PHENOTYPIC CHARACTERIZATION AND FUNCTIONAL POLYMORPHISMS AT PROLACTIN AND *VIPR1* GENES IN EMERGING POULTRY SPECIES FROM WESTERN KENYA

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Phenotypic Characterization and Functional Polymorphism at Prolactin and *VIPR1* Genes in Emerging Poultry Species from Western Kenya

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DECLARATION

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

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DEDICATION

This work is dedicated to my husband Xavier Bowen, my daughter Nicole and my son Nathan. You have been a great source of support and borne with my absence during the duration of my studies. Thank you!

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LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation	Description
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
bp	Base Pair
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
HPG axis	Hypothalamus-Pituitary-Gonadal axis
FAO	Food and Agricultural Organization
IBR	Institute for Biotechnology Research
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MEGA	Molecular Evolutionary Genetics Analysis
MJ	Median Joining
mtDNA	Mitochondrial DNA
MUSCLE	Multiple Sequence Comparison by Log- Expectation

Abbreviation	Description
mv	Median vectors
ng	Nanogram
PCR	Polymerase Chain Reaction
PRL	Prolactin
QTL	Quantitative trait loci
RADseq	Restriction Associated DNA Sequencing
RAPD	Random amplified polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SSCP	Single Strand Conformation Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TBE	Tris Boric Ethylenediaminetetraacetic acid
µl	Microliter
VIP	Vasoactive Intestinal Peptide
VIPR1	Vasoactive Intestinal Peptide Receptor 1

ABSTRACT

Poultry are kept as a source of food as well as a source of income by millions of rural poor families globally. In addition to chicken, emerging poultry species including ducks, geese, pigeons, turkeys and quails are also reared. The aim of this study was to characterize the phenotypic traits of emerging poultry species from Western Kenya and to evaluate functional polymorphisms in Prolactin and Vasoactive Intestinal Peptide receptor 1 (*VIPR1*) candidate genes for egg production in these species. A cross sectional study design was employed with 214 poultry being sampled. R Core statistical package was used to investigate the relationship between sex and the morphometric measurements. Sexual dimorphism in favour of males was evident in ducks, geese, pigeons and turkey. The opposite was true for the wild quails where the females had higher body weights and longer shanks compared to the males. A total of 105 poultry were used for the molecular work, with 20-32 poultry per species. Prolactin and Vasoactive Intestinal Peptide Receptor 1 genes were amplified via PCR and the amplicons sequenced. The pigeon prolactin sequences clustered into four haplotypes while the quail *VIPR1* sequences formed eight haplotypes. The prolactin and *VIPR1* sequences for the other poultry species were all found to be monomorphic. Analysis of molecular variance (AMOVA) revealed variation among individuals to be 66.41% while within individuals to be 33.59% in the pigeons. In the wild quails variation amongst individuals variation was 88.41% while within individuals was 11.59%. The results from this study are the first step towards identifying and recording the animal genetic resources from emerging poultry species in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Poultry have served man in various capacities as food, pets and cultural symbols since their initial domestication. As poultry meat and eggs gained popularity, it gave rise to poultry breeding with the selection for traits such as high egg production and quality meat (FAO, 2008; Sonaiya & Swan, 2004). Chicken is the most widely kept poultry species. However, emerging poultry also play a part in supplying animal proteins in many parts of the world (Aslam et al., 2011; Xu et al., 2013; Yu et al., 2015). In Kenya, interest in ducks, geese, pigeons, quail and turkey is growing rapidly. Locally adapted Muscovy ducks are kept in small numbers in various parts of the country (FAO, 2008). They are good foragers and their meat has a unique rich flavor and a low calorie count (FAO, 2008). Geese are also kept mainly as ornamental poultry besides being eaten. In some households they also play the role of security as they are very loud and hostile towards strangers. Pigeons are kept throughout the country in small numbers and can be found in public areas such as city estates and around mosques in Nairobi city, where they are fed grains by volunteers (FAO, 2008). Globally many people keep the pigeons as a hobby. Wild quail are deeply rooted in the traditions of communities from Western Kenya. The quails are trapped from the wild and kept for breeding or consumed directly after trapping (FAO, 2008). Japanese quails (*Coturnix japonica*) which are kept as domestic poultry have also gained popularity. Their eggs though small in size have three times the nutritional value of chicken eggs (Tunsaringkarn et al., 2013). Small-scale

farmers in the rural areas rear bronze and buff local turkey varieties (FAO, 2008). The Broad Breasted White variety and Buff variety are the two most common commercial varieties in the country. Emerging poultry are reared mostly using backyard production systems, mostly in mixed flocks with chicken. They roam freely and forage for food including household wastes, farm waste, worms and insects. The birds provide meat and eggs for family consumption and also for sale. No attempts have been made to document the local ecotypes of emerging poultry species in the country or to improve their productivity. The aim of this study was to phenotypically characterize the emerging poultry and to evaluate functional polymorphisms in their Prolactin and *VIPR1* genes.

1.2 Statement of the problem

Emerging poultry species are found in many parts of Kenya. However, phenotypic characterization of ducks, geese, pigeons, turkey and wild quail has not been done. Genetics greatly influences most livestock production traits including egg production in poultry. Prolactin and *VIPR1* genes have been found to influence egg production in poultry. Characterization of these two genes in the emerging poultry species has not yet been done. This lack of phenotypic and genetic information limits their utilization.

1.3 Justification of the study

United Nations projections indicate that Kenya's population will rise from the current 44 million to about 85 million by 2050 (World Bank, 2010) . Larger quantities of poultry products need to be produced annually in order to meet the demands of the ever increasing population.

Currently, there is an over reliance on chicken as the main source of poultry products in the country. This study will document the morphology of the emerging poultry species, as well as identify any functional polymorphisms in the Prolactin and *VIPR1* genes that could prove to be instrumental in increasing egg production. This information will change the status of the emerging poultry species from ornamental birds to genetic resources making a contribution towards food security in the country.

1.4 Objectives

1.4.1 General Objective

To assess the phenotypic characteristics and to analyze functional polymorphisms in Prolactin and Vasoactive Intestinal Peptide Receptor 1 genes for egg production in emerging poultry species from Western Kenya.

1.4.2 Specific objectives

1. To evaluate the phenotypic characteristics of emerging poultry species from Western Kenya.
2. To evaluate functional polymorphism in Prolactin and Vasoactive Intestinal Peptide Receptor 1 as candidate genes for egg production in emerging poultry species from Western Kenya.
3. To compare the genetic diversity of Prolactin and Vasoactive Intestinal Peptide Receptor 1 egg production genes in emerging poultry species from Western Kenya.

1.5 Research Hypothesis

1. Emerging poultry species from Western Kenya have diverse phenotypic characteristics.
2. There are functional polymorphisms in Prolactin and Vasoactive Intestinal Peptide Receptor 1 candidate genes for egg production in emerging poultry species from Western Kenya.
3. There is genetic diversity in Prolactin and Vasoactive Intestinal Peptide Receptor 1 genes in the emerging poultry species from Western Kenya.

1.6 Research Questions

1. Are there diverse phenotypes evident in emerging poultry species from Western Kenya?
2. Are there functional polymorphisms in Prolactin and Vasoactive Intestinal Peptide receptor 1 genes in emerging poultry species from Western Kenya?
3. Do Prolactin and Vasoactive Intestinal Peptide Receptor 1 genes from the emerging poultry species from Western Kenya show any diversity?

CHAPTER TWO

LITERATURE REVIEW

2.1 Family Poultry

Family poultry refers to local breeds of poultry reared in small scale scavenging based production systems, common in rural areas of developing countries (FAO, 2014). Family poultry play vital role in both the provision of food and income generation. About 90% of rural families keep one or more poultry species (Guèye, 2005). These poultry species include local breeds of chicken, ducks, geese, turkey, pigeons, quails and guinea fowls. Many developing countries have included family poultry production in their poverty alleviation strategies geared towards meeting the Millennium Development Goals (MDGs) (Sonaiya, 2007). These poultry are reared mostly by women and children. The low cost of maintenance, adaptability to local conditions and disease resistance makes them highly suitable for rural poor families (FAO, 2014). The poultry are housed in rudimentary structures at night to keep them safe from predators (Guèye, 2000).

2.2 Adaptation of local poultry ecotypes to local conditions

Poultry kept in rural areas in backyard systems are indigenous/local ecotypes which are known to be hardy as well as being able to tolerate harsh conditions (Guèye, 2000). However, the last 50 years have seen rapid development of hybrid breeds specialized for maximizing egg and meat production (Sonaiya & Swan, 2004). Farmers sometimes cross their local breeds with the commercial hybrids in an attempt to increase their

productivity. This may cause the extinction of local breeds that are well adapted to the local conditions where they live (FAO, 2011). Native breeds are important for maintaining genetic diversity while also acting as reservoirs for potentially valuable economic characteristics (Seo et al., 2016) and therefore need to be conserved.

Phenotypic characterization is first step towards the documentation of local ecotypes of emerging poultry in order to come up with strategies for their conservation. Genetic characterization may discover polymorphisms linked to advantageous traits such as high egg production. Egg production in local poultry ecotypes may be improved without crossing them with exotic breeds by use of breeding strategies that select for reduced broodiness (Hossain, 1992). Breeding programs established based on this data would result in increasing the production potential of local poultry breeds without the loss of the many advantageous traits they possess.

2.3 Overreliance on chicken

Out of an estimated 32 million birds reared in Kenya, 98% are chicken (76% consisting of free range indigenous chicken while 22% are commercial layers and broilers). The remaining 2% comprises other poultry species such as duck, geese, turkey, pigeon, guinea fowl, quail and farmed ostrich, which are becoming increasingly important (Government of Kenya, 2010). A catastrophic event such as a disease affecting chicken would result a complete collapse of the poultry industry in the country. Increased diversification in poultry rearing to include local ecotypes of ducks, geese, turkey, pigeons and guinea fowl is therefore advisable to mitigate such risks.

2.4 Emerging poultry in Kenya

2.4.1 Ducks

Domestic ducks can be categorized into two species- the common duck which was domesticated from the wild mallard (*Anas platyrhynchos*) and the Muscovy duck (*Carina moschata*) (Zeng et al., 2015). The common duck can be traced back to ancient Egypt, China, Mesopotamia and the Roman empire (Besbes et al., 2007). Muscovy ducks were first domesticated in South America and were introduced into Europe by the Spanish. Soon after, the Muscovy duck was rapidly introduced to other parts of the world (Stahl et al., 2006).

Ducks are hardy, resistant to most poultry diseases and able to withstand harsh environmental conditions (Adzitey & Adzitey, 2011). Duck production does not compete with chicken rearing as they are good foragers and therefore require low input for feeding as well as housing and veterinary care (Banga-Mboko et al., 2007). However, they exhibit strong nesting behavior and are generally poor layers (Wu et al., 2014).

Kenya is rich in wild duck species which include Fulvous Whistling Duck (*Dendrocygna bicolor*), the White-Faced Whistling Duck (*Dendrocygna viduata*), the White-Backed Duck (*Thalassornis l. leuconotus*), the Knobb-Billed Duck (*Sarkidiornic m. melanotos*), the African Black Duck (*Anas sparsaleucostigma*), the Cape Teal (*Anas capensis*), the Eurasian Wigeon (*Anas penelope*), the Gadwall (*Anas s. strepera*), the Common or Green-Winged Teal (*Anas c. crecca*), the Garganey (*Anas querquedula*), the

Yellow-Billed Duck (*Anas u. undulate*), the Northern Pintail (*Anas a. acuta*), the Red-Billed Teal (*Anas erythrorhyncha*), the Hottentot Teal (*Anas hottentota*), the Northern Shoveler (*Anas clypeata*), the Southern Pochard (*Netta erythropthalmabrunnea*), the Common Pochard (*Aythya ferina*), the Ferriginous duck or White-Eyed Pochard (*Aythya nyroca*), the Tufted Duck (*Aythya fuligula*) and the Maccoa Duck (*Oxyura maccoa*) (Zimmerman et al., 1999).

2.4.2 Geese

Domestic geese are the descendants of two wild species, the graylag goose (*Anser anser*) and the Swan goose (*Anser cygnoides*) (Buckland & Guy, 2002). Although geese are highly adaptable, grow rapidly and have low input requirements, they also exhibit strong broodiness which results in poor egg performance (Xu et al., 2013). In geese, egg laying genes exhibit low heritability (Xu et al., 2013; Yu et al., 2015) and thus selection based on parentage or phenotype is of limited use.

The wild goose species found in Kenya include Spur-Winged Goose (*Plectropterus g. gambensis*) and the Egyptian Goose (*Alopechen aegyptiacus*) (Zimmerman et al., 1999).

2.4.3 Pigeons

Pigeons are thought to have been domesticated 3000-5000 years ago or earlier in the Mediterranean region (Wang et al., 2015)**.** A number of pigeon and dove species are found in Kenya including the Rock Pigeon (*Columba livia),* the Speckled Pigeon (*Columba guinea*), the Lemon dove (*Columba larvata*) , the Rameron/Olive Pigeon (*Columba arquatrix*), the Delegorgues Pigeon (*Columba delegorguei*), the European

Turtle-Dove (*Streptopelia turtur*), the Dusky Turtle-Dove (*Streptopelia lugens*), the White-Winged Collared-Dove (*Streptopelia reinchenowi*), the Mourning Collared-Dove (*Streptopelia decipiens*), the Red-Eyed Dove (*Streptopelia semitorquata*), the Ring-Necked Dove (*Streptopelia capicola*), the Laughing Dove (*Streptopelia senegalensis*), the Emerald –Spotted Wood-Dove (*Streptopelia turturchalcospilos*), the Blue-Spotted Wood-Dove (*Turtur afer*), the Tambourine Dove (*Turtur tympanistria*), the Namaqua Dove (*Oena capensis*), the Bruce's Green Pigeon (*Treron waalia*) and the African Green Pigeon (*Treron calva*) (Bennun et al., 1996 ; Zimmerman et al., 1999) .

2.4.4 Turkey

Domestic turkeys are all descendants of the North American wild turkey *Meleagris gallopavo* (Crawford, 1992).They were first domesticated in Mexico in the pre-colonial era (Cockett & Kole, 2009). They are globally the second largest contributor of poultry meat after chicken (Aslam et al., 2011). Commercial breeds have undergone intensive selection to increase body weight that they are no longer able to breed naturally but are bred by Artificial Insemination (AI). They are the Broad Breasted White and the Broad Breasted Bronze varieties. In the US, the turkey is the centerpiece at Thanksgiving celebrations. Heritage breeds include the Black, Bronze, Bourbon Red, Slate, Royal Palm, Narragansett, White Holland, Beltsville Small White breeds (The Livestock Conservancy, 2015) and Buff (Heritage Turkeys, 2011). A turkey hen can raise four to six poults, once or twice annually depending on their feeding regimen (FAO, 2008).

2.4.5 Quails

Quail are migratory birds and travel as far as Europe, Turkey, and Central Asia to China (BirdLife International, 2012). They can be found in different parts of Africa, including Central Africa, the Nile River valley from Egypt to Kenya, and Angola. There are African races in Kenya, Tanzania, Malawi south to Namibia, South Africa, and Mozambique as well as in parts of Madagascar (Alderton, 2012). The Harlequin quail (*Coturnix delegorguei*), Common quail (*Coturnix coturnix*), African Blue Quail (*Coturnix adansonii*) and the Rain quail (*Coturnix coromandelica*) are all found in Kenya (MOLD., 2012; Muthoni et al., 2014; Zimmerman et al., 1999).

2.5 Phenotypic characterization of emerging poultry species in Kenya

Phenotypic and genetic characterization are important for planning and managing animal genetic resources efficiently (FAO, 2012). The vast majority of poultry characterization studies carried out in Africa have concentrated on chicken (Daikwo et al., 2011; Dessie, 2003; Hassaballah et al., 2015; Mwacharo et al., 2006). Moraa et al. (2015) investigated the phenotypic traits relevant to adaptation to hot environments in indigenous chicken in Kenya, while Aswani et al. (2015) characterized phenotypes associated with body growth and egg production in local chickens from three agro-climatic zones of Kenya. Recently, the scope of these studies in Africa has expanded to include other poultry species. Oyeyemi et al. (2012) analyzed sexual dimorphism in local turkeys while Raji et al. (2009) investigated the relationship between body measurements and live body weight in local Muscovy ducks, both in Nigeria. Djebbi et al. (2014) characterized indigenous turkeys from North West Tunisia. In Kenya, Panyako et al. (2016) studied

the phenotypic traits of domestic and wild guinea fowls. This study is the first to characterize the phenotypic traits of emerging poultry species in Kenya.

2.6 Genetic characterization of emerging poultry species in Kenya

Globally, numerous genetic characterization studies as well as association studies have been conducted in poultry to study traits of interest such as disease resistance, egg and meat production. Selection for hard to measure traits such as fertility, disease resistance, longevity, feed conversion can be done by genomic means by employing marker-assisted selection or genomic selection (Eggen, 2012). This is done with the use of various methods and molecular markers which reveal polymorphisms at DNA level.

2.6.1 Techniques for genetic characterization

The candidate gene approach is widely used to investigate the effect that genetic variations (SNP, insertions or deletions) in selected genes have on a phenotype of interest via association analysis. It has been used to identify genes responsible for disease, economically important, or even evolutionary traits. The gene to be investigated is selected based on prior knowledge about its physiological, biochemical or functional role (Zhu & Zhao, 2007). Candidate genes for egg production include prolactin, prolactin receptor, *VIP*, *VIPR1*, dopamine, dopamine D2 receptor, growth hormone, growth hormone receptor amongst others (Luan et al., 2014).

Single nucleotide polymorphisms occur when a nucleotide base at a specific locus in a DNA strand is replaced by any one of the other three nucleotide bases. SNPs are the most common form of genetic polymorphism and are routinely used as molecular

markers to study biodiversity of a species (Soller et al., 2006). Jiang et al. (2009) reported three SNPs in the 5'-proximal region of prolactin gene in Chinese Wanxi White geese and European Rhine goose.

Microsatellites, also known as simple sequence repeats (SSR), refer to short motifs a few nucleotides long found in DNA with the number of repeats varying in members of a species. Microsatellites are highly informative and are experimentally reproducible and transferable among related species (Vieira et al., 2016). Minvielle et al. (2005) used microsatellites to detect quantitative trait loci (QTL) for commercially important traits including clutch length, age at first egg, egg number and egg shell weight in Japanese quail. Takahashi et al. (2001) elucidated the relationship between egg type and egg and meat type duck breeds in Indonesia with the use of microsatellites.

Restriction Fragment Length Polymorphism (RFLP) is a method commonly used in variation analysis which detects differences in homologous DNA sequences by the generation of fragments of different lengths after digestion with specific restriction endonucleases. The different genotypes are identified by use of a fluorescent or radioactive probe that hybridizes with one or more products of the digest after gel electrophoresis. Yang et al. (2010) used PCR-RFLP to study intron 1 of the prolactin gene in Tianzhu Muscovy and identified one polymorphism.

Single Strand Conformation Polymorphism (SSCP) is used to detect mutations in a single strand of a given double stranded DNA sequence. A single nucleotide change at one locus in one strand results in different motilities in gel due to changes in the tertiary

structure of the strands. Fathi & Zarringhobaie (2014) used PCR-SSCP to identify three genotypes of the prolactin gene in native Iranian turkey, one of which was associated with higher egg production. Chen et al. (2011) employed the same technique to identify SNPs in prolactin Intron 2 of Wanxi Goose and Sichuan Goose in China.

Restriction-site Associated DNA Sequencing (RADseq) involves the digestion of the genome with restriction enzymes followed by the attachment of identifiable adapters. After PCR, sequencing of the fragments is carried out to identify genetic variants. It reduces the complexity of the genome by sub-sampling only at specific sites defined by restriction enzymes. This method enables genotyping and SNP discovery by the simultaneous identification, verification and scoring of markers (Davey & Blaxter, 2011). It is useful for detecting large-scale single nucleotide polymorphism (SNP) without having reference genomic information available (Yu et al., 2015). Yu et al. (2015) used RADseq and linear regression analysis to confirm three SNPs involved in a multiple gene network affecting laying performance in geese.

Transcriptome profiling utilizes high throughput techniques to examine mRNA expression levels in given tissues. This method has been extensively employed in poultry whose genomes are yet to be sequenced like geese. To investigate egg production in geese, mRNA (for candidate genes for egg production such as prolactin, prolactin receptor, *VIPR,VIPR1*, estrogen receptors 1& 2, follicle stimulating hormone receptor, etc) is extracted from the different goose tissues at the different stages of their reproductive cycles to investigate which genes are differentially expressed (Ding et al., 2015; Kang et al., 2009 ; Luan et al., 2014; Xu et al., 2013).

The candidate gene approach was chosen for this study as there is prior knowledge as to the role of prolactin and *VIPR1* in avian reproduction. Many studies have linked polymorphisms in these two genes to egg production traits in poultry. This study was the first to investigate polymorphism in the two genes in emerging poultry species from Kenya.

2.7 Functional polymorphisms in egg production genes

Egg laying performance is an economically important trait in poultry production. Production traits that are related to egg productivity include egg number (EN), egg laying rate (LR) and age at first egg (AFE) (Yuan et al., 2015). Egg productivity is greatly influenced by genetic factors, even though metabolic and environmental factors also play a major role. In poultry, the hypothalamus-pituitary-gonadal axis (HPG axis) strictly controls the reproductive endocrine system (Luan et al., 2014). The hypothalamus releases gonadotrophin-releasing hormone (*GnRH*) which binds to its receptor gonadotrophin-releasing hormone receptor (*GnRHR*). This causes the pituitary gland to release gonadotropins; luteinizing hormone *(LH*) and follicle-stimulating hormone (*FSH*). Luteinizing hormone and *FSH* then stimulate gametogenesis and sex steroid hormone production. An increase in *LH* levels in the ovary leads to ovulation and eventually oocyte maturation (Cao et al., 2015). Various association studies have linked genes expressed in the HPG axis to reproduction in poultry by their influence on egg laying and nesting behavior (Fathi & Zarringhobaie, 2014; Li et al., 2009; Pu et al., 2016; Zhao et al., 2011; Yin et al., 2017). The aim of this study was to evaluate

functional polymorphism in prolactin and *VIPR1* genes in emerging poultry species from Western Kenya.

2.7.1 Prolactin

Prolactin is a polypeptide hormone secreted by the pituitary gland. Prolactin (*PRL*) regulates a range of physiological functions like reproduction in mammals, osmoregulation in fish and broodiness in birds (Hui-Fang et al., 2012). In birds, prolactin has been associated to the onset and maintenance of incubation behavior (broodiness). Elevated levels of prolactin have been linked to reduced egg laying (Bhattacharya et al., 2011; Wilkanowska et al., 2014). It inhibits the secretion of gonadotropins and thus arrests ovarian follicle development (Luan et al., 2014). Broodiness can be interrupted by inhibiting prolactin secretion or blocking it from binding with its receptor (Cui et al., 2006). Polymorphisms in prolactin gene could alter its transcription influencing how it is expressed in the tissues (Bhattacharya et al., 2011).

Chang et al. (2009) discovered six SNPs in Tsaiya duck *PRL*, all of which were associated with at least one reproductive trait. Cui et al. (2005) identified a 24bp indel in chicken that was linked to differential prolactin mRNA expression in the different genotypes. Usman et al. (2014) found that this 24bp indel had a highly significant effect on egg production. Yousefi et al. (2012) discovered the same 24bp indel in prolactin in Japanese quails. Mutations in exon 10 of prolactin receptor (*PRLR*) gene of Wainjang geese have been associated to egg production (Chen et al., 2012). These mutations were suggested to interfere with the binding of prolactin to its receptor *PRLR*, altering its physiological effects on the HPG axis and thus causing variation in egg productivity.

Cloning of the prolactin gene has been done for several avian species. Liu et al. (2008) cloned prolactin in Magang goose and found that it shared 98.4%, 92.2%, 92%, and 91.9% sequence homology to duck, turkey, chicken and quail prolactin respectively.

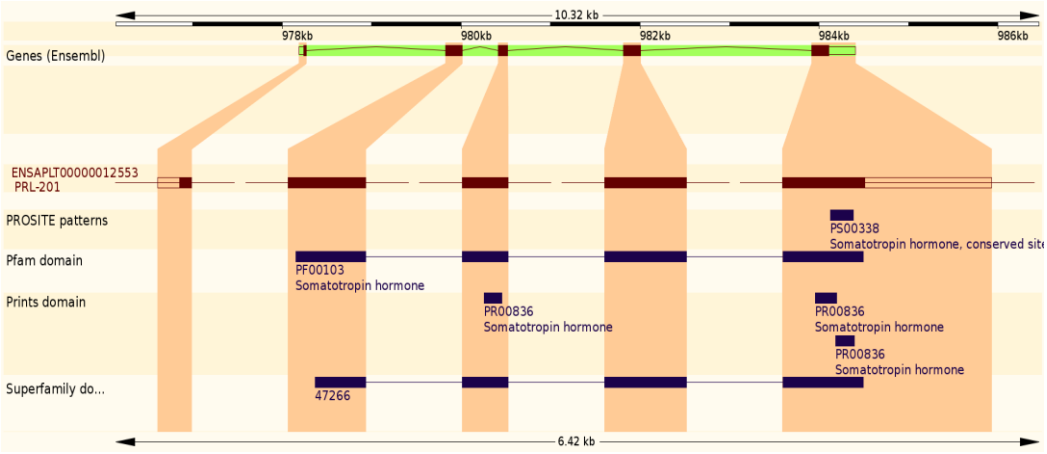


Figure 1.1: Structure of Prolactin gene from Ensembl database.

2.7.2 Vasoactive Intestinal Peptide Receptor 1

Vasoactive intestinal peptide receptor 1 (*VIPR1*) is a 7-transmembrane G-coupled receptor located on the surface of the anterior pituitary cells (Zhou et al., 2008). It affects reproduction in birds through its interaction with Vasoactive Intestinal Peptide (*VIP*) which is a prolactin releasing factor. Vasoactive Intestinal Peptide is a hormone whose function has been suggested to be the passing of information from the environment to the HPG axis. Teruyama & Beck (2001), in a study on Japanese quail, found that an increase in *VIP* levels resulted in elevation of prolactin as well and subsequent regression of the ovaries. Vasoactive intestinal peptide was proposed as a

transmitter of photoperiodic signals that control reproduction in Japanese quail. Pu et al. (2016) associated polymorphisms in *VIPR1* to egg production in laying quails. El-Halawani et al. (2000) managed to inhibit incubation behavior in turkey hens by immunization against *VIP*. Polymorphisms in *VIPR1* could cause conformational changes in the receptor which would subsequently cause variations in egg laying. You et al. (2001) found the mRNA levels of *VIPR1* to change in the pituitary gland during the reproductive cycle of turkey. Rozenboim and El-Halawani (1993) demonstrated that the affinity of *VIP* receptors for *VIP* in turkey hens varied throughout their reproductive cycle further confirming their involvement in egg production.

Zhou et al. (2012) cloned *VIPR1* gene in Japanese quail and found that it showed high amino acid sequence identity to that of chicken (99.1%) and turkey (92.2%).

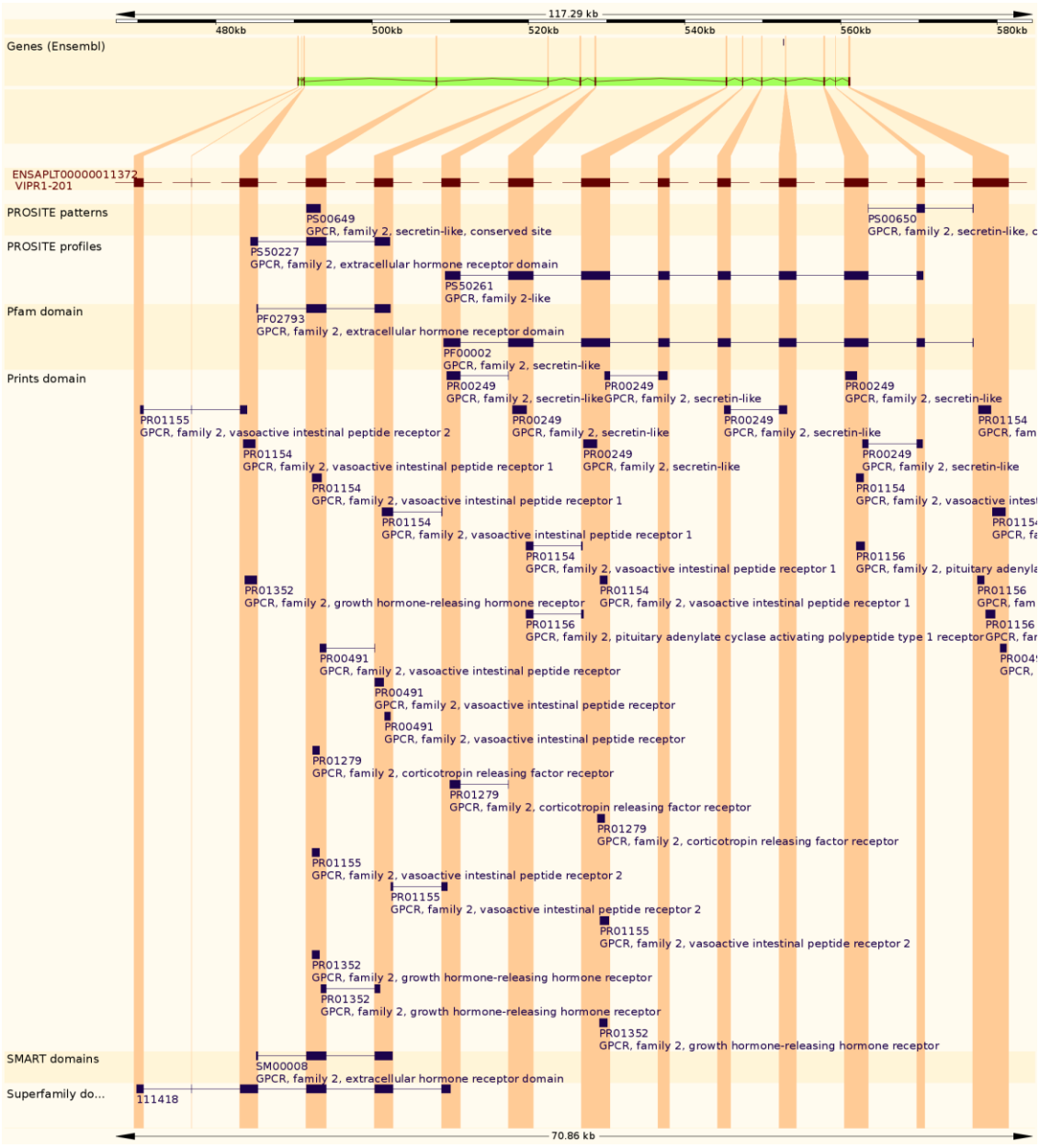


Figure 1.2: Structure of *VIPR1* gene from Ensembl database.

This study is the first to evaluate functional polymorphism in genes related to egg production in emerging poultry species from Western Kenya.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

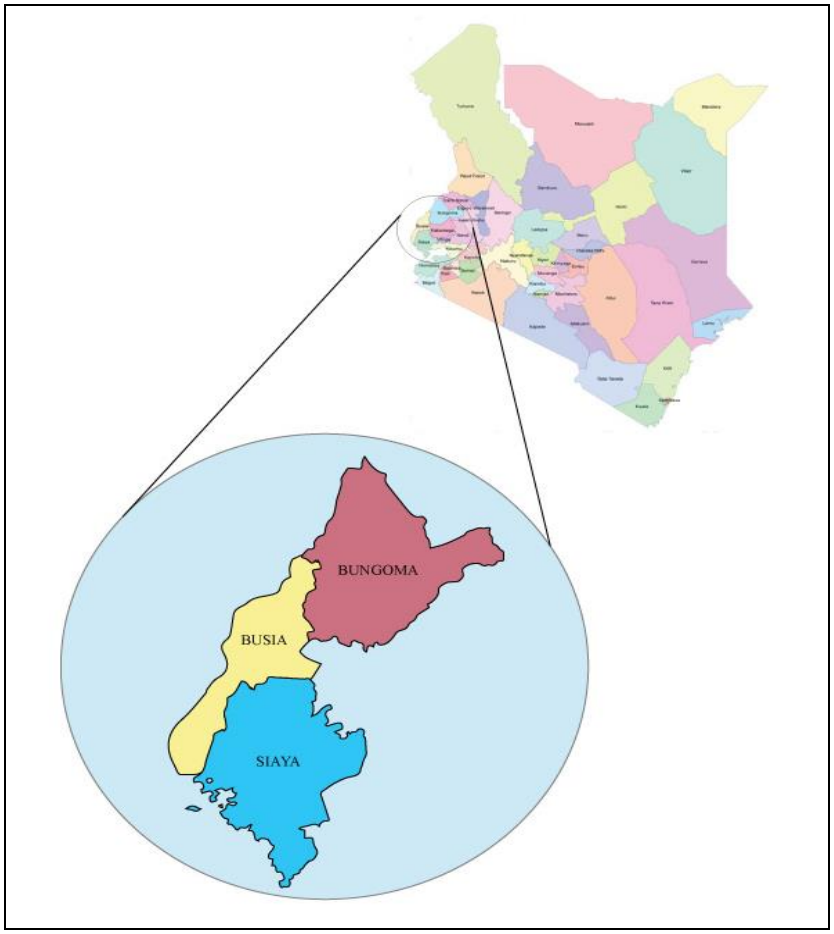


Figure 3.1: Map of Kenya showing the location of the sampling counties

(Source: www.wikimedia.org)

The study was conducted on emerging poultry species from Siaya (00^0 03'N; 34^0 25'E), Bungoma (00^0 34'N; 34^0 34'E) and Busia (00^0 34'N; 34^0 25E) counties of Western Kenya as shown in figure 3.1 above. This area was chosen due to the high poultry species diversity, minimal implementation of poultry improvement programs and the widespread use of backyard poultry farming systems in these counties. Table 3.1 shows the sampling locations in the counties.

Table 3.1: Summary of sampling locations

Poultry Species	County	Sub-county/Village	Sample number
Ducks	Siaya	Rarieda	
		Kametho A, Marang'any'a,Gingo, Ujwanga	13
	Bungoma	Bungoma West	
		Ngoli, Meno	16
		Bungoma Central	
		Chepkeki,Lutasi Chwele B	
	Busia	Teso-North	
		Kakapei,Oduruko	16
Total Ducks			45
Geese	Siaya	Rarieda	
		Manera, Rambugu	8
	Bungoma	Bungoma Central	
		Kapuchai, Sanandiki, Chwele B, Msemwa, Kutwa B, Lutasi	14
	Busia	Teso-North	
		Kakapel	2
Total Geese			24
Pigeons	Siaya	Rarieda	
		Sangla A, Ujwanga, Gingo	14
	Bungoma	Bungoma Central	
		Kutwa B,Chwele B, Makhonge	16
	Busia	Teso-North	
		Oduruko, Kapkapei B	16
Total Pigeons			46
Turkey	Siaya	Rarieda:	
		Ujwanga, Rambugu	6

Poultry Species	County	Sub-county/Villages	
	Bungoma	Bungoma Central:	
		Kapuchai, Kutwab, Mukunyuri	6
		Bungoma West: Ngoli,Meno	4
	Busia	Teso-North	
		Omajo, Okadil	5
Total Turkey			21
Quail	Siaya	Rarieda:	
		Nyayere, Gingo, Membe, Ganga, West-Asembo, Sagia, Tiga, Kamino-Gedo, Marang'any'a	78
Total Quail			78
Total Samples			**214**

3.2 Study design

A cross-sectional study design was adopted. Farmers were interviewed with the assistance of the local agricultural extension officers. Purposive sampling was carried out where only farmers who were found to rear ducks, geese, pigeons, turkey or quail were included in the survey. As the interest of the study was in local ecotypes of emerging poultry, only farms where backyard poultry systems were in use were considered.

3.3 Ethical Clearance

The relevant permits for sample collection were sought and obtained. The Ministry of Agriculture, Livestock and Fisheries, Directorate of Veterinary Services issued permit No. RES/POL/VOL XXVII/162 for sample collection from the domestic poultry whereas Kenya Wildlife Services permit No. KWS/BRM/5001 was for sample collection from the wild quails.

3.4 Sample and Data Collection

Qualitative traits including feather colour, skin colour and shank colour were observed and recorded. The quantitative traits measured and recorded were shank length and body weight. Individual birds were weighed using a scale and the live body weight recorded. For the shank length measurements, the distance from the upper most shank joint to the toe was measured using a flexible measuring tape graduated in centimeters and vernier calipers graduated in millimeters. The Open Data Kit (ODK) application accessible on android mobile phones was used to record and store the data collected. Blood samples for the molecular work were collected from 20-32 individuals per population (Hale et al., 2012) of ducks, geese, pigeons, quail and turkey from the sampled counties and stored at JKUAT metadata base. Two mature unrelated birds were randomly sampled per flock/household. Blood was drawn from the wing of the birds and spotted onto FTA Classic cards (Whatman Biosciences) which were air dried then stored as per the manufacturer's instructions.

3.5 Molecular Analysis

3.5.1 Genomic DNA extraction

Genomic DNA was extracted from the FTA classic cards as per the manufacturer's instructions. The Guanidine Thiocyanate protocol (Smith & Burgoyne, 2004) was used. Five 1.2 mm discs were punched from each FTA card sample and placed in 1.5ml Eppendorf tubes. 1ml of 100mM Tris (with 0.1% SDS at pH 8) was added and gently agitated after every five minutes on a vortex for 30 minutes at room temperature. After brief spinning in a centrifuge to settle the discs, the supernatant discarded. 500 µl of

1.5M guanidine thiocyanate was added and gently vortexed frequently for a total of 10 minutes then discarded. 500 µl of triple distilled water was added and this was gently vortexed frequently for 10 minutes. This step was repeated thrice and the water discarded. 50 µl of triple distilled water was added and placed in a water bath at 90^0C for 20 minutes. The tubes were left to cool at room temperature for 30 minutes. The supernatant containing the extracted DNA was spun and transferred into clean Eppendorf tubes. The DNA concentration was determined by NanoDrop 1000 spectrophotometer and the DNA integrity checked using 260/280 ratio. This showed a wide range of concentrations for the different samples. Dilution of the samples was carried out to standardize the concentrations at 50ng/µl and used to generate the working stock for PCR reaction. The remaining DNA was stored at -20^0C.

3.5.2 Polymerase Chain Reaction amplification for prolactin

PCR amplifications was carried out in 10µl reaction volumes containing 1.5µl genomic DNA, 5µl Thermo Scientific Dream Taq Mastermix, 0.2µl pM of each primer and 3.1µl of PCR water (Thermo Scientific). The forward primer used was 5'-TTTAATATTGGTGGGTGAAGAGACA-3' while the reverse primer was 5'-ATGCCACTGATCCTCGAAAACTC-3' (Akaboot et al., 2012). Thermal cycling conditions applied were initial denaturation at 94^0C for 3 minutes, 35 cycles of 94^0C for 1 minute, melting temperature (Tm) for 1 minute, 72^0C for 1 minute and a final extension step at 72^0C for 10 minutes. Applied Biosystems 9800 Fast Thermal Cycler was used to carry out PCR.

Table 3.2: PCR conditions for Prolactin

Poultry species	Melting Temperature (^{0}C)
Ducks	55, repeat PCR of the amplicons under same conditions
Geese	Step Down 58 to 53
Pigeons	53
Turkeys	58
Quails	58

3.5.2.1 Gel electrophoresis for prolactin

2% agarose gels were prepared by weighing 2g of agarose powder which was poured into a Pyrex beaker. 100ml of 1X TBE Buffer (89mM Tris, 89mM boric acid, 2mM Na_2 EDTA) was measured and added into the beaker. After stirring to dissolve the agarose, the beaker was placed in the microwave for one minute until all the agarose had dissolved. 2.5µl of GelRed^{TM} (Biotium) was added to the mixture while shaking to ensure equal distribution in the liquid gel. The electrophoresis tray was prepared by sealing the ends with tape and placing the combs appropriately. The gel was then allowed to cool to about 55^{0}C before pouring it into the tray of the electrophoresis unit. Once the gel had set, the combs were carefully removed and the tray placed into the electrophoresis tank. Enough 1X TBE buffer added so as to fully submerge the gel in the electrophoresis tank. 2µl of the PCR product was mixed with 4µl loading dye and pipetted into the wells in the gel. The gel was allowed to run at 80 Volts for 1 hour.

Visualization of the gels was done under UV light in a transilluminator (BTS-20 model, UVLtec Ltd., UK).

3.5.3 Polymerase Chain Reaction amplification for *VIPR1*

PCR amplifications was carried out in 10µl reaction volumes containing 1.5µl genomic DNA, 5µl Thermo Scientific Dream Taq Mastermix, 0.2µl pM of each primer and 3.1µl PCR water. The forward primer used was 5'-AGAGGAACGCAGCCAGTGC-3' while the reverse primer was 5'-CCCACCTAACATAAAAGCTCAAC-3' (Akaboot et al., 2012). Thermal cycling conditions applied were initial denaturation at 94^0C for 3 minutes, 35 cycles of 94^0C for 1 minute, melting temperature (Tm) for 1 minute, 72^0C for 1 minute and a final extension step at 72^0C for 10 minutes.

Table 3.3: PCR melting temperatures for *VIPR1*

Poultry species	**Melting Temperature (^{0}C)**
Ducks	54, repeat PCR of the amplicons under same conditions
Geese	56
Pigeons	55, repeat PCR of the amplicons under same conditions
Turkeys	58
Quails	58

3.5.3.1 Gel electrophoresis for *VIPR1*

2% agarose gels were prepared by weighing 2g of agarose powder which was poured into a Pyrex beaker. 100ml of 1X TBE Buffer (89mM Tris, 89mM boric acid, 2mM Na_2 EDTA) was measured and added into the beaker. After stirring to dissolve the agarose, the beaker was placed in the microwave for one minute until all the agarose had dissolved. 2.5µl of GelRed^{TM} (Biotium) was added to the mixture while shaking to ensure equal distribution in the liquid gel. The electrophoresis tray was prepared by sealing the ends with tape and placing the combs appropriately. The gel was then allowed to cool to about 55^0C before pouring it into the tray of the electrophoresis unit. Once the gel had set, the combs were carefully removed and the tray placed into the electrophoresis tank. Enough 1X TBE buffer added so as to fully submerge the gel in the electrophoresis tank. 2µl of the PCR product was mixed with 4µl loading dye and pipetted into the wells in the gel. The gel was allowed to run at 80 Volts for 1 hour. Visualization of the gels was done under UV light in a transilluminator (BTS-20 model, UVLtec Ltd., UK).

3.6 DNA sequencing and analysis

Purified PCR products were sequenced at Macrogen using ABI 3730 DNA analyzer (Applied Biosystems, USA) using Sanger sequencing method (Sanger & Coulson, 1975) (Appendix 3).

3.7 Data analysis

3.7.1 Phenotypic analysis

Phenotypic data was analyzed using Microsoft Excel to determine mean measurements of various quantitative traits in each species. ANOVA tests were done using R Core statistical software (R commander) version 3.1.2. Results are presented in the form of bar graphs, tables and percentages.

3.8 Bioinformatics analysis

3.8.1 Editing sequences

The DNA sequencing chromatograms were edited using Chromas Lite v2.1 software (Avin, 2012). The forward and reverse strands were used to generate an accurate consensus sequence. Multiple sequence alignment was done using ClustalX 2.1.1 (Thompson et al., 1997). The sample sequences were aligned against references from GenBank where they were available. MUSCLE version 3.8.31 (Edgar, 2004) in Seaview version 4 software (Gouy et al., 2010) was also used to perform the multiple sequence alignments.

3.8.2 Haplotype analysis

DnaSP 5.10 software (Librado & Rozas, 2009) was used to assign haplotypes and establish the haplotypes distributions for the various emerging poultry species populations. The results were presented in form of spreadsheets and pie charts prepared using MS Excel (Liengme, 2013).

3.8.3 Diversity Indices

Nucleotide diversity, allele frequencies, number of segregating sites and haplotype frequencies and were calculated using DnaSP 5.10 software (Librado & Rozas, 2009) and Arlequin 3.5.2.2 software (Excoffier et al., 2005).

3.8.4 Analysis of Molecular Variance

Arlequin 3.5.2.2 software (Excoffier et al., 2005) and DnaSP software (Librado & Rozas, 2009) were to perform the analysis of molecular variance by analyzing whether observed genotype frequencies agree with those expected under the Hardy-Weinberg equilibrium.

3.8.5 Comparison of the genes in the emerging poultry species

MatGat v2.0 software (Campanella et al., 2003) was used to compare the percentage similarity and percentage identity of the prolactin and *VIPR1* gene fragments in the emerging poultry species to the same gene fragment in chicken.

3.8.6 Phylogeny

Based on the sequence alignments done in MUSCLE version 3.8.31 (Edgar, 2004) rooted phylogenetic trees for the Prolactin and *VIPR1* gene fragments were constructed in MEGA6 (Tamura et al., 2013) using the Maximum Likelihood algorithm. The confidence of the trees was retested by bootstrapping with 1000 replications being done. SplitsTree version 4.13.1 (Huson & Bryant, 2006) was used to confirm the phylogenetic analyses sequence clusters by the construction of a splits decomposition network.

CHAPTER FOUR

RESULTS

4.1 Phenotypic characterization of emerging poultry species from Western Kenya

The first objective of this study aimed to characterize emerging poultry species found in Western Kenya. Qualitative traits recorded included skin and shank colour while the quantitative traits measured were body weight and shank length. The results for each species are presented below.

4.1.1 Phenotypic characterization of ducks

All ducks encountered were Muscovy ducks (*Carina moschata*) as identified by red fleshy outgrowths around the eyes and beak. All the ducks had black and/or white and brown coloured feathers. 98% had white skin and the remaining 2% had pink skin colour. The shanks in ducks showed six different colours which included spotted black, spotted grey, plain grey, plain yellow, plain black and spotted yellow which represented 25%, 21%, 20%, 18%, 14% and 2% respectively. Male Muscovy ducks had a mean live weight of 3.38kg (0.61) while the females mean weight was 1.97kg (0.40). The average shank length for the male Muscovy ducks was 8.42 cm (0.72) while that for females was 7.02 cm (0.46).

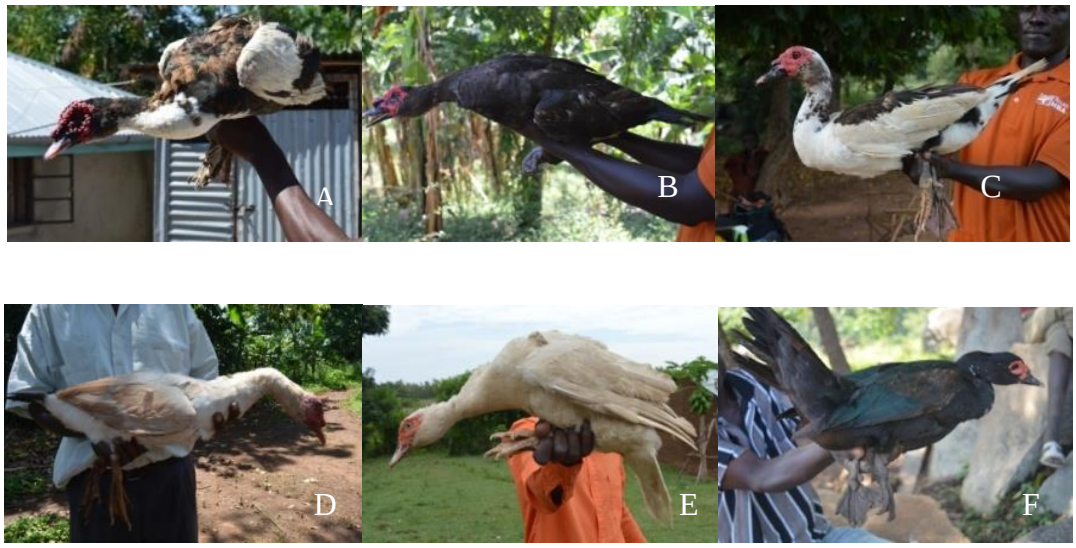


Figure 4.1: Sampled duck phenotypes

Key A: White, brown & black feathers
B: Black feathers
C: White with black feathers
D: White with brown feathers
E: White feathers
F: Black with brown feathers

4.1.2 Phenotypic characterization of geese

All the geese were of the *Anser anser* species. The geese sampled had plain white feathers, white with brown feathers and grey with some white feathers. No variation was observed in terms of skin and shank colour. All the geese had white skin and yellow shanks. The mean weight for male geese was 3.73kg (0.49) while that for females was 3.27kg (0.46). The males had an average shank length of 10.78cm (0.83) while the average for the females was 10.16cm (0.61).

Figure 4.2: Sampled geese phenotypes

Key A: White feathers B: Grey feathers C: White with grey feathers

4.1.3 Phenotypic characterization of pigeons

The pigeons exhibited a wide range of feather colours including white, grey, brown and black. Other than one rock pigeon, the pigeons could not be classified into exact breeds as they exhibited a mixture of characteristics. 87% of the pigeons had pink skin, 11% had white skin and the remaining 2% had spotted pink skin. Similarly pink was the predominant shank colour at 94% while spotted pink, black and grey accounted for 2% each. Male pigeons had a mean live weight of 0.85kg (0.03) while the females mean weight was 0.26kg (0.04). The average shank length for the male pigeons was 3.70 cm (0.29) while that for females was 3.51cm (0.29).

Figure 4.3: Sampled pigeon phenotypes

Key A: White feathers D: Black feathers

B: Brown feathers E: Grey Feathers

C: Mixed colour feathers F: Grey, black and white feathers

4.1.4 Phenotypic characterization of turkeys

The feather colours observed in the turkeys were white and black mixed with white. Pink skin was observed in 56% of the turkeys. 33% had grey skin while 11% had spotted pink skin. The most prominent shank colour was pink at 72% followed by white at 17% while the least common was red at 11%. The male turkeys mean weight was 7.20kg (1.86) while that for the females was 3.39kg (0.89). The average shank length for the male turkeys was 16.94cm (0.72) while the females had an average of 13.5cm (0.72).

Figure 4.4: Sampled turkey phenotypes

Key A: White feathers C: Black with white feathers

B: White with black feathers D: Black feathers

4.1.5 Phenotypic characterization of wild quails

The wild quail sampled were all harlequin quail. The feather colour observed was speckled brown with distinct white and brown face markings. All the quails had yellow skin colour and pink shanks. The mean weight for the female quails was 70.03g (10.59) while that of the males was 65.03g (6.06). The average shank lengths for the females 31.38mm (1.067) was while that for the males was 30.67mm (1.034)

Figure 4.5: Sampled quail phenotypes

Key A: Male harlequin quail B: Female harlequin quail

4.1.6 Skin colour distribution in emerging poultry species from Western Kenya

The turkey and pigeons sampled showed the most variation in skin colour with three different colours being observed in each species. Ducks had two skin colours while geese and wild quail exhibited no variation in skin colour. These results are represented in the graph below.

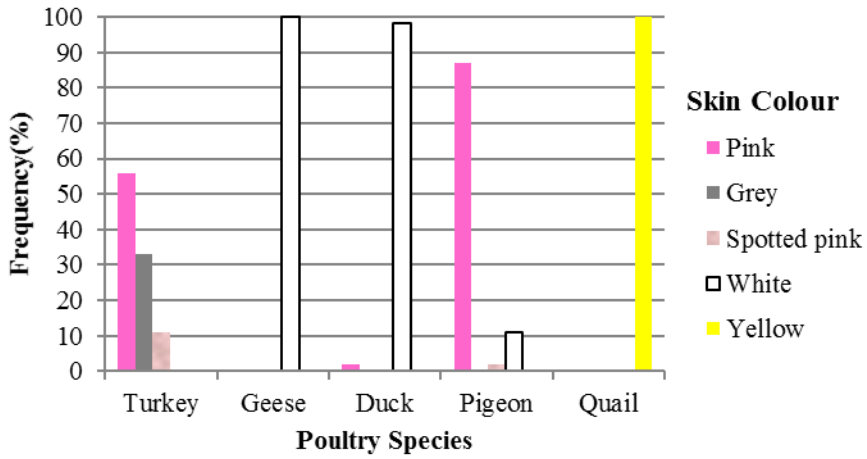


Figure 4.6: Skin colour distribution amongst the emerging poultry species from Kenya

4.1.7 Shank colour distribution in emerging poultry species from Western Kenya

Ducks exhibited the largest diversity with six different shank colours being identified. Turkeys and pigeons followed with three shank colours each being recorded. As was the case with skin colour, there was no variation in shank colour in geese and wild quails. The distribution of shank colours is shown in figure 4.7 below.

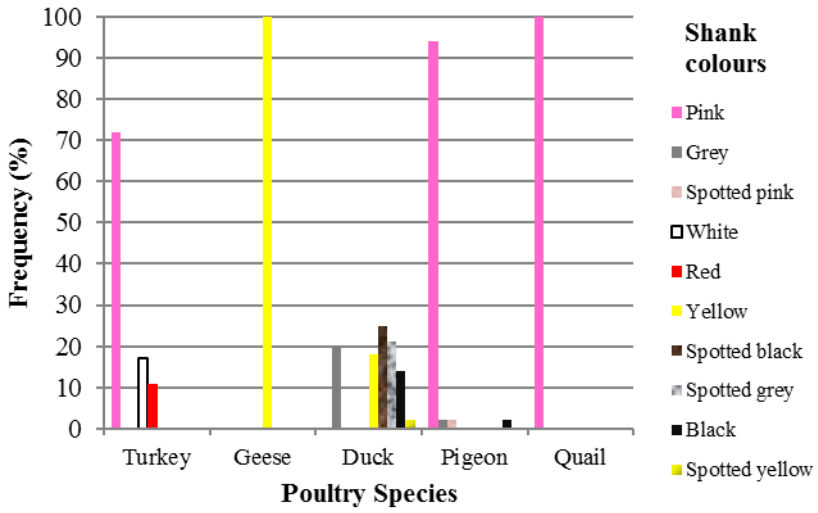


Figure 4.7: Shank colour distribution amongst emerging poultry species in Kenya

4.1.8 Relationship between sex, body weight and shank length of the emerging poultry species

R Core statistical package was used to carry out statistical analysis to determine if there was any relationship between the quantitative traits measured and the sex of the birds. The ANOVA p values indicate significant interaction between sex and both body weight and shank length in all the poultry species. The mean body weight (kg) was significantly higher in males than in females for ducks ($p \leq 0.001$), geese ($p \leq 0.05$), pigeons ($p \leq 0.05$) and turkeys ($p \leq 0.001$) with the largest difference between male and female body weight recorded in turkey. Male shanks were significantly longer than those of females in ducks ($p \leq 0.001$), pigeons ($p \leq 0.05$) and turkey ($p \leq 0.001$). The exception was geese where the shank length did not differ significantly between the sexes. Female quails had significantly higher body weight ($p \leq 0.05$) and shank length ($p \leq 0.01$) compared to the

male quails. Table 4.1 below presents a summary of these results.

Table 4.1: Interaction between sex, body weight and shank length in emerging poultry species from Western Kenya

Poultry species	Trait	Sex	Mean (Std. dev.)	P
Duck	Body weight (kg)	Female N=23	1.97 (0.40)	1.27e-11 ***
		Male N=22	3.38 (0.61)	
	Shank length (cm)	Female N=23	7.02 (0.46)	9.89e-10 ***
		Male N=22	8.42 (0.72)	
Geese	Body weight (kg)	Female N=12	3.27 (0.46)	0.0293 *
		Male N=12	3.73 (0.49)	
	Shank length (cm)	Female N=12	10.16 (0.61)	0.0517
		Male N=12	10.78 (0.83)	
Turkey	Body weight (kg)	Female N=9	3.39 (0.89)	4.55e-05 ***
		Male N=9	7.20 (1.86)	
	Shank length (cm)	Female N=9	13.5 (0.72)	3.18e-08 ***
		Male N=9	16.94 (0.72)	
Pigeons	Body weight (kg)	Female N=23	0.26 (0.04)	0.0391 *
		Male N=23	0.28 (0.03)	
	Shank length (cm)	Female N=23	3.51 (0.29)	0.0337 *
		Male N=23	3.70 (0.29)	
Quails	Body Weight (g)	Female N=39	70.03 (10.59)	0.01472*
		Male N=39	65.03 (6.06)	
	Shank length (mm)	Female N=39	31.38 (1.067)	0.003467**
		Male N=39	30.67 (1.034)	

*Significant at 0.05, **Significant at 0.01 and ***Significant at 0.001. All the surveyed birds were adults over 12 months old.

4.2 Evaluation of functional polymorphisms in prolactin

The prolactin gene was amplified in the emerging poultry species samples using chicken primers. Chicken samples from ILRI were used as the positive control while PCR water was the negative control. A 1kb plus ladder was used to estimate the fragment size.

4.2.1 Characterization of prolactin in ducks

The PCR amplicons of duck prolactin were separated in a 2% agarose gel. The results of the gel electrophoresis are show in figure 4.8.

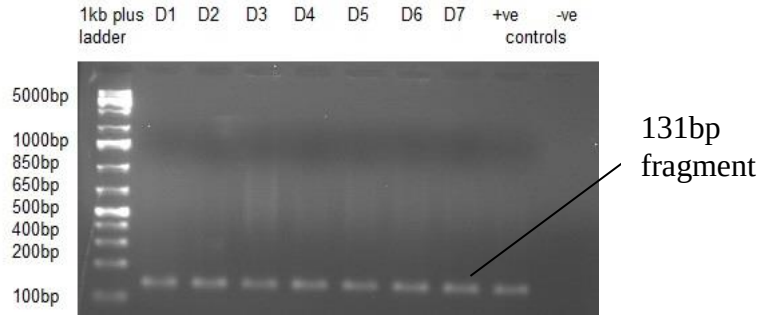


Figure 4.8: Gel image showing the amplified prolactin fragments from the duck samples

Editing of the DNA sequence chromatograms using Chromas Lite v2.1 software revealed that the amplified region was 131 bp and monomorphic in all the samples. The duck prolactin sequences were aligned against the Mallard duck (*Anas platyrhynochos*) prolactin sequence from GenBank (Accession No. KB 742775.1). The sequences were found to be conserved at all the loci as shown in figure 4.9.

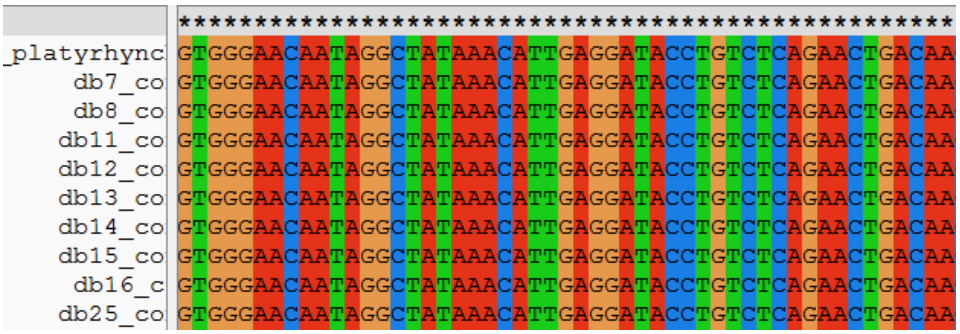


Figure 4.9: Sequence alignment of the sample duck prolactin sequences against the Mallard duck reference sequence from GenBank done using ClustalX 2.1

4.2.2 Characterization of prolactin in geese

The amplified goose prolactin fragments were run on a 2% agarose gel as shown in figure 4.10.

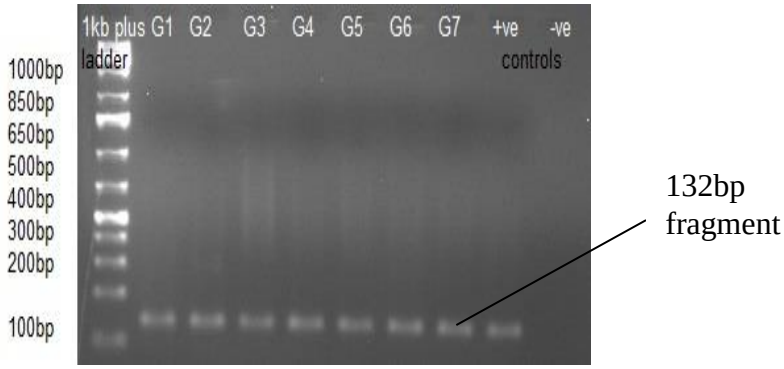


Figure 4.10: Gel image showing the amplified prolactin fragments from the goose samples

A 132 bp fragment was amplified from the goose samples. Once editing was done, the amplified region was found to be monomorphic. The goose sequences were aligned against the domestic goose (*Anser cygnoides*) reference from GenBank (Accession No. NW_013185719.1) and were found to be conserved at all loci (figure 4.11).

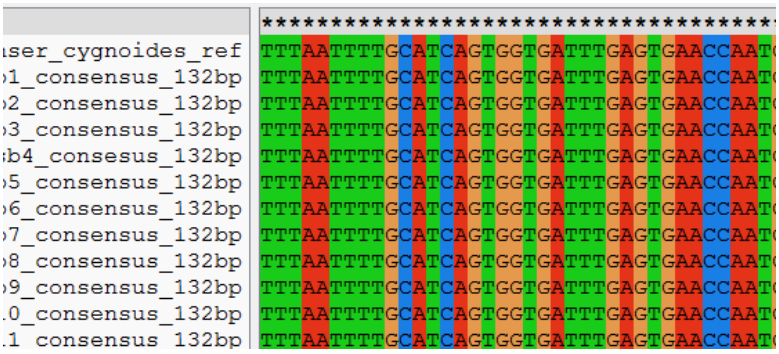


Figure 4.11: Sequence alignment of the sample goose prolactin sequences against the domestic goose reference sequence from GenBank done using ClustalX 2.1

4.2.3 Characterization of prolactin in pigeons

The PCR amplicons of pigeon prolactin were separated in a 2% agarose gel. The 1kb ladder showed the fragment size to be about 300bp (figure 4.12).

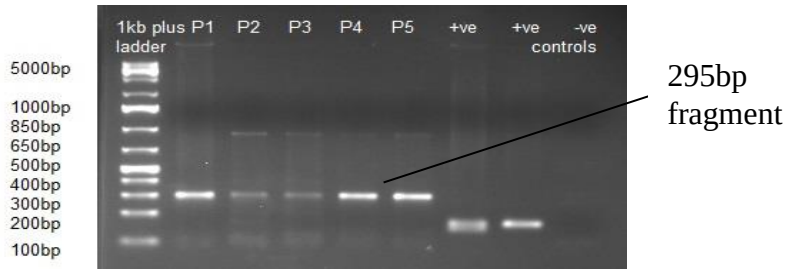


Figure 4.12: Gel image of the amplified prolactin fragments from the pigeon samples

4.2.3.1 Editing of pigeon prolactin chromatograms

Editing with Chomas Lite v2.1 revealed a 295bp fragment as opposed to the expected 130bp fragment. Three polymorphic sites were identified in the pigeon prolactin sequences. The first polymorphic site was a transition at position 171 where guanine was replaced by adenine. The second polymorphic site was a transversion at position 214 where cysteine replaced adenine. The site was heterozygous in some samples with both cysteine and adenine being present. The third polymorphism was a transition at position 233 which was also heterozygous with either cysteine, thymine or both nucleotides appearing. Figure 4.13 shows the polymorphic sites in the chromatograms.

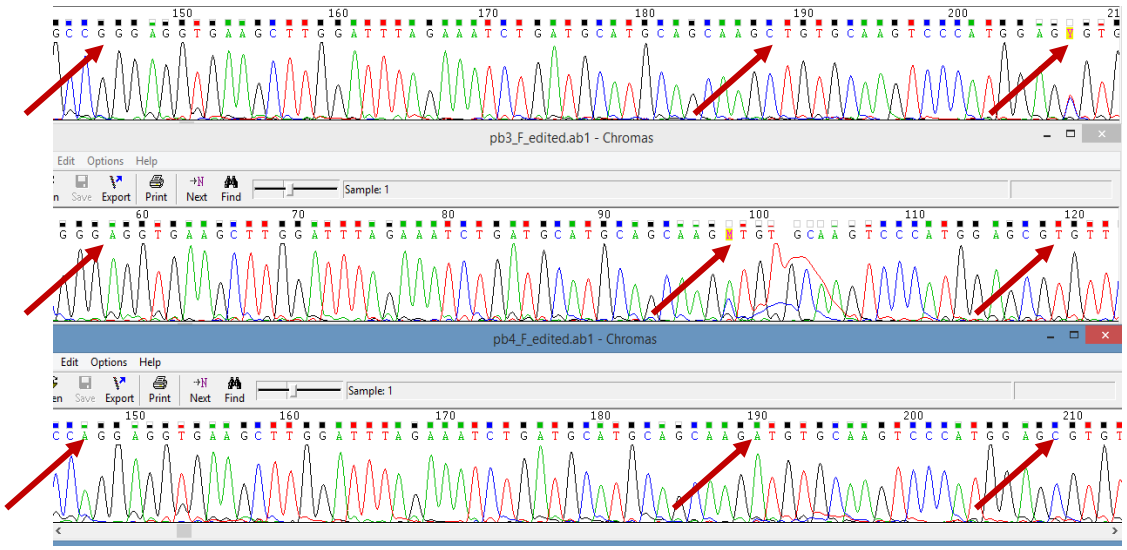


Figure 4.13: Chromatograms of pigeon prolactin gene fragment showing the polymorphic sites edited using Chromas Lite v2.1

Multiple sequence alignment showed the presence of both homozygous and heterozygous strands within the amplified fragments as shown in figure 4.14.

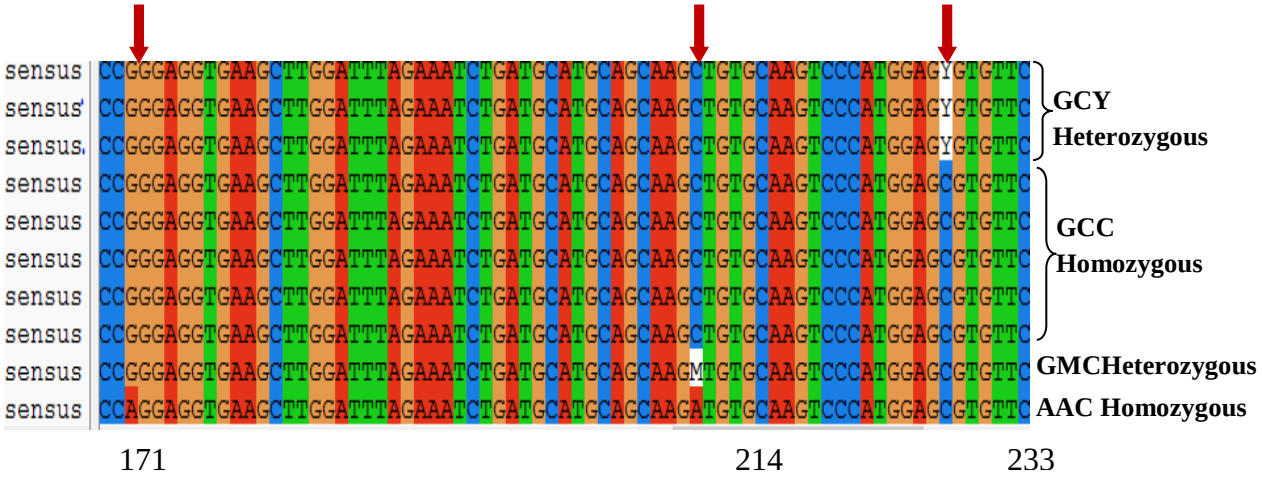


Figure 4.14: Sequence alignment of pigeon prolactin sequences done using ClustalX 2.1

N.B: Y=C or T; M=A or C

The four identified pigeon prolactin haplotypes were GAC, AAC, GCC and GCT. Haplotype 4 was the most common, with 57.5% of the pigeons belonging to this haplotype. Haplotype 2 followed with 35% whereas haplotypes 3 and 4 accounted for 5 and 2.5% respectively. The four pigeon haplotypes are shown in figure 4.15.

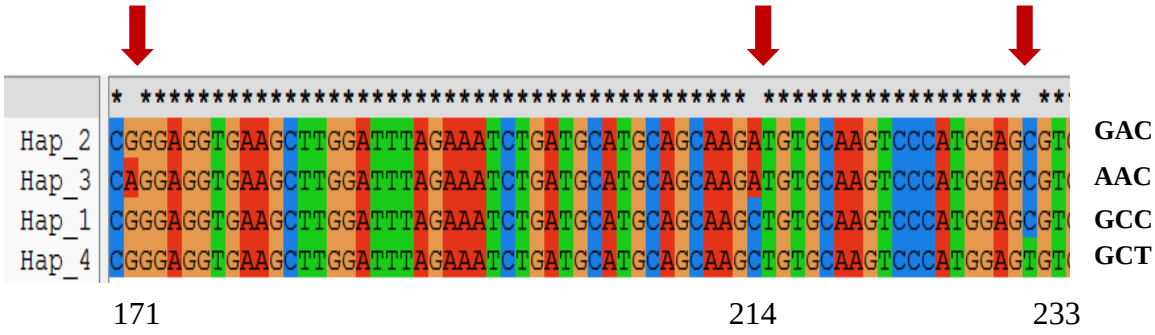


Figure 4.15: Sequence alignment of pigeon prolactin haplotypes done using ClustalX 2.1

4.3.2.3 Molecular Diversity Indices

Molecular diversity indices for the pigeon population were calculated using Arlequin version.3.5.1.2. Of the 40 sample pigeon prolactin alleles, 10 were found to be heterozygous while the remaining 30 were homozygous. The nucleotide diversity observed was C: 17.16%, T: 29.37%, A: 28.80% and G: 24.68%. Table 4.2 summarizes the molecular diversity indices.

Table 4.2: Diversity Indices for Pigeon Prolactin

Gene	H_E	H_O	Gene diversity
Pigeon *PRL*	0.24599	0.08333	0.5608+/-0.0327

H_E: Expected heterozygosity; H_O: Observed heterozygosity

4.2.3.4 Analysis of Molecular Variance of pigeon population based on prolactin

Arlequin v.3.5.1.2 was used to assess the variance among and within individuals in the pigeon population. Variance among individuals (66.41%) was found to be more than within individuals (33.59%). These results are presented in Table 4.3.

Table 4.3: Pigeon Prolactin genetic structure based on AMOVA

Source of variance	df	Sum of squares	Variance components	% variation	F_{IS}	P value
Among Individuals	39	24.15	0.24714	66.41	0.66408	0.0000
Within Individuals	40	5	0.125	33.59		

F_{IS}: Deviation from HWE in a subpopulation

4.2.4 Characterization of prolactin in turkey

The amplified turkey prolactin fragments were run on a 2% agarose gel as shown in figure 4.16.

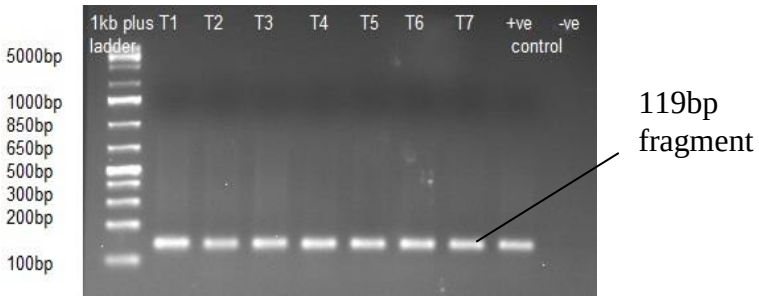


Figure 4.16: Gel image showing amplified prolactin fragments from turkey samples

A 119 bp monomorphic fragment was amplified. The edited DNA sequences of turkey prolactin were aligned against that of *Meleagris gallopavo* from GenBank (Accession No.: 015013.2). The forward and reverse primer regions were found to be conserved. However, two gaps of 11bp and 2bp respectively were present in the sample sequences.

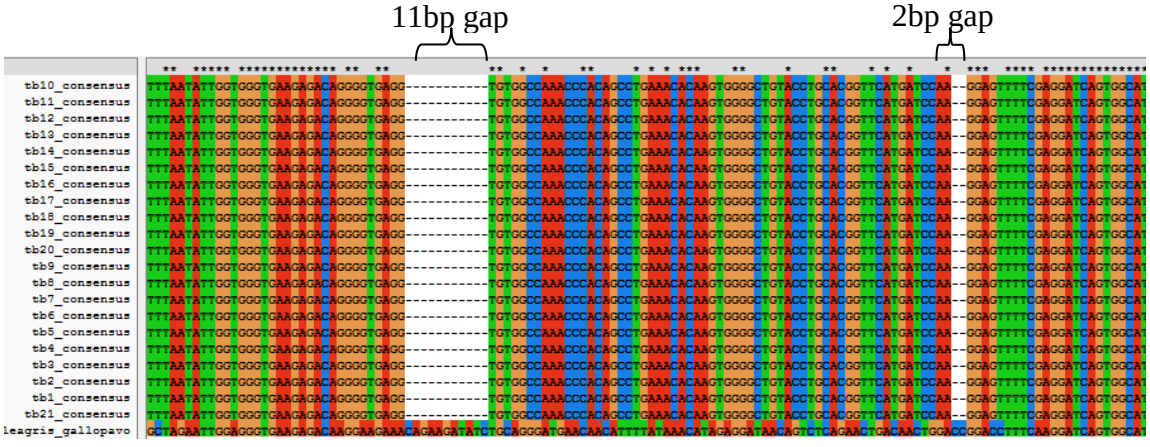


Figure 4.17: Sequence alignment of the sample turkey prolactin sequences against the domestic turkey reference sequence from GenBank done using ClustalX 2.1

4.2.5 Characterization of prolactin in quail

The PCR amplicons of quail prolactin were separated in a 2% agarose gel as shown in Figure 4.15.

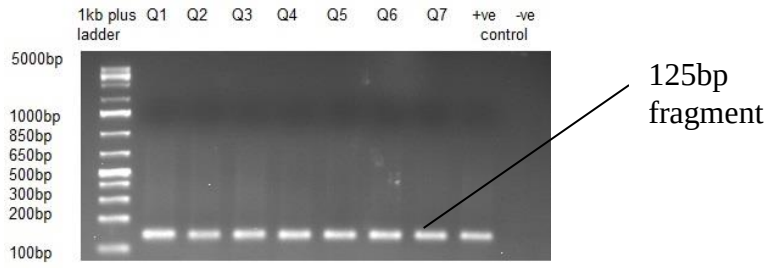


Figure 4.18: Gel image of amplified prolactin fragments from the quail samples

DNA sequencing revealed a 125 bp amplicon which was monomorphic. The wild quail sequences were aligned against that of the Japanese quail (*Coturnix japonica*) from GenBank (accession No.: AB162005.1). The region of the forward primer had only few conserved bases. However the second half of the sequences up to the reverse primer contained conserved blocks as seen in figure 4.19.

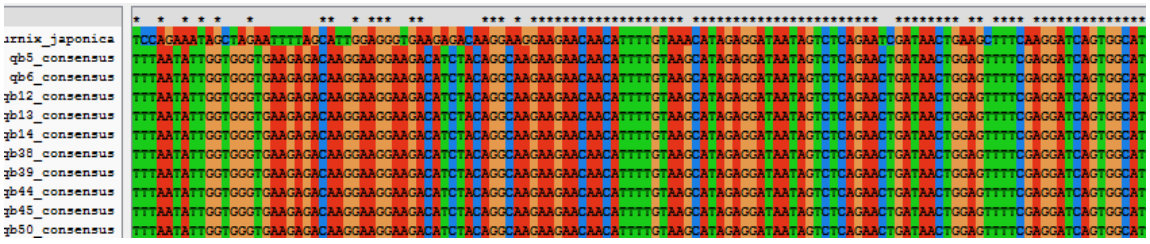


Figure 4.19: Sequence alignment of quail prolactin sequences against the Japanese quail reference sequence from GenBank done using ClustalX 2.1

4.2.6 Haplotype distribution of prolactin in emerging poultry species

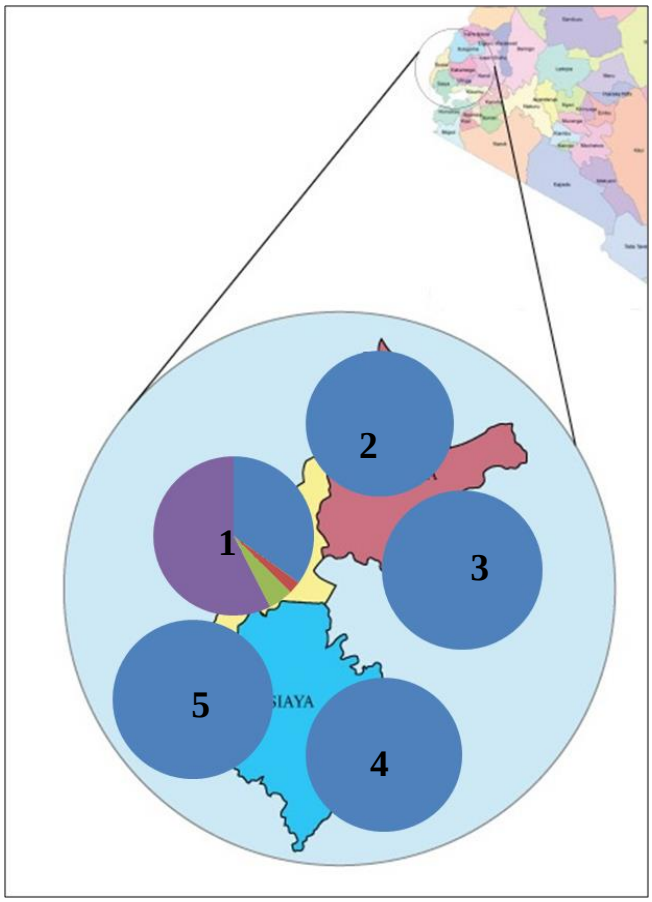
Ducks, geese, turkey and quail samples were monomorphic and therefore had a single haplotypes each. The pigeon prolactin samples clustered into four haplotypes as shown in table 4.4.

Table 4.4: Percentage Haplotype distribution of Prolactin in Emerging Poultry Species from Western Kenya

Species	Hap 1	Hap 2	Hap 3	Hap 4	Total
Ducks	100				100
Geese	100				100
Pigeons	35	2.5	5	57.5	100
Turkey	100				100
Quail	100				100

4.2.7 Percentage haplotype distribution of prolactin in emerging poultry species from Western Kenya

The distribution of the discovered haplotypes in the emerging poultry species populations from Western Kenya was presented in form of pie charts (figure 4.20).



Key

Species represented by pie charts

1. Pigeons
2. Quails
3. Turkey
4. Geese
5. Ducks

Haplotypes identified in the different species

- Haplotype 1
- Haplotype 2
- Haplotype 3
- Haplotype 4

Figure 4.20: Pie charts showing the percentage haplotype distribution in the prolactin gene fragment of the emerging poultry species from Western Kenya

4.3 Evaluation of functional polymorphism of *VIPR1* in emerging poultry species from Western Kenya

The *VIPR1* gene was amplified in the emerging poultry species samples using chicken primers. Chicken samples from ILRI were used as the positive control while PCR water was the negative control. A 1kb plus ladder was used to estimate the fragment size.

4.3.1 Characterization of *VIPR1* in ducks

The PCR amplicons of duck *VIPR1* were separated in a 2% agarose gel. The results of the gel electrophoresis are shown in figure 4.21.

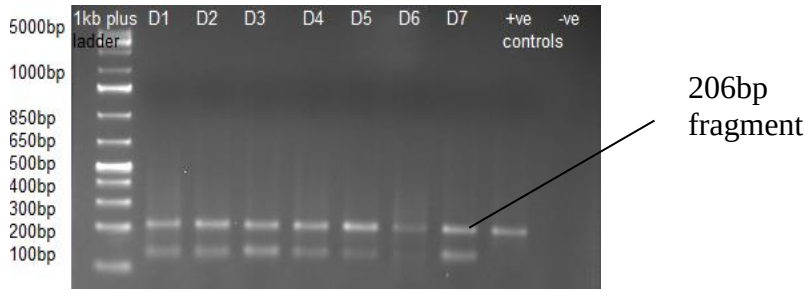


Figure 4.21: Gel image showing amplified *VIPR1* fragments from the duck samples

Two bands were visible in the gel, the first one of approximately 200 bp and the second about 150 bp. The expected fragment size was 203 bp. The 200 bp fragment was excised out of the gel in a dark room under UV light and sent for sequencing. The fragment was monomorphic in all the samples. The sample duck *VIPR1* consensus sequences were aligned against a reference sequence for Mallard duck (*Anas platyrhynochos*) prolactin from GenBank (Accession No. KB 742775.1) using ClustalX software. The multiple

sequence alignment showed some highly conserved regions. However, the sample sequences differed from the reference sequence at 15 sites (figure 4.22).

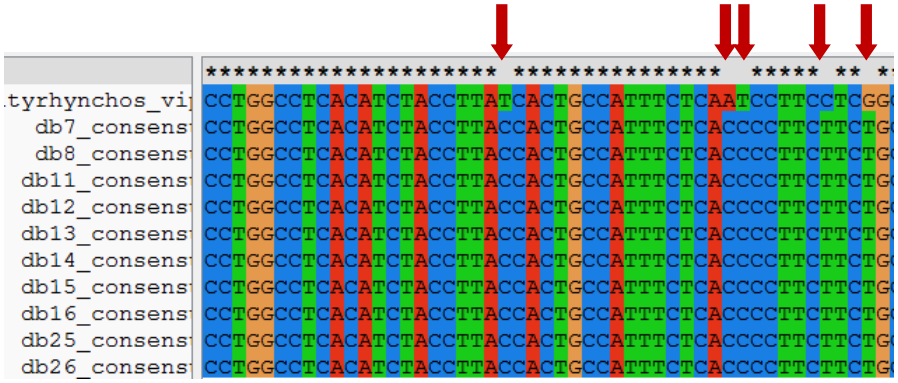


Figure 4.22: Sequence alignment of the sample duck *VIPR1* sequences against the Mallard duck reference from GenBank done using ClustalX 2.1

4.3.2 Characterization of *VIPR1* in geese

The amplified goose *VIPR1* fragments were run on a 2% agarose gel as shown in figure 4.23.

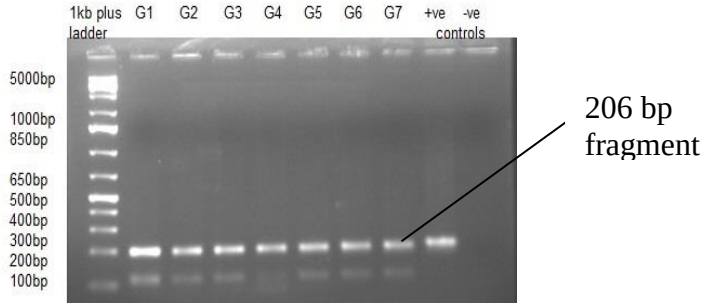


Figure 4.23: Gel image showing amplified *VIPR1* fragments from goose samples

The amplification of goose *VIPR1* revealed a 206 bp fragment. The fragments were monomorphic as shown in the multiple sequence alignment (figure 4.24).

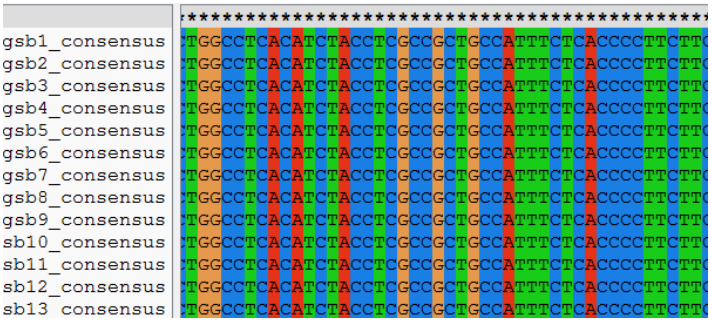


Figure 4.24: Sequence alignment of goose *VIPR1* sequences done using ClustalX 2.1

4.3.3 Characterization of *VIPR1* in pigeons

The PCR amplicons for pigeon prolactin were run on a 2% agarose gel. Multiple fragment sizes were observed on the gel as shown in Figure 4.25.

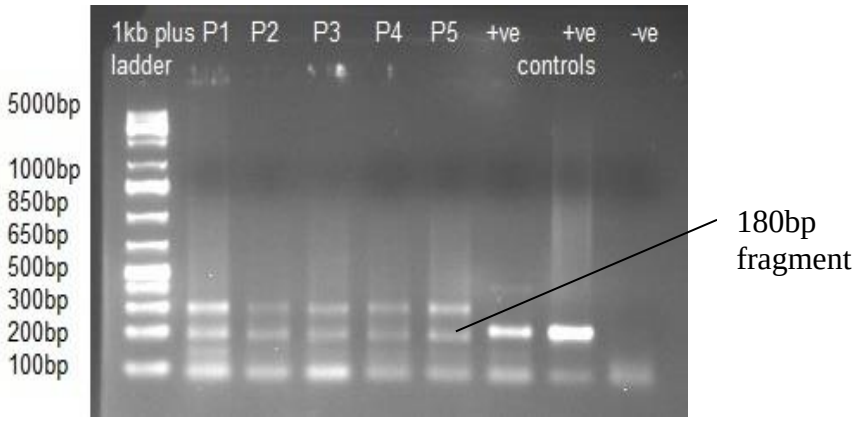


Figure 4.25: Gel image of the amplified *VIPR1* fragments from the pigeon samples

The fragment size that was about 200 bp was excised from the gel in a dark room under UV light. Sequencing revealed this fragment to be 180 bp long and monomorphic as shown in figure 4.26.

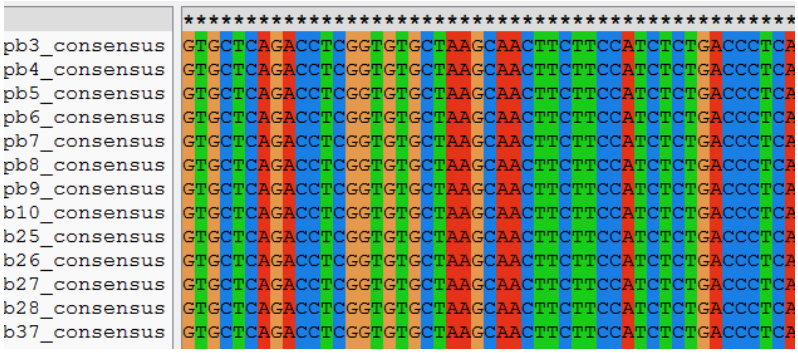


Figure 4.26: Sequence alignment of monomorphic pigeon *VIPR1* sequences done using ClustalX 2.1

4.3.4 Characterization of *VIPR1* in turkey

The amplified turkey *VIPR1* fragments were run on a 2% agarose gel as shown in figure 4.27.

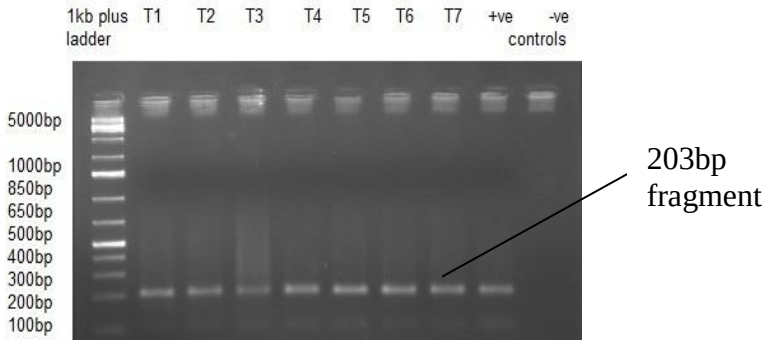


Figure 4.27: Gel image of amplified of *VIPR1* fragments from turkey samples

Editing the DNA sequences with Chromas Lite 2.1 revealed the fragment size to be 203 bp. The fragment was monomorphic. The sample turkey *VIPR1* sequences were aligned against the reference sequence of turkey (*Meleagris gallopavo*) from GenBank (Accession No. NC_015016.2). Four sites differed in the reference sequence and the sample sequences. Position 165 was G in the reference and A in the sample sequences. Position 190 was a C/T transition and position 201 was an A/T transversion. Position 202 was an A/G transition (figure 4.28).

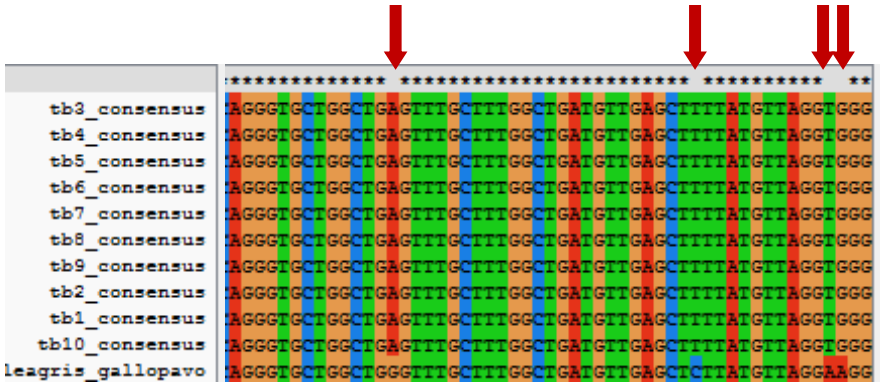


Figure 4.28: Sequence alignment of turkey*VIPR1* sequences against the domestic turkey reference sequence from GenBank done using in ClustalX 2.1

4.3.5 Characterization of *VIPR1* in quail

The PCR amplicons of wild quail *VIPR1* were separated in a 2% agarose gel as shown in figure 4.29.

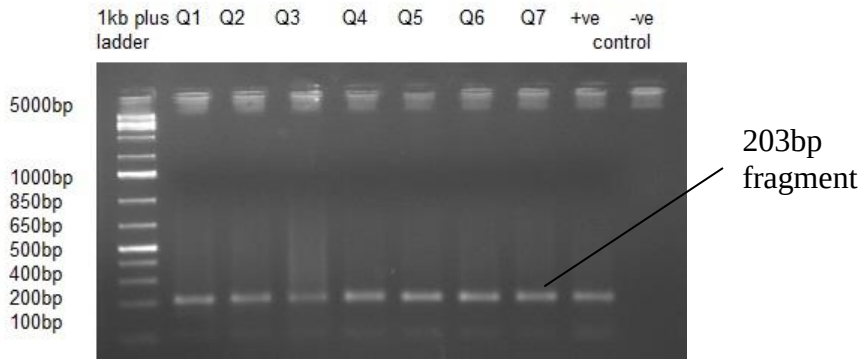


Figure 4.29: Gel image of amplified *VIPR1* fragments from quail samples

4.3.5.1 Editing of quail *VIPR1* chromatograms

Editing with Chromas Lite v2.1 revealed 19 polymorphic sites. 11 sites were transitions while the remaining eight sites were transversions. Some of the sequences were found to be homozygous while others were heterozygous. The chromatograms presented in figure 4.30 indicate the position of the polymorphic loci

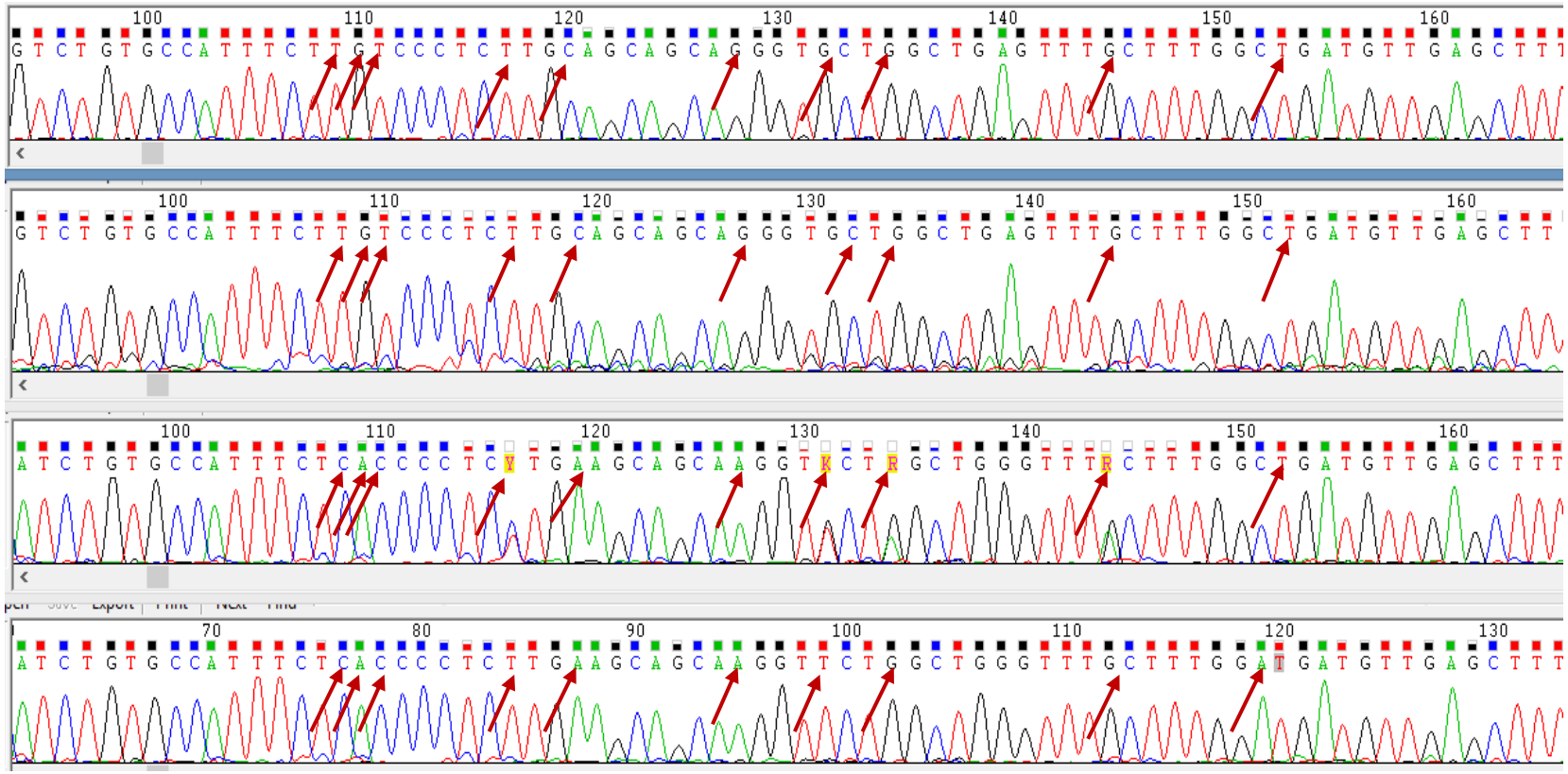


Figure 4.30: Chromatograms showing homozygote and heterozygote sequences of the quail *VIPR1* fragments edited using Chromas Lite v2.1

Multiple sequence alignment of the quail *VIPR1* sequences showed 28 samples to be heterozygous and 12 to be homozygous. Figure 4.31 shows the homozygotes and heterozygotes as well as some of the polymorphic sites.

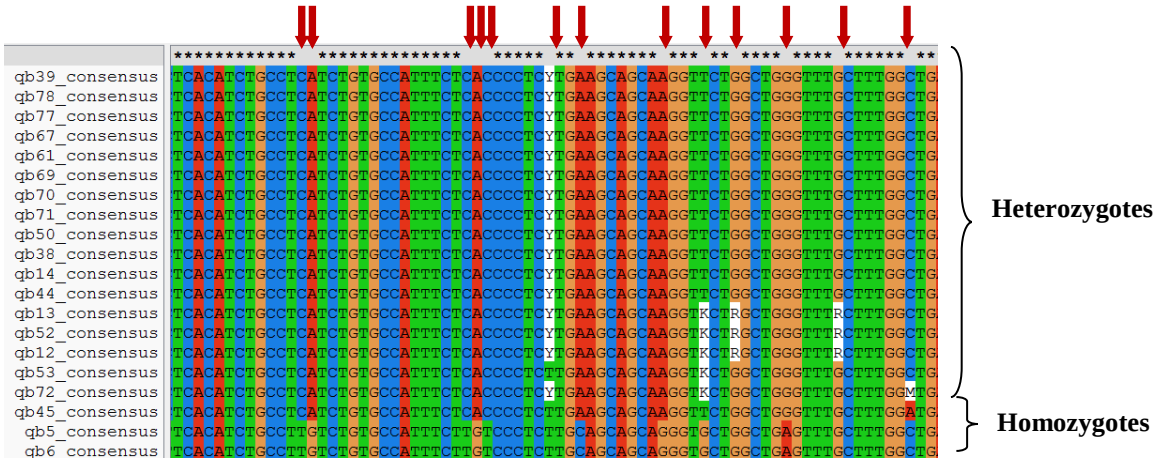


Figure 4.31: Sequence alignment of quail *VIPR1* fragments done using ClustalX 2.1

NB: Y=C or T; K=T or G; R=G or A; M=C or A

The polymorphic wild quail DNA fragments clustered into eight haplotypes. Haplotype 4 accounted for 72.5% of all the samples. This was followed by haplotype 5 at 7.5%. Haplotypes 1 and 2 accounted for 5% each whereas, haplotypes 3, 6, 7 and 8 accounted for 2.5%. Alignment of the wild quail sequences against the Japanese quail (*Coturnix japonica*) reference sequence from GenBank (Accession No. NC_029517.1) revealed some conserved regions (figure 4.32).

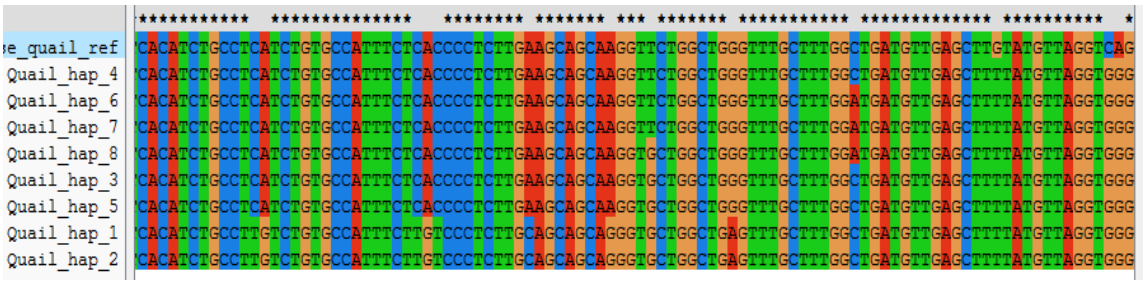


Figure 4.32: **Sequence alignment showing the eight quail *VIPR1* haplotypes and the Japanese quail *VIPR1* reference sequence from GenBank done using ClustalX**

Of the eight haplotypes, haplotype 4 was most similar to that of the domestic *Coturnix japonica*, differing at only four bases in the reverse primer. Conversely, haplotypes 1 and 2 differed the most from the Japanese quail sequence. The list of the eight haplotypes is presented in table 4.6.

Table 4.5: List of Quail *VIPR1* Haplotypes

Haplotype	Polymorphic sites
Haplotype 1	GTGCGCAATTGTGTCGGAC
Haplotype 2	ATGCGCAATTGTGTCGGAC
Haplotype 3	GCTTTATCCCACACAAGGC
Haplotype 4	GTTTTATCCCACACAATGC
Haplotype 5	GTTTTATCCCACACAAGGC
Haplotype 6	GTTTTATACCACACAATGA
Haplotype 7	GTTTTATCCCACACAATGA
Haplotype 8	GTTTTATCCCACACAAGGA

4.3.5.2 Molecular Diversity Indices

Molecular diversity indices for the quail population were calculated using Arlequin version 3.5.1.2. Of the 40 sample quail *VIPR1* alleles, 28 were homozygous while 12 were found to be heterozygous. The nucleotide diversity observed was C: 29.64%, T: 29.66%, A: 18.60% and G: 22.10%. The summary of the molecular diversity indices are shown in table 4.7 below.

Table 4.6: Diversity indices for quail *VIPR1*

Gene	H_E	H_O	Gene diversity
Pigeon *PRL*	0.24599	0.08333	0.5608+/-0.0327

H_E: Expected heterozygosity; H_O: Observed heterozygosity

4.3.5.3 Analysis of Molecular Variance of quail population based on *VIPR1*

Arlequin v.3.5.1.2 was used to assess the variance among and within individuals in the wild quail population. Variance among individuals (88.41%) was found to be more than within individuals (11.59%).

Table 4.7: Quail *VIPR1* genetic structure based on AMOVA

Source of variance	df	Sum of squares	Variance components	% variation	F_{IS}	P value
Among Individuals	39	126	1.52564	88.41	0.8841	0.0000
Within Individuals	40	8	0.2	11.59		

F_{IS}: Deviation from HWE within a subpopulation

4.3.5.4 Haplotype distribution of *VIPR1* in the emerging poultry species

With the exception of quail *VIPR1*, the amplified fragments for all other species were monomorphic with one haplotype each. Quail *VIPR1* however had 8 haplotypes as shown in Table 4.9.

Table 4.8: Percentage haplotype distribution of *VIPR1* in the emerging poultry species from Western Kenya

	Hap 1	Hap 2	Hap 3	Hap 4	Hap 5	Hap 6	Hap 7	Hap 8	Total
Ducks	100								**100**
Geese	100								**100**
Pigeons	100								**100**
Turkey	100								**100**
Quail	5	5	2.5	72.5	7.5	2.5	2.5	2.5	**100**

4.3.5.5 Percentage haplotype distribution of *VIPR1* in the emerging poultry species from Western Kenya

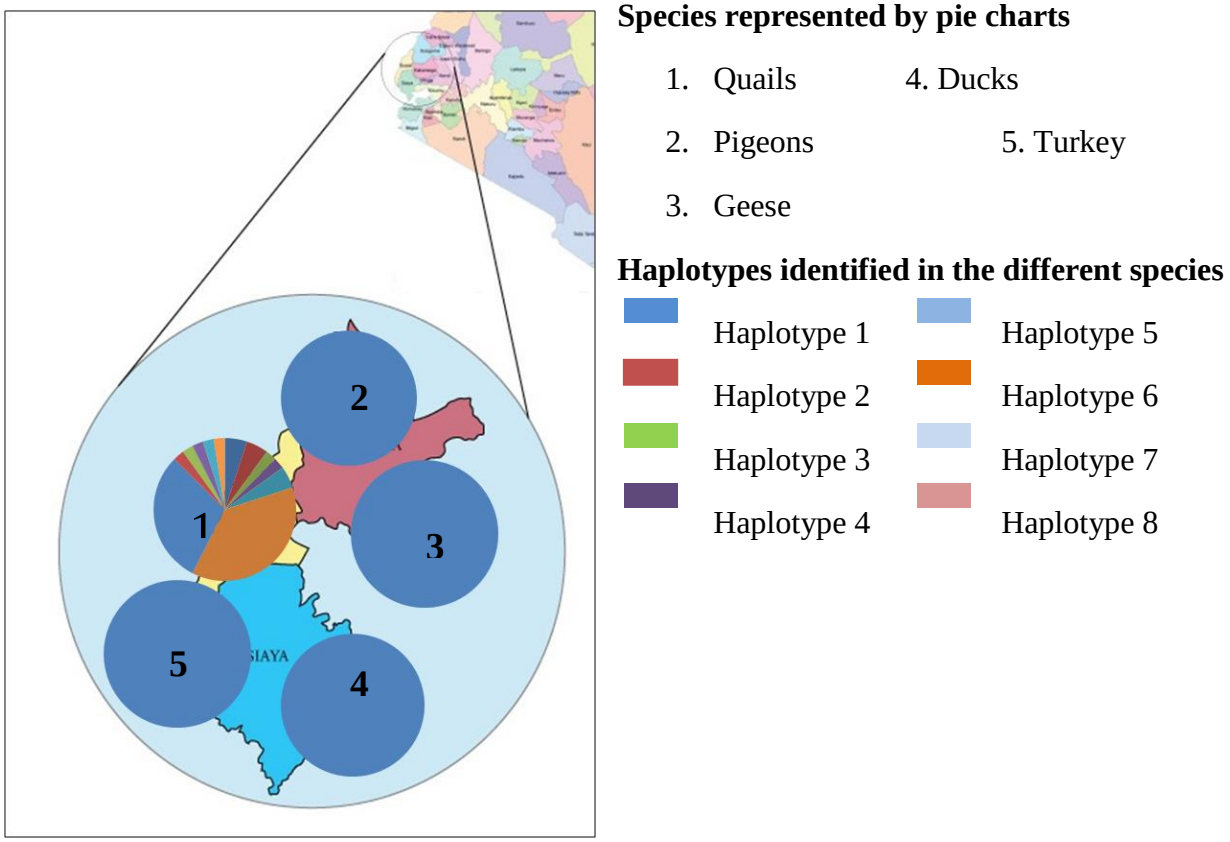


Figure 4.33: Pie charts showing percentage haplotype distribution of *VIPR1* haplotypes of emerging poultry species from Western Kenya

4.4 Genetic diversity in Prolactin and Vasoactive Intestinal Peptide Receptor 1 genes between the emerging poultry species from Western Kenya

A comparison was made of prolactin and *VIPR1* orthologs in the five emerging poultry species that were under study.

4.4.1 Comparison of prolactin fragments in the emerging poultry species

The prolactin amplicons from the five emerging poultry species were ran on a single gel. The fragments separated according to their molecular weights. The PCR products for duck, goose, quail and turkey had around the same molecular weight compared to the chicken prolactin positive control (about 130bp). Pigeon prolactin however showed an estimated weight of 300bp as shown in figure 4.34.

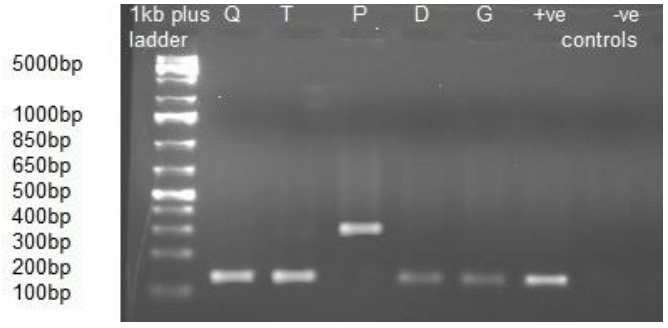


Figure 4.34: Gel image showing amplified prolactin fragments from all the emerging poultry species.

Key: Q=Quail, T=Turkey, P=Pigeon, D= Duck, G=Goose

A multiple sequence alignment of the prolactin fragment from all emerging poultry species and that of chicken were done using ClustalX software. The alignment did not

include the four pigeon haplotypes as they were much longer at 295 bp compared to duck, goose, turkey and quail which were about half this length. Gaps were observed in the turkey and quail sequences which were 119bp and 125bp respectively. They were shorter than those of duck (131bp) and goose (132bp). Some regions were conserved in some of the species as shown in figure 4.35.

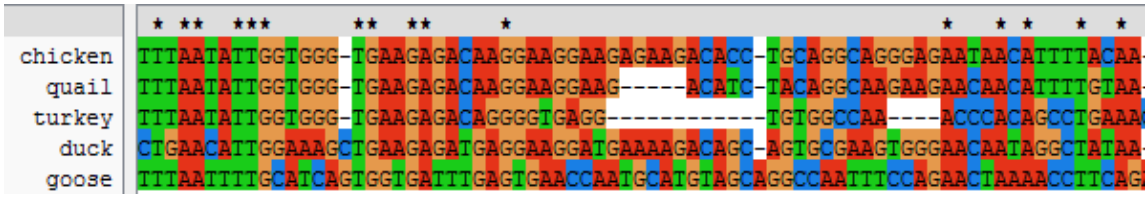


Figure 4.35: Sequence alignment of prolactin fragments from quail, turkey, duck and goose against the chicken sequence from GenBank done in ClustalX 2.1

A comparison of the sequence similarity and identity of the emerging poultry prolactin fragments was done against chicken prolactin. The wild quail fragment had the highest similarity values at 87.7%. Duck, turkey and goose followed with 74.8%, 64.6% and 51.9% respectively. The pigeon haplotypes had the lowest similarity value of 36.6% when compared to chicken prolactin. Table 4.10 shows the percentage similarity and identity of prolactin from the emerging poultry species compared to the chicken reference sequence**.**

Table 4.9: Comparison of % similarity and % identity of prolactin of chicken and emerging poultry species from Western Kenya

	1	2	3	4	5	6	7	8	9
1.Chicken		74.8	49.6	36.6	36.6	36.6	36.6	87.7	64.6
2.Duck	74.8		49.6	34.1	34.1	32.9	34.1	72.5	55.4
3.Goose	51.9	51.9		32.5	32.5	32.5	32.5	52.6	46.7
4.Pigeon hap 1	36.6	34.2	32.5		99.7	99.3	99.7	36.9	32.5
5.Pigeon hap 2	36.6	34.2	32.5	99.7		99.7	99.3	36.9	32.5
6.Pigeon hap 3	36.6	32.9	32.5	99.3	99.7		99.0	36.9	32.5
7.Pigeon hap 4	36.6	34.2	32.5	99.7	99.3	99.0		36.9	32.5
8.Quail	87.7	72.5	54.2	36.9	36.9	36.9	36.9		63.4
9.Turkey	64.6	58.8	48.1	32.5	32.5	32.5	32.5	66.4	

4.4.1.1 Phylogeny of prolactin gene fragment

A rooted phylogenetic tree was generated using MEGA 6. The gamma shape parameter of more than 1 indicates low rate heterogeneity. The ostrich prolactin sequence was downloaded from GenBank and used to root the tree. The ostrich was chosen as it is closely related to the emerging poultry species. The pigeon haplotypes clustered together. The gene tree produced was not in agreement with the species tree of the poultry species as shown in figure 4.36.

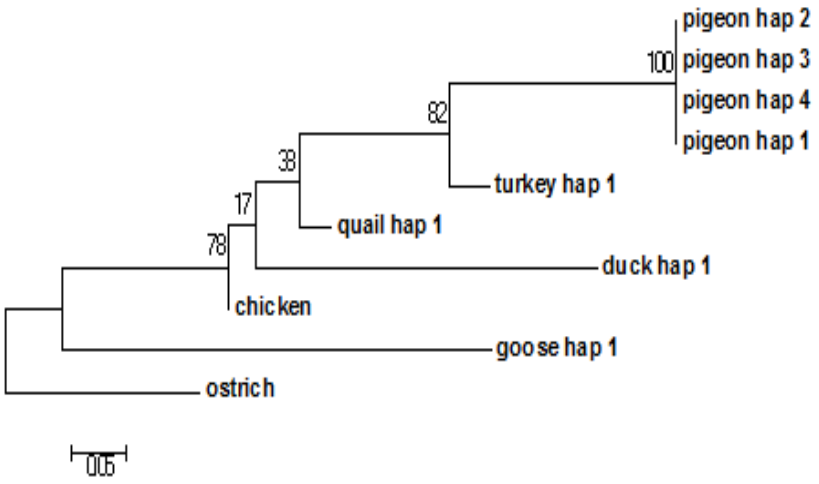


Figure 4.36: Phylogenetic tree of the prolactin sequences from the emerging poultry species done using MEGA. Algorithm: Maximum Likelihood; Bootstrap cutoff: 1000 replications; Model: T92 model (Tamura, 1992); Gamma Shape Parameter: 1.6347

4.4.1.2 Splits decomposition of prolactin gene fragment

A splits decomposition network of the prolactin gene fragments from the emerging poultry species was generated using SplitsTree 4.14.4 software (figure 4.37). The software uses NeighbourNet algorithm which is distance based. Splitstree uses the splits decomposition method to construct tree-like network that accommodates data with differing and conflicting phylogenetic signals.

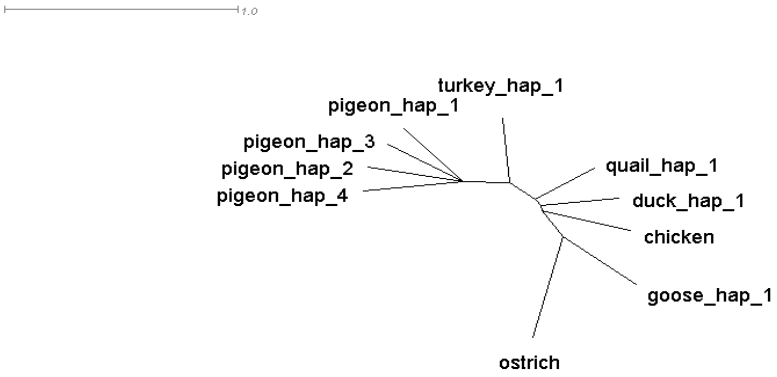


Figure 4.37: Splits tree showing the prolactin gene in the emerging poultry species done using Splitstree 4.14.4 (NeighborNet algorithm)

4.4.2 Comparison of *VIPR1* fragments in emerging poultry species

The *VIPR1* amplicons from the five emerging poultry species were ran on a single gel in which the fragments separated according to their molecular weights. All five species produced fragments of about 200bp in size. This is the same size as the chicken *VIPR1* fragment used as the positive control as shown in figure 4.38.

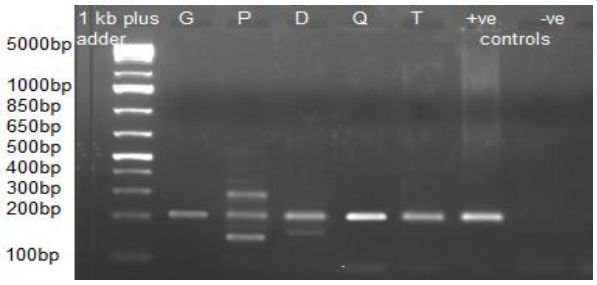


Figure 4.38: Gel image showing the amplified *VIPR1* fragments from all the emerging poultry species from Western Kenya

A multiple sequence alignment of the fragments from the different species was done using ClustalX software. The alignments included *VIPR1* sequences from chicken and guinea fowl as well as the emerging poultry species being studied. Some region of the sequence alignment showed conserved blocks across these poultry species as seen in figure 4.39.

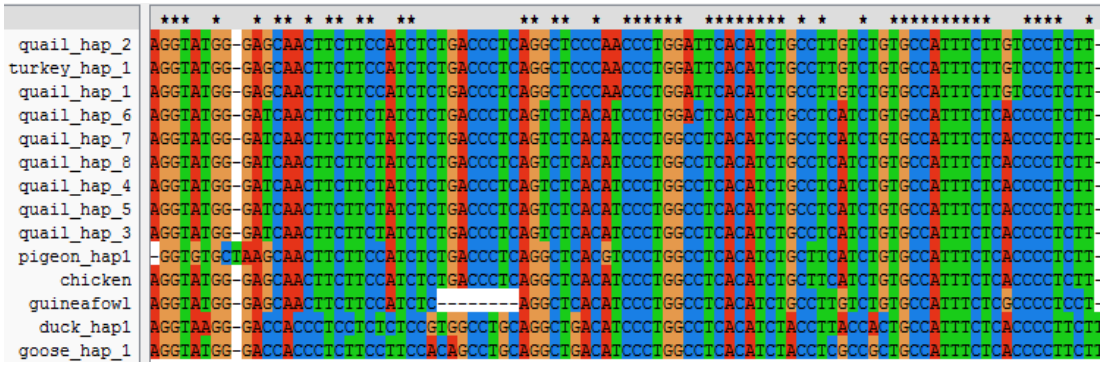


Figure 4.39: Sequence alignment of *VIPR1* fragment from all emerging poultry species and chicken and guinea fowl *VIPR1* sequences done using ClustalX 2.1

A comparison of the sequence similarity and identity of the emerging poultry *VIPR1* fragments was done against chicken *VIPR1*. The wild quail haplotype 5 had the highest similarity values at 96.6%. Similarity for the other quail haplotypes ranged from 96.1% to 93.1%. Turkey *VIPR1* had 93.6% sequence similarity with chicken. The pigeon, duck and goose sequences followed at 86.2%, 80.6% and 78.6% respectively. Table 4.11 shows the similarity and identity percentage comparisons for the poultry species.

Table 4.10: Comparison % similarity and % identity of chicken *VIPR1* and the emerging poultry species from Western Kenya

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.Chicken		80.6	78.6	85.8	93.1	93.6	96.1	96.1	96.6	95.1	95.6	96.1	93.6	67.0
2.Duck	80.6		90.8	69.4	78.6	78.2	81.1	81.6	81.6	80.6	81.1	81.1	78.2	59.1
3.Goose	78.6	90.8		67.0	76.7	76.2	78.2	78.6	78.6	77.7	78.2	78.2	76.2	62.1
4.Pigeon	86.2	69.4	67.0		79.4	79.4	82.8	82.4	82.8	81.4	81.9	82.4	79.4	64.0
5. Quail hap 1	93.1	78.6	76.7	79.8		99.5	92.1	92.1	92.6	92.1	91.6	92.1	99.5	67.0
6. Quail hap 2	93.6	78.2	76.2	79.8	99.5		91.6	91.6	92.1	91.6	91.1	91.6	100	66.5
7. Quail hap 3	96.1	81.1	78.2	83.3	92.1	91.6		99.0	99.5	99.0	98.5	99.0	91.6	66.0
8. Quail hap 4	96.1	81.6	78.6	82.8	92.1	91.6	99.0		99.5	99.0	99.5	99.0	91.6	66.5
9. Quail hap 5	96.6	81.6	78.6	83.3	92.6	92.1	99.5	99.5		99.5	99.0	99.5	92.1	66.5
10.Quail hap 6	95.1	80.6	77.7	81.8	92.1	91.6	98.0	99.0	98.5		99.5	99.0	96.1	65.5
11.Quail hap 7	95.6	81.1	78.2	82.3	91.6	91.1	98.5	99.5	99.0	99.5		99.5	91.1	66.0
12.Quail hap 8	96.1	81.1	78.2	82.8	92.1	91.6	99.0	99.0	99.5	99.0	99.5		91.6	66.0
13.Turkey	93.6	78.2	76.2	79.8	99.5	100	91.6	91.6	92.1	91.6	91.1	96.1		66.5
14.Guinea fowl	67.0	59.7	62.1	66.1	67.0	66.5	66.0	66.5	66.0	65.5	66.0	66.0	66.5	

4.4.2.1 Phylogenetic analysis of *VIPR1* gene fragment

A rooted phylogenetic tree was generated using MEGA 6. The gamma shape parameter of more than 1 indicates low rate heterogeneity. The ostrich *VIPR1* sequence was downloaded from GenBank and used to root the tree. The ostrich was chosen as it is closely related to the emerging poultry species. Six of the eight quail haplotypes clustered together in the tree as shown in figure 4.40. The other two clustered with the turkey haplotypes. The gene tree produced was not in agreement with the species tree of the poultry species.

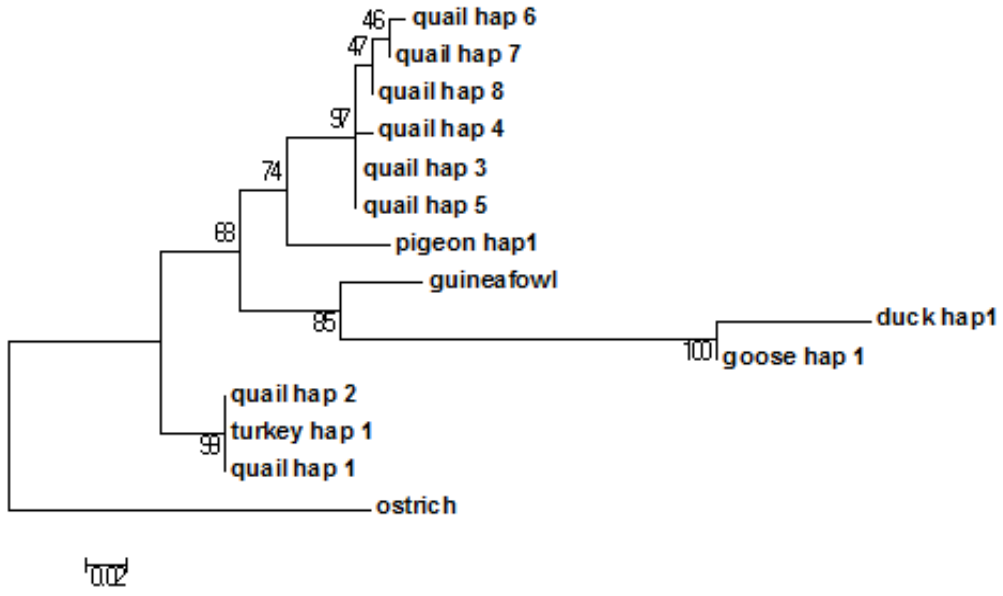


Figure 4.40: Phylogenetic tree showing the relationship of *VIPR1* gene in the emerging poultry species from Western Kenya. Algorithm: Maximum likelihood; Bootstrap cutoff: 1000 replications; Model: K2 model (Kimura, 1980); Gamma parameter: 3.01

4.4.2.2 Splits decomposition of *VIPR1* gene fragment

A splits decomposition network of the *VIPR1* gene fragments from the emerging poultry species was generated using SplitsTree 4.14.4 software (figure 4.41). The software uses NeighbourNet algorithm which is a distance based method.

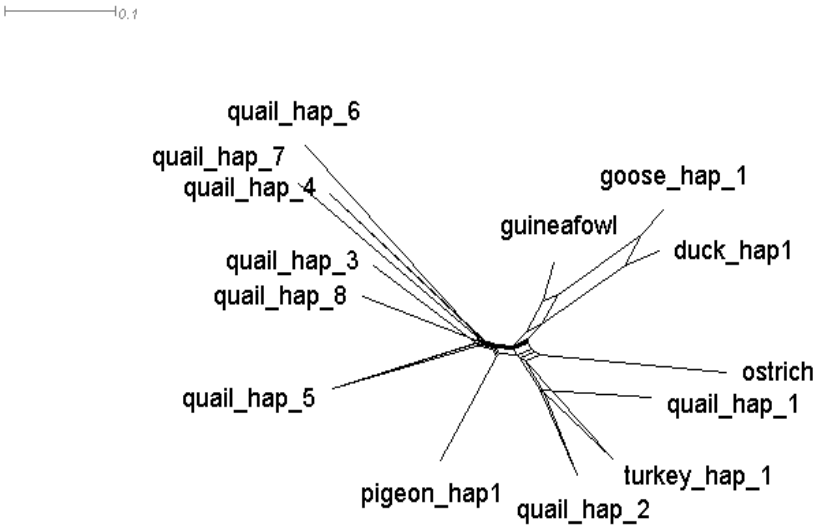


Figure 4.41: Splits tree of *VIPR1* sequences from emerging poultry species, guinea fowl and chicken done using SplitsTree 4.14.4 (NeighborNet algorithm)

CHAPTER FIVE

DISCUSSION

5.1 Characterization of the phenotypes of emerging poultry species

Phenotypic characterization is an important first step in evaluating the production potential of emerging poultry species in Kenya. This study therefore aimed to identify local breeds of emerging poultry species from Western Kenya by describing their morphological characteristics. Variations in plumage, skin and shank colours were noted in the emerging poultry species. Sexual dimorphism was detected in all the emerging poultry species. These findings are vital towards the documenting of the animal genetic resources from emerging poultry in the country with an aim of fully utilizing these resources.

5.1.1 Phenotypic characterization of ducks

All ducks sampled were Muscovy ducks (*Carina moschata*) which are identifiable by their fleshy red outgrowths found around the eyes and beaks as described by Yakubu (2013). This is consistent with the assessment of Banga-Mboko, et al. (2007) who found Muscovy ducks to be well adapted in Africa. Muscovy ducks are the predominant waterfowl reared in Africa because they are well suited to free range husbandry and therefore attractive to rural farmers (Sonaiya & Swan 2004). Female Muscovy ducks are excellent foster-brooder mothers for eggs from other poultry species. This is highly

advantageous to the local farmers who were found to rear Muscovy ducks in mixed flocks with other poultry species.

The feather colours observed included white, black, black and white. The same feather colours were observed in Muscovy ducks from India (Banerjee, 2013). In addition to these colours, Raji, et al. (2009) reported multicoloured plumage in Muscovy ducks in Nigeria. All the ducks had white skin colour with one exception having pink skin colour.

The shank colour in ducks showed the most variation with six different colours: yellow, spotted yellow, black, spotted black, grey and spotted grey. Some of these variations in shank colour that have previously been reported are grey with yellow markings, plain grey and yellow as described by Banerjee (2013).

The male ducks had significantly higher body weight with a mean of 3.38±0.61 kg ($p \leq 0.001$) as compared to the females mean weight of 0.97±0.40 kg. The shank length was also significantly longer in males with a mean of 8.42±0.72cm ($p \leq 0.001$) as compared to females whose mean length was 7.02±0.46 cm. This sexual dimorphism was also reported by Ogah (2009) in his study on the morphological traits of geographically separated populations of Indigenous Muscovy Duck in Nigeria. Yakubu (2013) also observed sexual dimorphism in his study to characterize local Muscovy ducks in Nigeria with males having significantly higher body weight, breast circumference, bill length and wing length as compared to the females.

5.1.2 Phenotypic characterization of geese

All the geese were of the *Anser anser* species which are descendants of the graylag goose (FAO, 2002; Albarella, 2005). The most common feather colours were white, white with brown and grey with some white feathers (figure 4.2).

There was no variation in skin and shank colour. All sampled geese had white skin colour and yellow shank colour. This is in partial agreement with Banerjee (2013) who reported white skin colour but both yellow and orange shanks in geese from India.

Sexual dimorphism was evident from the significantly higher body weight of the male geese whose mean was 3.73±0.49kg ($p \leq 0.5$) was compared to the females 3.27±0.26kg. This is in agreement with the assessment of Yarza (2014) that male Canadian geese were heavier than their female counterparts. However, the shank lengths in the male and female geese showed no significant difference at 10.78±0.83cm and 10.16±0.61cm respectively.

5.1.3 Phenotypic characterization of pigeons

The pigeons were feral and had a wide range of feather colours including white, grey, brown and black. Pink was the predominant colour noted for the shank and skin colour of pigeons. Other than one Rock Pigeon, the pigeons could not be classified into exact breeds as they exhibited a mixture of characteristics due to cross breeding. Pigeons are able to travel great distances as they scavenge for food. They are therefore likely to encounter pigeons from different populations. This phenotypic diversity could also indicate genetic diversity which can be attributed to pairing of pigeons from different

breeds. A number of pigeon and dove species are found in Kenya including the Rock Pigeon (*Columba livia),* the Speckled Pigeon (*Columba guinea*), the Lemon dove (*Columba larvata*) , the Rameron/Olive Pigeon (*Columba arquatrix*) , the Delegorgues Pigeon (*Columba delegorguei*) among others (Zimmerman et al., 1999) .

The pigeons were sexually dimorphic as the males had significantly higher mean body weights at 0.35±0.03kg ($p \leq 0.05$) compared to females whose average was 0.26±0.04kg. Omojola et al. (2012) also found male pigeons (278.67g) from Nigeria to weigh more than the females (239.75g). Male shank lengths averaged 3.70±0.29cm ($p \leq 0.05$) as compared to the females 3.51±0.29cm. Similarly, Hassan and Adam (1997) found male indigenous pigeons to have significantly higher body weight and morphometric measurements than females (Assan, 2013).

5.1.4 Phenotypic characterization of turkeys

The feather colours observed were white and black mixed with white. Djebbi et al. (2014) reported white, black, red and bronze feather colours in indigenous Tunisian turkeys. Camacho-Escobar et al. (2008) reported a total of 18 colours/colour combinations in Mexican turkey including brown, yellow red, grey and combinations of two or more colours. Over half of the turkeys in the three counties had white skin colour. The rest had grey and red skin.

The most prominent shank colour was pink, then white and the least common was red. Pinkish white , grey , black , white, brown pink and dark pink shanks were reported by Djebbi et al. (2014) in Tunisian indigenous turkeys. Camacho-Escobar et al. (2008)

observed white, pink, black, grey, yellow and brown shanks in Mexican turkey. From these qualitative traits, it is evident that Mexican turkeys are more phenotypically diverse than those found in Africa. This could be due to the fact that turkeys were domesticated in Mexico and therefore the gene pool here is larger.

Sexual dimorphism was evident with male turkeys weighing significantly more ($p \leq 0.001$) than female turkeys. Male turkeys weighed a mean of 7.20±1.86kg while the females had a mean weight of 3.39±0.89kg. The males also had significantly ($p \leq 0.001$) longer shank lengths as compared to the females, 16.94±0.72cm and 13.5±0.72cm respectively. Similarly, Oyeyemi et al. (2012) also reported sexual dimorphism in their study on turkeys from Nigeria in which males recorded higher values in the eight morphometric traits they evaluated. The same was found to be true in indigenous turkeys from Tunisia where sexual dimorphism in favor of the males was observed Djebbi et al. (2014).

5.1.5 Phenotypic characterization of quail

All wild quails encountered were harlequin quail. The males and females could be distinguished from the feathers with males having more vibrant colouration. All had yellow skin colour and pink shanks. On the other hand, Wamuyu et al. (2017) found farm-reared quails in the country to have pink skin and white shank colour respectively.

In contrast to the other four emerging poultry species, female quails had significantly higher body weight compared to males ($p \leq 0.05$). The mean weight for the females was 70.03±10.59g while that of the males was 65.03±6.06g. The same trend was observed

for the shank lengths of the quails with the female average being 31.38±1.07mm ($p \leq 0.001$) while the average for males was 30.67±1.04mm. This is in agreement with the findings of Malarmathi et al. (2012) who found females to weigh significantly more than the males in their study on the production traits of Japanese quail in India. Similarly, Raji et al. (2015) found the same to be true for Japanese quail in Nigeria. Shanaway (1994) found female quails to be slightly larger than males measuring 18.5cm and 16cm respectively.

Plumage, skin and shank colours in birds are influenced by specific genes that determine carotenoid and melanin pigmentation. All verterbrates do not synthesis carotenoids and therefore acquire them from their diet. In wild birds, carotenoid levels in skin can be used as an estimator of health and foraging efficiency (Eriksson et al., 2008). Gladbach et al. (2010) found female upland geese whose head and shank colours were more intense to have higher egg and clutch volumes when compared to the females exhibiting paler colouration. Some genes involved in the melanogenesis such as *MC1R* have pleotropic effects and have been found to also affect behavior, immune and stress responses (Zhang et al., 2015). In theory, skin and shank colouration in emerging poultry species could be used as a basis of selection in the absence of molecular markers by associating pigmentation to traits of economic interest such as egg production.

5.1.6 Sexual dimorphism in emerging poultry

Sexual dimorphism refers to difference in size of the males and females in a given species during the various developmental stages. In birds and mammals, sexual dimorphism is mostly in favor of males (Blanckenhorn, 2005). This is evident from the

findings of this study with reference to ducks, geese, pigeons and turkeys. Sexual selection favors larger males who are more likely to mate with the female of their choice. Larger males have an advantage in mating competition and displays of agility that are used to convey their degree of fitness to the females (Tamás et al., 2017). However, female biased sexual size dimorphism occurs when selection for fecundity of the females is stronger than sexual selection for the size of the males (Blanckenhorn, 2005). This could explain the larger size of female quails compared to males observed in the wild quail population in this study.

Assan (2016) discussed the importance of morphometric body measurements to livestock and poultry farmers. In the absence of weighing scales, linear measurements such as shank length could be used to estimate the weight of an animal. They could also be used as an indicator of the age of the animal in a situation where no records have been kept by the farmer.

5.2 Characterization of functional polymorphism in prolactin in emerging poultry species from Western Kenya

The availability of genetic variation in production traits is important for selection. This study was the first to undertake the genetic characterization of prolactin and *VIPR1* genes in ducks, geese, pigeons, turkey and quails in Kenya. The data collected could be the first step towards increasing egg production in emerging poultry species in the country.

5.2.1 Characterization of duck prolactin

All the ducks sampled were of the same haplotype with no variations being present in the amplified region of the prolactin gene. This could be due to the absence of genetic variants of prolactin in the sampled population. It is also possible that a different region of the gene could have polymorphisms. Other studies have identified polymorphisms in duck prolactin. Wang et al. (2009) suggested prolactin could be used as a marker for reproductive traits in Muscovy ducks after their study identified two SNPS in prolactin exon 4 that were found to significantly affect broodiness. Zhang et al. (2015) also identified two SNPs in prolactin from female Muscovy ducks that were significantly associated with both egg number and age at first egg. Wang et al. (2011) identified 12 SNPs in native Chinese ducks, one of which was found to be significantly associated with egg production and egg weight. This study was the first to characterize duck prolactin in Kenya and could be the first step towards increasing egg production in local ducks.

5.2.2 Characterization of goose prolactin

The amplified region of the prolactin gene was monomorphic. In contrast, Jiang et al. (2009) identified three SNPs in the 5'-proximal region of *PRL* gene of Chinese and European geese. Genotype AA laid more eggs than AB and BB ($p<0.05$) indicating that Genotype AA has the potential to be utilized in molecular breeding for egg production.

Geese are known to be poor layers that exhibit strong broodiness (Yu et al., 2015). Other approaches that have been used to investigate egg production in geese include transcriptome profiling. Xu et al., (2013) carried out transcriptome profiling of goose (*Anser cygnoides*) ovaries in the laying and broodiness periods and did not find prolactin to be differentially expressed in the two periods. However, Luan et al, (2014) found expression of prolactin receptor gene in Huoyan geese to be significantly reduced during the laying period compared to the ceased period and suggested this might reduce the inhibitory effect of prolactin on egg production.

5.2.3 Characterization of pigeon prolactin

The pigeon prolactin fragments amplified was 295bp and clustered into four haplotypes. Haplotype GCT was the most abundant followed by haplotype GCC. Haplotypes AAC and GAC had the fewest members.

The pigeon population was not in Hardy-Weinberg equilibrium (HWE). The observed heterozygosity was found to be 0.08333 whereas the expected heterozygosity was 0.24599. The conditions required for a population to be in HWE include a large population with random mating and the absence of evolutionary influences like

mutations, gene flow, genetic drift and natural selections. These conditions are unattainable in natural populations.

AMOVA results indicate that most variation in the population to be due to variation among individuals (88.41%) as opposed to within individuals (33.59%).

Pigeons typically lay two eggs per clutch. They become broody when the second egg is laid and ovulation ceases (Farner, 1960). Prolactin in pigeons is also responsible for the production of crop milk on which the squabs are fed. This is unique to columbiformes (Goldsmith, 1983).

Most studies on reproduction in pigeons have dealt with physiological and behavioral aspects of pigeon reproduction (Fabricius & Jansson, 1963; Michel & Moore, 1986; Saldanha & Silver, 1995; Horseman & Buntin, 1995). More recently, however, the molecular aspects of pigeon reproduction are being investigated. For instance, Wang et al. (2015) studied pigeon ovaries transcriptome to identify genes involved in light regulation which also affects reproduction. This study was the first to undertake the characterization of prolactin in pigeons in Kenya.

5.2.4 Characterization of turkey prolactin

All turkey sampled were monomorphic with no variations identified in the prolactin fragment amplified. In contrast, Fathi and Zarringhobaie (2014) characterized the prolactin promoter from native turkey from Iran and identified two allelic variants and three genotypes. Genotype II was statistically associated with higher egg production as compared to genotypes ID and DD. Kansaku et al. (2008) also found the turkey prolactin

promoter region to have two genetic variants. Wong et al. (1991) cloned turkey prolactin and found levels to increase from photo stimulated to laying and finally to the incubation stage. El Halawani et al. (1990) had similar findings suggesting that prolactin influences egg production in turkeys. Comparison of the sample turkey prolactin sequence and that of *Meleagris gallopavo* (domestic turkey) from GenBank did not align well except at the area covering the primers. This could be due to the fact that domestic turkeys are reared primarily for meat and have undergone intensive selection to increase their body size. The differences in the prolactin gene could be due to changes in the gene in commercial poultry that occurred during the selective breeding process. This study was the first to characterize turkey prolactin in Kenya.

5.2.5 Characterization of quail prolactin

The prolactin fragment amplified from the wild quails was monomorphic. Other studies have however found prolactin in both wild and domestic quails to be polymorphic. Yousef et al. (2012) studied the prolactin promoter region of Japanese quail and identified two alleles (A and B) and three genotypes (AA, AB and BB). Allele A had a 24bp insertion at position 358. Lofti et al. (2013) carried out an association study on Japanese quail in which two alleles (I and D) and three genotypes (II, ID and DD) were identified. Genotypes II and ID were found to be significantly associated with the number of eggs laid by the quails ($p<0.01$). Eichie et al. (2016) found both domestic and wild quails to be polymorphic with respect to this 24bp indel. Marker trait association analysis in chicken also found significant correlation between the 24bp indel and egg production (Cui et al., 2005; Emamgholi-Begli et al., 2010; Usman et al., 2014). This

24bp insertion in the promoter region of prolactin gene is thought to affect egg production by inhibiting pituitary transcription factor 1 (*Pit-1*),Vasoactive Intestinal Peptide (*VIP*) and other transcriptional factor binding sites for *PRL* (Reddy et al,. 2006). It has also been suggested to decrease the expression of prolactin by reducing secretion of stimulatory factors like thyrotropin- releasing hormone (Yousefi et al., 2012). The 24-bp insertion introduces a possible ecotropic viral integration site-1 (*Evi-1*) binding site (Sharp et al., 1988). *Evi-1* could represses the expression of prolactin gene in chickens by binding the *Evi-1* binding site, preventing broodiness thus improving egg production (Jiang et al., 2005). This study is the first in Kenya to characterize prolactin in quails.

5.3 Characterization of Vasoactive Intestinal Peptide receptor 1 in emerging poultry species from Western Kenya

5.3.1 Characterization of duck *VIPR1*

The amplified duck *VIPR1* fragment was monomorphic. In contrast, other studies have identified polymorphisms in duck *VIPR1*. Xin et al. (2013) discovered eight mutations in black Muscovy duck *VIPR1* and suggested the association of these polymorphisms and broodiness studies be further investigated. In addition, Zheng et al. (2012) detected significant changes in levels of *VIPR1* during the different stages of egg laying, with the incubation stage having the highest levels. Two bands were visible in the gel for duck *VIPR1* amplicons. The use of species specific primers could be employed to prevent amplification of multiple fragments. This is the first study to characterize duck *VIPR1* in Kenya and could be the first step in improving productivity in local Muscovies.

5.3.2 Characterization of goose *VIPR1*

No polymorphisms were discovered in the goose *VIPR1* amplified fragment. No other studies on the characterization of goose *VIPR1* seem to have been conducted. However, transcription profiling by Luan et al, (2014) revealed a rise in expression levels *VIPR2* in the ovaries of Huoyan geese during the laying period. The action of *VIP* is mediated by its interaction with both *VIPR1* and *VIPR2*.

5.3.3 Characterization of pigeon *VIPR1*

All pigeon *VIPR1* fragments clustered into a single haplotype. The gel of the amplicons revealed bands of different molecular weights. The use of primers designed specifically for pigeon prolactin could eliminate this problem. This appears to be the first attempt at the characterization of pigeon *VIPR1* as no other studies were found. Studies in other poultry species have however shown that dopamine inhibits prolactin secretion by acting through *VIP* (Yin et al., 2017). Polymorphisms in its receptor would therefore also be expected to have some influence in the reproductive cycle.

5.3.4 Characterization of turkey *VIPR1*

All the turkeys sampled were of the same haplotype with no variations being present in the amplified region of the *VIPR1* gene. In previous studies, immunization against *VIP* has been proven to influence egg production in turkey hens (El Halawani et al., 1998; Kulick et al., 2005; Pitts et al., 1994; Tong et al., 1998). Chaiseha et al. (2004) noted significant changes in *VIPR1* mRNA expression during the different reproductive stages in turkeys. Polymorphisms in *VIPR1* could cause conformational changes in the structure of the protein interfering with its coupling with *VIP*.

5.3.5 Characterization of quail *VIPR1*

The *VIPR1* fragments amplified from the wild quail samples revealed eight haplotypes and 19 polymorphic sites. Haplotype 4 (GTTTTATCCCACACAATGC) was the most abundant with 29 alleles. This haplotype was also the most similar to the Japanese quail which could be due to mating with domestic quails that have escaped captivity. The wild quail population was not in Hardy-Weinberg equilibrium, with the H_E being 0.17961 and the H_O being 0.02105. Among population variation was 88.41% whereas variation within individuals was 11.59%.

Pu et al. (2016) identified two SNPs in their characterization study of *VIPR1* gene in two domestic lines of quail as well as wild quails. The two domestic lines were chosen for different breeding goals- high egg number and high egg weight respectively. For the second SNP locus, they found the dominant allele for the high egg number line to be the same in the wild quails. This led them to speculate that wild quails had the potential to produce more eggs if domesticated and given a suitable breeding environment. The 19 SNPs discovered by the current study in wild quail from Western Kenya could very well harbor potential for high egg production.

5.4 Comparison of prolactin and *VIPR1* fragments from emerging poultry species

Comparative genomics entails the comparison of genomic features of different organisms. Segments of DNA from different organisms can be compared by aligning them against each other. These comparisons can help us to establish the general function of a DNA segment such as coding and non-coding areas and regulatory elements as well as the evolutionary history of a species.

5.4.1 Percentage similarity/identity matrices for prolactin

On comparison with chicken prolactin, the gene fragment from quail was found to be the most similar at 87.7%. Unexpectedly, duck prolactin scored 74.8% while turkey prolactin had 64.6% similarity to chicken. Chicken, quails and turkey are classified as gallifomes while ducks and geese are anseriformes. The higher percentage similarity of duck to chicken compared to turkey and chicken could be due to the difference in fragment lengths with the duck fragment having 125bp and the turkey fragment 119bp. The four pigeon haplotypes showed the least similarity at 36%. The pigeon prolactin fragment amplified was 295bp long, almost twice the length of the chicken fragment. Liu et al. (2008) cloned goose prolactin cDNA and found the highest similarity values to be between duck and goose at 98.4%. Turkey, chicken and quail shared 92.2%, 92%, and 91.9% similarity to the goose respectively. In a comparison that did not include geese, Kansaku et al. (2005) found duck prolactin cDNA to shared 92.0%, 91.7%, and 91.4% sequence identity with chicken, turkey and quail respectively. The current study used genomic transcripts which might explain the lower percentage values observed. High sequence similarity percentages of chicken, quail, turkey, ducks and goose would suggest similar biological functions of prolactin as well as common ancestry of the prolactin gene in the poultry species under study. Prolactin plays the additional role of regulating the production of crop milk in pigeons which is an avian feeding mechanism unique to columbiformes (Horseman & Buntin, 1995). Crop milk is produced by both male and female pigeons. Prolactin stimulates the development of specialized epithelial cells in the lining of the crop sac of pigeons. These cells produce crop milk which is fed

to young pigeons after hatching. The difference in sequence length could be linked to this function.

5.4.2 Percentage similarity/identity matrices for *VIPR1*

Quail *VIPR1* had the highest similarity to chicken prolactin ranging 96.9% from for haplotype 5 to 93.1% for haplotype 1. Turkey, pigeon, duck and goose *VIPR1* had 93.6%, 86.2%, 80.6% and 78.6% similarity when compared to chicken prolactin. Zhou et al. (2012) found quail *VIPR1* to show high amino acid sequence identity with chicken (99.1%) and turkey (92.2%). The multiple sequence alignment of chicken *VIPR*1 and the emerging poultry species *VIPR1* fragments showed some highly conserved regions. High sequence homology implies similar biological function as well as a common ancestor of chicken and the emerging poultry species.

5.4.3 Phylogeny of prolactin gene fragment from emerging poultry species

A maximum likelihood tree was constructed using the sequence data from the prolactin fragments from the emerging poultry species. Ostrich prolactin was used as an out group to root the tree. The four pigeon haplotypes clustered together in the tree. The splits decomposition showed similar positioning of the branches. The branch for duck prolactin occurred between the quail and chicken branches. The generated phylogenetic tree shows the evolutionary history of prolactin gene in the emerging poultry species. It is however not in congruence with the species tree for the studied species.

5.4.4 Phylogeny of Vasoactive Intestinal Peptide Receptor 1 gene fragment

A maximum likelihood tree was constructed using the *VIPR1* fragments from the emerging poultry species. Ostrich prolactin was used as an outgroup to root the tree. Of the eight quail haplotypes, six clustered together while other two clustered with the turkey *VIPR1* fragment. The gene tree produced was not in agreement with the poultry species tree.

5.4.3 Incongruence of the gene trees with the species tree for poultry

Gene trees and species trees can be incongruent for a number of reasons including unequal rates of evolution, gene flow between lineages after their separation or recombination between neighboring regions can also lead to species phylogenies and gene histories that do not match.

Gene loss and gene duplication, horizontal gene transfer and incomplete lineage sorting may also cause the gene and species tree to show different topologies (Maddison, 1997).

In addition to biological reasons, the length of the fragments used to construct the tree also affects the accuracy of the generated tree. Saitou and Nei (1986) recommended 2600-2700 nucleotides for sequence data with two out groups. The fragment lengths used in this study were less than 300bp in length. The use of many different loci that have evolved independently to increase probability that the gene tree produced will represent the evolutionary pathway of the species under study (Pamilo and Nei, 1988).

In summary, the results of this study indicate the presence of local ecotypes of emerging poultry species in Western Kenya. The range of qualitative and quantitative traits

observed indicates underlying genetic diversity in the emerging poultry species from Western Kenya. These poultry species were found to be diverse in terms of phenotype with differences in plumage colour, skin and shank colours being observed. As is the case in most avian species, sexual size dimorphism was in favour of the males as compared to the female ducks, geese, pigeons and turkeys. Quail was the exception with the females being larger than the males. The polymorphisms discovered in the amplified regions of pigeon prolactin and quail *VIPR1* suggest the presence of genetic variants that could be linked to increased egg production.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The emerging poultry from Western Kenya exhibited wide phenotypic diversity. Variations in plumage, skin and shank colours were noted in the different species, indicating underlying genetic diversity. The study shows that in as much as there is an overreliance on chicken as a source of poultry meat and eggs in Kenya, emerging poultry do play a part in supplying both food and income to rural farmers in Western Kenya. Rural farmers have began to diversify their poultry flocks to include ducks, geese, pigeons, turkeys and quail in addition to indigenous chicken. It was evident that farmers preferred Muscovy ducks (*Carina moschata*) to the common duck (*Anas platyrynchos*) as all ducks encountered in the farms were Muscovy ducks. Sexual dimorphism was evident in all the emerging poultry species studied. The body weight of males was significantly higher than that of females in ducks, geese, pigeons and turkeys. The same trend was observed in the shank length measurements except in the case of geese where the difference was not significant. In the quails, sexual dimorphism was in favor of the females.

The results of this study revealed the presence of polymorphisms in pigeon prolactin and quail *VIPR1,* suggesting differing production potentials based on the genetics of the

emerging poultry species. Interestingly, polymorphisms in prolactin and *VIPR1* genes were discovered in the wild emerging poultry species- wild quails and feral pigeons. This could be due to the fact that these two species are able to fly long distances in search of food. During these scavenging trips, they encounter birds from different populations giving them access to a wider pool of mating partners. The resultant diversity in these populations is evident from the presence of polymorphisms in the two candidate genes studied.

The sequence homology exhibited by the prolactin and *VIPR1* genes in the emerging poultry species and chicken suggests similar function and common ancestry of the two genes.

Overall, the results of this study lead me to accept the hypothesis that there is phenotypic diversity in emerging poultry species from Western Kenya and there are functional polymorphisms in prolactin and *VIPR1* genes in emerging poultry species from Western Kenya.

6.2 Recommendations

The findings of this study could be the basis for establishment of breeding programs for emerging poultry species in Kenya. In the case of pigeons and quails where genetic polymorphisms were discovered in prolactin and *VIPR1* respectively, association studies should be conducted to compare the effects of the different genotypes on egg production.

This study characterized fragments of prolactin and *VIPR1* genes from the emerging poultry species. Characterization of the whole genes should be carried out so as to fully investigate possible polymorphisms that could have an effect on egg production. The use of Next Generation Sequencing technologies would allow the parallel analysis of massive amounts of DNA fast and cheaply. Other genes that have been found to influence egg production in chicken should also be sequenced in the emerging poultry species.

Exotic breeds of ducks, geese, turkey and quail are also present in Kenya, predominantly in urban and peri-urban areas. Cross breeding of these commercial breeds which are more productive could result in the extinction of the local ecotypes. Some haplotypes of wild quails were found to be distinctly different from the Japanese quail. An effort should be made to conserve wild quails in order to preserve unique genotypes as a source of future breeding material. Initiatives to conserve the local ecotypes of all emerging poultry species should be established.

All sampled poultry from the five emerging poultry species were from Western Kenya. Phenotypic and genetic studies need to be carried out with emerging poultry from all the agro-climatic zones in Kenya to investigate phenotypes and genetic polymorphisms that could be associated with egg production.

Chicken primers were used as catch-all primers to amplify prolactin and *VIPR1* genes in all the emerging poultry species. Species specific primers should be used to amplify these two genes in order to eliminate the amplification of multiple fragments as was the case in duck and pigeon *VIPR1* genes.

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APPENDICES

Appendix 1: Sanger sequencing method

This method was developed by Frederick Sanger and his colleagues in 1977. First, the PCR product is first purified to remove excess primers and the 4 dNTPs. Purification is done enzymatically. Classical Sanger sequencing requires a single-stranded DNA template, a DNA polymerase, a DNA primer, normal deoxynucleotide triphosphates (dNTPs), and dideoxynucleotide triphosphates (ddNTPs) that terminate DNA strand elongation. These ddNTPs lack the 3′-OH group that is required for the formation of a phosphodiester bond between two nucleotides, causing the extension of the DNA strand to stop when a ddNTP is added. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard dNTPs (dATP, dGTP, dCTP, and dTTP), the DNA polymerase, and only one of the four ddNTPs (ddATP, ddGTP, ddCTP, or ddTTP) for each reaction. The ddNTP is fluorescently labeled either with ^{32}P or ^{35}S. The result of these reactions is a set of molecules of different lengths where the length of the molecule is tied directly to its fluorescent emission. After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called capillary gel electrophoresis**.** Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment reaches the end of the tube, it's illuminated by a laser, allowing the attached dye to be detected. The smallest fragment (ending just one nucleotide after the primer) crosses the finish line

first, followed by the next-smallest fragment (ending two nucleotides after the primer), and so forth. Thus, from the colors of dyes registered one after another on the detector, the sequence of the original piece of DNA can be built up one nucleotide at a time. The data recorded by the detector consist of a series of peaks in fluorescence intensity on a chromatogram. The DNA sequence is read from the peaks in the chromatogram.

Appendix 2: Accession numbers for Reference sequences from GenBank

Organism	Accession Number
Prolactin	
Mallard Duck	KB_742775.1
Domestic Goose	NW_013185719.1
Domestic Turkey	0.015013.2
Japanese Quail	AB 162005.1
Southern Ostrich	XM_009688738.1
VIPR1	
Mallard Duck	KB 742775.1
Domestic Turkey	NC_015015.2
Japanese Quail	NC_029517.1
Southern Ostrich	NW_009271904.1

Appendix 3: List of polymorphic sites for wild quail *VIPR1* samples

Locus No.	Transition/Transversion
13	G/A
15	T/C
43	G/T
54	C/T
70	G/T
74	C/A
77	A/T
84	A/C
85	T/C
98	T/C
99	G/A
114	T/C
115	G/A
116	T/C
125	C/A
133	G/A
137	G/T
145	A/G

157 C/A

Appendix 4: Publication from this research

J W Macharia, S Ogada, M Mberu, J K Lichoti and S C Ommeh (2017).

Phenotypic analysis of Underutilized Poultry Species from Western Kenya. *Livestock Research for Rural Development, Volume 29, Issue 5. Article # 99.*

Appendix 5: Questionnaire for the phenotypic characterization of emerging poultry species from Western Kenya

Farmer's Name: …… Region: ……

Location: …… GPS: ……

Enumerator's Name: …….. Date of Interview: ……

Poultry species kept:

……

Number of birds: Ducks ( ), Geese ( ), Pigeons ( ), Turkey ( ), Quail ( )

Others (Please specify):.....

How long have emerging poultry been kept in the household? ……..

Source of foundation stock: ……

Age of emerging poultry birds: …….

Do you feel the need to improve emerging poultry production? Yes ( ) No ( )

Traits to improve in emerging poultry: ……...

What type of management system do you practice? Extensive ( ), semi-intensive ( ), intensive ( ) others,

specify……

Do you give supplementary food to your emerging poultry? Yes ( ) No ( ) If you give feeds, how frequently do you feed your birds each day?

.....

How often do you cull your birds? …….

For what purpose do you cull your poultry? ( ) for consumption, ( ) sale, ( ) sacrifice,

( ) Others, specify ……

Which factors determine which bird you will cull? ( ) poor productivity, ( ) old age, ( ) sickness, ( ) others, specify …….

Have you heard about the improved poultry production practices? Yes ( ), No ( )

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

Morphometry

Age in months ……..

Sex: male ( ) female ( )

Shank color: White ( ), Yellow ( ), Green ( ), Grey ( ), Others

……

Skin color: White ( ), Yellow ( ), Cream ( ), Grey ( ),

others……

Other general issues

Do you intend to extend poultry production? Yes ( ), No ( ).

If yes, to how many?

…….

What are your barriers to future expansion of poultry production?

.....

What traits do you wish to see improved in the emerging poultry species?

……