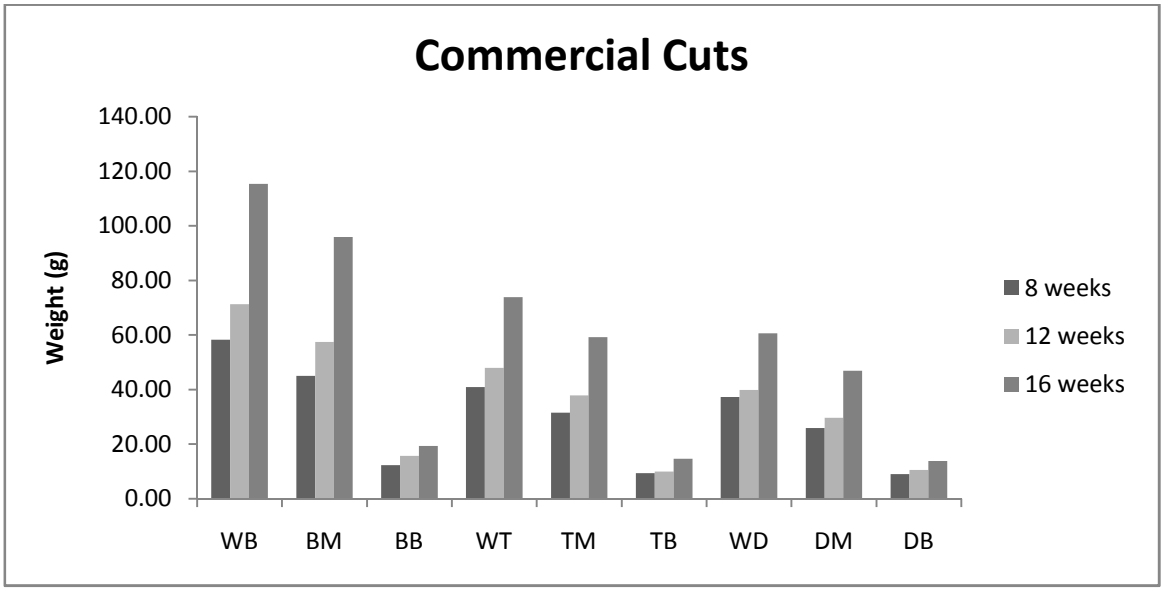


Figure 4.6. Commerial Cuts (Breast)

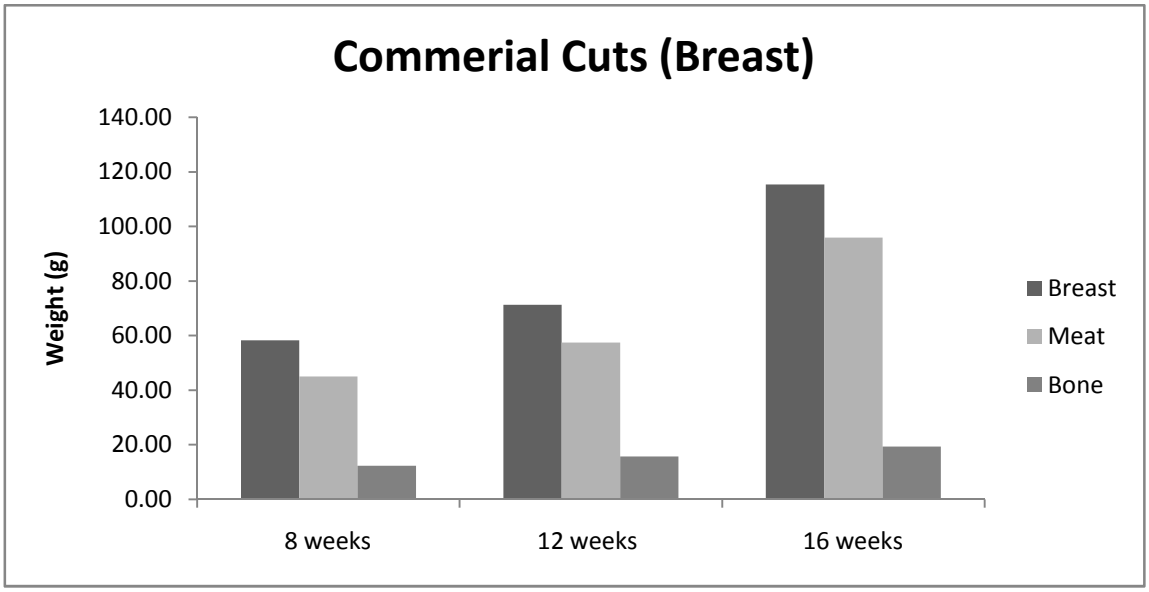


Figure 4.7. Commerial Cuts (Thigh)

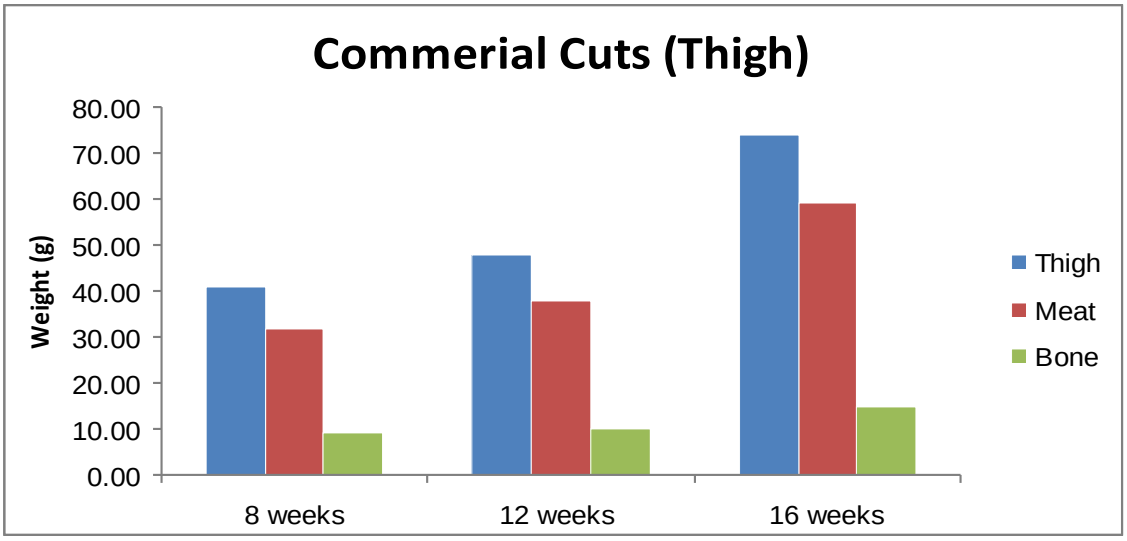
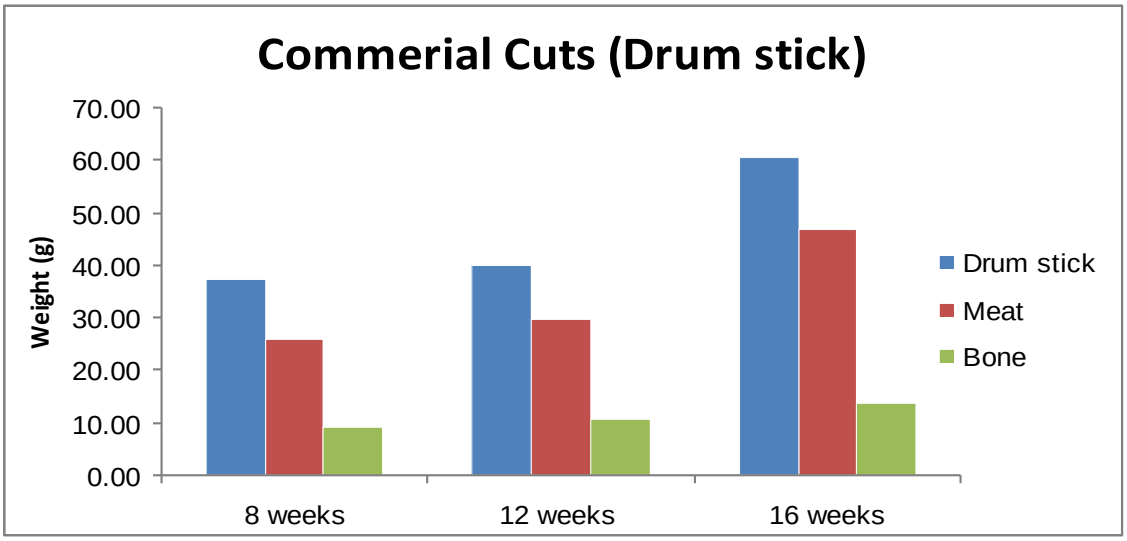


Figure 4.8. Commerial Cuts (Drum stick)



4.8 Chemical analysis

The results in Table (4.22) on chemical analysis (percentage) revealed that the mean value of moisture was 77.06±0.70%, protein was 19.60±0.59% fat was2.07±0.01% and ash was1.04±0.01%.

Table 4.21. Means percentage composition of meat (moisture, protein, fat and ash)

	N	Minimum	Maximum	Mean	SE	Std.
MOISTURE	3	76.30	77.60	76.8000	.4041	.7000
PROTEIN	3	18.50	19.60	19.1667	.3383	.5859
FAT	3	1.94	2.07	2.0133	0.03844	0.06658
ASH	3	1.03	1.05	1.0433	0.006667[3]	0.01155
Valid N (listwise)	3					

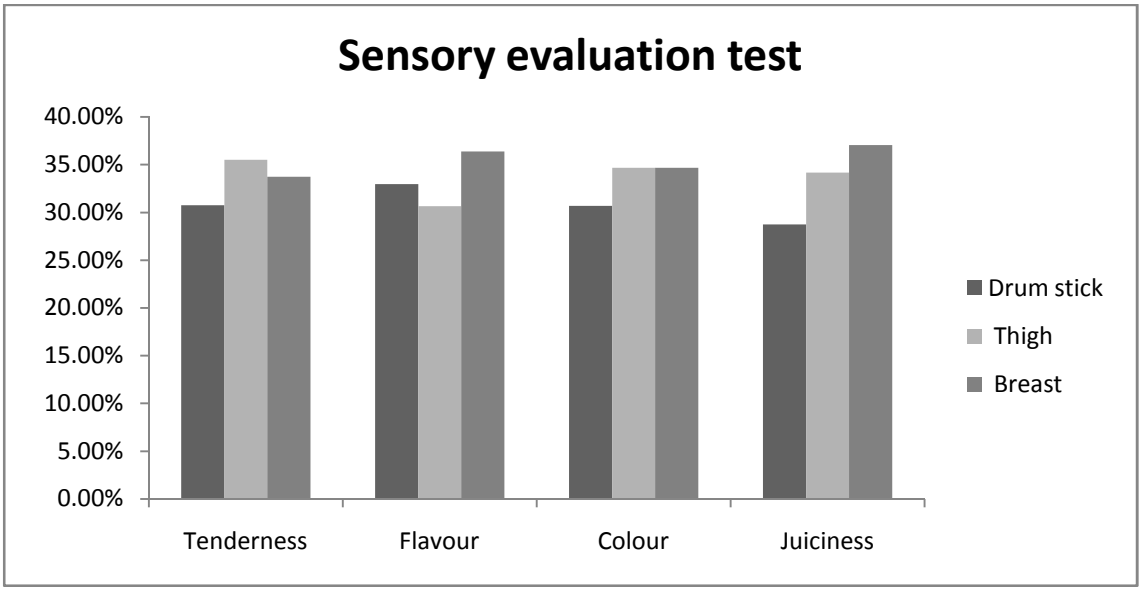
4.9 Sensory evaluation test or taste penal

Results in Table (2.21 and Figure 4.9) of sensory evaluation revealed that thigh tenderness scored (35.52%) followed by breast (33.75%) and drumstick (30.75%). The flavor of breast scored (36.39%), drumstick (32.95%) and thigh (30.66%). With respect to colour thigh and breast scored the same percentage (34.66%) and drumstick had the lowest score of (30.68%). The juiciness of breast was the highest (37.06%), followed by thigh (34.19%) and drumstick was the lowest (28.75%).

Table 4.22. Sensory evaluation parameters (tenderness, flavor, colour, juiciness)

	Tenderness	Flavour	Colour	Juiciness
Drum stick	30.75%	32.95%	30.68%	28.75%
Thigh	35.52%	30.66%	34.66%	34.19%
Breast	33.73%	36.39%	34.66%	37.06%

Figure 4.9. Sensory evaluation test



4.10 Molecular studies

4.10.1 Prolactin receptor gene

Amplification of prolactin receptor gene (PRLR) produced a band size of 250 bp (Fig. 4.10), after cutting with BamH1 restriction enzyme, two bands size of 195 and 55 (representing allele B) were observed in all the line samples studied (Fig. 4.11), meaning the presence of only one allele (B) and one homozygous genotype (BB), subsequently the absence of polymorphism in this gene in all the lines studied.

Figure 4.10. PCR of prolactin receptor on the lanes

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

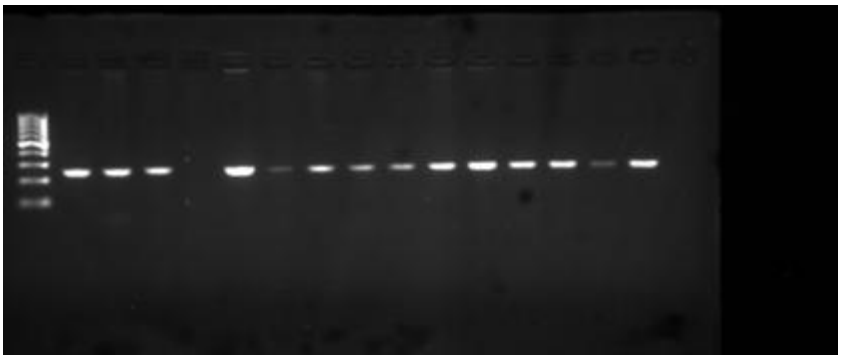
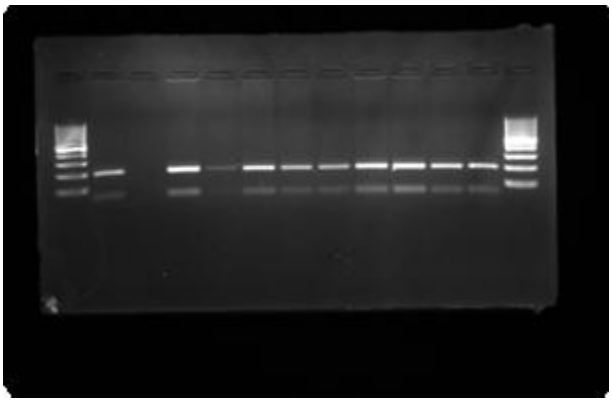


Figure 4.11. Restriction PRLR by enzyme BamH1 showed no Polymorphism on the lanes

1 2 3 4 5 6 7 8 9 10 11



BB BB BB BB BB BB BB BB BB

4.10.2 Growth hormone gene

The growth hormone gene has shown polymorphism in all the lines studied. Amplification of growth hormone gene (GH) produced a band of 1050 bp (Fig.4.12), after cutting with Sac1 restriction enzyme, two bands (representing allele B) were observed in some line samples studied (Fig. 4.13), meaning the presence of two alleles (A, B) two homozygous genotypes (AA; BB), and one heterozygous genotype (AB). Results of allele frequencies as well as genotype frequencies are presented in table 4.24.

Figure 4.12. PCR of growth hormone gene (GHG) on the lanes

1 2 3 4 5 6 7 8 9 10 1112 13 14 15

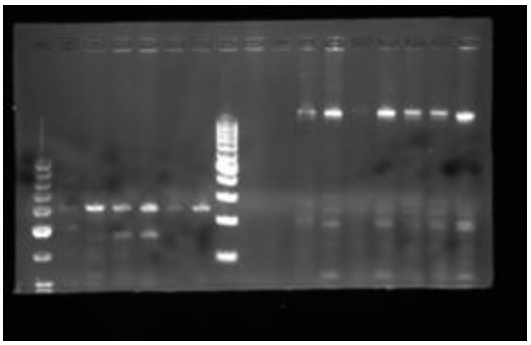
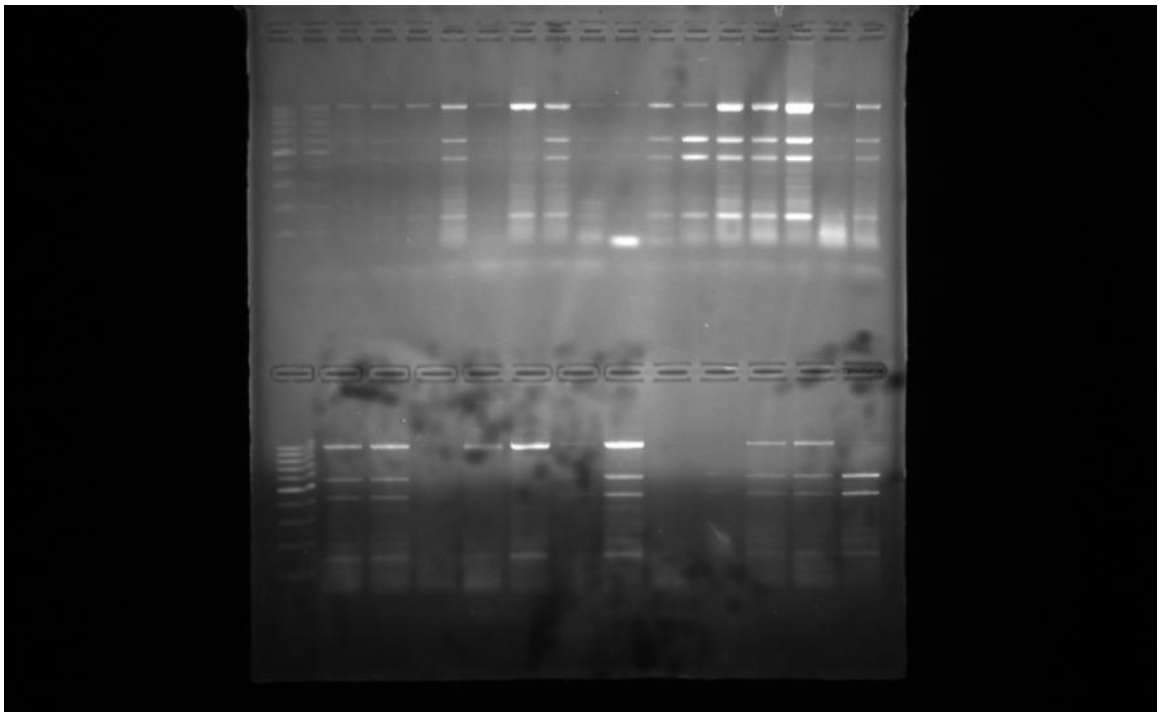


Figure 4.13. Restriction of growth hormone by restriction enzyme Sac1 showing polymorphism on the lanes

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17



AB AA AB AB AB AB AB AB AB

4.10.3 Pituitary specific transcription factor1 gene

Pituitary specific transcription factor 1 gene (PIT1) amplification produced a band of 599 bp (Fig.4.14), after cutting with Taq1 restriction enzyme, two bands (representing allele B) were observed in some line samples studied (Fig. 4.15), meaning the presence of two alleles (A, B). Only two genotypes were observed, the first was the homozygous genotype (AA) while the second genotype was the heterozygous genotype (AB), the homozygous genotype (BB) was not observed in any line. Results of allele frequencies as well as genotype frequencies are presented in table 4.25.

Figure 4.14. PCR of PIT1 Gene on the lanes

1 2 3 4 5 6 7 8 9 10 11

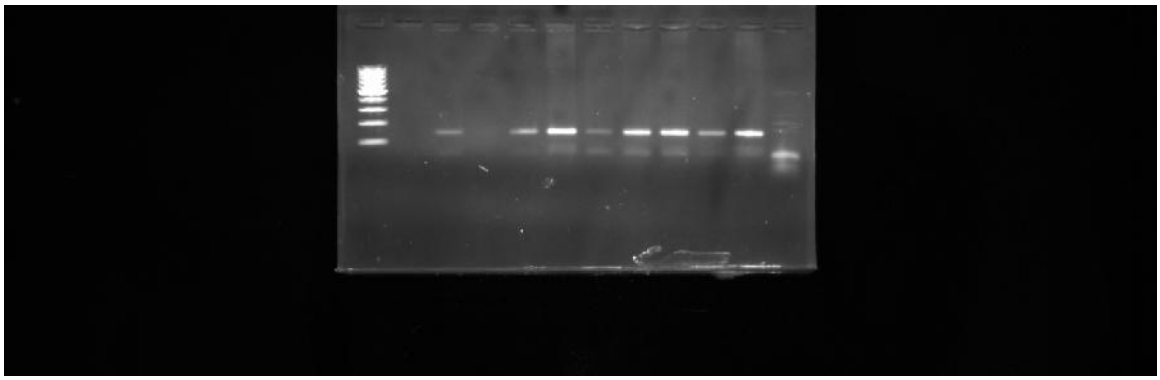
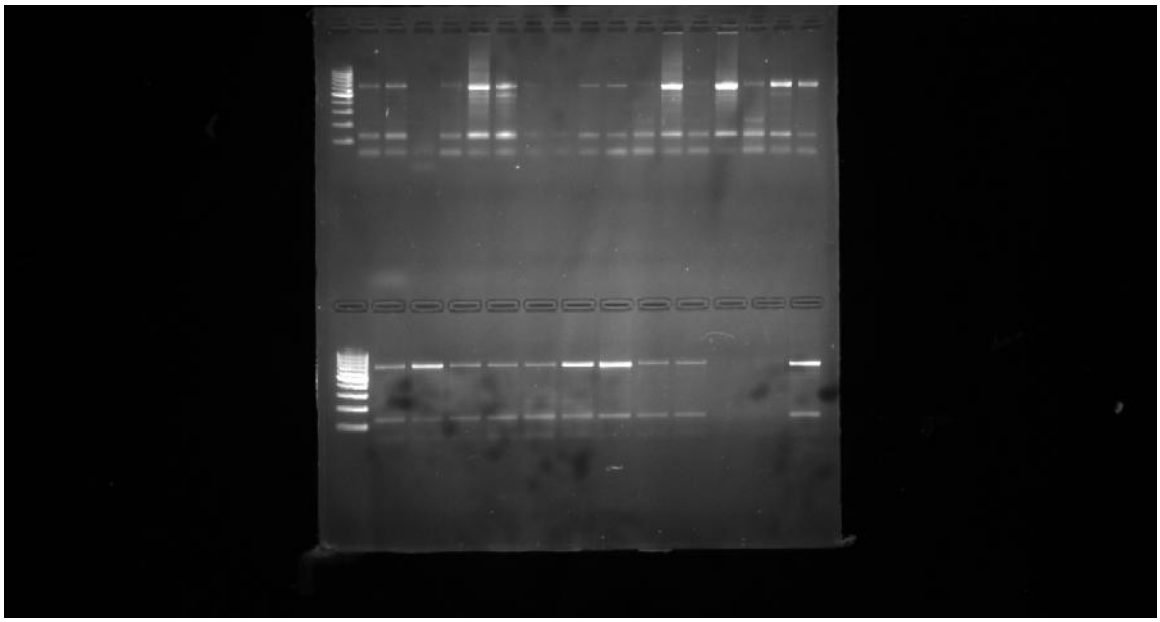


Figure 4.15. Restriction of PIT1 Gene with restriction enzyme Taq1 showing Polymorphism on the lanes

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15



AA AA AA AA AA BB

Table 4.23 PCR primers, cycle conditions, amplicon size and RFLP restriction enzymes used for the three genes studied.

Gene name (amplified part)	Primer sequence	PCR cycle	Amplicon size	Restriction enzyme	Reference
GH (PM1)	CTAAAGGACCTGGAAGAAGGG AACTTGTCGTAGGTGGGTCTG	initial denaturation at 95°C for 4 min, 35 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and an extension at 72°C for 7 min	1050 bp	Sac1	Makhsous *et al.,* 2013
PIT 1 (intron 5)	GGA CCC TCT CTA ACA GCT CTC GGG AAG AAT ACA GGG AAA GG	initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 62°C for 45 sec, 72°C for 1 min, and an extension at 72°C for 5 min	599 bp	Taq1	Rodbari *et al.,* 2011
PRLR (Exon 5)	TTGTCTGCTTTGATTCATTTCC TGCATTTCATTCTTCCCTTTTT	initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 59°C for 1 min, 72°C for 1 min, and an extension at 72°C for 5 min	250 bp	BamH1	Rashidi *et al.,* 2012

Both the GH and Pit1 loci were polymorphic with two alleles each (Table 4.23). The frequency of the A allele in the GH locus varied from a low of 0.375 in the BN2 line to a high of 0.782 in the FZ line (χ^2=20.61, P<0.01) In the Pit locus the frequency of the A allele ranged between 0.900 and 0.967(χ^2=10.129870, P>0.05)

Table 4.24. Frequencies of genotypes and alleles of GH gene in Sudanese indigenous Chicken lines.

Line	Allele frequency		Genotype frequency		
	A	B	AA	AB	BB
BN1	0.500	0.500	0.313	0.375	0.313
BN2	0.375	0.625	0.090	0.580	0.330
LB1	0.500	0.500	0.330	0.330	0.330
LB2	0.500	0.500	0.270	0.460	0.270
FZ	0.782	0.438	0.125	0.875	0.000
BT	0.429	0.571	0.143	0.571	0.286

Table 4.25. Frequencies of genotypes and alleles of Pit1 gene in Sudanese indigenous Chicken lines.

Line	Allele frequency		Genotype frequency		
	A	B	AA	AB	BB
BN1	0.938	0.062	0.875	0.125	0.000
BN2	0.967	0.033	0.933	0.067	0.000
LB1	0.941	0.059	0.882	0.118	0.000
LB2	0.938	0.062	0.875	0.125	0.000
FZ	0.900	0.100	0.800	0.200	0.000
BT	0.967	0.033	0.933	0.067	0.000

Table 4.26. Allele counts in the six populations in two loci

Population	GH Locus		PIT Locus	
	Allele A	Allele B	Allele A	Allele B
Pop 1	12	14	24	2
Pop 2	13	7	26	0
Pop 3	24	0	26	0
Pop 4	10	10	26	0
Pop 5	9	9	26	0
Pop 6	7	7	26	0
χ^2	20.613408		10.129870	
Probability	0.000958		0.199320	

The allele and genotype frequencies over all lines for the two loci are shown in Table 4.27. The frequencies of the A allele across all lines in the GH locus was 0.6148. In the Pit locus the frequency of allele A was 0.9872. The Chi square test indicated that the total chicken population was in equilibrium with regard to the GH locus while there were significant differences from Hardy-Weinberg expectations with regard to the Pit locus.

Table 4.27. Overall allele and genotype frequencies and Chi-square test

Locus	Genotypic frequency			Allelic frequency		χ_{cal}	Prob
	AA	BA	BB	A	B		
GH	0.3279	0.5738	0.0983	0.6148	0.3852	2.5207	0.1124
Pit	0.9871	0	0.1429	0.9872	0.0128	155.0065	0.0000

Table 4.28.presents the fixation index estimates (FIS) which is a measure of heterozygote deficiency or excess (Wright,1978).

Observed heterozygosity (Obs-Het) is the proportion of observed heterozygotes at the given locus while expected heterozygosity (Exp-Het) is the expected number of heterozygotes under random mating

Table 4.28. Summary of heterozygosity statistics for all loci averaged over all populations

Locus	Sample Size	Obs_Het	Exp_Het*	Nei**	Ave_Het	Fis	Fit	Fst	Nm***
GH	122	0.5738	0.4776	0.4737	0.4087	0.4614	0.2463	0.1472	1.4482
PIT	156	0.0000	0.0255	0.0253	0.0237	1.0000	1.0000	0.0649	3.6000
Mean	139	0.2869	0.2515	0.2495	0.2162	-0.3805	-0.1831	0.1430	1.4977
S.D.		0.4057	0.3197	0.3170	0.2722				

* Expected homozygosty and heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

*** Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

Table 4.29 presents the observed and effective number of alleles(Kimura and Crow (1964) as the reciprocal of homozygosity and estimates of Shannon's information index as a measure of gene diversity (Lewontin 1972).

Table 4.29. Effective number of alleles and shannon's Information index

Locus	Sample Size	na*	ne*	I*
GH	122	2.0000	1.8999	0.6666
PIT	156	2.0000	1.0260	0.0686
Mean	139	2.0000	1.4629	0.3676
St. Dev		0.0000	0.6180	0.4228

* na = Observed number of alleles

* ne = Effective number of alleles

* I = Shannon's Information index

Tables 4.30 show estimates of Nei's (1972) genetic identity and genetic distance and table 4.9 presents Nei's (1978) unbiased genetic identity and genetic distance estimates.

Table 4.30.Nei'soriginal measures of genetic identity and genetic distance

pop ID	BN1	BN2	LB1	LB2	FZ	BT
BN1	****	0.9734	0.8393	0.9960	0.9960	0.9960
BN2	0.0269	****	0.9387	0.9853	0.9853	0.9853
LB1	0.1752	0.0633	****	0.8660	0.8660	0.8660
LB2	0.0040	0.0148	0.1438	****	1.0000	1.0000
FZ	0.0040	0.0148	0.1438	0.0000	****	1.0000
BT	0.0040	0.0148	0.1438	0.0000	0.0000	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 4.31. Nei's unbiased measures of genetic identity and genetic distance

pop ID	BN1	BN2	LB1	LB2	FZ	BT
BN1	****	0.9817	0.8431	1.0049	1.0054	1.0069
BN2	0.0185	****	0.9422	0.9933	0.9938	0.9952
LB1	0.1706	0.0595	****	0.8698	0.8702	0.8714
LB2	-0.0049	0.0067	0.1395	****	1.0091	1.0000
FZ	-0.0054	0.0062	0.1391	-0.0091	****	1.0111
BT	-0.0068	0.0048	0.1376	-0.0105	-0.0110	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Figures 4.16 and 4.17 show the phylogenetic relationship among the six Sudanese fowl populations. The calculations were based on Nei's (1972) Genetic distance (the Neighbor-Joining, modified from NEIGHBOR procedure of PHYLIP Version 3.5) and the UPGMA method of clustering.

Figure 4.16.GH polymorphism.Phylogenetic relationships among six populations of Sudanese

Figure 4.17. Pit polymorphism. Phylogenetic relationships among six populations of Sudanesefowl

CHAPER FIVE

5.1 Discussion

In this study the indigenous bare neck chicken was characterized phenotypically using production performance data. The performance traits studied were body weight, body weight again, feed consumption, feed conversion ratio, age at first egg, egg quality characteristics, inedible carcass (blood, feather, head, legs, intestine, abdominal fat, wings, shank weight, shank length and back), edible carcass cuts (giblets) gizzard, heart and liver, edible carcass commercial cuts (breast, thigh and drumstick), Inaddition chemical composition and sensory evaluation were studied.

The results on body weight of Sudanese native chicken ecotypes of Bare neck, Large Baladi and Betwil in this study agreed with the results obtained by Binda, (2009). The body weight estimates were (27.83±4.24, 141.53±33.75 and 341.73±6.39g), (24.68±2.60, 109.28±25.77 and 271.90±25.18g) and (25.42±3.27, 121.23±26.68 and 301.80±62.36g) respectively during the periods of 0, 4 and 8 weeks of age. This showed that the Bare neck chickens were heavier than the Large Baladi and Betwil. They were inferior to the exotic breeds of Hybro and Hubbard which reached body weight of (37.85±2.23, 516.25±107.95 and 1269.63±242.16g) for Hybro and (39.76±3.77, 497.37±101.50 and 1230.46±258.06g) for Hubbard at the ages 0, 4 and 8 weeks in the study conducted by Tibin and Mohammed (1990). The difference in body weights between local and exotic breeds was remarkable and the reason for that was because the local breeds were not subjected to any improvement as the exotic breeds. Although the indigenous bare neck chickens had heavier body weights than their counterpart local chickens, they were not exposed to any improvement. Considering the body weight on the basis of sex, males were heavier than females throughout the rearing period (Table 4.2 and Figures 4.1, 4.2 and 4.3). These results are in agreement with the results of Yousif *et al*. (2006) who reported that the overall mean body weight ranged between 28.0±2.5g at hatching and 840.2±14.0g at 18 weeks of age with average body weight of males at the same period of 926.3±125.5g, whereas the corresponding weights of females was 769.8±97.0g during the same period. Sexual dimorphism was evident at all

ages with males being significantly (P< 0.01) heavier than females (Yousif *et al*., 2006). The indigenous chicken breeds in the tropics have been described as having slow growth rate. The study on Large Baladi chicken types of Sudan revealed that the birds can hardly attain 2 kg at adult stage (Desai 1962, Yousif and Osman, 1994). Considering the growth curve pattern Oluyemi and Oyenugo (1974) stated that the growth curve of the native chicken seemed to follow the normal sigmoid pattern and complete its rapid growth phase earlier than exotic breeds. Yousif *et al.* (2006) reported that from 10 to 12 weeks, the growth curve tended to increase and the birds attained maximum growth at the 12^{th} weeks. This was the point of inflection, where males gained higher body weight than females. Pavlovski *et al.,* (2009) using the naked neck-autochthonous breed of chickens in Serbia concluded that in general, chickens of autochthonous naked neck breed varieties of white, black and gray in extensive production systems at the duration of fattening of 98 days (14weeks) did not realize the body weight required by the present standards for fattening.

The results of body weight gains in this study agreed with the results reported by Binda (2009) on body weight gains at 4 and 8 weeks respectively were (113 and 200.2g) for Bare neck, (84.60 and 162.62g) for Large baladi and (95.81 and 180.57g) for Betwil. Bare neck chickens had better body weight gain than the Large Baladi and Betwil local chickens.

Feed consumption in this study is in agreement with the work of Binda (2009) who reported that feed intake of 282.59 and 704.39g for Bare neck, 243.88 and 675.19g for Large Baladi and 272.46 and 712.92g for Betwil at 4 and 8 weeks of age respectively, whereas the Bare neck of this study consumed 401.24g and 702.92g at the same period. The difference with the present study with the bare neck chickens during this period 4 weeks of age in which the bare neck chickens in the study consumed more feed when compared with Bare neck, Large Baladi and betwil in the study carried out by Binda but the Large Baladi consumed slightly higher feed.

The results of feed conversion ratios were close to the results obtained by Binda (2009) which were 2.77, 3.33 and 3.34 for bare neck, Large Baladi and Betwil Sudanese local chickens at 8 weeks of age.

The results of age at first egg were in agreement with the results obtained by El faki (2000) who reported that the age at first egg for Sudanese Large Baladi type ranged between 136.00 and 148.25 days. Elamin *et al*., (2004) found that the age at sexual maturity for Large Baladi type birds was 180.52±18.85 days. Likewise Howlider *et al*. (2003) found higher age at first egg which ranged between 169.00 and 170.60 days for Deshi normal and dwarf indigenous chickens of Bangladesh which was higher than the results of this study. Ershad (2005) also found higher age at first egg for Bangladesh Native Chicken of 175.00 days. The difference in age at first egg between the normal and dwarf chicken may have been due to difference in body weight prior to onset of sexual maturity (Khawaja *et al.*, 2013). This implies that age at first egg is correlated with body weight according to ecotypes, the smaller the body weight the earlier the age at sexual maturity, reported that within the same level of management; genetically heavier birds attained sexual maturity later than light body weight birds. The attainment of sexual maturity varied from breed to breed or strain and occurred at certain age and body weight. Adenowo *et al*. (1995) reported that sire influence as well as additive gene effects were important in the inheritance of age at first egg in crossbred chickens. A sex-linked gene and an autosomal one were ascertained by Greenwood and Blyth (1951) to be involved in the inheritance of sexual maturity. This character is also influenced by many environmental factors such as temperature, nutrition and lighting intensity, etc. Moreover, the modern poultry industry has succeeded in reducing age at first egg in layers up to 20 weeks (Moreng and Avens, 1984), which has been economically important. However, this reduction in age of layers must be considered with certain precaustion, because it may lead to the increase in the incidence of vaginal prolapse and hence would increase mortality within the flock.

Results of egg quality and characteristics in this study showed that egg weight was lower than that obtained by Yousif and Eltayeb (2011) and El tayeb (2009) from stocks of bare neck and Betwil (45.40±0.31 and 41.71±0.31g) respectively. Also Rajkumar *et al.* (2009) found egg weight for Indian indigenous chicken genotypes (NaNa, Nana and nana) were 56.45g, 56.72g and 56.11g, respectively. The egg weight of Nigerian indigenous chicken genotypes (Na na) naked neck and (nana) fully feathered were 45.04g and 40.83g (Yakubu *et al.* 2008). Hussain *et al.* (2013) found egg weight of

Pakistan indigenous chicken was 41.05±0.63g. All these mean values were higher than the value obtained in this study. The differences could be attributed to the fact that egg weight is largely affected by environmental factors, feed restriction and parental average body weight, evidence of genetic improvement could equally be observed (Cary *et al*. 1993).

The results of egg length in the present study were close to those obtained by Yousif and Eltayeb (2011). Rajkumar *et al.* (2009) reported the egg length of three Indian indigenous genotypes (NaNa, Nana and nana) was 57.92, 58.12 and 50.21mm respectively. Also Mohammed *et al*., (2005) obtained from Sudanese Bare neck and Betwil an egg length of 50.86±0.21 and 50.15±0.21 mm respectively and was close to the length found in this study and lower than that of Indian indigenous genotypes mentioned above. Yakubu *et al*. (2008) reported that egg length of Nigerian indigenous chickens (Na Na and na na) was 5.15 and 4.87 cm respectively which were also close to the value in this study. Hussain *et al.* (2013) found a higher egg length of 55.00±0.07mm in Pakistani indigenous chickens. The difference in egg length with Pakistani indigenous chickens may be due to the smaller size of eggs in this study.

The results of egg diameter in this study was in agreement with the results reported by Yakubu *et al.* (2008) on Nigerian indigenous chicken genotypes of NaNa and nana which were 3.64 cm and 3.54 cm. Also the mean values of 38.07±0.16 mm and 38.17±0.16 mm for bare neck and Betwil as reported by Yousif and Etayeb (2011) showed that the egg diameter of bare neck was higher and that of Betwil was close to the results of the present study. Yousif and Eltayeb (2011) reported the higher egg diameter of 40.07±0.16 mm.

Albumen height was significantly ($P< 0.01$ and $P< 0.05$) correlated with egg weight, yolk weight and Haugh unit. Albumen height mean value of 4.31±0.39 mm was lower than the value obtained by Yousif and Eltayeb (2011) which was 7.57±0.06 mm. Rajkumar *et al* .(2009) in Indian genotypes (NaNa, Nana and nana) found values of 5.97, 6.03 and 5.64mm. These were close to the results found in the present study with the exception of the genotype Nana which score a higher albumen height. El tayeb (2009) reported higher albumen height than the value found in this study.

The albumen weight results were in agreement with results of Yakubu *et al.* (2008) who reported albumen weight of 20.53 and 17.61g for NaNa and nana Nigerian indigenous genotypes and Hussain *et al.* (2013) who reported albumen weight of 21.56±0.63 which is close to the finding of the present study. While Yousif and Eltayeb (2011) reported higher albumen weight of 24.42±0.22g. Akhtar (2007) also found a higher albumen weight in Pakistani genotypes of Lyallpur Silver Black, Fayoumi and Rhode Island Red of 25.50±2.423, 23.99±2.926 and 25.14±1.911g, respectively. El tayeb (2009) reported higher albumen weight of Sudanese Bare neck and Betwil of 24.41±0.22 and 22.09±0.22g respectively. Rajkumar *et al*. (2009) also reported higher albumen weight of Indian genotypes of NaNa, Nana and nana to be 34.24, 34.65 and 33.26g respectively. The higher or the lower proportion of albumen may be because of larger or lower size of eggs recorded in the study.

The yolk weight results were close to the results obtained by Hussain *et al*. (2013). Whereas Yousif and Eltayeb (2011) reported a higher yolk weight of 15.02±0.14g and also El tayeb (2009) found a higher yolk weight of 15.02±0.14 and 14.13±0.14g in Bare neck and Betwil indigenous Sudanese chickens. Yakubu *et al.* (2008) found higher values of 16.95 and 16.05g in NaNa and nana Nigerian genotypes. The yolk weight of Indian genotypes NaNa, Nana and nana was 17.09, 17.05 and 17.78 g and showed also higher values of egg weight (Rajkumar *et al.*, 2009). Akhtar (2007) reported higher values of yolk weight (15.55±1.05, 16.29±1.205 and 16.83±1.380g) in Lyallpur Siliver Black, Fayoumi and Rhode Island Red of Pakistan.

Results of yolk height in agreement with our estimates amounting to 13.85, 14.24 and 14.98 mm for NaNa, Nana and nana genotypes were obtained by Rajkumar *et al.* (2009). The values of 1.19 and 1.05 cm of NaNa and nana of Nigerian indigenous chicken reported by Yakubu *et al.* (2008) were lower than estimates. Higher yolk heights were reported by (Yousif and Eltayeb, 2011) (16.75±0.08mm) and (El tayeb, 2009). (16.49±0.08mm).

The results of yolk index were in agreement with the results of Akhtar, (2007) who obtained yolk index of 0.476±0.034, 0.446±0.034 and 0.454±0.030 mm for Lyallpur Siliver Black, Fayoumi and Rhode Island Red genotypes of Pakistan. Yakubu *et al.*

(2008) found yolk index of Nigerian indigenous chickens in NaNa and nana genotypes of 50.60 and 48.77% which was close to the results of this study. Hussain *et al.* (2013) found a lower yolk index value of 30.90±0.91 in Pakistan local chicken. Rajkumar *et al.* (2009) also reported lower yolk index of 34.82, 36.79 and 38.29mm in NaNa, Nana and nana genotypes.

Shell weight was significantly ($P< 0.05$) correlated with egg weight, yolk weight and shell thickness (Table). Chatterjee *et al.* (2007) reported no significant breed difference in shell weight for six indigenous chicken breeds from Andamans. The results of shell weight in the present study were in agreement with the values of 4.48 and 4.65 g reported by Yakubu *et al.* (2008). Higher shell weights were reported by Yousif and Eltayeb (2011) (5.97±0.06), Hussain *et al.* (2013), (4.89±0.06), Rajkumar *et al.* (2009), (5.12, 5.02 and 5.07g for NaNa, Nana and nana) Tibin and Mohammed (2009), (5.97±0.06 and 5.48±0.05g in Bare neck and Betwil). Akhtar (2007) also reported higher shell weight of 7.29±0.620, 6.29±0.478 and 6.50±0.50g in Lyallpur Siliver Black, Fayoumi and Rhode Island Red.

The results of shell thickness in this study were in agreement with the findings obtained by Yousif and Eltayeb (2011) (0.385±0.005mm) and Yakubu *et al.* (2008), in NaNa and nana, (0.38 and 0.34mm).

The results of Haugh unit were in agreement with the results reported by Rajkumar *et al*. (2009) (75.94, 76.36 and 73.99 for NaNa, Nana and nana genotypes). Akhtar (2007) reported Haugh units values of 75.8±7.355, 75.1±6.533 and 72.2±5.99 in a study of Lyallpur Siliver Black, Fayoumi and Rhode Island Red. Yakubu *et al.* (2008) reported lower Haugh unit of 73.22 and 71.22 in NaNa and nana genotypes of Nigerian indigenous free range chickens. Likewise, Hussain *et al.* (2013) reported a lower Haugh unit of 71.40±1.48 in Pakistan indigenous chicken. Yousif and Eltayeb (2011) reported a higher Haugh unit of 77.81±0.46 in Sudanese indigenous stock. Akhtar, (2007) reported that Haugh unit determines albumen quality; higher Haugh unit mean better albumen contents.

The results of egg shape index in our present study were lower than the values of 76.36, 75.77 and 74.31 for NaNa, Nana and nana genotypes reported by Rajkumar *et al.* (2009). Hussain *et al.* (2013) also reported egg shell index as high as 80.40±1.19.

The blood weight was significantly (P< 0.01 and P< 0.05) correlated with live weight and feather weight at 12 weeks of age (Table 4.10). The blood percentage was determined on live weight and was in line with the results obtained by Zein-El Dein *et al.,* (1981) for Na/na and na na genotypes. They were also close to the results reported by Munira *et al*., (2006) on blood percentages of Barred Plymouth Rock (BPR), White Leghorn (WLH), Rhode Island Red (RIR) and White Rock (WR). Conversely, the blood percentage reported by Hagan and Agei (2012) on cockerel phenotypes and Thutwa, (2012) on chickens raised under intensive systems in South East District of Botswana were slightly higher than in the present study. This slight difference may be due to the size and age of the stock in the different studies. *Mahrous et al*., (2008) reported that the presence of the Na gene significantly increased blood percentage compared to normally feathered genotypes. This higher blood content in naked neck genotypes was probably due to greater haemoglobin concentrations and packed cell volumes associated with naked neck gene (Luger *et al.*, 1998; Raju *et al.*, 2004; Galal *et al.*, 2007) as a consequence of greater oxygen demand. This higher blood proportion may be due to the higher blood supply to organs and muscles (Galal and Fathi, 2001; Mahrous 2003 and Galal, 2007).

The feather weight was significantly (P< 0.01) and (P< 0.05) correlated with live body weight and blood weight at 12 weeks of age (Table 4.10). The feather weight percentage was determined on live weight basis and was in line with the results reported by Zein-El Dein *et al*., (1981) on Na/na and nana males and females genotypes. Munira *et al*., (2006) found similar results on Barred Plymouth Rock (BPR), White Leghorn (WLH), Rhode Island Red (RIR) and White Rock and are in conformity with the present results of the study. On the other hand, Hagan and Agei, (2012) and Thutwa *et al.*, (2012) found slightly higher feather percentage. This difference may be due to differences in genotypes and age between stocks studied The Na gene is associated with the reduction of feather on naked neck chickens. Fathi *et al*., (2013) stated that Na gene either in single

or double segregation state significantly reduced feather coverage compared to normally feathered counterparts. This reduced feathering is advantageous in facilitating better heat dissipation and tolerance to low protein diet.

The mean values of head weights were 19.87±3.38, 23.38±2.80 and 29.06±12.44g at 8, 12 and 16 weeks of age (Table 4.11). The head weights were significantly ($P< 0.01$) correlated with live body weight at 8 and 12 weeks of age and significantly ($P< 0.05$) correlated with the neck weight at 8 weeks of age. Also it was significantly ($P< 0.05$) correlated with leg weight at 16 weeks, and significantly ($P< 0.01$) correlated with gizzard weight at 8 weeks. It was significantly ($P< 0.05$ and $P< 0.01$) correlated with the heart weight at 8 weeks and 12 weeks of age (Table 4.12). The results in the present study are in agreement with the results reported by Binda (2009) on Sudanese indigenous chicken of Bare neck, Large Baladi, and Betwil which were 17.66±2.48g, 15.92±2.52g and 16.19±2.57g.

The mean values of leg weights were 23.33±6.70, 25.13±4.38 and 27.62±.94g (Table 4.11).The leg weight were significantly ($P< 0.01$) correlated with the live body weight, head, neck and heart weight at 16 weeks of age (Table 4.12). The results in the present study are lower than the results reported by Binda on Bare neck, Large Baladi and Betwil which were 33.01%, 32.84% and 32.41%.

The mean values of intestine weights were 27.47±4.9, 37.94±7.75 and 47.94±7.1g at 8, 12 and 16 weeks of age (Table 4.11). The intestine weight was significantly ($P< 0.05$ and $P< 0.01$) correlated with live body weight at 8 and 12 and 16 weeks of age respectively. It was significantly ($P<0.01$ and $P< 0.01$) correlated with r weight at 8 and 16 weeks of age and significantly ($P< 0.05$) correlated with back at 16 weeks of age (Table 4.13). The results of the present study are in agreement with Binda (2009) who reprted intestine weights of Bare neck, Large Baladi and Betwil were 28.60±5.58g, 23.23±5.03g and 21.60±4.43 respectively.

The mean values of abdominal fat weight were 0, 2.50±1.67 and 3.19±2.12g (Table 4.11). This showed low fat content in Bare neck chicken in the present study

which agrees with the findings of (Binda 2009). The abdominal fat showed significant (P< 0.01) correlation with the liver weight at 12 weeks of age (Table 4.13).

The mean values of wing weights were 24.93±4.13, 42.81±9.00 and 57.62±12.76g (Table4.11). The wing weights were significantly (P< 0.01) correlated with live body weight, liver and intestine weight at 12 and 16 weeks of age and also significantly (P< 0.05) correlated with intestine weight and shank length at 12 weeks and shank weight at 16 weeks of age (Table 4.13).

The mean values of shank weights were 5.87±2.17, 6.13±2.42 and 6.87±1.93g at 8, 12 and 16 weeks of age (Table 4.11).Shank weight showed significant (P< 0.05) correlation with live body weight, liver, intestine, wings and back weights at 16 weeks of age (Table 4.13).

The mean values of shank length were 4.53±0.74, 5.3±.0.48 and 5.94±0.00 cm (Table 4.11).Shank length was significantly (P< 0.05) correlated with live body weight at 8 and 12 weeks of age and wings and back at 12 and 8 weeks of age (Table 4.12). The results in the present study are in agreement with results reported by Binda (2009) on Bare neck, Large Baladi and Betwil which were 4.33±0.50cm, 3.88±0.55cm and 3.55±0.64cm.

The mean values of back weights were 41.33±10.07, 87.13±24.29 and 138.26± at 8, 12 and 16 weeks of age (Table 4.11). The back was significantly (P< 0.05) and (P< 0.01) correlated with live body weight at 8 and 12 and at 16 weeks of age. Also it was significantly (P< 0.05) correlated with liver, wings and shank weight at 16 and intestine at 8 weeks of age (Table 4.14). The results in the present study are lower than the results reported by Binda (2009) which were 26.81±3.22%, 25.18±3.88% and 27.32±3.64% respectively.

The mean values of edible carcass cuts weights (giblets) gizzard weights were 12.73±2.84, 15.88±2.06 and 17.25±3.62g (Table 4.14). The gizzard weight was significantly (P< 0.01 and P< 0.05) correlated with eviscerated carcass weight at 8 weeks and at 16 weeks of age, whereas it was significantly (P< 0.05) correlated with heart and liver at 8 and 16 weeks of age respectively (Table 4.15). The results in the present study

are close to the the results reported by Binda (2009) on Bare neck, Large Baladi and Betwil which were 16.73±5.16g, 12.23±2.29g and 13.52±3.18g respectively.

The mean values of heart weight were 3.00±1.36, 3.44±1.63 and 4.50±1.71g (Table 4.14). The heart was significantly (P< 0.01) correlated with eviscerated carcass weight at 8 and 16 weeks, whereas it was significantly (P< 0.05) correlated with gizzard weight at 8 and liver weight at 8 and 16 weeks of age (Table 4.15). The results in the present study are close to the results of Binda (2009) on Bare neck, Large Baladi and Betwil which were 1.93±0.77g, 1.61±0.53g and 1.67±0.49 respectively.

The mean weights of liver were 14.60±2.80, 20.81±4.02 and 21.94±6.31g (Table 4.14). The liver weight was significantly (P< 0.05) and (P< 0.01) correlated with eviscerated carcass weight at 8 and 16 weeks of age, whereas it was significantly (P< 0.05) correlated with gizzard and heart weight at 16 weeks and significantly (P< 0.01) correlated with heart weight at 8 weeks of age (Table 4.15). The results in the present study are lower than the results reported by Binda (2009) on Bare neck, Large Baladi and Betwil which were 8.94±1.81g, 7.39±1.56g and 7.36±1.93g respectively.

Results on eviscerated carcass weight showed mean weight values of 216.33±58.02, 323.75±68.48 and 449.06±84.07g (Table 4.16). Eviscerated carcass weight was significantly (P< 0.01 and P< 0.05) correlated with breast weight at 8and 16 and 12 weeks of age respectively, whereas it was significantly (P< 0.01) correlated with thigh weight at 8, 12 and 16 weeks of age and significantly (P< 0.01) correlated with drumstick at 8 and 16 weeks of age (Table 4.17).

The mean values of breast weight were 58.27±39.41g (16.25%), 71.25±14.14g (13.69%) and 115.38±0.75g (16.32%). Breast weight was significantly (P< 0.01 and P< 0.05) correlated with eviscerated carcass at 8,16 and 12 weeks of age, whereas it was significantly (P< 0.01) correlated with thigh weight at 8, 12 and 16 weeks of age and significantly (P< 0.01) correlated with drumstick weight at 8 and 16 weeks of age. The results in the present study are lower than results reported by Binda (2009) on bare neck, Large Baladi and Betwit which were 22.70±3.40%, 23.74±3.20% and 23.14±3.62% respectively.

The mean values of thigh weights were 40.93±25.06g (11.41%), 48.00±11.08g (9.23%) and 73.88±16.12g (10.45%). Thigh weight was significantly (P< 0.01) correlated with eviscerated carcass weight, breast weight and drumstick weight at 8, 12 and 16 weeks of age. The results in the present study are lower than that reported by Binda (2009)on Bare neck, Large Baladi and Betwil which were16.87±1.44%, 17.41±1.26% and 17.02±1.81% respectively.

The mean weights of drumstick were 37.33±32.50g (10.41%), 39.88±6.98g (7.66%) and 60.63±4.66g (8.57%). Drumstick was significantly (P<0.05) correlated with eviscerated carcass weight, breast weight and thigh weight at 8 and 16 weeks and was correlated with thigh weight at 12 weeks of age (Table 4.17).

The mean weights of breast meat were 45.07±36.32g (77.34%), 56.56±14.76g (79.20%) and 95.87±25.44g (83.09%) and corresponding breast bone weights were 12.27±4.33g (21.06%), 15.75±5.36g (22.11%) and 19.38±8.66g (16.80%). Breast weight was significantly (P<0.01) correlated with breast meat weight at 8, 12 and 16 weeks and significantly (P< 0.01and P< 0.05) with breast bone weight at 8 and 16 weeks of age respectively. Breast meat weight was significantly (P<0.01) correlated with breast weight at 8, 12 and 16 weeks of age and (P<0.05) correlated with breast bone weight at 16 weeks of age. The results in the present study are lower than results reported by Binda (2009) on Bare neck, Large Balad and Betwil which were 16.14±1.34%, 15.43±1.11% and 15.39±1.625.

The mean weights of thigh meat were 31.53±21.68g (78.01%), 37.88±9.65g (7892%) and 59.25±14.25g (14.25%) and corresponding mean weights of thigh bone were 9.33±8.06g (22.80%), 10.00±2.63g (20.83%) and 14.63±4.66g (19.80%). Thigh weight was significantly (P<0.01) correlated with thigh meat weights at 8, 12 and 16 weeks of age and (P< 0.01 and P<0.05) correlated with thigh bone weights at 8, 12 and 16 weeks of age, Thigh meat weight was significantly (P<0.01) correlated with thigh weight at 8, 12 and 16 weeks of age and (P<0.05) correlated with thigh bone weight at 8 weeks of age.

The mean weights of drumstick meat were 25.86±18.49g (77.69%), 29.63±6.21g (74.30%) and 46.88±11.31g (77.32%). The corresponding mean weights of drumstick were 9.00±4.52g (24.11%), 10.50±3.46g (24.12%) and 13.87±4.76g (22.88%) (Table 4.16). Drumstick weight was significantly ($P<0.01$) correlated with drumstick meat weight at 8, 12 and 16 weeks and correlated with drumstick bone weight at 8 weeks of age. Drumstick meat weight was significantly ($P<0.05$) correlated with drumstick bone weight at 8 weeks at age (Table 4.20).

The chemical composition of the chickens' meat under study revealed that the meat contained 77.06% moisture, 19.60% protein, 2.07% fat and 1.04% ash (Table 4.21). Compared with the previous studies made in similar indigenous chickens by (Binda, 2009) on Bare neck, Large Baladi and Betwil, (Zakeria, 2009) on Large Bladi Sported colour and (Mudir, 2011) on Betwil. The fat and ash are in agreement whereas the moisture and protein were slightly different. Moisture was slightly high and protein was slightly low in the present study. The slight difference that existed could be attributed to the stock management. Chicken meat was reported to contain 16.44-23.31% protein, 0.37-7.20% fat, 0.19-6.25% ash and 72.8-80.82% moisture content (Smith *et al.*, 1993; Xiong *et al.*, 1999; Abeni and Bergoglio, 2001; Al-Najdawi and Abdullah, 2002; Van Heerden *et al.*, 2002; Wattanachant *et al.*, 2004 and Chuaynukool *et al*., 2007). The estimates obtained in the present study are all within these ranges of values. However, it appear that the indigenous chicken contains low fat and high protein content. Ding *et al.*, (1999) showed significant differences in fat contents between broiler and local chickens. Wattanachant *et al*., (2002) found that Thai indigenous chicken muscle contained higher protein but lower fat and ash contents compared to broiler chickens.

Sensory evaluation of breast, thigh and drumstick for the attributes tenderness, flavor, colour and juiciness revealed that thigh scored 35.52% in tenderness followed by breast (33.73%) and drumstick (30.75%) scored the least, which indicated that thigh was more tender than breast and drumstick was the least in tenderness. With regard to flavor, the highest score was that of breast (36.39%) followed by drumstick (32.95%) and thigh was the least (30.66%). This showed that breast had better flavour than drumstick and thigh. Never the less, for the colour, breast and thigh had the highest score (34.66%)

and drumstick was the least (30.68%). This indicated that the breast and thigh had better colour than drumstick, while for juiciness the breast had highest score (37.06%) followed by thigh with (34.19%) and drumstick was least (28.75%). This showed that the thigh was juicier than breast and drumstick (Table 4.22 and Figure 4.9).Generally the percentages of the taste panel showed little variations in subjective judgments. The results in the present study are within the range of those reprted by Binda (2009) who reported no significant difference (P> 0.01) between the exotic meat type strains and native chicken ecotypes interms of flavor, tenderness, juciness and general acceptability, the only significant difference was in colour. Also Tibin and Mohamed (1990) found no significant differences between the exotic breeds and the indigenous chicken in all sensory parameters evaluated.

Five main characteristics which contribute to the overall eating quality of meat are taste, texture, juiciness, appearance and odor. Among these characteristics, texture is probably considered to be the most important attribute by the average consumer (Dransfield, 1994; Chrystall, 1994). Mechanical factors (tenderness) and juiciness (succulence) contribute to different meat textures. The tenderness of meat is the sum total of the mechanical strength of the skeletal muscle tissue and it's weakening during the post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animals and fowls (Takahashi, 1996). Meat tenderness originates in structural and biochemical properties of skeletal muscle fibers, especially myofibrils and intermediate filaments and of the intramuscular connective tissue, the endomysium and perimysium which are composed of collagen fibrils and fibers. The attractive appearance to consumer of indigenous chicken meat is result by its carcass conformation, skin or meat color which might be related to chicken genotypes, feeds, rearing system or even processing conditions. There are many intrinsic and extrinsic factors including genotype or breed, age, rearing system, feeds, chemical composition, structure and properties of muscle and processing conditions which can have an influence on different quality characteristics of chicken meat (Wattanachant, 2004a).

The tenderness of chicken meat decreased during muscle growth (Nakamura *et al*., 1975; Wattanachant, 2007). Probably because of the structural changes of collagen (Fang et al., 1999; Nakamura *et al*., 2004). Wattanachant and Wattanachant (2007) stated that the appropriate age for indigenous chickens to possess economical live weight and high quality was in the 16-18 weeks of age.

The genetic information on polymorphisms in this study were obtained through subjecting PRLR, GH and PIT1 gene sequences to restriction enzyme BamH1, Sac1 and Taq1 respectively to get information on the six lines of Sudanese indigenous chickens.

The results of genotyping of PRLR gene in the six Sudanese chicken lines for the PRLR gene did not showed any polymorphism and shows only one allele (B) and consequently one genotype (BB). The heterozygous genotype AB was absent. The absence of heterozygous individuals among females was expected due to the localization of the gene on the Z chromosome and consequently it is a sex linked gene (Cheng *et al.*, 1995). In chickens, previous studies have suggested that the PRLR gene was attractive as a candidate gene for broodiness, egg production and age of first egg in chicken (Romanov *et al.*, 2002; Liu *et al.*, 2012; Zhang *et al*., 2012). Dunn *et al.* (1998) have mapped the chicken PRLR gene as a candidate gene for the control of broodiness, because a major gene involved in susceptibility to broodiness is thought to reside on the chicken Z chromosome (Tanaka *et al.,* 1992) and manifest as a sex-linked trait. Rashidi *et al.* (2012) found that individuals with AA genotype produced higher eggs than BB genotype. They found that the AA genotype produced five eggs higher than BB genotype during recorded laying time. This may partly explain why studied Sudanese chicken lines produced less eggs. The frequency of allele B was 1.0 while the allele A was absent completely, which is at variance with the results reported by Rashidi *et al.* (2012) who found that the frequency of A allele was higher (0.72) than B allele (0.28) at PRLR5 marker site in the Mazandaran Iranian chickens. Prolactin receptor gene is mediating the work of prolactin and growth hormone receptor genes (Kitamura *et al.,* 1994; Bole-Feysot *et al.,* 1998). Presence of one allele for the PRLR gene in the chicken lines under study will probably reflect on the productive and reproductive traits of these chickens.

Regarding the growth hormone gene, it showed polymorphism with two alleles (A, B) in all the lines genotyped. This gene was localized by Shaw *et al.* (1991) on the long arm of chromosome 1. Earlier studies reported the association between growth hormone alleles and productive traits. Feng *et al.* (1997) found that GH genotype was significantly associated with age at first egg as well as the hen-day rate of egg production. Kulibaba and Podstreshnyi (2012); Makhsous *et al.* (2013), Su *et al.* (2014) reported that polymorphism of the GH gene affects egg laying hens; egg production and rate of laying eggs. On the other hand, Yan *et al.* (2003) Ghelghachi et *al.* (2013), Mehdi and Reza (2012) found an association between the polymorphism of this gene and carcasses composition, growth and fatness. In the present it was study we found almost equal distribution of the two alleles (A, B). This is different from what was reported earlier by Enayati and Rahimi-Mianji, (2009) who found that the allele A was the most frequent and ranged from 0.99 to 0.79. They attributed this observation to the long term selection strategy used in the population they studied.

The PIT1 gene demonstrated polymorphism in the six lines studied. According to the chicken genome sequence, this gene is localized on chromosome 1 (**M**GC/ORFeome, 2006). Our results are similar to earlier reports of polymorphism in this gene (Nie et al., 2008; Rodbari *et al.,* 2011; Bhattacharya *et al.,* 2012).

Regarding the absence of BB genotype, in this study lines and the presence of AA and AB genotypes only this may be due to the effect of seclion or it could be a sampling antefact The predominance of allele A over allele B was very clear at this studied lines since allele A frequency exceeded 0.9 in the chicken lines studied. The PIT1 gene is reported to be significantly associated with body growth and body composition traits (Rodbari *et al.,* 2013). PIT 1 seemed to have higher effects on chicken early growth, it was associated with average daily gain at 0-4 and 4-8 weeks, body weight at 21, 28, 35 and 42 days, and shank diameters at 63 days (Nie *et al.,* 2008). In view of these previous observations the chicken Pit1 gene is regarded as a key candidate gene for production traits (Nie *et al.,* 2008).

The GH locus was reasonably informative with regard to polymorphism information content (0.6666) in contrast with the Pit locus (0.0686) (Table 4.7). The observed proportion of heterozygosity for the GH locus was 0.5738 while the Pit locus showed no heterozygosity. On the other hand expected heterozygosity for the GH locus

was 0.4776 and that of the Pit locus was 0.0255. The GH locus did not deviate significantly from Hardy-Weinberg expectations(χ^2_{cal} = 2.5207, P=0.1124) while the PIT did deviate significantly from expectations (χ^2_{cal} =155.01, P=0.00) The values of observed and expected heterozygosity among loci may vary as a result of evolutionary forces, such as mutation and random genetic drift, affecting loci differently and thus creating differences in the amount of heterozygosity (Nei, 1978).

F statistics are used to clarify the reasons for deviations from Hardy Weinberg expectations. The mean FIT, FST and FIS estimates among the six Sudanese chicken populations, are reported in table 4.6. The fixation index is the probability of two alleles taken from the same subpopulation (or the same or different individuals) are identical by descent.The value of FST was estimated to be 0.1430. This value indicates the presence of average genetic differentiation. FST (for sub-population S relative to metapopulation T) is the fixation index and it is the proportion of the total inbreeding in a population due to differentiation among sub-populations. It varies between 0 and 1 and is calculated by the formula: FST = (FIT - FIS)/(1 - FIS). The average inbreeding value at the total sample level (FIT) was -0.1831. Negative estimates of this parameter indicate either the presence of selection favouring heterozygotes or outbreeding. The mean Fis was-0.3805 which again indicates non random mating.

Phylogenetic relationships based on Nei's (1972) genetic distance among the populations were visualised through a Neighbourjoining tree (Figures 1 and 2). The least genetic identity was between BN1 and LB1 (0.8393). LB1showed comparatively lower genetic identity estimates with all other five populations (0.8660) in comparison to the genetic identity estimates among the other populations (0.8393-1.00). The UPGMA dendrogram among the six Suanese domestic chicken populations showed that LB1 was distinct from the other populations and formed a separate cluster, whereas the remaining five groups of domestic chicken were clustered together into another group (Fig.1). The clustering of LB2 with FZ suggests a close relationship between these two populations.

5.2 Conclusions and recommendations

There are a number of studies dealing with the phenotypic characterization of Sudanese indigenous chicken including bare neck chicken. However, the indigenous chicken in general and bare neck chicken in particular have slow growth rate and hardly reach market weight at 16 weeks of age when compared with the exotic breeds which reach market weight at 35 days. Male chickens were heavier than female counterparts.

Age at first egg was significantly ($P<0.01$) correlated with egg weight, and the body weight was also significantly ($P<0.01$) correlated with egg weight.

Egg quality characteristics showed some positive and other negative correlations with other traits. The positive correlation of egg weight was significantly ($P<0.01$) correlated with egg diameter, albumen weight, yolk weight, yolk height, egg shape index and yolk index and significantly ($P<0.05$) correlated with albumen weight and negatively correlated with shell weight. The positive correlations between traits, if they are a result of positive genetic relationships may mean the possibility of positive selection responses while the negative ones may mean improvements in one trait may result in reduction in others.

The chemical composition of the bare neck showed low fat content as well as the abdominal fat. This low fat content may be advantageous to human nutrition in avoiding heart diseases which are correlated with high cholesterol levels.

There were generally positive significant correlations among the commercial cuts (breast, thigh and drumstick). Also positive correlations were detected between breast meat and bone, thigh meat and bone, drumstick meat and bone.

Few studies have been done on the genetics of Sudanese indigenous chicken. In this study prolactin receptor gene, growth hormone gene and PIT1 gene were studied. Prolactin receptor gene after cutting with BamH1restriction enzyme showed one allele (B) representing one homozygous genotype (BB). Growth hormone gene after cutting with Sac1 restriction enzyme showed two alleles (A, B) representing two homozygous genotypes (AA, BB) and one heterozygous genotype (AB).

Pituitary specific transcription factor1 gene after cutting with Taq1 restriction enzyme showed two alleles (A, B) representing homozygous genotype (AA) and heterozygous genotype (AB).

Due to the presence of polymorphism in the Pit1 and GH genes, their association with egg production and growth traits, they can be used as candidate genes in poultry breeding programs while PRLR cannot be due to the absence of polymorphism in it.

More studies are needed to characterize the six lines of Sudanese indigenous chicken genetically in order to determine the genetic potential of each line in terms of growth rate and egg production.

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(http://www.ias.ac.in/currsci/oct 25/ articles19.htm)

Appendix

Appendix (I)

MIX COLOR FEATHER LARGE BALADI CHICKEN (LB1) BUTANA AREA

Appendix (II)

WHITE COLOR FEATHER LARGE BELADI (LB1) CHICKEN BUTANA AREA

Appendix (III)

CROWN FEATHER LARGE BALAI CHICKEN (LB1) BUTANA AREA

Appendix (IV)

BROWN COLOR FEATHER BARE NECK CHICKEN (BN2) BUTANA AREA

Appendix (V)

MIX COLOR FEATHER BARE NECK CHICKEN (BN2) BUTANA AREA

Appendix (VI)

BACK COLOR FEATHER BARE NECK CHICKEN (BN2) BUTANA AREA

Appendix (VII)

RED COLOR FEATHER BATWEL COCK (NUBA MOUNTAIN) (99((CHI) CKEN-SUDAN

Appendix (VIII)

MIXED COLOUR FEATHER BETWIL COCK (NUBA MOUNTAINS)

APPENDIX (IX)

LIGHT BROWN FEATHER BETWIL CHICKEN (NUBA MOUNTAIN) MIX COLOR FEATHE

Appendix(X)

FRIZZLE SUDANESE CHICKEN

Appendix(X1)

SENSORY EVALUATION CARD

Evaluate these samples for colour, flavor, juiciness, and tenderness. For each sample the appropriate scale to show your attitude by checking at the point that best describes your

Feeling about the sample, if you have any question please ask. Thank you for your cooperation.

Name:..... Date.....

Tenderness	Flavour	Clour	Juciness
8- Extremely tender	8- Extremely intense	8- Extremely desirable	8- Extremely juicy
7-Very tender	7- Very intense	7- Very desirable	7- Very juicy
6- Moderately tender	6- Moderately intense	6- Moderately desirable	6- Moderately juicy
5- Slightly tender	5- Slightly intense	5- Slightly desirable	5- Slightly juicy
4- Slightly tough	4- Slightly bland	4- Slightly undesirable	4- Slightly dry
3- Moderately tough	3- Moderately bland	3- Moderately undesirable	3- Moderately dry
2- Very tough	2-Very bland	2- Very undesirable	2- Very dry
1- Extremely tough	1- Extremely bland	1- Extremely undesirable	1- Extremely dry

Scale	score					Comment
1						
2						
3						
4						
5						

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13						
14						
15						