FUNCTIONAL POLYMORPHISMS AT INSULIN GROWTH FACTOR-1 AND PROLACTIN CANDIDATE GENES FOR MEAT AND EGG PRODUCTION IN INDIGENOUS CHICKENS OF KENYA

PATRICIA BEVERLY ASWANI

MASTER OF SCIENCE

(Biotechnology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

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Functional polymorphisms at insulin growth factor-1 and prolactin candidate genes for meat and egg production in indigenous chickens of Kenya

Patricia Beverly Aswani

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DECLARATION

This research thesis is my original work and has not been presented for a degree in this or any other university.

Signature: …… Date: ...……

Patricia Beverly Aswani

This thesis has been submitted for examination with our approval as university supervisors.

Signature: …… Date: ...……

Dr. Sheila Ommeh, PhD

JKUAT, Kenya

Signature: …… Date: ...……

Dr. Jacqueline Lichoti

Ministry of Agriculture, Livestock and Fisheries, Kenya

DEDICATION

This work is dedicated to, D’vock, D’vania, Philip and Risper

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LIST OF ACRONYMS AND ABBREVIATIONS

ASALs Arid and Semi-Arid Lands

AMOVA Analysis of Molecular Variance

ANOVA Analysis of Variance

bp Base Pair

d.f degrees of freedom

DNA Deoxyribonucleic acid

EDTA Ethylenediaminetetraacetic acid

GPS Global Positioning System

FAO Food and Agricultural Organization

IBR Institute for Biotechnology Research

IGF-1 Insulin Growth Factor 1

JKUAT Jomo Kenyatta University of Agriculture and Technology

MEGA Molecular Evolutionary Genetics Analysis

MJ Median Joining

MUPID Mini Electrophoresis Unit

MUSCLE Multiple Sequence Comparison by Log- Expectation

mv	Median vectors
NJ	Neighbor Joining
PCR	Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism
TBE	Tris Boric Ethylenediaminetetraacetic acid
µl	Micro liter

ABSTRACT

Indigenous chickens are important livestock in Kenya since they provide a large proportion of quality protein in human diet as of meat and eggs. Indigenous chicken are primarily reared under the free range system where they are left to scavenge for food, with minimal feed supplementation or veterinary inputs. This study aimed to 1) characterize the phenotypes associated with meat and egg production in indigenous chickens in Kenya, 2) assess the functional polymorphisms at *IGF-1* gene, a candidate for meat production in indigenous chickens in Kenya 3) assess the functional polymorphisms at *PRL* gene, a candidate for egg production in indigenous chickens in Kenya from Lake Turkana basin, Lamu archipelago and Mt. Elgon catchment. Data on 296 indigenous chickens was collected from farmers by the use of questionnaires. Blood samples were stored on FTA® cards. Analysis of variance (ANOVA) for the phenotypic traits was carried out across the agro-climatic zones. There were significant interactions between shank length, body length and live weight for meat production and the number of yolks per egg for egg production. All the other traits (numbers of eggs laid per hen per clutch, the number of eggs incubated per hen per clutch and the number of eggs hatched per hen per clutch) had no significant difference in the various agro-climatic zones. Despite being the lightest and small bodied, chickens from Lake Turkana basin produced more eggs than the heaviest and big bodied. Lamu Archipelago produced the highest number of double-yolked eggs. For molecular work, Insulin like growth factor 1(*IGF-1*) gene and Prolactin gene (*PRL*) were amplified using PCR, purified and sequenced. Editing of the sequences was done and the sequences aligned. A total of 3 *IGF-1* haplotypes were mapped. Phylogenetic analysis displayed haplotype 9C as the ancestral haplotype since it was in the same clade with the ancestral red jungle fowl and it dominated in Lake Turkana basin. Haplotype 11C dominated Mt.Elgon catchment and Lamu archipelago zones while haplotype 9A was least in all the zones. Analyses of molecular variance (AMOVA) values were highest within individuals at 87.85% an indication of high phenotypic variation for potential genotypic phenotype association.

No recombination events were observed. Two *PRL* haplotypes; 154 and 130 were mapped. Phylogenetic analysis displayed haplotype 130 as the ancestral haplotype since it was in the same clade with the ancestral red jungle fowl and it dominated in Lake Turkana basin. Haplogroup 154 was dominant in all the agro-climatic zones. The AMOVA values were highest within individuals, an indication of high phenotypic variation for potential genotypic phenotype association. No recombination events were observed. In conclusion this study successfully characterized the phenotypes associated with meat and egg production. Functional Polymorphisms in *IGF-1* and *PRL* for meat and egg production were established. These initial results should pave way towards the development of molecular breeding tools for meat and eggs in indigenous chicken and their conservation by sustainable use.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Indigenous chicken is one of the most important livestock worldwide because it provides a large proportion of protein in human diet in terms of eggs and meat. Chickens have also been used for ornamental purposes (e.g. silkie or bantams) and entertainment (e.g. gamecocks used for cockfighting). A survey carried out in 2010 in Kenya showed the population of chickens to be approximately 32 million and out of these, almost 70% were indigenous chicken whereas the commercial and hybrid constitute the remaining 30% (Kenya National bureau of Statistics 2009). Most of the indigenous chicken were kept by the majority rural poor to fulfill multiple functions among them supply of cheap affordable protein in their nutrition and source of income (Magothe, Okeno, Muhuyi, & Kahi., 2012).

The diversity within indigenous chicken is extensive, which should provide an excellent base for breeding animals that are well adapted to a variety of local environmental conditions. However, the industrialization and globalization of chicken production in the 20th century adversely affected the distribution of chicken genetic resources worldwide, practically limiting the breed composition to commercial stocks of broilers and egg-type, laying hens. Consequently, many chicken breeds have already become extinct or are seriously endangered with extinction (Magothe et al., 2012).

In the 1900s, commercial chickens were produced from a small founder population (Muir et al., 2008). The broiler industry is comprised of three founders which are Plymouth Rocks/Cornish cross, Cornish/White Rock cross and White Rock /Plymouth Rock cross, whereas the layer comprises of two population founders which are White leg horn and Rhode Island Red (Muir et al., 2008) see figure1.1. Genetically these founder populations represent only five individuals which are the parental stock of the

billions of commercial chickens. The aim of developing these commercial chickens was to increase the production of meat and eggs (Muir et al., 2008). Though this was successful, there was a trade- off of production traits with undesirable traits, and these led to loss of adaptability and resistant traits (Muir et al., 2008). But more important for this study is the reduced quality of meat and eggs in terms of flavour and taste and the micro nutrients which were lost (Muir et al., 2008). Consequently, it is feared that the continuous cross-breeding of the indigenous chicken with exotic chickens to increase meat and egg production as is the current practice of the national poultry breeding program will lead to the loss of diversity as has been seen in the Western countries (Muir et al., 2008).

Broilers-3 founder populations

Plymouth Rocks

Cornish

White Rock Chicken

Layers-2- founder populations

Rhode Island Red

White leghorn

Figure 1.1 Pictures of broilers and layers founder populations

1.1.1 Chicken production systems

Kenyan indigenous chickens have been known to have high genetic diversity, which may be of great value as a genetic resource for genetic improvement (Mwacharo et al., 2011). Indigenous chicken have been ignored and exotic chickens given priorities because indigenous chicken produce less meat or eggs (Okeno, Magothe, Kahi, & Peters., 2012). The preference for indigenous chicken meat is attributed to its characteristic leanness; flavour and presumed organic product, with an assumption that they have got a high nutritional value and are less associated with increasing lifestyle diseases (Kingori, Wachira, & Tuitoek., 2010).

A bio-economic model developed by Okeno, Kahi, & Peters, (2012) accounting for the risk attitude of the farmers was used to evaluate the utilization of indigenous chickens under different production systems. The model classified the production systems into three categories based on the level of management; 1.Free-range system, where chickens scavenge for feed resources with no supplementation and healthcare the common system of poultry production practiced by many in the rural areas; 2. Intensive system, where the chickens are permanently confined and supplied with rationed feed and healthcare for example Kenchic, Sigma and Muguga production systems; 3. Semi-intensive system, a hybrid of free-range system and intensive system, where the chickens are partially confined, supplemented with rationed feeds, provided with healthcare and allowed to scavenge within the homestead or in runs common around the peri-urban areas like Mombasa, Nakuru, Wangige, and Kikuyu among others (figure 1.2). The results suggested that utilization of indigenous chickens in their current genetic merit and production environment was more profitable under and free-range and semi-intensive systems but not economically viable under intensive system (Okeno et al., 2012). Little effort has been made to characterize indigenous chicken and the environment in which they are produced. Indigenous chicken performance has been evaluated but their unique physiological and behavioral characteristics and their sociocultural values have been ignored. Currently the National Poultry Breeding programs are using traditional methods

of crossbreeding indigenous chicken and commercial chicken which will eventually lead to the loss of the genetic diversity that is found in indigenous chicken.


Free range system: no feed supplement, no veterinary input


Semi intensive free to roam, minimal feed supplement minimal or no veterinary inFigure


Intensive system strictly confined, full veterinary input and feed supplem

Figure 1.2 showing different production systems in Kenyaput

1.1.2 Meat industry

By the second half of the 20th century a five-fold increase in meat production has been recorded. Due to the growing human population of about seven billion, man is forced to share the planet and its resources with nearly 1.0 billion pigs, 1.3 billion cattle, 15.4 billion chickens, 1.8 billion sheep and goats (Fayemi & Muchenje, 2012). Diverse vegetation patterns and human preferences in various agro-ecological zones have been implicated for the variation in animal population all over the world (Fayemi & Muchenje, 2012). The total world production of four major types of meat has been estimated to be 83.2 million tons for pork, 53.9 million tons for poultry, 53.2 million tons for beef and 7.0 million tons for mutton (Warriss, 2010). A total of 20,889 metric tonnes of chicken meat is consumed in Kenya annually. (Behnke & Muthami, 2011)

1.1.3 Egg industry

Eggs are a great source of protein and they contain numerous vitamins, and potassium (Messens 2005). It is best to acquire any source of protein from an environment that is as natural as possible, meaning the animal was able to feed on foods that its body could tolerate, in conditions that were not overly stressful. For egg-producing chickens, this environment is often called “cage-free” or “free-range. This means the chicken was allowed to roam, picking what it wanted to eat. Research has shown that cage-free hens produce eggs higher in various vitamins. Free-range eggs actually show greater resistance to pathogens like salmonella (Messens 2005). Annually Kenya produces about1.3 billion eggs worth 9.7 billion Kenyan shillings (Republic of Kenya, 2010).

1.2 Statement of the problem

The current National poultry breeding program encourages the use of conventional breeding methods such as cross breeding with exotic breeds to increase meat and egg production, consequently these will lead to genetic dilution among the indigenous chicken and this may affect future production traits such as quality of meat and eggs in terms of nutrition and taste.

1.3 Justification of study

Indigenous chicken are known to have diverse production traits and produce quality meat that has high micronutrient and a preferred taste. In Kenya, there are indigenous chicken ecotypes that are known to have high body weight hence produce a lot of meat and also are known to lay a lot of eggs. This research will examine the polymorphisms of two underlying candidate genes for egg and meat production among indigenous chicken. Based on the results obtained from this study independently or with other findings, molecular breeding of indigenous chicken will be adopted by the national breeding program and the farmers.

1.4 Objectives

1.4.1 Main objective

To investigate the polymorphism at insulin growth factor-1 and prolactin candidate genes for meat and egg production in indigenous chickens in Kenya

1.4.2 Specific objectives

1. To characterize the Phenotypes associated with meat and egg production in indigenous chickens of Kenya.

2. To assess the functional polymorphisms at Insulin Growth Factor-1 candidate gene polymorphisms for with increased meat production in indigenous chickens of Kenya.

3. To assess the functional polymorphisms at Prolactin candidate gene polymorphisms for with increased egg production in indigenous chickens of Kenya.

1.5 Hypotheses

1.5.1 Null hypotheses

1. Indigenous chickens in Kenya do not have phenotypes associated with increased meat and egg production.

2. There are no polymorphisms at Insulin Growth Factor-1 candidate gene and Prolactin candidate gene for increased meat and increased egg respectively among indigenous chicken in Kenya.

1.5.2 Alternative hypotheses

1. Indigenous chickens in Kenya have phenotypes associated with increased meat and egg production.

2. There are polymorphisms at Insulin Growth Factor-1 candidate gene and Prolactin candidate gene for increased meat and increased egg respectively among indigenous chicken in Kenya.

1.6 Research questions

1. What are the phenotypes associated with increased meat and egg production in indigenous chicken in Kenya?

2. Are there polymorphisms at Insulin Growth factor-1 candidate gene for with increased meat among indigenous chicken in Kenya that can be used in molecular selection breeding?

3. Are there polymorphisms at Prolactin candidate gene for with increased egg among indigenous chicken in Kenya that can be used in molecular selection breeding?

CHAPTER TWO

LITERATURE REVIEW

2.1 Production phenotypes

Characterization of phenotypes is necessary for important traits like adaptation traits and production traits. A study by (Moraa et al., 2015) was able reveal the ability to cope with heat stress as an important adaptation trait.

Different studies in Africa have revealed the existence of considerable variation of different traits within and among local chicken populations. Halima, Neser, van Marle-Koster, and de Kock. (2007) reported the existence of variations between genetic groups of local chicken in Ethiopia as indicated by high heterozygosity values. On the other hand, Ajayi (2010) reported the heritability estimates for body weight in the Nigerian local chicken populations that indicated the dual potential for development into a meat or egg breed.

Studies in Tanzania have been conducted with the aim of identifying and improving the performance of local chickens (Msoffe et al., 2004; Lwelamira, Kifaro, & Gwakisa., 2008). The Results from these studies showed existence of many genotypes, phenotypes and varied productivity potential within local chicken populations hence indicating the possibility of improving genetic potential through selective breeding within and between local chicken populations. Msoffe et al., (2004) reported large variations in reproduction and production performance of local chickens in Tanzania. Recently Guni, Katule and Mwakilembe., (2013) characterized selected indigenous chickens from southern highlands of Tanzania.

In Kenya (Njenga, 2005 ; Okeno et al., 2012) studies revealed that farmers carry out chicken selection at household level based on growth rate, large body size, high egg production, hatchability and good mothering ability. Farmers, Marketers and Consumers

preferred the above mentioned traits for economic gain and such traits therefore should be given priority when developing breeding objectives for improvement of indigenous chicken. Entrepreneurship and Sufficiency, (2010) inferred that linear body measurements like shank length, drumstick length, drumstick circumference and chest circumference are easy to measure and suggested that these variables may be used to predict body weight of indigenous chicken especially under field conditions.

Olwande et al., (2010) reported significant clutch sizes and hatchability rates in Komolorume village in Kenya. Njenga, (2005) reported that the three major objectives of poultry rearing were for food, sale, and cultural uses and concentrated more on the hatchability so as to achieve the objectives.

2.2 Candidate genes

The candidate gene associated with increased meat production is Insulin-like growth factor I (IGF-I) also known as somatomedin is located at chromosome 1: 55,432,530-55,480,957 in the forward strand (Galgal4:CM000093.3) as shown in figure 2.1

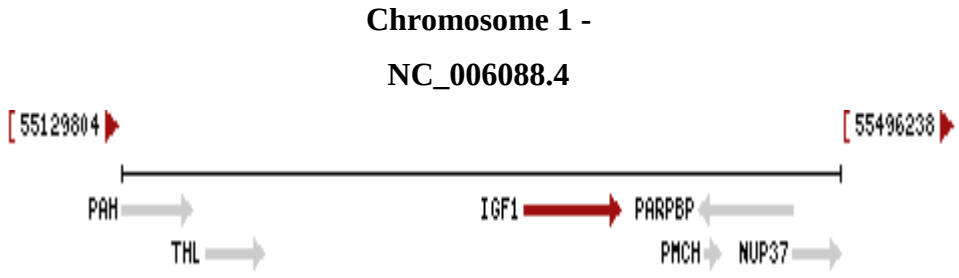

Figure 2.1- the position of *IGF-1* on chromosome 1- NC_006088.4 from ENSEMBL

IGF-1 protein consists of 70 amino acids in a single chain with three intermolecular disulfide bridges. *IGF-1* has a molecular weight of 7,649 Daltons (NCBI). *IGF-1*

encodes a protein similar in molecular structure to insulin (Akaboot, Duangjinda, Phasuk, & Kaenchan, 2012)).

Prolactin gene is the candidate gene associated with increased egg production in chicken. It is located at Chromosome 2: 58,655,089-58,661,243 forward strand (Galgal4:CM000094.3) as shown in figure 2.2.

Chromosome 2 -NC_006089.4

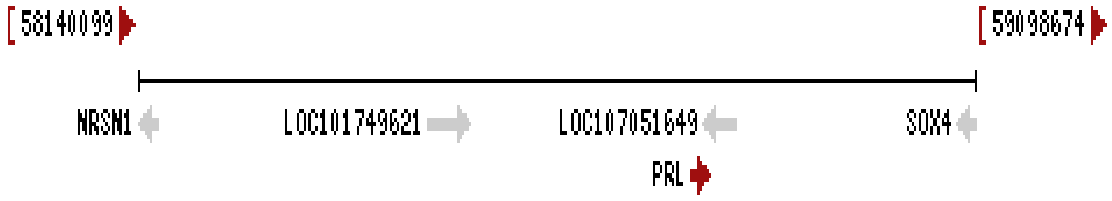

Figure 2.2- the position of *PRL* on chromosome 1- NC_006088.4 from ENSEMBL

Several candidate genes associated with increased egg production have been studied including prolactin gene; this gene encodes the anterior pituitary hormone prolactin. This secreted hormone is a growth regulator for many tissues.(Bhattacharya et al., 2011; Akaboot et al., 2012). Recently, the candidate gene approach has become a powerful technique for genetic improvement (Zhu & Zhao, 2007).

2.2.1 Candidate gene for meat production in chicken

IGF-1 main source is the liver and some other tissues, like kidney brain and muscle (Barton DeMeno, Lei, 2010). *IGF-1* is a major hormone required to support muscle development and normal growth (Scanes 2009; Promwatee et al., 2013). Biological effects are on cell growth, proliferation, differentiation, and survival against apoptosis (Khandwala, McCutcheon, Flyvbjerg, & Friend., 2000; Pollak, Schernhammer, & Hankinson., 2004; Promwatee et al., 2013). In poultry, *IGF-1* gene can influence body composition, lipid metabolism and growth rate (McMurtry, 1998 ; Promwatee et al.,

2013). It was reported by Bian et al., (2008) that polymorphism in the promoter region was associated with growth rate and feed efficiency. Meanwhile, (Bian et al., 2008; Zhou, Mitchell, , McMurtry , Ashwell, & Lamont., 2005) showed that polymorphism of the *IGF-1* gene in the promoter and 5'-untranslated region (5'-UTR) was associated with growth traits in chicken. Also, (Bian et al., 2008) reported that haplotypes based on three *IGF-1* polymorphisms (5'-flanking, exon 3, and 3'-flanking regions) were associated with body weight (BW) traits. (Promwatee et al., 2013 and Dorji, Daungjinda & Phasuk., 2011) found that polymorphism of the *IGF-1* gene at 5'-UTR was associated with body weight in studies done in the Thai native chicken population and reported that the *IGF-1* gene could be used as a positional candidate gene for growth traits.

Several studies have shown that circulating *IGF-1* affects growth rate in poultry (Goddard, Wilkie, & Dunn., 1988; Scanes, Dunnington, Buonomo, Donoghue, & Siegel., 1988; Ballard et al., 1990; Zhou et al., 2005). In chickens divergently selected for high or low growth rates, there were significantly higher *IGF-1* mRNA levels in the high growth rate line than in the low growth rate line (Beccavin, Chevalier, Cogburn, Simon, & Duclos, 2001). Duclos, (1998) indicated that *IGF-1* stimulated glucose uptake, amino acid uptake, and protein synthesis and inhibited protein degradation by satellite cell derived myotubes. In another experiment, a quality line selected for increased breast yield and decreased fatness had significantly higher circulating *IGF-1* concentration than the unselected control line (Tesseraud, Pym, Le Bihan-Duval, & Duclos 2003). Tomas et al (1998) showed that recombinant human *IGF-1* infusion in chickens enhanced growth and decreased carcass fat content. Associations of an *IGF-1* promoter polymorphism with average daily gain (ADG) and feed efficiency were found in 2 genetically diverse Black Penedesenca chicken strains (Amills et al., 2003). The *IGF-1* gene, therefore, was selected as a biological candidate gene to investigate growth, body composition, metabolic, and skeletal traits in chickens, hence *IGF-1* is also a positional candidate gene for growth and fat deposition in chicken (Zhou et al., 2005). *IGF-1* is linear has 63 orthologues, 1 paralogues and is a member of 1 ensemble protein family (Cunning et al., 2015) (Figure 2.3)

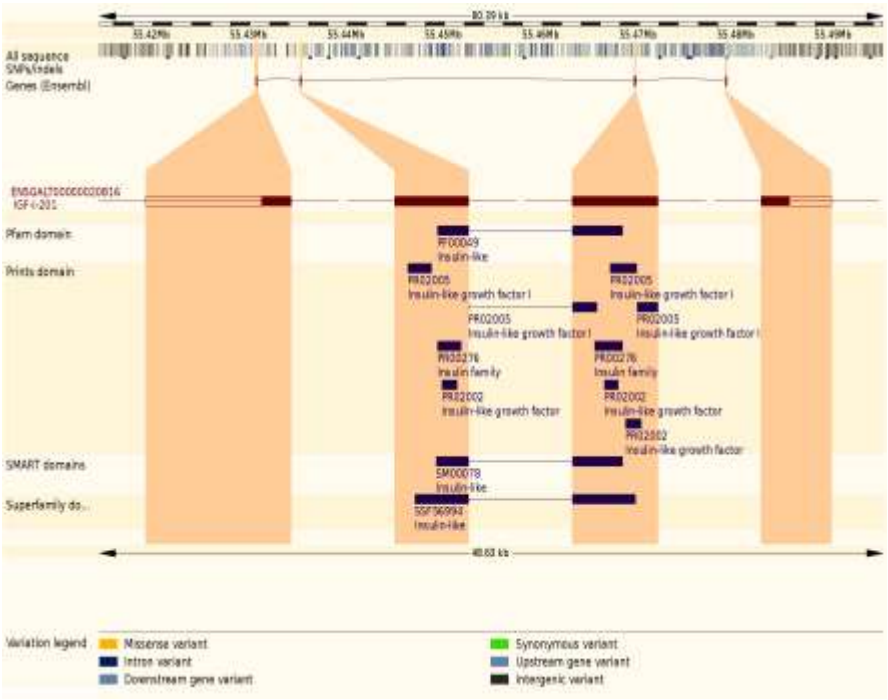

Figure 2.3 the structure of *IGF-1*gene from ENSEMBL

2.2.2 Candidate gene for egg production in chicken

Prolactin gene promoter is highly polymorphic, and has significant effects on egg quality traits in poultry (Liu et al., 2010). Prolactin (*PRL*) is a single-chain polypeptide hormone that belongs to the growth hormone gene family and is synthesized mainly by lactotropes of the anterior pituitary of all vertebrates. In avian species, *PRL* is a crucial hormone in induction and maintenance of incubation behaviour and regulation of the follicular development (Sharp, Macnamee, Talbot, Sterling, & Hall., 1984; Reddy, David, Sarma, & Singh, 2002). Recently, a large number of SNPs have been reported in the chicken *PRL* gene. Cui et al., (2006) obtained six SNPs (C-2402T, C-2161G, T-2101G, C-2062G, T-2054A and G-2040A) and a 24-bp Indel (insertion-deletion) from direct sequencing and association analysis showed that the 24-bp Indel was associated with egg production and chicken broodiness traits. Three mutations screened (C-1607T, C-5749T and T-5821C) by Cui, Du, Zang (2005) showed that there is a correlation

between different Haplotypes and egg production. All the above studies displayed that *PRL* is an important candidate gene on egg production.

The elevation of egg production and the inhibition of incubation behavior are the aims of poultry production. Prolactin (PRL) gene is confirmed to be critical for the onset and maintenance of these reproductive behaviors in birds. The prolactin gene promoter is highly polymorphic, and has significant effects on egg quality traits in White poultry hens (Bhattacharya, Chatterjee, Sharma, Rajkumar, & Niranjan., 2012).

A study done on the Chinese Qingyuan (Q line) chicken showed that non broody hens produce more eggs than broody hens. Higher laying rate during the non-broody period partially compensates egg loss from broodiness and the age of the first broody cycle is regarded as a phenotypic marker for intensity of broodiness in hens (Jiang, Chen, & Geng., 2010).

Genetic control of broodiness in the chicken is a considerable interest. Broodiness is one of the economically important traits which are controlled at least by two dominant autosomal genes that induce and inhibit the behavior, respectively, with equal influences (Romanov, Talbot, Wilson, & Sharp., 2002). Prolactin (*PRL*), has varied functions in vertebrate species Freeman, Kanyicska, Lerant, & Nagy., (2000), including the initiation of incubation in the avian species, resulting in regression of the ovary and cessation of egg production (Sharp, 1997). A study done by Shimada, Ishida, Sato, Seo, and Matsui., (1991) showed that the expression of *PRL* mRNA was significantly higher in the low egg production strains', indicating that egg production was related to *PRL* mRNA expression in chickens (Shimada et al., 1991). Cui et al., (2005) recently reported an association between *PRL* gene and egg production in chickens.

A study was done in Fars native chickens since they show a high frequency of broodiness, and they produce 20-70 eggs during each laying period of 8 months

Sarvestani & Taromsari, (2013), It indicated in the examined sample a 24-bp insertion-deletion polymorphic site 358 with two alleles, I and D.

In avian species, changes in plasma *PRL* level are associated with the expression of *PRL* mRNA In the anterior pituitary Wong., Ferrin, Silsby and El Halawani (1991). The expression of *PRL* is regulated by the 5′-flanking region sequence by binding with specific transcription factors Jiang, Xu, Zhang, & Yang., (2005). Studies in mammals and birds have shown that Pit-1/GHF-1 Frisch, Kim, Häusler, and Pfäffle., (2000), estrogen receptors Maurer & Notides, (1987), the CCAAT-enhancer binding protein-α Enwright, Kawecki-Crook, Voss, Schaufele, & Day, R. N., (2003) and other proteins are essential in regulating the expression of *PRL* via specific promoter binding sites. The sequence variation in the 5′-proximal region of *PRL* may lead to changes of transcription factor binding sites and alter the expression of *PRL* in terms of amounts/levels. A possible ecotropic viral integration site-1 encoded factor (Evi-1) binding site (score 93) was presented in the 5′ flanking region of the chicken *PRL* gene because of the 24-bp insertion Cui et al., (2005). The Evi-1 has 2 zinc-finger domains and Evi-1 has been shown to be involved in transcription of many genes as a repressor (Hirai, Izutsu, Kurokawa, & Mitani., 2001; Vinatzer, Taplick, Seiser, Fonatsch, & Wieser., 2001). Thus, Evi-1suppresses transforming growth factor-β (TGF-β) signaling and antagonizes the growth-inhibitory effects of TGF-β (Kurokawa et al., 1998; Izutsu et al., 2001). The presence of an Evi-1 binding site suggested the possible transcriptional regulation of the chicken *PRL* gene by Evi-1. It is possible that Evi-1 represses the expression of *PRL* gene in White Leghorn chickens by binding the Evi-1 binding site and further prevents broodiness Jiang et al., (2005), which can improve egg production to some extent.

Kansaku et al., (2005) studied the genomic structures of *PRL* in avian in order to elucidate the genetics of *PRL*. An abundance of SNP was reported in the 5′flanking region of chicken *PRL*, and the 24bp insert-deletion was significantly associated with broody behavior and egg production (Jiang et al., 2005 ;Cui et al., 2005), indicating its usefulness as a molecular marker for egg production. *PRL* is linear has 146 orthologues,

1 paralogues and is a member of 1 ensemble protein family (Cunning et al., 2015) (Figure 2.3)

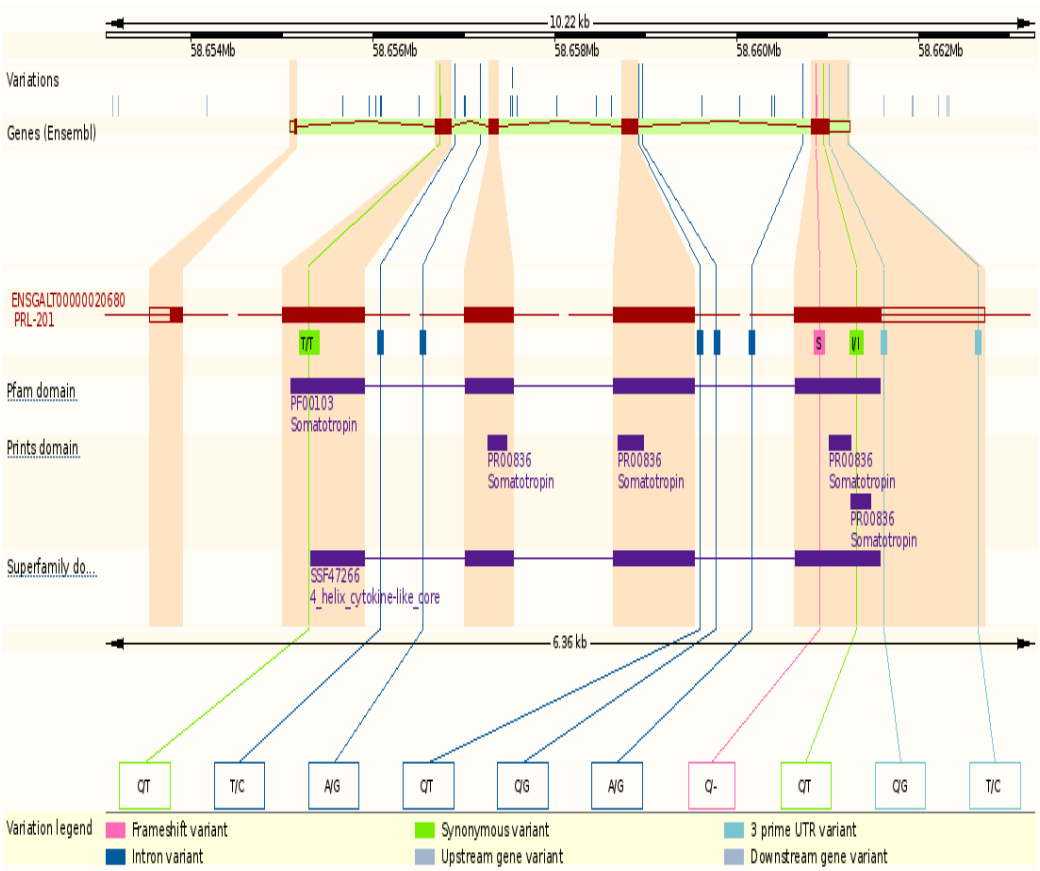

Figure 2.4 the structure of *PRL* gene from ENSEMBL

Although the performances of local chickens have been evaluated and documented in Kenya, most of the studies have been on phenotypic characterization. After reading through published literature it was noted that a lot of candidate genes associated with increased egg and meat production has been studied worldwide, few in Africa but there is no published research done in Kenya.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Indigenous chickens were sampled from Lake Turkana basin, Mount Elgon catchment and Lamu Archipelago areas as shown in figure 3.1.

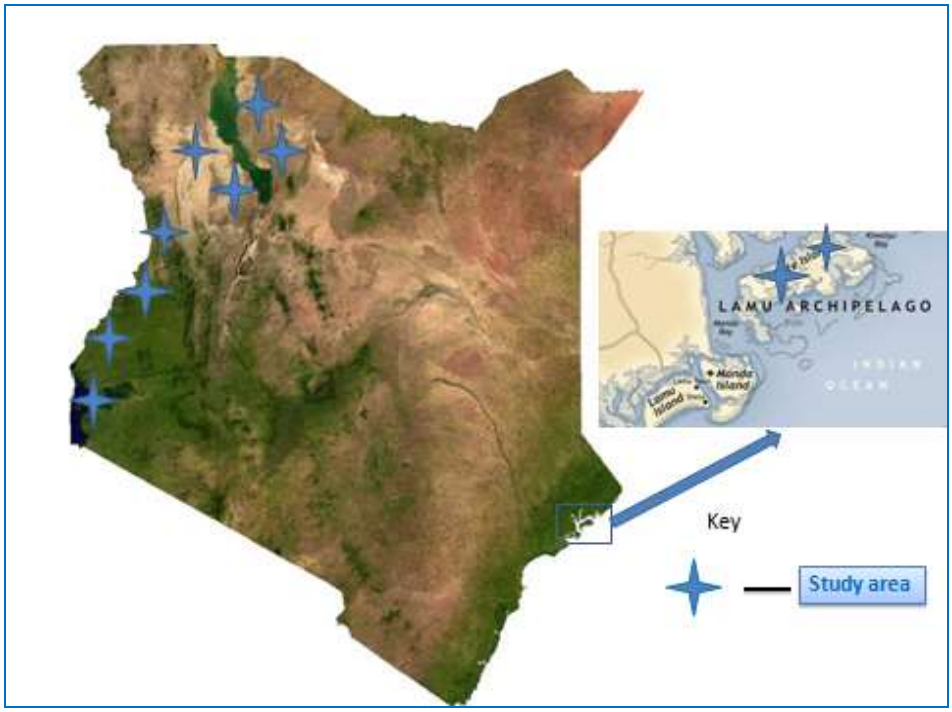

Figure 3.1: Map of study area

(Source: http://www.lahistoriaconmapas.com/atlas/map-satellite/Kenya-satellite-map.htm)

The selected regions were areas that were not affected by the cockerel/pullet exchange program (1976-1994) conducted by the government (Republic of Kenya, 2010). These areas were also livestock domestication/migration corridors into Kenya as suggested by

Mwacharo et al. (2013) (figure 3.2), hence they harbor distinct ecotypes (Aswani et al.,2015 ; Moraa et al.,2015).

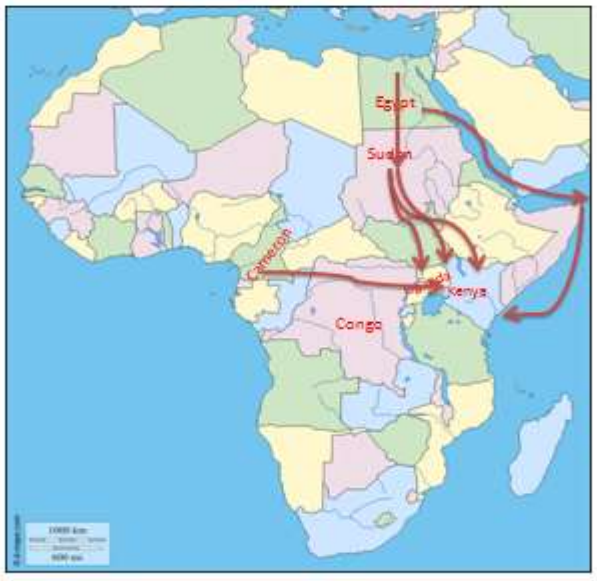

Figure 3.2: Migratory routes of indigenous chickens from Egypt and Cameroon to Kenya (Source: http://www.d-maps.com/carte.php?num_car=25456&lang=en)

3.1.1 Mt. Elgon catchment

Mt. Elgon catchment is located between 0°27'38.76", 0°24'41.74" South latitude and 34°6'41.26", 34°9'51.55" East longitude. It comprises zone I, II and III of the agro-ecological zones of Kenya. Zone I and II are wet and are characterized by an annual rainfall of 1000 mm per annum and an annual temperature of 21.0°C minimum and 22.7°C maximum. zone III receives an annual rainfall of 500-1000 mm with an annual temperature of 17.1°C minimum and to 29.4°C maximum. (Jaetzold & Schmidt, 1983).

3.1.2 Lamu Archipelago

Lamu lies within zones IV of the agro-ecological zones of Kenya, this zone is

considered dry and humid and receives an annual rainfall of 800 mm and an annual temperature of 24.1°C minimum and 29.2°C maximum. Lamu is located between 2°16'10.41" South latitude and 40°54'2.31" East longitude.

3.1.3 Lake Turkana basin

Turkana basin lies within zone VI and zone VII of the agro-ecological zones of Kenya. It is positioned between 3°37'36.55" South latitude and 36°0'8.35" East longitude. These zones receive annual rainfall of 200-600 mm. Lake Turkana is also characterized by an annual temperature of 23.7°C and 34.9°C (Paron, Olago & Omuto., 2013). These zones are considered as semi desert and comprise driest part of Kenya.

Table 3.1: Summary of sampled locations

Agro-climatic zones	Population	Number of sampled individuals
Lake Turkana basin	Lake Turkana East	33
	Lake Turkana West	61
Lamu Archipelago	Lamu North	27
	Lamu central	30
	Lamu South	46
Mt. Elgon catchment	Mt. Elgon north	31
	Mt. Elgon south	44
	Lake Victoria	24
	Total	**296**

3.2 Study design

A stratified cross-sectional study design was employed. Field surveys were carried out in remote villages that were not included in the cockerel and pullet exchange program in different counties of Kenya. With the assistance of local agricultural extension officers, a rural participatory approach was employed where interviews were conducted at the farmers' houses. A pre-tested questionnaire (appendix I) was used to obtain information

from the farmers as well as to record the various morphological data of the indigenous chickens. The key factors considered for inclusion were:

1. No vaccination
2. No supplementary feed (free to roam and search for their own food)
3. No cockerel or poultry were introduced to the flock by NGO's or government or any poultry improvement program.

3.2.1 Clearance

This study got a no objection for the research from Ministry of Agriculture, Livestock and Fisheries state department of veterinary services. A permit was issued under the permit number "RES/POL/VOL.XXVII/162" from Ministry of Agriculture, Livestock and Fisheries state department of veterinary services

3.3 Data collection

A total of 296 adult indigenous chickens from thirteen sub-populations in the different counties were phenotypically characterized. Data was collected on various phenotypic attributes such as number of eggs laid per hen, eggs seated on per clutch per hen, eggs hatched per clutch per hen, number of yolks in each egg and sitting times per hen per year. Physical measurements like body weight, body length, shank length, were also taken using a measuring tape and recorded as described by FAO (2012) (appendix II) . The weights of chickens were measured using portable sensitive weigh balance. The body weight was the individual live weight of the chicken. Body length was taken as the distance between the caudal (tail, exclusive of feathers) and tip of the rostrum maxillae (beak) when chicken was fully stretched while the shank length was measured from the spur to the hock joint of either leg according to Adeleke et al.,(2011). A global positioning system (GPS) was used to record coordinates for the study sites.

3.4 Molecular genetics analysis

3.4.1 Blood sample collection

Blood samples were collected from 296 genetically unrelated chickens and stored at JKUAT poultry metadata base. Blood was drawn from the chicken wing (figure 3.3) of two adults per flock, one male and one female, from different populations (appendix III). Each population comprised of 30 individuals per population following the recommendations of Hale, Burg and Stevers (2012). The blood was spotted on FTA classic cards and air-dried for preservation (figure 3.4).

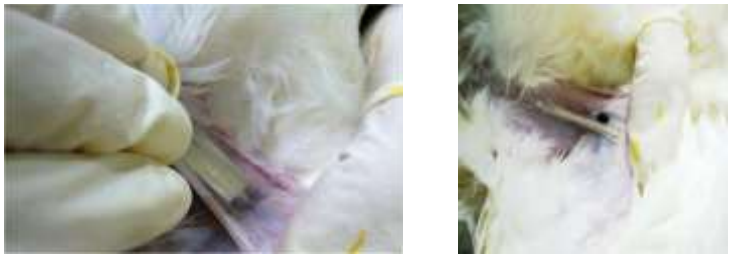

Figure 3.3: Puncturing of the wing vein

Figure 3.4: Air drying of blood spotted on FTA card

3.4.2 DNA extraction for *IGF-1* and *PRL*

Genomic DNA was extracted from air-dried blood preserved on FTA classic cards (Whitman Biosciences) Gutiérrez-Corchero et al., (2002), using the manufacturers protocol (see appendix IV). The correct concentration was determined by nanodrop 1000 spectrophotometer. The integrity was checked using 260/280 ratio. The samples were diluted to working stock of 50ng/µl for the purpose of PCR. The rest of the DNA was stored as stock solution at -80°C.

3.4.3 PCR amplification for *IGF-1* and *PRL*

PCR reactions were performed in a final volume of 10µl containing 3.8µl of double distilled water; 1µl of template genomic DNA, 5µl of thermoscientific™ DreamTaq™ Green Master Mix (2X), 0.2µl Primer (forward + reverse, 20pmol/µl). The first 813bp in the coding region of *IGF-1* gene was amplified via primers *IGF-1*F (5′-CATTGCGCAGGCTCTATCTG-3′) and *IGF-1*R (5′-TCAAGAGAAGCCCTTCAAG C-3′) (Moody, Haynie, Schreiweis, & Hester, 2003; Akaboot., 2012). The first 154bp

and 130bp region of *PRL* gene was amplified via primers *PRL*F (5′-TTTAATATTGGTGGGTGAAGAGACA-3′) and *PRL*R (5′-ATGCCACTGATCCTCGAAAACTC-3′) (Cui,et al., 2005 and Akaboot et al., 2012) at the promoter region. Amplification was carried out in a Veriti 9901 96 Well Fast gradient Systems thermo cycler. The amplification program started with initial denaturation at 94^0C for 3min followed by 35 cycles of 15 seconds at 94^0C, 30 seconds at 60^0C for primer annealing and 30 seconds at 72^0C for elongation. The final extension was done at 72^0C for 10 minutes.

For electrophoresis analysis, 2% agarose gel in 1X Tris Boric Ethylenediaminetetraacetic acid (TBE) buffer was prepared by adding 2g of agarose to 100 ml 1X TBE and weighed. The solution was then heated in a microwave at short intervals of 15-30 sec with occasional shaking until it boiled and became clear. This was left to cool to 55^0C. The gel was then poured on the tray of the mini electrophoresis unit (MUPID) which contained fixed combs to solidify, bubbles were removed, and the gel allowed was to set. After solidifying, the combs were removed and 1X TBE Buffer added on the mini electrophoresis unit to cover the gel. DNA preparations were loaded onto the 2% agarose gel using 1X TBE buffer (89Mm Tris, 89mM boric acid, 2Mm Na_2 EDTA) in a voltage of 70V for 1 hour. The gels were stained with gel red and visualized under UV light (BTS-20 model, UVLtec Ltd., UK). Cleaning and sequencing was done at Macrogen using the Applied Biosystems 3730*xl* DNA analyzer (Sanger & Coulson, 1975).

3.5 Data analysis

3.5.1 Phenotypic data analysis

Phenotypic data were analyzed using R core statistics software version 3.0.1, Graph Pad Prism™ version 6.0 and Microsoft excel 2013 to compute frequencies of occurrence of each trait. Significant differences within means were determined using Tukey's test at 95% confidence level. ANOVA tests in R core statistics software version 3.1.2 Ihaka

and Gentleman, (1996) was used to determine significant relationships between various traits and the ecosystems.

3.5.2 *IGF-1* and *PRL* data analysis

3.5.2.1 Editing *IGF-1* gene and *PRL* gene trace files

The raw *IGF-1* sequences were edited manually using Chromas Lite 2.1 Avin, (2012) and aligned using Clustal X 2.1.1 Thompson, Gibson, Plewniak, Jeanmougin and Higgins (1997) and MUSCLE version 3.8.31 Edgar, (2004). The forward and reverse sequences were aligned to determine the accurate sequence and the accurate sequence was aligned against reference sequences obtained from GenBank (see appendix V). These were visualized using seaview version 4 Gouy, Guindon and Gascuel., (2010) and Clustal X version 2.1 (Thompson et al., 1997). Subsequent analyses for *IGF-1* were restricted to 813bp region which corresponds to position 55,432,530-55,480,957 of the Galgal4 Chromosome 1 (CM000093.3). The primer sequences were trimmed out and consensii sequences generated. These consensii sequences were used for subsequent analysis.

3.5.2.2 Haplotype analysis for *IGF-1* gene and *PRL* gene

IGF-1 haplotypes were constructed manually and confirmed with DnaSP 5.10 software (Librado & Rozas, 2009). The distribution frequencies of the various haplotypes in various populations were displayed on pie chats constructed using Excel spreadsheet 2013 (Liengme, 2015).

3.5.2.3 Phylogenetic analysis for *IGF-1* gene and *PRL* gene

A multiple sequence alignment was first done on the haplotypes using MUSCLE version 3.8.31. Thereafter, a phylogenetic tree involving the *IGF-1* haplotypes observed in the three agro-climatic zones and the ancestral red jungle fowl and other avian species was inferred using the maximum likelihood tree algorithm as implemented in MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The model used was T92:

Tamura 3-parameter with a gamma shape parameter of 3.1250 to model the nucleotide substitution pattern and rate of evolution following 1000 bootstrap replications.

The affinity of indigenous chickens to the other avian species was revealed by the maximum likelihood tree incorporating nine avian species downloaded from GenBank (see appendix VII). The resultant tree was viewed and edited by Dendroscope version 3.3 (Huson & Scornavacca, 2012). To test the robustness of the phylogenetic analysis sequence clusters were detected by the analysis of phylogenetic networks from uncorrected p-distances with the phylogenetic splits decomposition network implemented with Splits Tree version 4.13.1 (Huson & Bryant, 2006). The phylogenetic network diagrams produced from these analyses were used to validate the haplotypes.

3.5.2.4 Population genetic diversity for *IGF-1* gene and *PRL* gene

Genetic variation (nucleotide diversity, number of homozygotes, number of heterozygotes, expected heterozygosity and observed heterozygosity) for each population were calculated using Arlequin v 3.5.2.2 software (Excoffier , Laval & Schneider, 2005). The population genetic structure was assessed by the nested analysis of molecular variance (AMOVA). The groupings used for AMOVA were as follows: Among populations, among individuals within populations and within individuals. Significance testing was performed using 10000 coalescent simulations in Arlequin 3.5.2.2 software (Excoffier et al., 2005).

3.5.2.5 Mantel test for *IGF-1* gene and *PRL* gene

Arlequin version 3.11 Excoffier et al., (2005) was used to perform the simple Mantel test between genetic and geographic distance matrices. The regression graph between the genetic and the geographic distances was plotted using GenAlEx version 6.5 Peakall and Smouse, (2006) as an add-on in Microsoft Excel.

CHAPTER FOUR

RESULTS

4.1 Characterization of the phenotypes associated with meat and egg production in indigenous chickens

The characterization of the phenotypes associated with meat and egg production in indigenous chickens was the first objective of the study and the results were obtained by getting the morphometric measurements and the body weight as indicators of meat production. Different aspects of the hatchability, number of eggs laid per clutch, number of sitting cycles per year, number of yolks per egg were analyzed as indicators of egg production.

4.1.1 Analysis of body and shank length in indigenous chickens of Kenya.

Body parameters in indigenous chickens are crucial to any improvement program since these are useful traits for selection. Therefore, body and shank lengths were measured as well as the body live weight of indigenous chickens in the 3 agro-climatic zones of Kenya. The average body lengths with a range of 71cm -50cm long and shank lengths with a range of 3cm and 10cm are outlined in figure 4.1. Chickens from Lamu archipelago were found to have the longest average shanks, followed by those from Mt Elgon while those from Lake Turkana recorded the shortest average shank (Figure 4.1 A). A similar pattern was observed for the body length of chickens in the agro-climatic zones. Chickens from Lamu Archipelago recorded the highest body lengths followed by those from Mt Elgon and Lake Turkana (Figure 4.1 B). The results differed from Guni et al., (2013) who reported a range of 40.2cm and 45.7cm in body length. They were higher than the ones reported by Magothe et al., (2012) who reported a range of 8.2cm-8.7cm for Lamu archipelago and Mt Elgon but lower for Lake Turkana. The observed variation in body length between agro-climatic zones indicates the existence of different diverse subpopulations within the indigenous chicken population.

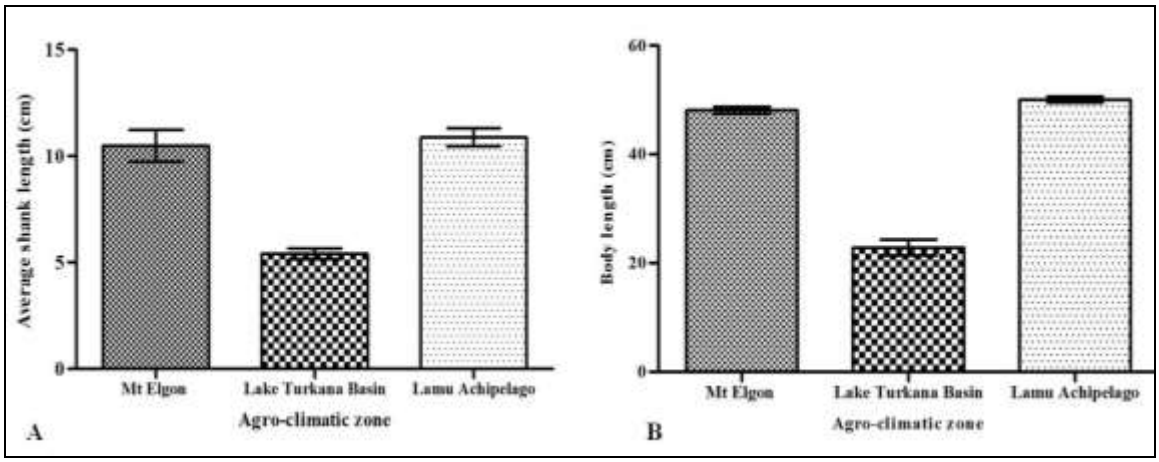

Figure 4.1: The average shank length (A) and body length (B) of chickens sampled in 3 agro-climatic zones of Kenya. Vertical bars represent standard deviation of the mean according to Tukey's test (P<0.05).

4.1.2 Analysis of Body Weight of Chickens Sampled

The live body weight was also measured and the average body weight presented in the bar graph (figure 4.2). The average live body weight recorded was within a range of 600g-4150g. Lamu archipelago recorded the heaviest chickens, based on body live weight, followed by Mt Elgon catchment with Lake Turkana basin recording the lowest average weight. The average body weights observed in the present study fell within the range of 1030g to 2860g, 1108g to 2915g and 1525g to 2095g as reported by Katule (1998), Msoffe et al., (2004) ; Guni et al., (2013) respectively. The results were higher than those reported by Dana, Dessie, Waaij and Arendonk., (2010); Olawunmi, Salako, and Afuwape., (2008); Mwalusanya et al., (2002); Guèye, Ndiaye and Branckaert. (1998). In Kenya, they were higher than the results reported by Magothe et al., (2012) who reported a range of 1330.2g-1741.0g, slightly different from Olwande et al., (2010) who reported a range of 1599g-2096g respectively though they were lower than the ones reported by King'ori, (2004) who reported a range of 1950g-3150g.

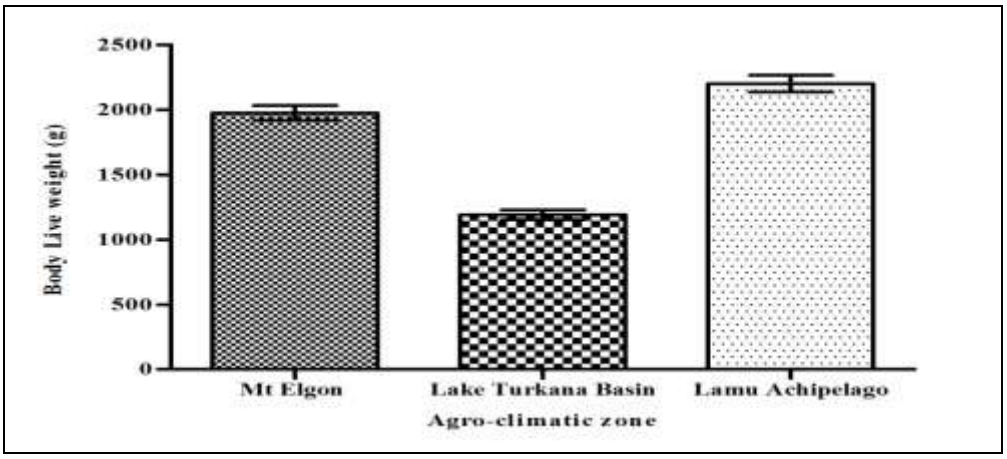

Figure 4.2. Analysis of average body weight of chickens sampled in 3 agro-climatic zones in Kenya. Vertical bars represent standard errors of the mean according to Tukey's test (P<0.05).

The observed large variation in body weight between agro-climatic zones indicates the existence of different diverse subpopulations within the indigenous chicken population. Chickens with small body lengths and weights and shank lengths need to be improved to help alleviate production problems associated with larger parameters (Adeleke et al., 2011). Similarly, those with larger suitable attributes need to be identified and conserved to avoid loss of these traits.

4.1.3 Regions and the various traits: Shank length, body length and body weight

The analysis of variance on the obtained data was carried out and presented in the table 4.1. There was a significant relationship between the shank length, body length and live weight and the agro-climatic zones (Table 4.1). The significant interaction between body weight and other body measurements imply that these easily measured parameters can be used for estimation of body weight and hence important in selection criteria that can be used to improve body weight. The existence of positive and significant correlations between body weight and body measurement traits have also been reported by Alabi, Ng`ambi, Norris and Egena., (2012); Guèye et al.,(1998) from Nigeria and Senegal respectively for local chickens.

Table 4.1: ANOVA Summary results of analysis of agro-climatic zones and various traits attributed to meat production in indigenous chickens of Kenya

Traits	*p*
Agro-climatic zones and Shank Length	***
Agro-climatic zones and Body Length	***
Agro-climatic zones and Live weight	***

Significant codes: 0 '***' 0.001 '**' 0.01 '*' ns-not significant $p<0.001$

4.1.4 Distribution of Production Traits Associated with egg production among chickens in Kenya

Frequencies of eggs seated on and eggs hatched in chicken sampled from 3 agro-climatic zones in Kenya were compared as shown in Figure 4.3.

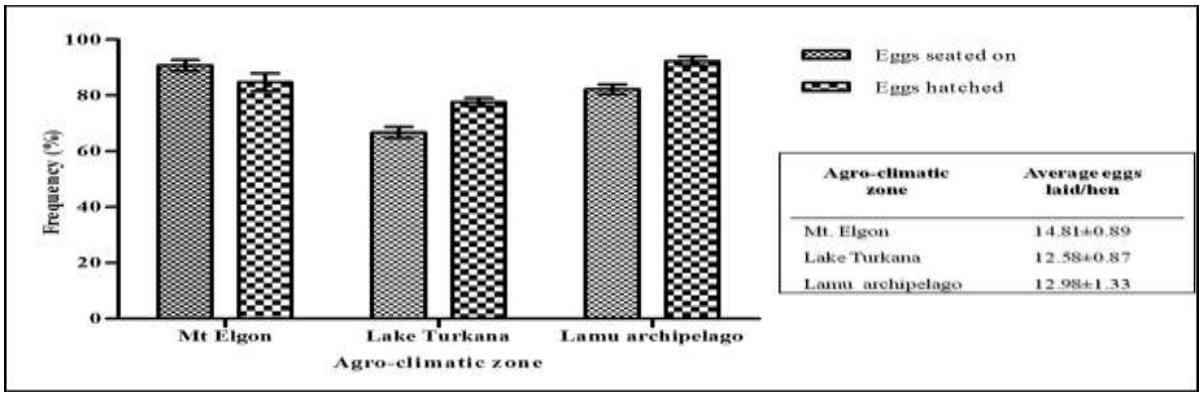

Figure 4.3: Comparison of frequencies of eggs seated on and eggs hatched in chicken sampled from 3 agro-climatic zones in Kenya. Vertical bars represent standard errors of the mean according to Tukey's test (P<0.05). The average number of eggs laid per hen in each zone are shown inset.

The study reported no significant differences between the agro-climatic zones with respect to this variable. These results were in consistent to those reported by Guni et al., (2013) who reported 13.7 eggs. Mwalusanya et al., (2002) and Olwande et al., (2010) reported the mean clutch size to be 11.8 eggs and 11 eggs respectively, lower than the mean value reported in the study.

The frequencies of eggs sat on showed a slight variation within the agro-climatic zones of Kenya. For instance, Mt Elgon catchment recorded the highest frequency of eggs seated on by the indigenous chickens while Lake Turkana basin had the lowest frequency of eggs seated on as illustrated in (Figure 4.3). The difference in the frequencies of eggs sat on was mainly based upon the farmers' decision to give out the eggs to the hens. The study found out that the farmers give out a certain fraction to the hens to lie on and consume or sell the rest for cash.

Analysis of hatchability among the 3 zones similarly revealed slight differences in the frequencies of eggs hatched. High hatchability was obtained in Lamu archipelago followed by Mt Elgon and Lake Turkana at 84.6% and 77.62% respectively (Figure 4.3). These results were consistent with Guni et al., (2013); Kugonza, Kyarisiima, & Iisa., (2008); Development and Veterinary, (2005) and Mengesha, Tamir, and Dessie., (2008) who reported a hatchability of between 83.2% and 92.6% in chickens from different districts in Tanzania with an exception for Lake Turkana basin which had lower hatchability. In Kenya Olwande et al.,(2010); King'ori (2004); Okitoi and Mukisira (2001) reported lower results than the ones reported in this study as follows: 70%-80%, 43%-47% and 46%-48% respectively.

The average sitting cycles of indigenous chickens sampled from 3 agro-climatic zones of Kenya were calculated and show in Figure4.4.

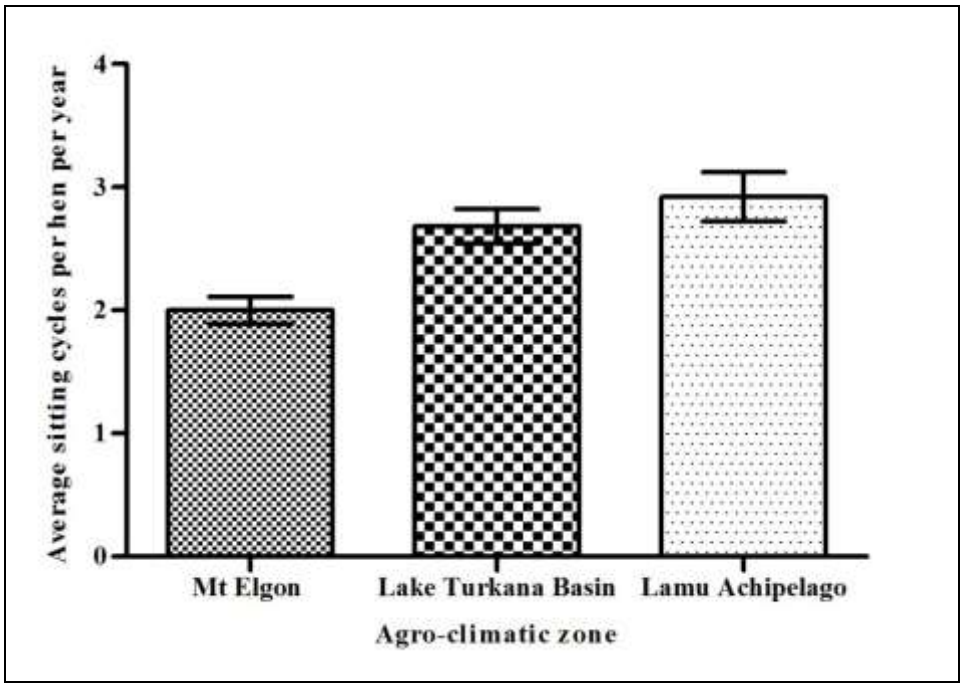

Figure4.4. Average sitting cycles of indigenous chickens sampled from 3 agro-climatic zones of Kenya. Vertical bars represent standard deviation of the mean according to Tukey's test P<0.05

The number of sitting cycles per hen per year was further estimated. Results of the present study showed that Lamu archipelago had the highest average sitting cycles per hen per year followed by Lake Turkana basin and Mt. Elgon catchment (Figure 4.4). An average mean number of 1.78 clutches per year in the present study was observed which was lower as compared to the ones that were reported from other studies in different countries such as Tanzania Guni et al., (2013); Hossen, (2010); Moreki, (2010); Petrus, (2011) and India Iqbal and Pampori, (2008). The latter reported a sitting cycle of 4 clutches per year. The study postulates that the differences observed in sitting cycles between countries could be attributed to genetic as well as environmental differences in populations across the different regions (Figure 4.4). Lamu Archipelago had heavy-bodied chickens though they produced fewer eggs which were often double-yolked. The study found out that despite the fact that Indigenous chickens in Lake Turkana basin were small but produced more eggs as compared to the ones in Lamu Archipelago which

were heavy-bodied though they produced fewer eggs that were often double-yolked. Double yolk is attributed of no hatchabilty. These is an ecotype that needs to be conserved for the fact that it is heavy and big bodied but lack of chick production means loss of the chickens in Lamu Archipelago (Figure 4.5).

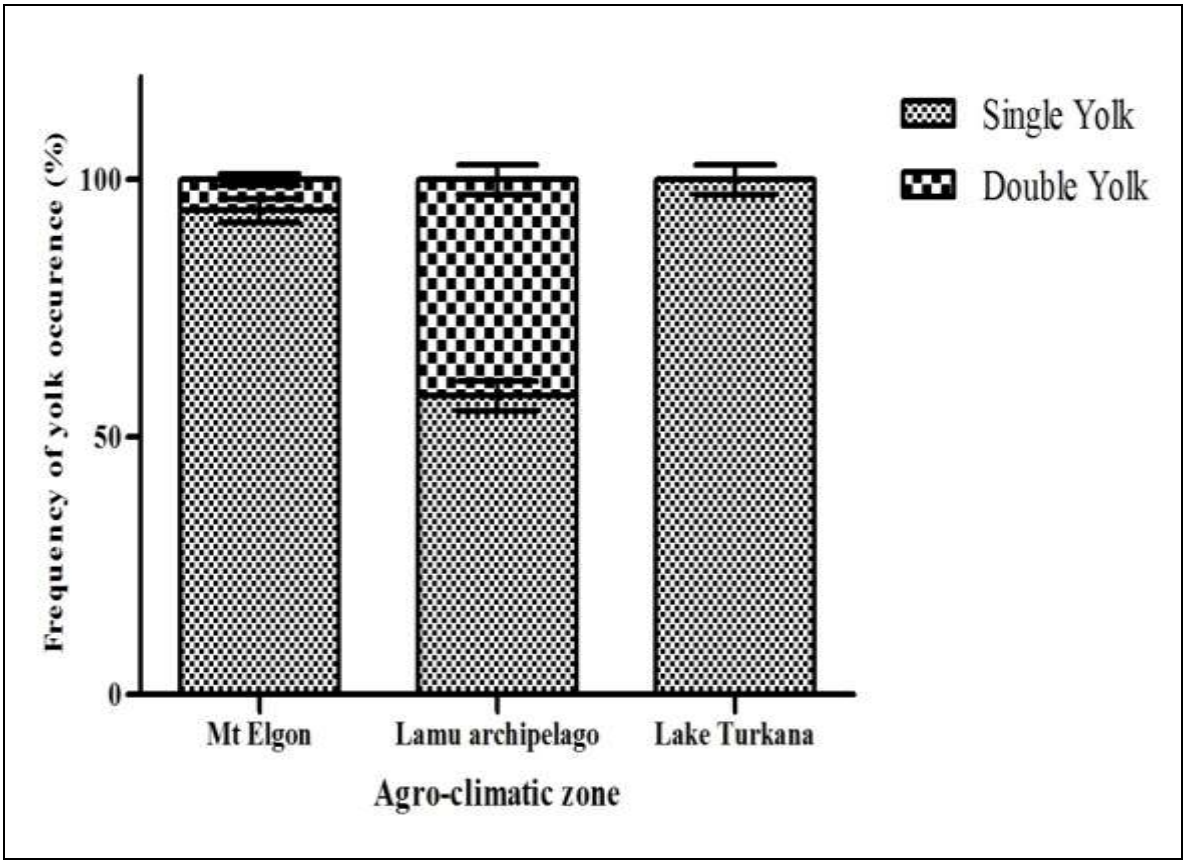

Figure 4.5: Analysis of the number of yolks per egg in chickens from 3 agro-climatic zones of Kenya. Vertical bars represent standard deviation of the mean according to Tukey's test at P<0.05. A summary of analysis of variance significant interactions was recorded in table 4.2.

Table 4.2: ANOVA Summary results of analysis of agro-climatic zones and various traits

Traits	***p***
Agro-climatic zones and number of yolk in an egg	**
Agro-climatic zones and number of eggs laid	ns
Agro-climatic zones and number of eggs sat on	ns
Agro-climatic zones and number of eggs hatched	ns
Agro-climatic zones and number of sitting times per year	**

Significant codes: 0 '***' 0.001 '**' 0.01 '*' ns-not significant *P<0.05*

This study also sought to find out whether there existed significant interactions between the numbers of eggs laid per clutch, sat on, hatched and the number of sitting times per year in different agro-climatic zones and the results were summarized in table 4.2. It was clear that there was a significant relationship between the agro-climatic zones and the number of yolks in an egg and the number of sitting times per year at 95% confidence interval level. The study further found out that there was no significant relationship between the agro-climatic zones and the number of eggs laid, number of eggs seated on and number of eggs hatched per hen per clutch (table 4.2).

4.2. Assessment of functional polymorphisms at *IGF-1* candidate gene for meat production in indigenous chickens of Kenya

4.2.1 Gel electrophoresis

The DNA samples were amplified for *IGF-1* candidate gene with the relevant primers and visualized under UV light. The gel image in plate 4.1- 4.3 displays a 2% agarose gel electrophoresis showing 813bp fragment of representative results.

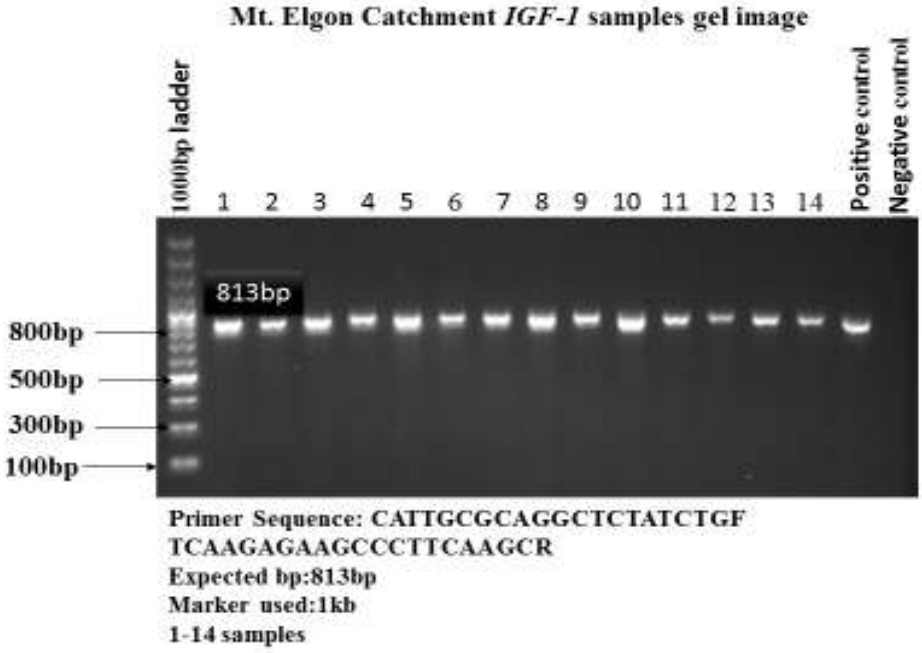

Plate 4.1: Gel image of *IGF-1* gene representaive sample of indigenous chicken from Mt.Elgon catchment.

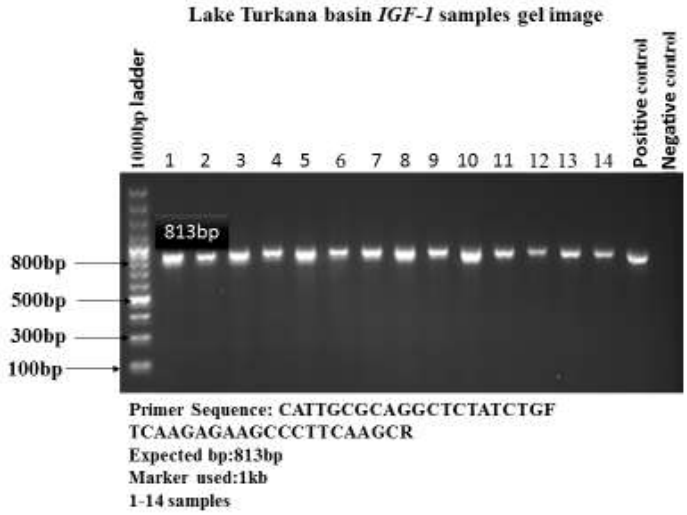

Plate 4.2: Gel image of *IGF-1* gene representaive sample of indigenous chicken from Lake Turkana basin

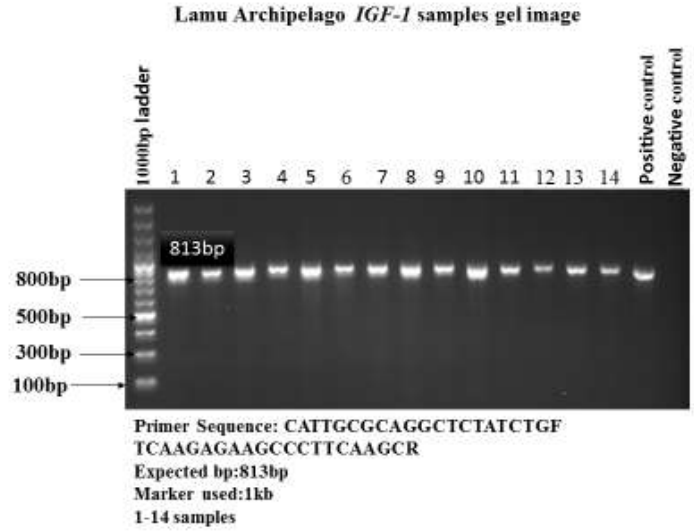

Plate 4.3: Gel image of *IGF-1* gene representative sample of indigenous chicken from Lamu archipelago

The specific gene of interest was amplified through PCR using specific primers: *IGF-1*F (5′-CATTGCGCAGGCTCTATCTG-3′) and *IGF-1*R (5′-TCAAGAGAAGCCCTTCAAG C-3′) (Moody et al., 2003), (Akaboot et al., 2012). The

amplified product was about 813bp (Plate 4.1- 4.3) this was in line with other earlier findings reported by (Zhou., 2005; Promwatee et al., 2013). The primers targeted a region of 813bp which is clearly indicated by the 1000 base pair molecular weight DNA marker. The amplified region corresponds to chromosome 1: 55,432,530-55,480,957 in the forward strand (Galgal4:CM000093.3) of *IGF-1* gene. All the samples amplified. Water was used as a negative control. The positive control of a known avian species with an amplified region of interest was used and was obtained from the International Livestock Research Institute samples.

4.2.2 Editing of *IGF-1* chromatograms

Quality of the underlying trace data was evaluated and the most accurate consensus sequence possible generated as shown in the chromatograms (fig 4.6, 4.7 and 4.8). Editing of the chromatograms was done manually using Chromas Lite 2.1 (Avin, 2012).

Indels were seen (Fig 4.6) at position 285 and 286 where there was either insertion or deletion of adenine base.

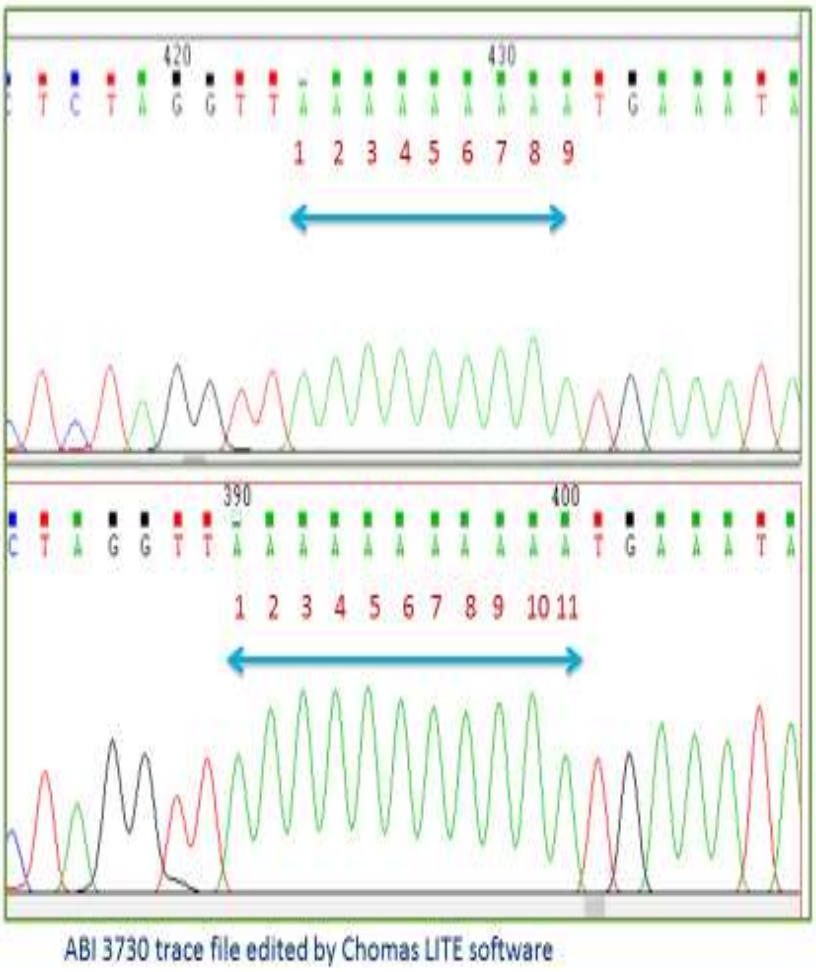

Figure 4.6: Chromatogram of *IGF-1* sequence showing monomeric pair expansion between position 285and 286.

There was evidence of multibase pair expansion which were clearly detected between position 273 and 276 as shown in figure 4.7

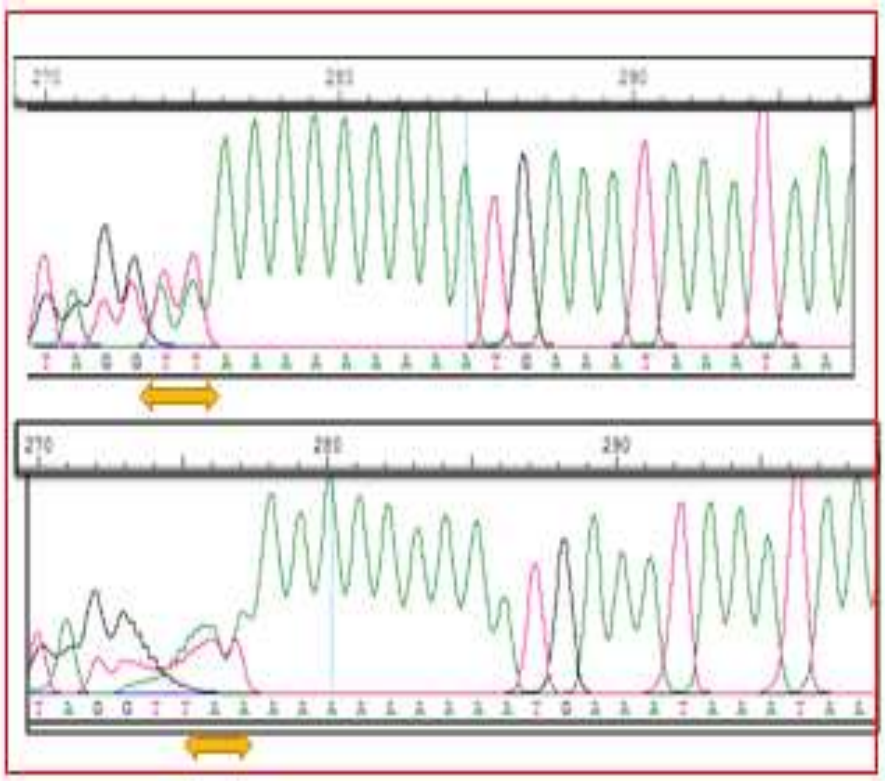

Figure 4.7: Chromatograms of *IGF-1* sequence showing heterozygous Indel between position 273 and 277

Position 570 showed a transversion between purine base (adenine) with a pyrimidine base (cytosine). SNP A → C at base 570 (accession number M74176) in the promoter region was identified, which was the same point mutation previously detected in other studies (Amills, et al., 2003 and Zhou et al., 2012).

The arrows show positions of nucleotide differences (fig 4.8). The various samples varied at various locations along the chromatogram.

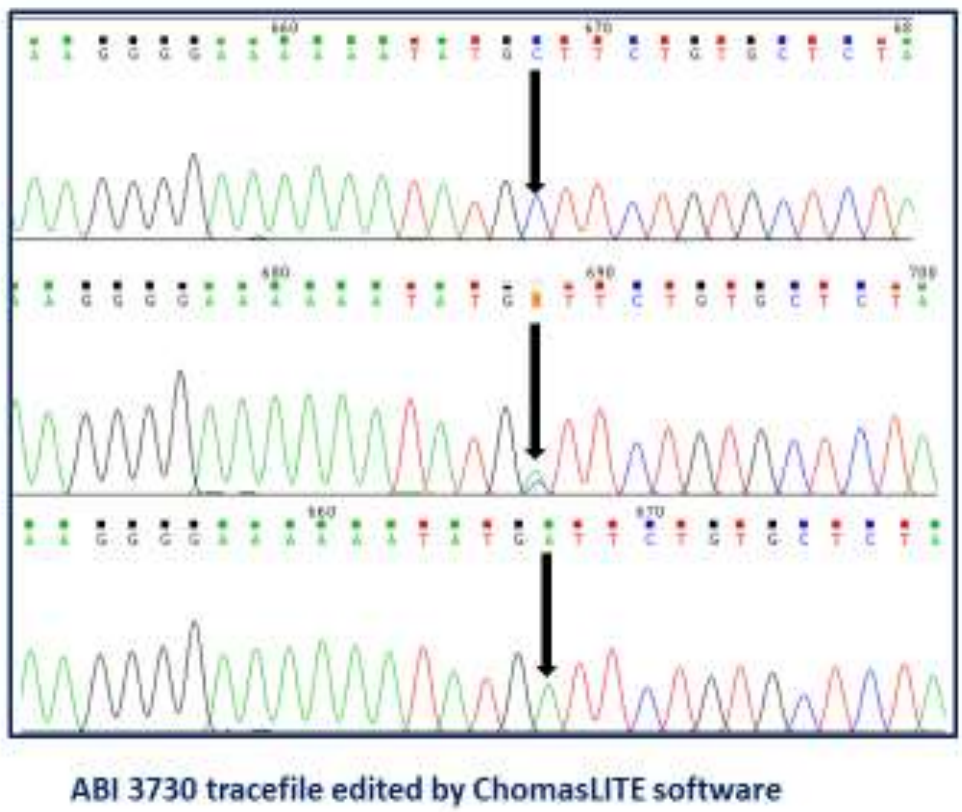

Figure 4.8: Chromatogram of *IGF-1* sequence showing point mutation A → C at base 570.

Position 570 showed a transversion between purine base (adenine) with a pyrimidine base (cytosine). SNP A → C at base 570 (accession number M74176) in the promoter region was identified, which was the same point mutation previously detected in other studies (Amills, et al., 2003 ; Zhou et al., 2012).

4.2.3 Multiple sequence alignment of *IGF-1* of the homozygotes, heterozygotes and red jungle fowl sequences

A multiple sequence alignment of all the samples plus reference sequences from GenBank(see appendix V) was done with Clustal X2 software .

This was done to confirm variable regions that were earlier shown in the sequence chromatograms (figure 4.6 and 4.7). Variable sites were clearly indicated as Indels as displayed in figure 4.9. This confirmed the Indels as was previously shown in (figure 4.6) by the chromatograms.

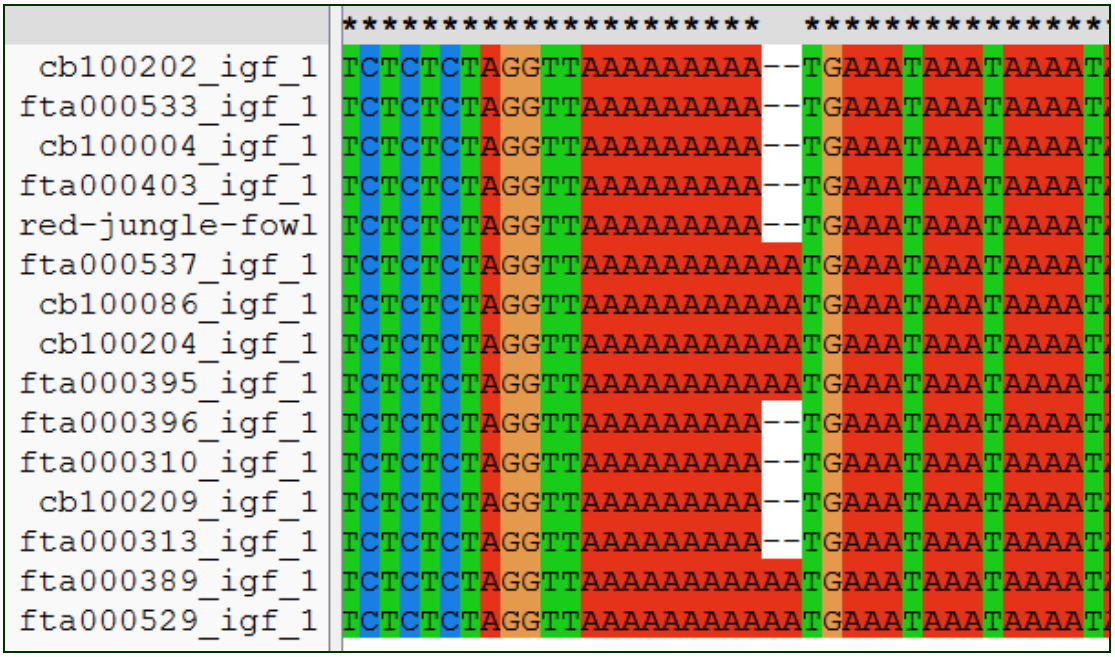

Figure 4.9-Sample multiple sequence alignment of *IGF-1* and red jungle showing Indel

Multiple sequence alignment of *IGF-1* and red jungle revealed point mutation, homozygotes and, heterozygotes chicken sequences as displayed in figure 4.10. Variable sites were clearly displayed and the point mutation at position 570 was displayed in figure 4.10. This confirmed the transitions in the purine and pyrimidine bases at position 570 (figure 4.8) by the chromatograms. Heterozygotes and homozygotes were clearly indicated in figure 4.10

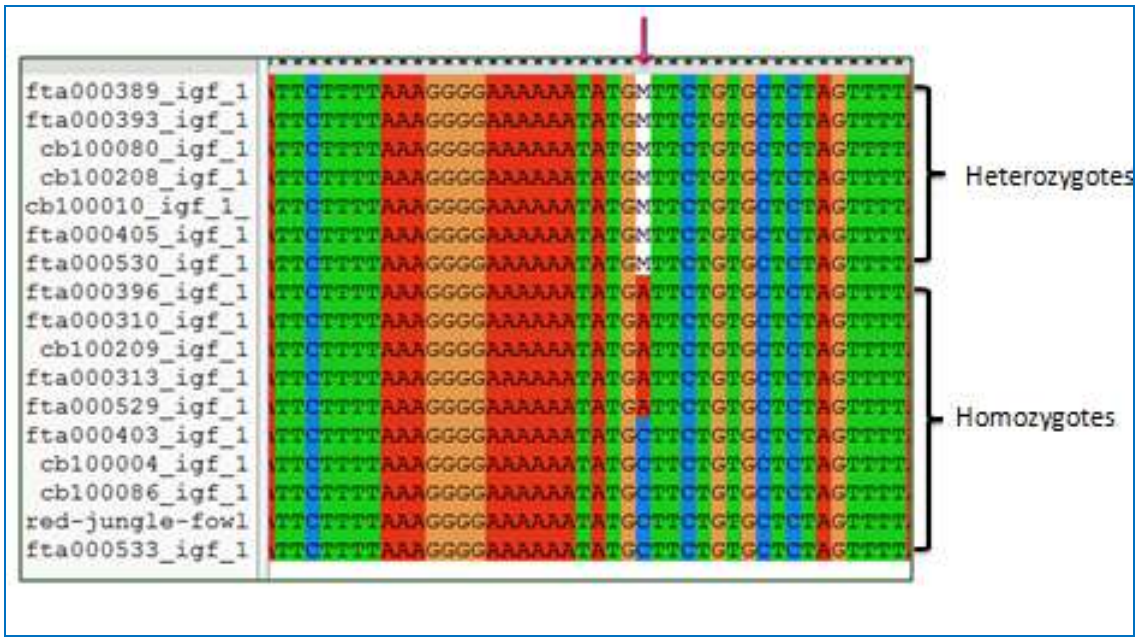

Figure 4.10- multiple sequence alignment of *IGF-1* and red jungle showing point mutation, homozygotes and, heterozygotes chicken sequences

Upon multiple sequences alignment, indigenous chicken haplotypes were generated as shown in figure 4.11. Three haplotypes were generated upon alignment using Clustal X and DnaSP software's, as shown in figure 4.11 namely: Chicken haplotype 9AC, Chicken haplotype 11AC, Chicken haplotype 9AA and they are the ones which were used for further downstream analysis.

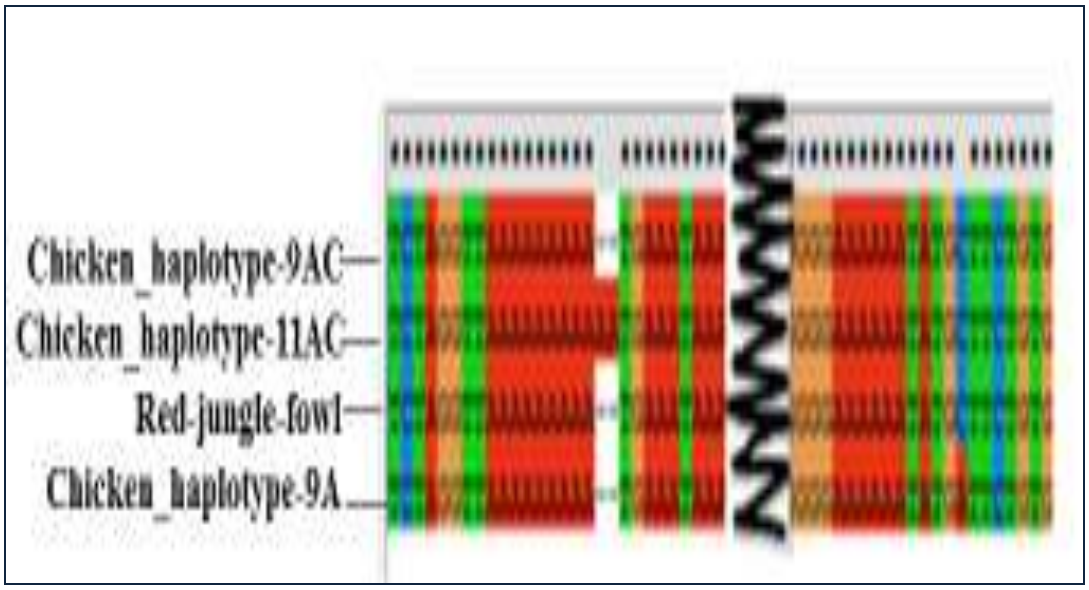

Fig 4.11 Multiple sequence alignment showing the three *IGF-1* chicken haplotypes and the red jungle fowl IGF

4.2.4 *IGF-1* chicken candidate gene haplotype distribution in three agro-climatic zones of Kenya

Frequencies of the *IGF-1* chicken candidate gene haplotype distribution in three agro-climatic zones of Kenya were recorded in the table 4.3.

Table 4.3: Frequencies of the three *IGF-1* chicken haplotypes distribution in the three agro-climatic zones of Kenya

Population	**Haplotype**	**Haplotype number**	**Haplotype frequency**	**%**
Lamu archipelago	haplotype 9A	15	0.27	27%
	haplotype 9C	14	0.25	25%
	haplotype 11C	27	0.48	48%
Lake turkana basin	haplotype 9A	23	0.35	35%
	haplotype 9C	31	0.47	47%
	haplotype 11C	12	0.18	18%
Mt.Elgon catchment	haplotype 9A	11	0.22	22%
	haplotype 9C	13	0.26	26%
	haplotype 11C	26	0.52	52%

Haplotype 11C was widely distributed in Lamu archipelago (48%) and Mt Elgon Catchment (52%) as compared to Lake Turkana basin (18%). Haplotype 9C was widely distributed in Lake Turkana basin (47%) followed by Mt Elgon Catchment (26%) and Lamu archipelago (25%) respectively. Haplotype 9A was averagely distributed in all the sampled areas as follows: Lamu archipelago (27%) and Mt Elgon Catchment (22%) as compared to Lake Turkana basin (35%). (Table 4.3). Pie charts were used to display the frequencies of the *IGF-1* chicken candidate gene haplotype distribution in three agro-climatic zones of Kenya figure 4.12.

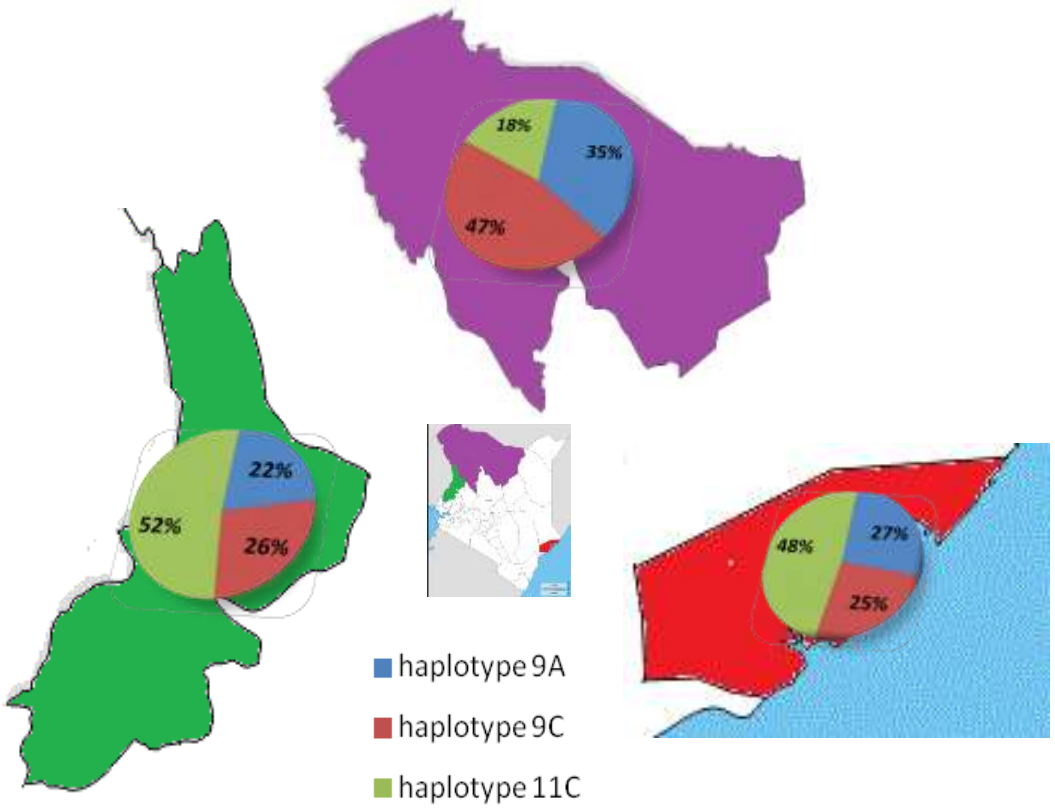

Figure 4.12: Pie chart distribution of the three *IGF-1* chicken haplotypes in the three agro-climatic zones of Kenya

4.2.5 Phylogenetic analysis of *IGF-1* haplotypes with other avian species

The phylogenetic relationship of the *IGF-1* haplotypes with other avian species was determined by the construction of a phylogenetic tree and splits decomposition network.

Maximum likelihood tree of *IGF-1* and other avian species was constructed to determine the model of sequence evolution and the rate of heterogeneity of the sequences, MEGA Version 6 software was used. Phylogenetic analysis of the three *IGF-1* haplotypes with other avian *IGF-1* sequences downloaded from GenBank showed clustering of the haplotype 9C to the ancestral red jungle fowl (Figure 4.13), while haplotype 11C and 9A clustered together in the same clade. The tree was rooted

with southern Ostrich *IGF-1* sequence.

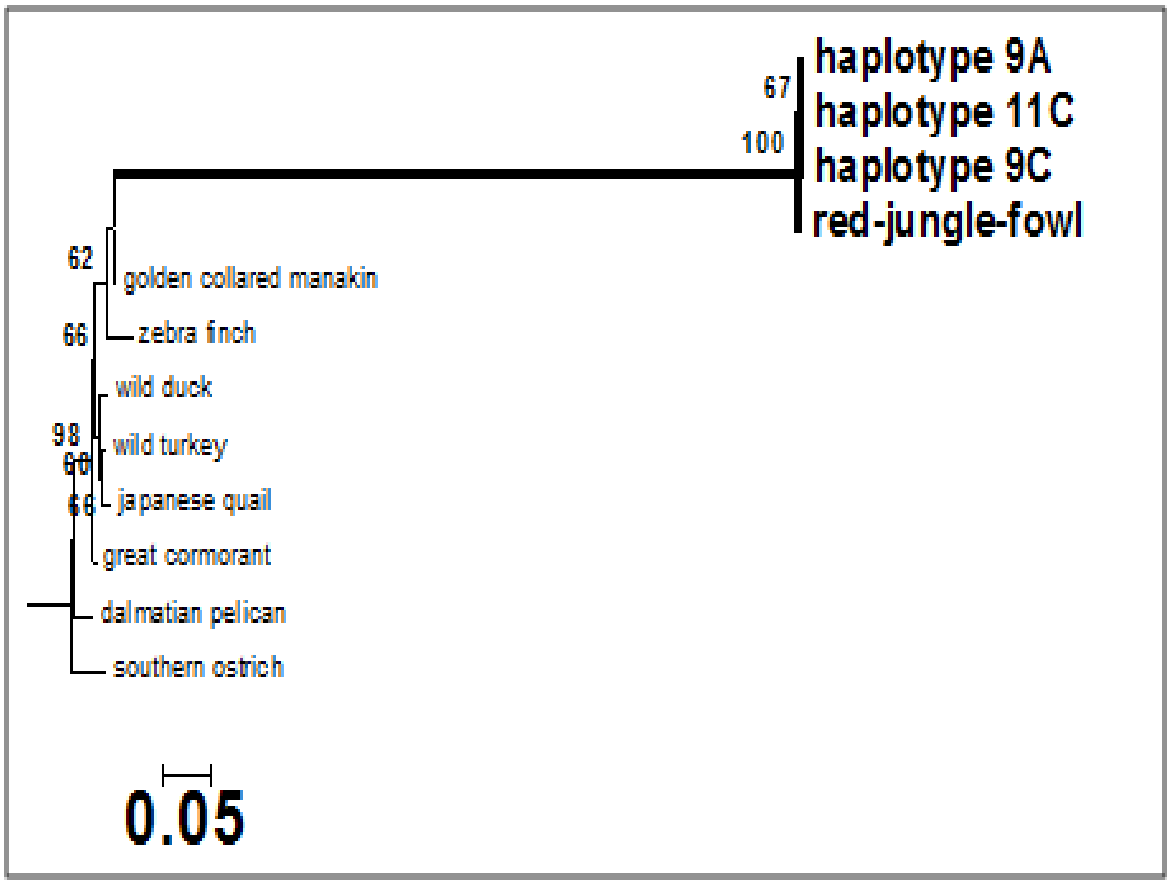

Figure 4.13: A rooted maximum likelihood tree of the three *IGF-1* chicken haplotypes and other avian *IGF-1*

A splits decomposition network of the three *IGF-1* chicken haplotypes and the red jungle fowl haplotype clustered with haplotype 9C. Haplotype 11C was too close in the same clade but were distant from haplotype 9A whereby both clustered together in the same Taxa. The other avian species were distant from the three chicken *IGF-1* haplotypes (figure 4.14).

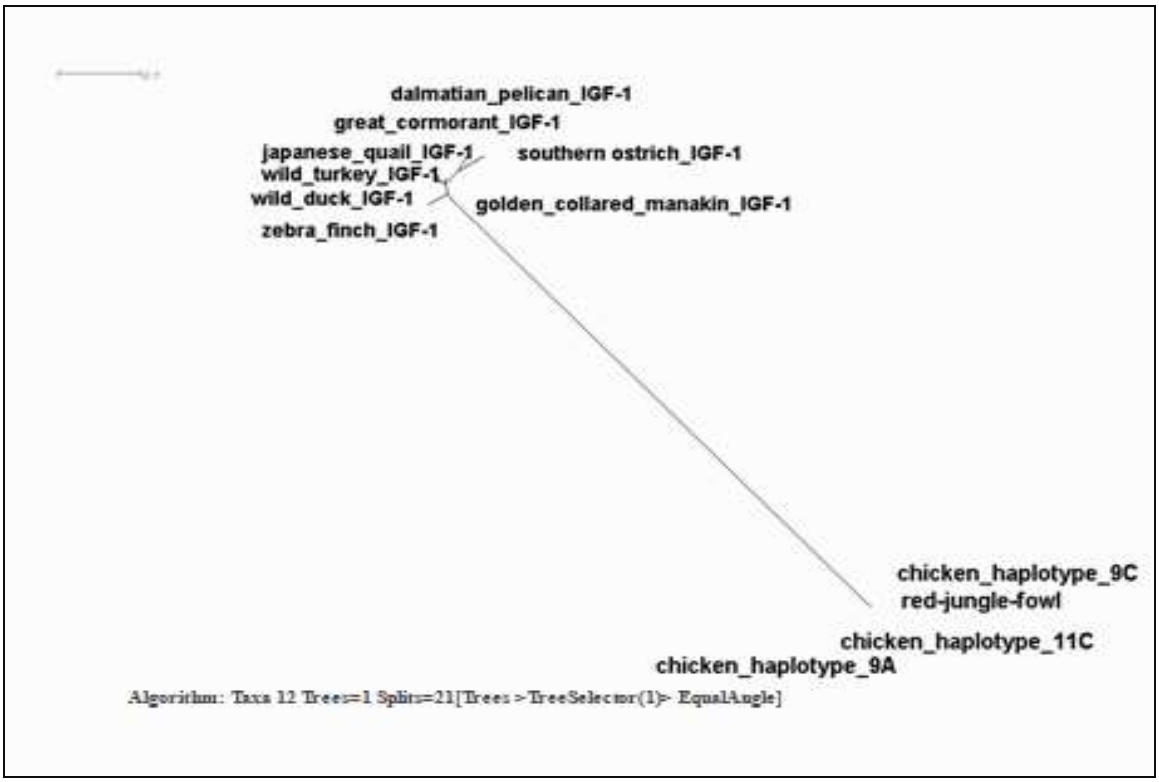

Figure 4.14: Phylogenetic network tree for the three *IGF-1* haplotypes with other avian *IGF-1* sequences.

4.2.6 *IGF-1* diversity indices in the three populations from three agro-climatic zones of Kenya

The summary of the *IGF-1* diversity indices was computed using Arlequin version.3.5.1.2. The observed heterozygosity (H_O) values ranged from 0.424 - 0.714 (Table 4.4). Lamu archipelago and Mt.Elgon catchment had the highest estimates of observed heterozygosity of 0.714 and 0.680 respectively and these values were higher than the expected heterozygosity values of 0.658 and 0.630 respectively. The least nucleotide diversity was observed in Lake Turkana Basin (0.001) while Lamu archipelago had the highest estimate of nucleotide diversity (0.002). Observed

heterozygosity was higher than the expected heterozygosity in all the populations except for Lake Turkana basin.

Table 4.4: Diversity indices of *IGF-1* candidate gene in the sampled populations

Populations	Number of Heterozygotes	Number of Homozygotes	H_O	H_E	Pi
Lake Turkana basin	28	38	0.424	0.630	0.001 +/- 0.001
Lamu archipelago	40	16	0.714	0.658	0.002 +/- 0.001
Mt. Elgon catchment	34	16	0.680	0.630	0.002 +/- 0.001

H_E = Expected heterozygosity; H_O = observed heterozygosity; Pi=Nucleotide diversity

4.2.7 Analysis of Molecular Variance of *IGF-1* candidate gene in indigenous chicken populations

Analysis of Molecular Variance (AMOVA) was carried out in Arlequin version 3.5.1.2 (Excoffier et al., 2005) to assess hierarchical partitioning among and within populations of genetic variability and assuming Hardy-Weinberg proportions within populations. Based on pairwise differences in AMOVA, variations within individuals was highest (87.85%) as opposed to variations among the three populations within the two groups (8.62%) and variations among individuals within the three populations (3.91%). Among

groups was (-0.38%), (Table 4.5). A significant sub-population structuring was evident with a *p*-value of 0.0 among sub-populations within groups.

Table 4.5: *IGF-1* population genetic structure from AMOVA

Source of variation	Hierarchy	d.f.	Variation	Percentage variation	*p*-value
Among groups	1	1	-0.003	-0.38	0.670+-0.014
Among sub-populations within groups	2	1	0.059	8.62	0.000+-0.000
Among individuals within populations	3	169	0.027	3.91	0.272+-0.014
Within individuals	4	172	0.599	87.85	0.065+-0.006

4.2.8 Association by distance model revealed by Mantel test for *IGF-1* Candidate gene

A Mantel test was used to assess the non-random association between genetic differentiation (F_{ST}) and geographic distances between populations by plotting the regression graph of the genetic and geographic distances using GenAlEx v6.501 software (Peakall & Smouse, 2006) which is an add-on in Microsoft Excel (Figure 4.15).

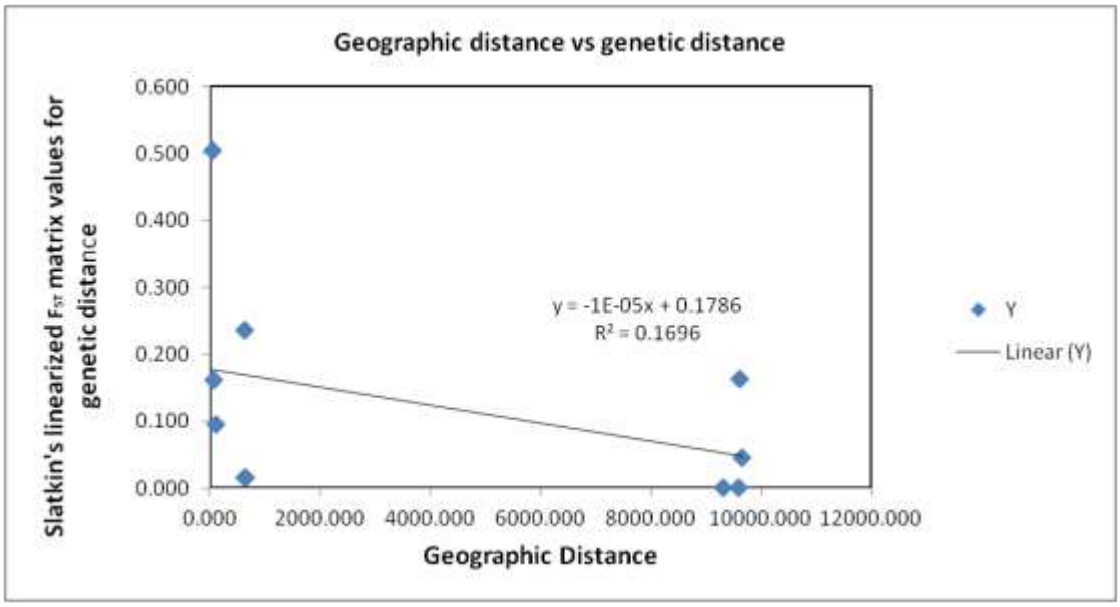

Figure 4.15: A regression graph showing the relationship between geographic and *IGF-1*candidate gene genetic distance matrices in the three populations from three agro-climatic zones of Kenya.

A weak non-significant negative correlation (r = 0.4118, P>0.05) was observed between genetic variations and the geographic location in the three populations from three agro-climatic zones of Kenya.

4.3 Assessment of functional polymorphisms at Prolactin (*PRL*) candidate gene for egg production in indigenous chickens of Kenya

4.3.1 Gel electrophoresis

The DNA was amplified for *PRL* candidate gene with the relevant primers and PCR products visualized under UV light. The gel image in plate 4.5 - 4.8 displays a 2% agarose gel electrophoresis showing a 154bp fragment and 130bp fragment of representative results. The specific gene of interest was amplified through PCR using specific primers: forward5′-TTTAATATTGGTGGGTGAAGAGACA-3′;reverse5′-ATGCCACTGATCCTCGAAAACTC-3′.for chicken *PRL* gene were used (Cui et

al.,2006 and Akaboot et al., 2012). The amplified product was about 154 bp insertion and 130 bp deletion (Plate4.2), this was in line with other earlier findings reported by (Cui et al.,2006 and Akaboot et al., 2012). The primers targeted a region of 154bp and 130bp which is clearly indicated by the 1000 base pair molecular weight DNA marker. The amplified region corresponds to Chromosome 2: 58,655,089-58,661,243. Galgal4:CM000094.3.PRl. All the samples amplified. Water was used as a negative control. The positive control of a known avian species with an amplified region of interest was used and was obtained from the International Livestock Research Institute samples

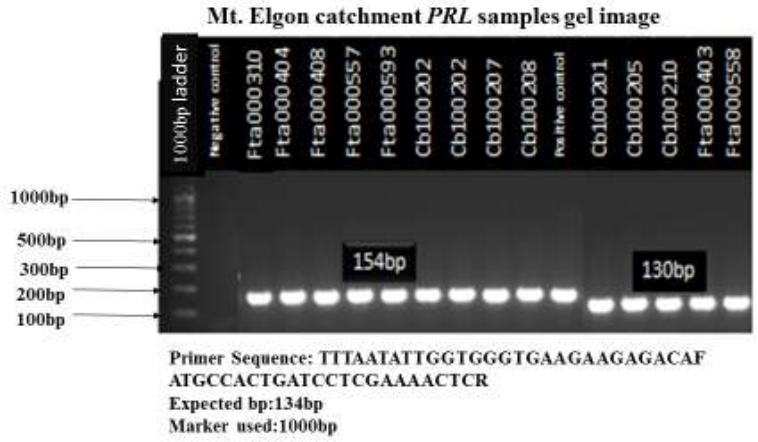

Plate 4.4: Gel image of *PRL* gene of indigenous chicken from Mt. Elgon catchment (representative sample)

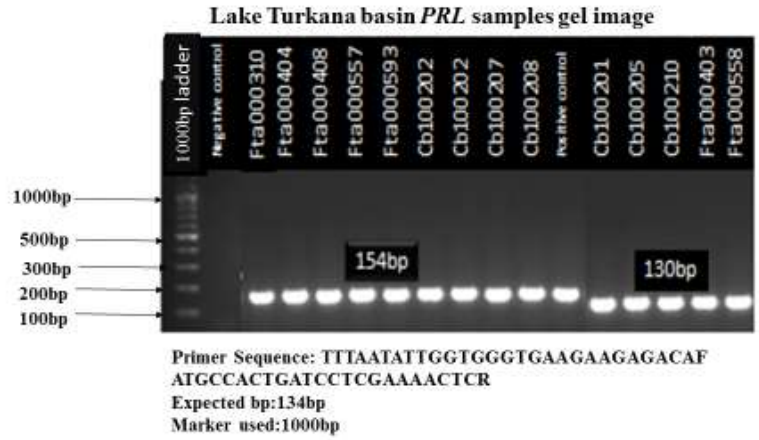

Plate 4.5: Gel image of *PRL* gene of indigenous chicken from Lake Turkana basin (representative sample)

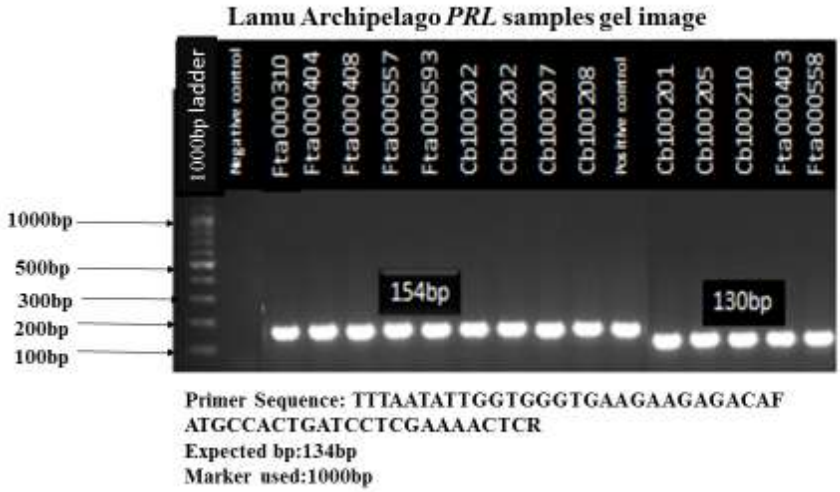

Plate 4.6: Gel image of PRL gene of indigenous chicken from Lamu Archipelago (representative sample)

4.3.2 Editing of *PRL* chromatograms

Quality of the underlying trace data was evaluated and the most accurate consensus sequence possible generated as shown in the chromatograms (fig 4.16, 4.17) this was done manually using Chromas Lite 2.1 (Avin, 2012). 24bp Insertions were illustrated in the sequences and hence identified as possible SNPs (Fig 4.16) at site 358 as previously reported by (Cui et al.,2006 and Akaboot et al., 2012). Possible SNPs were identified and illustrated as 24bp deletions in the sequences (Fig 4.17) at site 358 as previously reported by (Cui et al.,2006 and Akaboot et al., 2012). Polymorphism within the chromatogram sequence was evident as illustrated in figure 4.16.

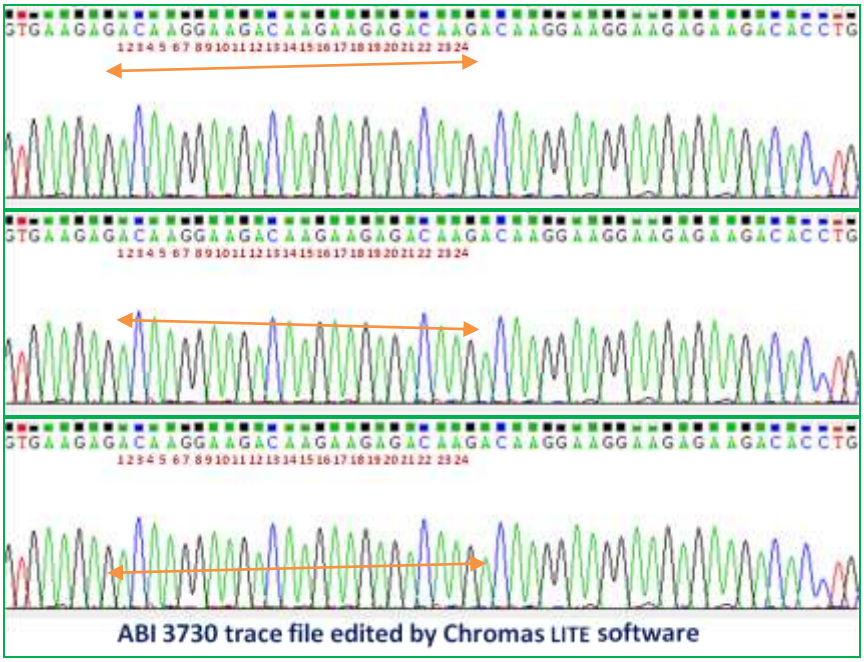

Figure 4.16 –chromatograms showing 24 bp insertions

Polymorphism within the chromatogram sequence was evident as illustrated in figure 4.17.

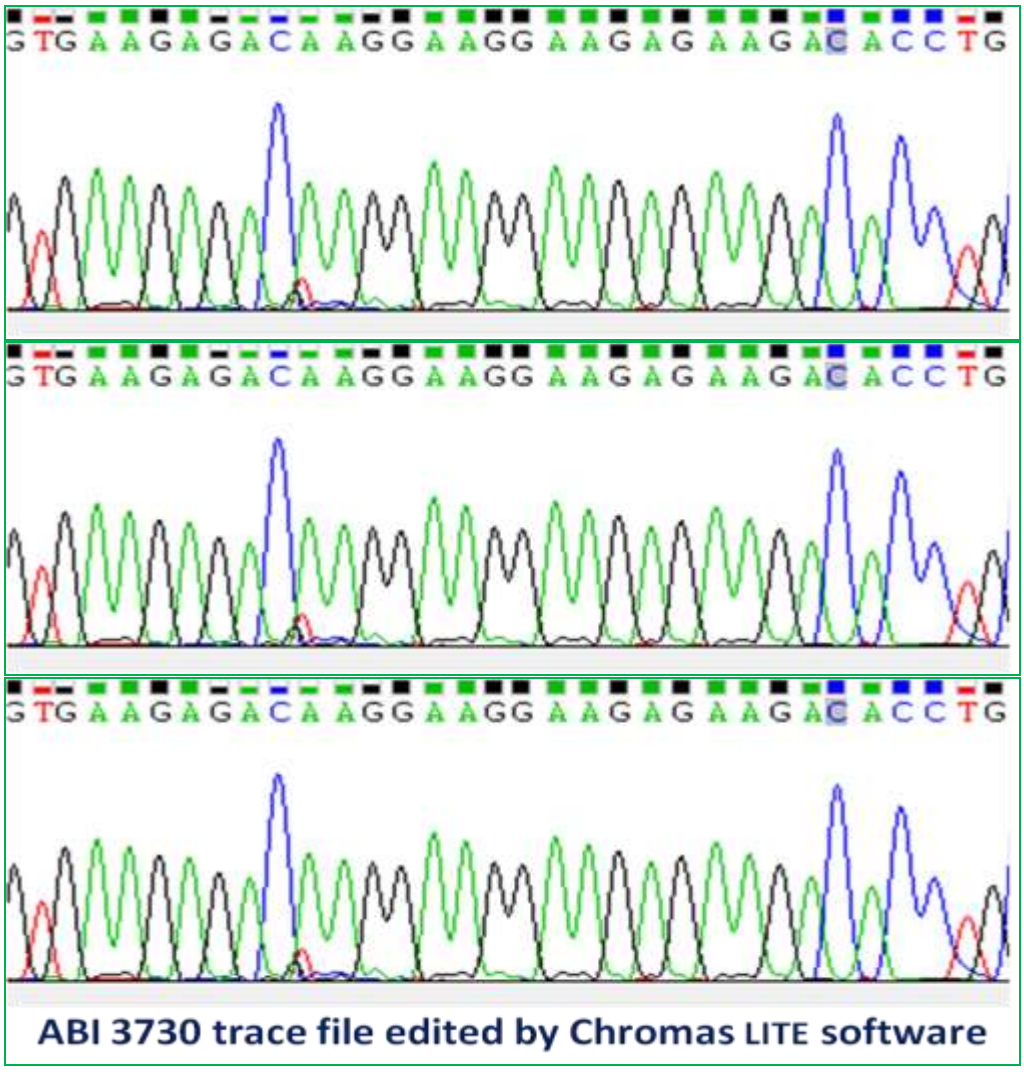

Figure 4.17– chromatograms showing 24 bp deletions

4.3.3 Multiple sequence alignment of *PRL* with the red jungle fowl

A multiple sequence alignment of all the samples plus reference sequences from GenBank (appendix VI) was done with Clustal X2 software. This sequences were aligned to identify regions of similarity that indicated functional relationships between the sequences. The aligned sequences were able to show the 24 bp Indel (Cui et al.,2006 and Akaboot et al., 2012) Figure 4.18. Alignment of the sequences generated two haplotypes i.e. Haplotype 154, Haplotype 130 (Figure 4.18). Haplotype 154bp included

the 24bp insertions whereas Haplotype 130 had the 24bp deletion. The red jungle fowl was used as the reference *PRL* sequence. This shows areas of similarities and where the sequences vary.

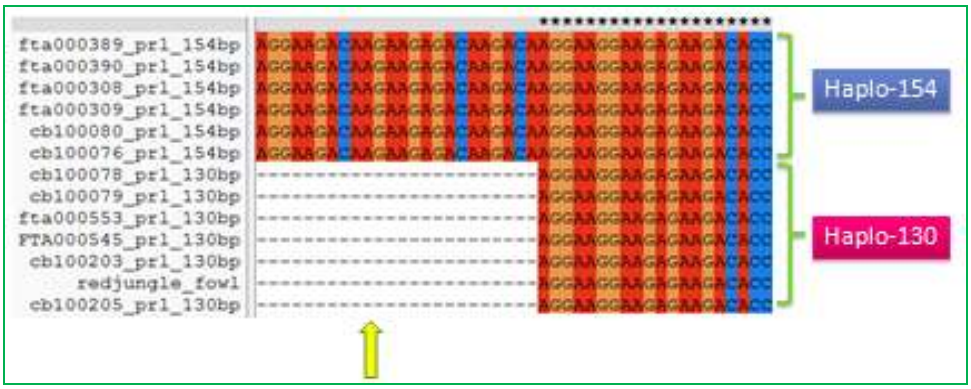

Figure 4.18 multiple sequence alignment of *PRL* and red jungle fowl showing 24 bp Indel (Clustal X version 2.1)

4.3.4 *PRL* haplotype distribution in the three agro-climatic zones in Kenya

The frequencies of the two *PRL* chicken haplotypes distribution in the three agro-climatic zones of Kenya were recorded in table 4.6. Haplotype 154 and Haplotype 130 were distributed in all the population sampled. Haplotype 154 had the highest percentage frequencies of 71%, 78% and 63% in Lake Turkana basin, Lamu archipelago and Mt. Elgon catchment respectively as compared to Haplotype 130 which had the lowest percentage frequencies of 29%, 22%, and 37% in Lake Turkana basin, Lamu archipelago and Mt. Elgon catchment respectively. The presence of the 130 haplotypes in all the populations' infered to the fact that *PRL* gene is polymorphic. (Table 4.6)

Table 4.6: Frequencies of the two *PRL* chicken haplotypes distribution in the three agro-climatic zones of Kenya

Population	**Haplotype**	**Haplotype number**	**Haplotype frequency**	**%**
Lake Turkana basin	Haplotype 154	20	0.7140	71%
	Haplotype 130	8	0.285	29%
Lamu archipelago	Haplotype 154	21	0.778	78%
	Haplotype 130	6	0.222	22%
Mt.Elgon Catchment	Haplotype 154	17	0.629	63%
	Haplotype 130	10	0.370	37%

The two *PRL* haplotypes were distribution in different percentages in the three agro-climatic zones of Kenya as shown in figure 4.19. Haplotype 154 and Haplotype 130 were distributed in all the population sampled. Haplotype 154 had the highest percentage frequencies in all the populations as compared to Haplotype 130 which had the lowest percentage frequencies. The presence of the 130 haplotypes in all the populations was able to infer to the fact that *PRL* gene is polymorphic. (Figure: 4.19)

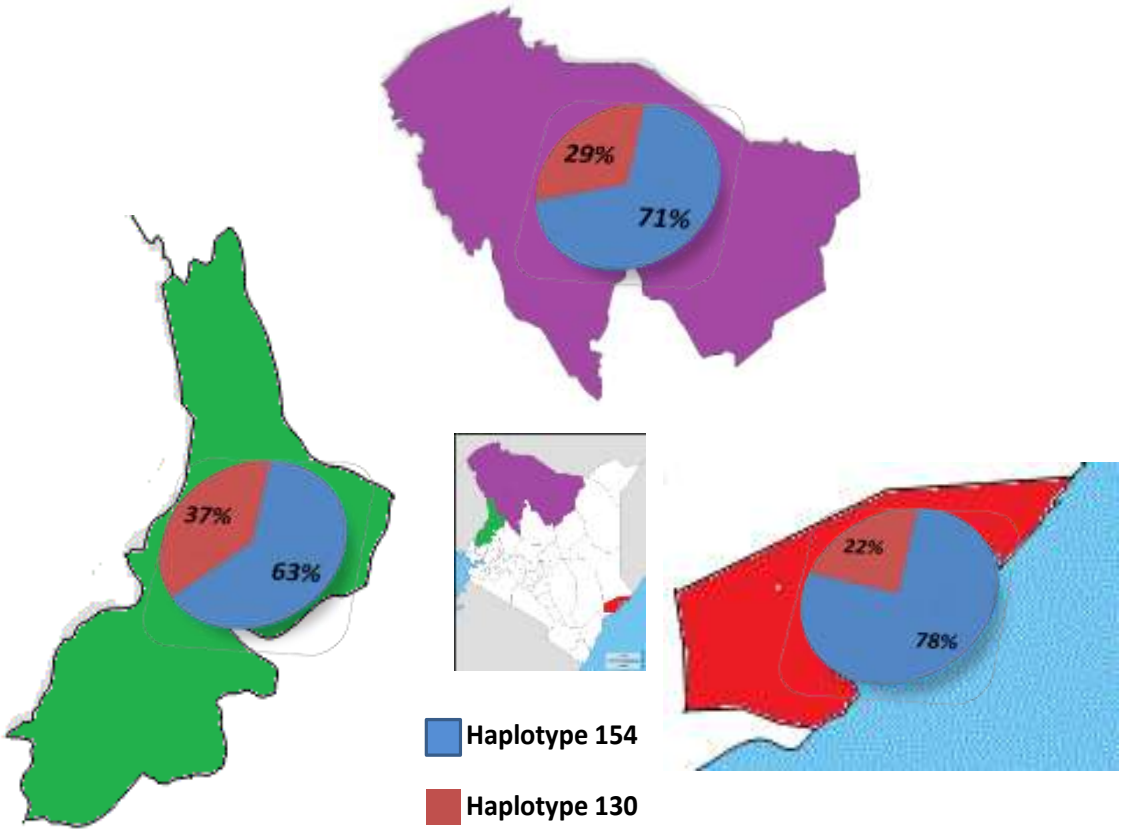

Figure 4.19– Pie chart distribution of the two *PRL* haplotypes in the three agro-climatic zones of Kenya

4.3.5 Phylogenetic analysis of *PRL* haplotypes with other avian species

The phylogenetic analysis of *PRL* haplotypes with other avian species was determined by the construction of a phylogenetic tree and construction of a splits decomposition network of the two *PRL* chicken haplotypes with other *PRL* avian sequences were generated in splits tree.

Maximum likelihood tree of *PRL* and other avian species was constructed to determine the model of sequence evolution and the rate of heterogeneity of the sequences MEGA Version 6 software was used (Figure 4.20)

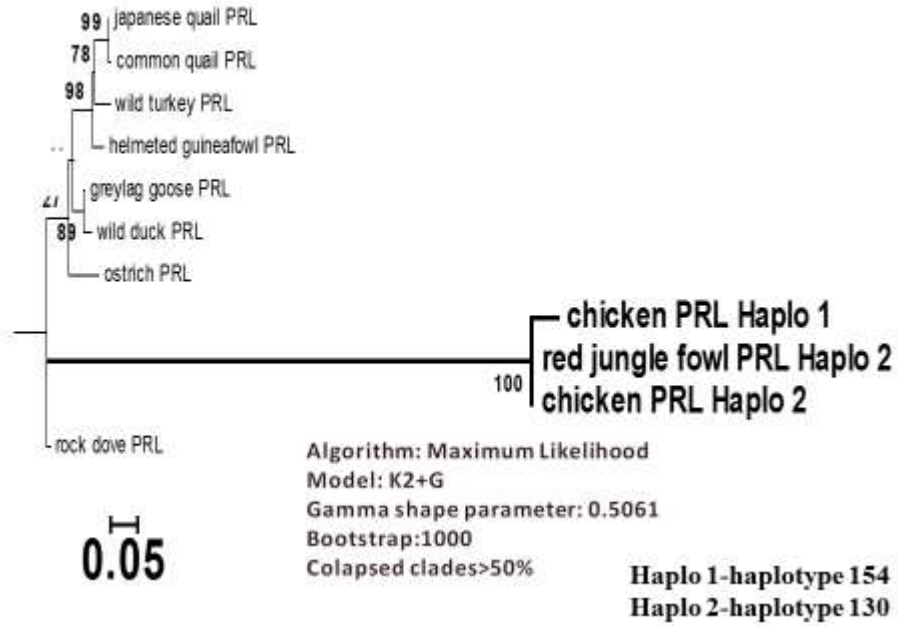

Figure 4.20: A rooted maximum likelihood tree of the two *PRL* haplotypes and other avian *PRL*

Phylogenetic analysis of the two *PRL* haplotypes with other avian *PRL* sequences downloaded from GenBank (appendix VIII) showed clustering of the haplotype 130 to the ancestral red jungle fowl (Figure: 4.20) whereas haplotype 154 was in the same clade as the latter. The tree was rooted with rock dove *PRL* sequence.

A splits decomposition network of the two *PRL* chicken haplotypes with other *PRL* avian sequences were generated in splits tree. The algorithim used was the Trees_ TreeSelector with Equal Angle. The red jungle fowl clustered with the haplotype 130 and were distant from haplotypes 154. The other avian species were distant from the two chicken *PRL* haplotypes (Figure: 4.21).

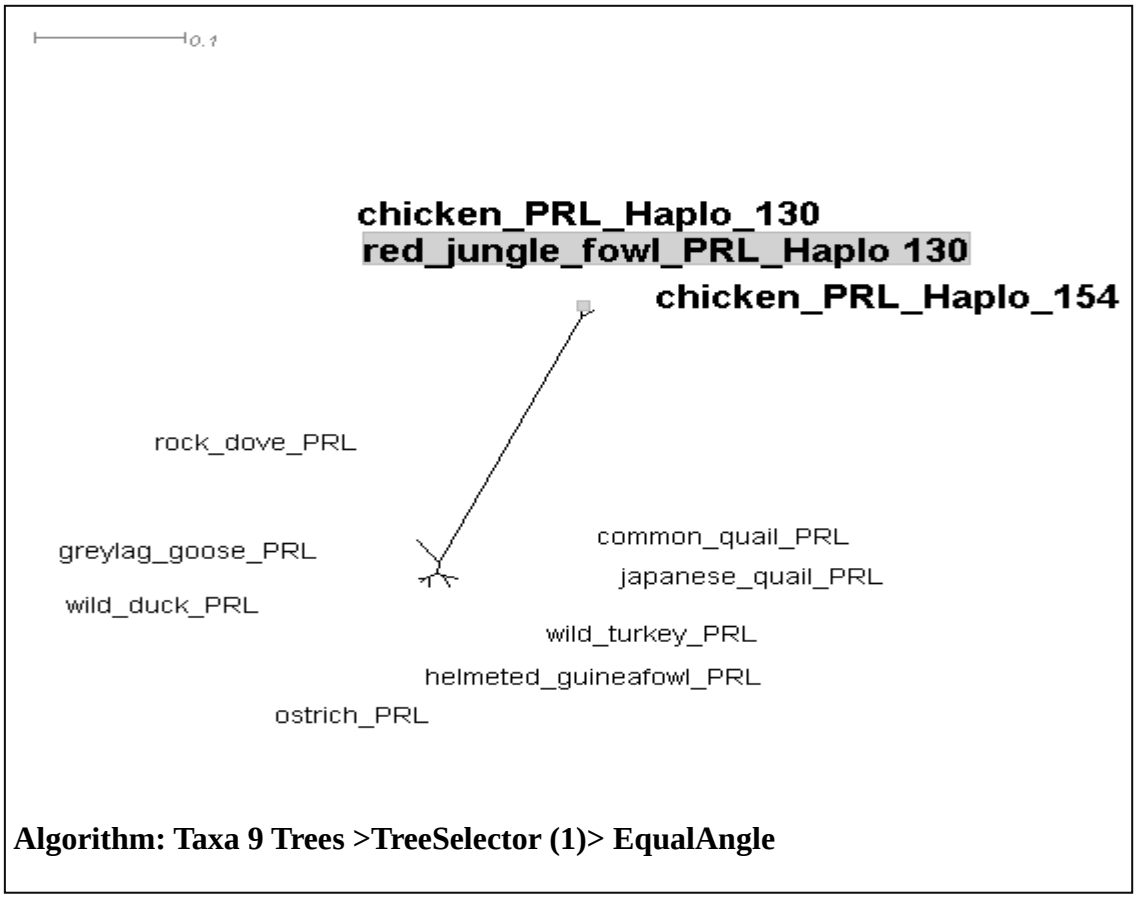

Figure 4.21: Phylogenetic network tree for the two *PRL* haplotypes with other avian *PRL* sequences.

4.3.6 *PRL* diversity indices in the three populations from three agro-climatic zones of Kenya

The summary of the *PRL* diversity indices were calculated using Arlequin version.3.5.1.2 and recorded in table 4.7. Lower estimates of expected heterozygosity and observed heterozygosity were calculated for Lamu archipelago (0.222) and (0.199) respectively compared to the other populations (Table 4.7). Mt. Elgon catchment population had the highest estimates of expected heterozygosity at (0.370) and also observed heterozygosity (0.305); Lamu archipelago had average estimates as compared to the other populations (Table 4.7). The least nucleotide diversity was observed in Lamu archipelago (0.045) while Mt. Elgon had the highest estimate of nucleotide

diversity (0.069).

Table: 4.7: Diversity indices of *PRL* haplotypes in the sampled populations

Populations	Number of heterozygotes	Number of Homozygotes	H_O	H_E	Pi
Lake Turkana basin	16	40	0.286	0.247	**0.056+/- 0.030**
Lamu archipelago	12	42	0.222	0.199	**0.045+/- 0.025**
Mt. Elgon catchment	20	34	0.370	0.305	**0.069+/- 0.036**

***H_E* = Expected heterozygosity*; H_O* = observed heterozygosity*; Pi*=Nucleotide diversity**

4.3.7 Analysis of Molecular Variance of *PRL* indigenous chicken populations

The variation in *PRL* among three populations was assessed at four hierarchical levels: among groups, among populations within groups, among individuals within populations and within individuals was measured with AMOVA of pairwise differences as implemented in Arlequin v.3.5.1.2 (Excoffier et al., 2005). Based on pairwise differences in AMOVA, variations within individuals was highest (116.31%) as opposed to variations among the three populations within two groups (-0.35%) and variations among individuals within the three populations (-16.99%) as illustrated in (Table: 4.8). Variation within individuals was very significant followed by variation among the three populations within the sampled areas.

Table: 4.8: *PRL* population genetic structure from AMOVA

Source of variation	Hierarchy	d.f.	Variation	Percentage of variation	*p*-value
Among groups	1	1	0.0310	1.03	0.320+-0.013
Among populations within groups	2	1	-0.011	-0.35	0.512+-0.014
Among individuals within populations	3	161	-0.513	-16.99	1.000+-0.000
Within individuals	4	164	3.512	116.31	1.000+-0.000

4.3.8 Association by distance model revealed by Mantel test for *PRL* Candidate gene

A Mantel test was used to assess the non-random association between genetic differentiation (F_{ST}) and geographic distances between populations by plotting the regression graph of the genetic and geographic distances using GenAlEx v6.501 software Peakall & Smouse, (2006) which is an add-on in Microsoft Excel (Figure 4.22).

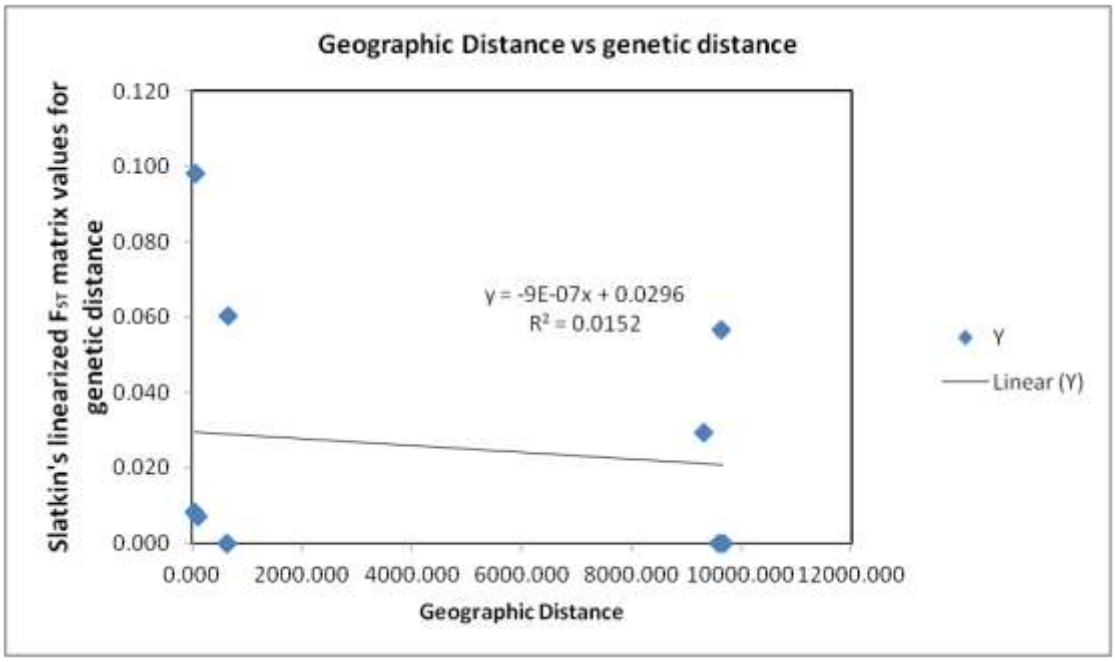

Figure 4.22: A regression graph showing the relationship between geographic and *PRL* candidate gene genetic distance matrices in the three populations from three agro-climatic zones of Kenya.

A weak non-significant negative correlation (r = 0.1233, P>0.05) was observed between genetic variations and the geographic location in the three populations from three agro-climatic zones of Kenya.

CHAPTER FIVE

DISCUSSION

5.1 Characterization of the phenotypes associated with meat and egg production in indigenous chickens

Knowledge of relevant morphological traits in terms of body parameters in indigenous chickens is crucial to any improvement program since these are used for selection. Indigenous chickens from Lamu archipelago were found to have the longest average shank length, while the ones in Lake Turkana basin recorded the shortest average shank. A similar pattern was observed for body length of chickens in the agro-climatic zones. The results differed from Guni et al., (2013) in Tanzania as they were higher than the ones reported by Magothe et al., (2012) who reported a range of 8.2cm-8.7cm for Lamu archipelago and Mt Elgon catchment but lower for Lake Turkana basin. The observed variation in body length and shank length between agro-climatic zones may indicate the existence of different diverse sub-populations within the indigenous chicken population. Bantam phenotype is characterized by a small body size and commonly found in Lake Turkana basin (Moraa et al., 2015). The small body has been associated with better heat tolerance and heat evacuation Chang et al., (2012), this may explain the reason why this phenotype was common in Lake Turkana basin which is characterized by high ambient temperature (Moraa et al., 2015). This further explains why chickens in Lake Turkana basin had smaller body lengths and shank length for adaptation to the climate.

Lamu archipelago recorded the heaviest chickens, based on body live weight compared to Lake Turkana Basin which recorded the lowest average weight. Previous studies have recorded similar results to the current study Katule (1998), Msoffe et al., (2001) and (Guni et al., 2013). Though the results were higher than those reported by Dana (2010), Olawunmi et al., (2008), Mwalusanya et al., (2002) and Guèye (1998). In Kenya, they were higher than the results reported by Magothe et al (2010) though slightly different from Olwande et al., (2010) and lower than the ones reported by King'ori (2004).

The study observed large variation in body weight between agro-climatic zones indicating the existence of different diverse subpopulations within the indigenous chicken population. Chickens with small body length, weight and shank length need to be improved to help alleviate production problems associated with larger parameters (Adeleke et al., 2011). Similarly, those with larger suitable attributes need to be identified and conserved to avoid loss of these traits.

The existence of positive and significant correlations between body weight and body measurement traits have been reported by Alabi et al., (2012) and Guèye et al.,(1998) from Nigeria and Senegal respectively for local chickens. The significant interaction between body weight and other body measurements imply that these easily measured morphological traits can be used for estimation of body weight and hence important in selection criteria that can be used to improve body weight Aswani et al., (2017).

The study reported no significant differences between the agro-climatic zones with respect to the clutch size. These results were inconsistent with those reported by Guni et al., (2013) who reported a clutch size of 11. Mwalusanya et al., (2002) and Olwande et al., (2010) reported a lower value. The frequencies of eggs sat on showed a slight variation within the agro-climatic zones in Kenya. For instance, Mt Elgon catchment recorded the highest frequency of eggs seated on by the indigenous chickens while Lake Turkana basin had the lowest frequency of eggs seated on. The difference in the frequencies of eggs sat on was mainly based on the farmers' decision to give out the eggs to the hens. The study found out that the farmers give out a certain fraction to the hens to lie on and consume or sell the rest for cash.

Analysis of hatchability among the 3 zones similarly revealed slight differences in the frequencies of eggs hatched. High hatchability was obtained in Lamu archipelago followed by Mt Elgon and Lake Turkana. These results were consistent with Guni et al., (2013), Kugonza et al., (2008), Development & Veterinary, (2005) and Mengesha et al., (2008), with an exception for Lake Turkana basin which had lower hatchability. In

Kenya Olwande et al., (2010), King'ori (2004) and Okitoi and Mukisira (2001) reported lower results than the ones reported in this study. The lower hatchability in Lake Turkana basin was attributed to the fact that it is a hot region experiencing an annual temperature of 34°C and a minimum of 30°C (Paron, et al., 2013), so when the eggs are laid, exposure to these high temperatures they ruin the hatching of the eggs.

Lamu Archipelago had heavy-bodied chickens though they produced fewer eggs which were often double-yolked. The study found out that despite the fact that Indigenous chickens in Lake Turkana basin were small-bodied but produced more eggs as compared to the ones in Lamu Archipelago which were heavy-bodied though they produced fewer eggs that were often double-yolked. The double-yolk is attributed to no production of chicks. This is an ecotype that needs to be conserved for the fact that it is heavy and big-bodied but lack of chick production means loss of the chickens in Lamu Archipelago.

The study also sought to find out whether there existed significant interactions between the numbers of eggs laid per clutch, sat on, hatched and the number of sitting times per year in different agro-climatic zones. It was clear that there was a significant relationship between the agro-climatic zones and the number of yolks in an egg and the number of sitting times per year at 95% confidence interval level. The positive significant variation indicates the existence of different diverse subpopulations within the indigenous chicken population in Kenya.

5.2 Assessment of the polymorphisms at *IGF-1* candidate gene in indigenous chickens in Kenya

5.2.1 *IGF-1* haplotype distribution in the three agro-climatic zones in Kenya

There were three *IGF-1* haplotypes that were discovered in this study that was chicken haplotype 9C, chicken haplotype 11C and chicken haplotype 9A. All the haplotypes were present in all the three agro-climatic zones but varied in their frequencies. Chicken haplotype 9C dominated in Lake Turkana basin while chicken haplotype 11C dominated

in Mt. Elgon catchment and Lamu archipelago. Chicken haplotype 9A was the least distributed in the various agro-climatic zones. The haplotype variations observed in the various agro-climatic zones of Kenya could be attributed to the varying polymorphism nature of the gene (Cui et al., 2006). Various studies have shown that polymorphisms in chicken *IGF-1* gene in different breeds has been found to be associated with egg production (Akaboot et al., 2012; Bhattacharya et al., 2011; Wang, Li, Yan Kwok, Ge, & Leung., 2010 and Cui et al., 2006).

5.2.2 *IGF-1* haplotype phylogeny

A maximum likelihood tree with the three chicken *IGF-1* haplotypes and other avian *IGF-1* was constructed. The phylogeny revealed that the chicken *IGF-1* haplotype 9C falls in the same clade with the red jungle fowl which is the ancestral haplotype while haplotype 11C though in the same Taxa whereas chicken haplotype 9A was a little bit distant in a different clade. Similar results were also evident in the splits decomposition network of the chicken *IGF-1* haplotypes and the other avian species *IGF-1*. Chicken haplotype 9A could be a recent introduction derived from the chicken haplotype 9C and chicken haplotype 11C. All the other avian species *IGF-1* were distant from the three chicken *IGF-1* haplotypes and the red jungle fowl. There still are some ancestral alleles present in indigenous chickens in Kenya as clearly indicated with these results.

5.2.3 *IGF-1* haplotype diversity

Low observed heterozygosity (H_O) was noted compared to expected heterozygosity (H_E). These results could be attributed to the selection for economic traits that may have caused the low observed heterozygosity (Akaboot et al., 2012). Low H_O may lead to positive assortment or a situation of high homozygosity. Reduction of heterozygosity can occur from inbreeding and may result in a loss of alleles; commercial breeds have shown a lack of some alleles found in Red Jungle fowl (Muir et al., 2008). The high observed heterozygosity in Mt. Elgon compared to the expected heterozygosity and these may be attributed to suspecting an isolate-breaking effect that is the mixing of two

previously isolated populations. This might be as a result of exportation of kuchi chicken from Lamu archipelago to Mt Elgon. The study yielded low values of observed heterozygosity in all the study populations an indication of low or no inbreeding as well as a bottle neck of founder population effect because most of the farmer have turned to commercial chickens.

5.2.4 Genetic structure revealed by *IGF-1* variations

Analysis of molecular variance indicated a significant amount of genetic variance within the populations. This inferred that the populations are constituted by genetically distinct individuals which is normally an indication of sexual reproduction ongoing in the population. There were also variations among the three populations within groups which was an indication that the populations are genetically different, suggesting limited gene flow between them.

5.2.5 Association by distance model revealed by Mantel test for *IGF-1*

To test whether genetic differentiation was directly proportional to geographic proximity, a Mantel test involving pair-wise F_{ST} values against geographic distance between populations was performed. From the results, a weak, negative and non-significant correlation between the geographic distance and genetic distance was observed ($r = 0.4118$, $P>0.05$). The Mantel test thus reveals the presence of a phylogeographic structure within Kenya for *IGF-1* chicken haplotypes.

5.3 Assessment of the functional polymorphisms on the *PRL* candidate gene in indigenous chickens in Kenya

5.3.1 *PRL* haplotype distribution in the three agro-climatic zones in Kenya

There were two *PRL* haplotypes that were discovered in this study that was Haplotype 154 and haplotype 130. All the haplotypes were present in all the three agro-climatic zones but varied in their frequencies. Haplotype 154 and Haplotype 130 were distributed in all the population sampled. Haplotype 154 had the highest percentage

frequencies of 71%, 78% and 63% in Lake Turkana basin, Lamu archipelago and Mt. Elgon catchment respectively as compared to Haplotype 130 which had the lowest percentage frequencies of 29%, 22%, and 37% in Lake Turkana basin, Lamu archipelago and Mt. Elgon catchment respectively. The presence of the 130 haplotypes in all the populations inferred to the fact that *PRL* gene is polymorphic, (Table 4.7). Haplotype 154 dominated in the three agro-climatic zones having more than a half of the entire populations sampled as compared to haplotype 130. The haplotype variations observed in the various agro-climatic zones of Kenya could be attributed to the varying polymorphism nature of the gene (Cui et al., 2006). Various studies have shown that polymorphisms in chicken *PRL* gene in different breeds has been found to be associated with egg production (Bhattacharya et al., 2011 and Cui et al., 2006)

5.3.2 *PRL* haplotype phylogeny

A maximum likelihood tree with the two *PRL* haplotypes and other avian *PRL* was constructed. The phylogeny revealed that the chicken *PRL* haplotype 130 falls in the same clade with the red jungle fowl while haplotype 154 was a little bit distant in a different clade. Similar results were also evident in the splits decomposition network of the *PRL* haplotypes and the other avian species *PRL*. Haplotype 154 could be a recent introduction derived from the haplotype 130. All the other avian species *PRL* were distant from the two *PRL* haplotypes and the red jungle fowl. There still are some ancestral alleles present in indigenous chickens in Kenya as clearly indicated with these results.

5.3.3 *PRL* haplotype diversity

The study recorded low H_O as compared to the H_E. These results could be attributed to the selection for economic traits that may have caused the low observed heterozygosity (Akaboot et al., 2012). Lake Turkana basin had the lowest observed heterozygosity and the highest egg production in the phenotypic analysis this could be an indication there could be an association between *PRL* gene and egg production as shown in other studied

(Bhattacharya et al., 2011 and Cui et al., 2006). The high observed heterozygosity in Mt. Elgon compared to the expected heterozygosity and these may be attributed to suspecting an isolate-breaking effect that is the mixing of two previously isolated populations. This might be as a result of exportation of kuch chicken from Lamu archipelago to Mt Elgon. The study yielded low values of observed heterozygosity in all the study populations an indication of low or no inbreeding as well as a bottle neck of founder population effect because most of the farmer have turned to commercial chickens.

5.3.4 Genetic structure revealed by *PRL* variations

Analysis of molecular variance indicated a significant amount of genetic variance within the populations this inferred that the populations are constituted by genetically distinct individuals this is normally an indication of sexual reproduction ongoing in the population. There were also variations among the five populations within groups an indication that the populations are genetically different, suggesting limited gene flow between them.

5.3.5 Association by distance model revealed by Mantel test for *PRL*

To test whether genetic differentiation was directly proportional to geographic proximity, a Mantel test involving pair-wise F_{ST} values against geographic distance between populations was performed. From the results, a weak, negative and non-significant correlation between the geographic distance and the genetic distance was observed. ($r = 0.1233$, $P>0.05$). The Mantel test thus reveals the presence of a phylogeographic structure within Kenya for *PRL* chicken haplotypes.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Characterization of indigenous chicken phenotypes associated with meat and egg production in selected agro-climatic zones of Kenya was successful. The study was able to show that these sites still contained distinct ecotypes that need to be conserved and incorporated in the poultry breeding programs, for instance, the kuchi phenotype from Lamu for increased weight and mixed phenotype from Turkana basin for increased egg production. The observed interactions between the traits and the agro-climatic zones showed that there could be genetic differences within these populations that if harnessed and integrated into other indigenous chickens would aid in improvement of poultry breeding programs.

Haplotype diversity analysis revealed three unique haplotypes in *IGF-1*. In Lamu Archipelago, chicken haplotype 9C, which could be a recent introduction derived from chicken haplotype 9A and chicken haplotype 11C, dominated as compared to Lake Turkana basin and Mt. Elgon Catchment. It had the heavy bodied indigenous chickens that are the Kuchi phenotype. This could be attributed to the dispersion of the kuchi phenotype to other parts of the country and the production of double-yolked eggs which are not capable of producing chicks. In Lake Turkana basin, chicken haplotype 9A, which was the ancestral haplotype, was dominant an indication that Lake Turkana basin may contain important ancestral ecotypes. A significant subpopulation structuring was evident among subpopulations within groups an important finding in the study as the meat and growth parameters also showed structuring inferring the presence of polymorphisms at Insulin Growth Factor-1 candidate gene.

Polymorphisms associated with increased egg production at prolactin candidate gene in indigenous chickens of Kenya were detected hence the third objective was fully met.

Haplotype analysis revealed two chicken haplotypes for *PRL* (Haplotype 130 and haplotype 154). The ancestral haplotype 130 had low percentage distribution in the populations and chicken haplotype 154 which may be a new haplotype was highly distributed especially in Lake Turkana basin. And it was in Lake Turkana basin where indigenous chicken produced a lot of eggs though the hatchability was very low because of the temperatures. So there is need for genetic conservation of the indigenous chicken in Kenya.

In overall conclusion from these results it is clear that indigenous chickens of Kenya are diverse both in the phenotypic and genetic attributes. For instance the Kuchi phenotype in Lamu for meat production and the mixed phenotype in Lake Turkana basin for egg production. The double yolk phenomena in Lamu archipelago need to be conserved so that the ecotype is not lost because double yolks do not hatch to chicks. This shows that despite the ongoing cross breeding programs, there are still some indigenous chickens' ecotypes present in Kenya especially in Lake Turkana basin and Lamu archipelago that need to be conserved for the future.

6.2 Recommendations

1. The samples used in this study were from chickens in their natural ecological setting so as to investigate the local chicken ecotypes in their natural settings. An on-station controlled experiment to study further the genetic potential of these chicken ecotypes is recommended. Findings from this study should direct further research to investigate the role of factors such as genetics, epigenetics, feed intake, disease status and appetite mostly in their natural ecological setting and pave way for more studies on phenotypic characterization of indigenous chickens in all the agro-climatic zones in Kenya.
2. The *IGF-1* results have indicated that there are unique haplotypes that exist and it is polymorphic. All indigenous chickens from the other agro-climatic zones of Kenya should also be assessed to know if other unique haplotypes exist and to determine

their functional polymorphisms. The Kuchi phenotype from Lamu archipelago should be conserved because of its high weight and the disadvantageous trait of double yolking. Genome wide association studies should be done and should also incorporate other genes associated with meat production in poultry

3. The *PRL* results indicated that the *PRL* was polymorphic. Haplotypes 154 dominated in the tree agro zones and it was not the ancestral haplotype. Lake Turkana had the highest egg production had the least relative amount of the ancestral haplotype 130. Indeed there is need to conserve this ecotype. Genome wide association studies should be done and should also incorporate other genes associated with egg production in poultry

4. Molecular breeding should be incorporated in poultry breeding programs. The next level should be the use of next generation sequencing of indigenous chicken since SNPs for functional important traits are across the whole chicken genome hence genome wide association studies. This will enable a quick collection of genetic pool and breeding will be quickly and easily initiated. The cross breeding programs should be discouraged since they lead to erosion and dilution of indigenous chicken populations which are unique and have good production traits like the Kuchi for increased meat production and mixed phenotype for egg production. Indigenous chicken are known to have disease resistant and heat tolerant traits and they are also cheap to keep , yielding high returns to the farmer and still maintain the production rate as the commercial chickens. Efforts should also be put in place for the conservation of indigenous chickens since this study has clearly shown indigenous chicken ecotypes present in the country and hence could be utilized in indigenous chicken rearing for instance the KARI improved kienyeji.

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APPENDICES

Appendix I: Questionnaire for the phenotypic characterization of indigenous chickens in the three agro-climatic zones of Kenya

Farmers name…… Region …….

Location…… GPS……..

Enumerators name……. Date of interview……

Number of chickens……

1. How long has indigenous chickens been kept in the household? .....
2. Source of foundation stock…….
3. Age of indigenous chicken……
4. Do you feel the need to improve indigenous chicken production?
   Yes ( ) No ( )
5. Traits to improve in indigenous chickens……
6. What type of management system do you practice?
   Extensive ( ) semi-intensive ( ) intensive ( ) others, specify…….
7. Do you give supplementary food to your indigenous chickens?
   Yes ( ) No ( )
8. If you give feeds how frequently do you feed your birds daily? .....
9. How often do you cull your birds? .....
10. For what purpose do you cull your poultry?
    ( ) for consumption, ( ) sale, ( ) sacrifice, ( ) others, specify……

11. Which factors determine which bird you will cull?

    ( ) poor productivity ( ) old age ( ) sickness ( ) others, specify……

12. Have you heard about the improved poultry production practices

    yes ( ) no ( )

13. If yes what is your major source of information on the improved poultry production practices…….

14. Have you received any flock (cockerel or hen) from NGO's, Government or church as way of promoting improved poultry production practices

    yes ( ) no ( )

Morphometric measurements

Age in months……

Sex; male ( ) female ( )

Shank Length…… Body length……

Body weight……..

15. Does the hen sit on eggs?

    Yes ( ) No ( )

16. If yes how many eggs does the hen lay per clutch? ……..

17. What is the colour of the eggs laid? .....

18. How many eggs does the hen sit on per clutch? ……

19. How many eggs hatch per clutch per hen? .....

20. How many yolks are present in each egg? .....

21. Have you ever come across double yolked eggs?

……

22. How many times does this hen sit on eggs per year?

…….

Other general issues

23. Do you intend to extend poultry production?

Yes ( ) No ( )

If yes to what size……

24. What are your barriers to future expansion of poultry production? .....

25. What traits do you wish to see improved in indigenous chickens? .....

26. What do you think the government should do to improve poultry keeping particularly in the rural areas? .....

Appendix II- physical measurements

Chicken weight being measured
portable sensitive weigh balance

↔ - The shank length measured

↔ - Body length measured

Appendix III - Blood collection

Items required

1. Alcohol wipes(for sterilization of the wing vein prior to pricking)
2. Dry sterile cotton wool (to stop bleeding),
3. Disposable pipettes
4. Lancet
5. Sharps container for disposal
6. FTA cards.
7. Latex examination gloves

Procedure

1. Latex gloves were put on.
2. The chicken was held upside down with the wing extended to expose the wing vein.
3. A few small feathers were plucked out
4. The wing vein was swabbed with an alcohol wipe,
5. The lancet was used to prick the vein and discarded to the safety disposal container.
6. The pipette was used to draw the blood

Pressure was placed on the vein insertion site with the dry sterile cotton wool until bleeding stopped using the finger.

7. The blood was then spotted on the FTA card and left to air dry.

NOTE: A lancet and a pipette was used on each chicken and discarded.

Appendix IV: SOP for DNA extraction from FTA cards with whole blood from chicken with high concentration of RBCs

1. Punch five 1.2mm Discs from one FTA sample using the Harris punch and place all of them in a 1.5mm Eppendorf tube.
2. Add 1ml of (100mM (Tris with 0.1% SDS at pH 8) and gently agitate after every five minutes on a vortex for a total of 30 minutes at room temperature. Spin briefly to settle the discs and then discard the supernatant.
3. Add 500ul of 1.5 Molar Guanidine Thiocyanate and gently vortex frequently for a total of 10 minutes then discard
4. Add 500ul of triple distilled water and gently vortex frequently for a total of for 10 minutes, do this three times then discard the water.
5. Add 50ul of triple distilled water and place in a water bath at 90 degrees and heat for 20 minutes.
6. Leave to cool at room temperature for 30 minutes. Spin and then transfer the water that contains the DNA into clean Eppendorf tubes.
7. This will generate 50-70ul, Use 1ul for PCR reaction (or optimize accordingly) and the rest can be stored at -20 degrees and can be used within one month.

Punching of FTA cards using Harris punch

Placing the punched pieces into Eppendorf tubes and labeling

Appendix V: *IGF-1* Reference Sequences used for Editing

1. JX414254.1 Gallus gallus IGF1-3 gene, promoter region and 5' UTR
2. JX414253.1 Gallus gallus IGF1-2 gene, promoter region and 5' UTR
3. JX414252.1 Gallus gallus IGF1-1 gene, promoter region and 5' UTR
4. JF831880.1 Gallus gallus breed Betong IGF-I gene, promoter region and 5' UTR
5. M74176.1|CHKIGFIEX1 Chicken insulin-like growth factor I (IGF-I) exon 1, 5' end
6. gi|398775892|gb|JX414253.1| Gallus gallus IGF1-2 gene, promoter region and 5' UTR
7. gi|398775888|gb|JX414252.1| Gallus gallus IGF1-1 gene, promoter region and 5' UTR
8. gi|335884954|gb|JF831880.1| Gallus gallus breed Betong IGF-I gene, promoter region and 5' UTR
9. gi|211952|gb|M74176.1|CHKIGFIEX1 Chicken insulin-like growth factor I (IGF-I) exon 1, 5' end
10. gi|398775907|gb|JX414254.1| Gallus gallus IGF1-3 gene, promoter region and 5' UTR

Appendix VI: *PRL* Reference Sequences used for Editing

1. DQ536315.1 Gallus gallus clone 5FGS004 prolactin gene,
2. DQ536314.1 Gallus gallus clone 5FGS003 prolactin gene,
3. DQ536312.1 Gallus gallus clone 5FGS001 prolactin gene,
4. DQ536311.1 Gallus gallus clone 5FLK004 prolactin gene,

Appendix VII: GenBank accession numbers for *IGF-1* reference populations.

Organism	**GenBank accession number**
Red jungle fowl	NC_006088.4
southern ostrich	AAF34222.0
Wild turkey	XP 003202426.1
Japanese quail	AAF67202.1
Wild duck	ABS76279.1
Dalmatian pelican	AAF98765.0
Great cormorant	XP 008766769.2
Zebra finch	XP 006754322.0
Golden collared manikin	XP 006921111.4

Appendix VIII: GenBank accession numbers for *PRL* reference populations.

Species	GenBank accession number
Red jungle fowl	NC_006089
Common ostrich	BAF81528.1
Wild duck	BAD14942.1
Japanese quail	BAJ61717.1
Common quail	BAD10927.1
Wild turkey	AAB60604.1
Rock dove	ADK73557.1
Helmeted guinea fowl	BAG68294.1
Graylag goose	XP 007653890.0