PHENOTYPIC AND GENETIC CHARACTERIZATION OF DIFFERENT STRAINS OF INDIGENOUS TSWANA CHICKENS IN KWENENG AND SOUTHERN DISTRICTS OF BOTSWANA

DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE

(ANIMAL BREEDING AND REPRODUCTION)

By

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PHENOTYPIC AND GENETIC CHARACTERIZATION OF DIFFERENT STRAINS OF INDIGENOUS TSWANA CHICKENS IN KWENENG AND SOUTHERN DISTRICTS OF BOTSWANA

A thesis submitted to Department of Animal Sciences in partial fulfilment of the requirements for degree of Doctor of Philosophy (PhD) in Animal Science (Animal Breeding and Reproduction).

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DECLARATION

I declare that the thesis submitted for the award of Doctor of Philosophy degree in Animal Science at the Botswana University of Agriculture & Natural Resources is my independent work and has not previously been submitted to this or any other University. Specific contributions by others are acknowledged.

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DEDICATION

I dedicate this work to my beloved wife, and to my young brother, son and daughter who always gave me the courage to complete my studies.

LIST OF ABBREVIATIONS/ACRONYMS

AFLD	Amplified fragment length polymorphisms.
AMOVA	Analysis of molecular variance
AnGR	Animal genetic resource
AU-IBAR	African Union-Interafrican Bureau for Animal Resources
BIDPA	Botswana Institute for Development Policy Analysis
CHIP	Chromatin Immuno-precipitation
CV	Cross-validation
DAD-IS	Domestic Animal Diversity Information System
DNA	Deoxyribose Nucleic Acid
EFABIS	European Farm Animal Biodiversity Information System
ERFP	European Regional Focal Point for Animal Genetic Resources
EU	European Union
FAO	Food and Agriculture Organization of United Nations
F*IS*	Inbreeding coefficients of an individual
FST	Inbreeding coefficient of sub-population.
GDP	Gross Domestic Product
GLM	Generalized Linear Models
IBS	Pairwise identity by state
INDEL	Insertion and/or deletion of nucleotides into genomic DNA
INFPD	International Network for Family Poultry Development
H_e	Expected heterozygosity
H_o	Observed heterozygosity
HSP	Heat shock protein
HVI	Hypervariable region I
HWE	Hardy Weinberg Equilibrium
KB	Kilobytes
LD	Linkage disequilibrium

MAF	Minor Allele Frequency
MDS	Multidimensional scaling
Mt DNA	Mitochondrial Deoxyribonucleic Acid
Ne	Effective population size
NGOs	Non-governmental organizations
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
QC	Quality control
RFLP	Restriction fragment length polymorphisms
RAPDs	Random amplified polymorphic DNAs
SADC	Southern Africa Development Community
SAS	Statistical System Analysis
SD	Standard deviation
SE	Standard Error
SPSS	Statistical Package for Social Sciences
SNP	Single Nucleotide Polymorphism
STRs	Short tandem repeats
USD	United States Dollar
UTR	Untranslated region

GENERAL ABSTRACT

The aim was to characterise indigenous Tswana chicken populations in Kweneng and Southern districts of Botswana. The qualitative traits involved in the study included tail colour, breast colour, back colour, neck colour, comb type, shank colour, earlobe colour and head shape. Data were subjected to frequency and cross tabulation procedures of descriptive statistics in Statistical Package for Social Sciences (SPSS) to compute frequencies of occurrence of each qualitative trait. The five strains of indigenous Tswana chickens under scavenging management system showed distinct physical variations for most of the qualitative traits. Black was the most predominant tail colour across the strains (51.6%) followed by brown (27.9%). The frequency of brown breast colour and brown back colour were significantly higher in those respective regions. Brown and black were the predominant neck colours across the strains. The single comb type (81.7%), featherless shank (65.4%), red ear lobes (67.6%) and grey shank colour (32.9%) were the most predominant phenotypes across the strains.

A total of eight (8) quantitative traits were measured using flexible measuring tape, and live body weight was measured using a Spring-Dial Hoist weighing scale. Data were analysed using mixed model's procedures of SAS and the model included fixed effects of strain and sex and their interaction. Normal-feathered males had significantly higher shank length (9.94±0.23 versus 8.35±0.20), shank circumference (0.99±0.02 versus 0.84±0.02) wing length (20.61±0.51 versus 18.60±0.48), wingspan (41.22±1.03 versus 37.19±0.96), comb length (6.30±0.30 versus 3.48±0.26) and wattle length (3.44±0.16 versus 2.40±0.14) than their female counterparts. Among males, there were no significant strain differences in spur length, wing length, wingspan, comb length, wattle length and live weight. Normal-feathered males had the highest live weight and rumpless males had the lowest live weight. Only naked neck and normal-feathered females had significantly higher wingspan and wing length than dwarf females. Finally, it was noted from the study that various strains of Tswana chickens had similar

qualitative traits except for shank length and shank circumference which were significantly shorter/smaller in dwarf strain compared to the other four strains.

SNP genotyping was carried out using the Illumina chicken iselect SNP 60 Bead chip using the Infinium assay compatible with the Illumina HiScan SQ genotyping platform on 96 samples in total for both indigenous Tswana and commercial broiler chickens. Principal component analysis (PCA) was used to obtain insight into the population structure of indigenous Tswana chickens. The first two principal components revealed a set of three clusters such as normal/naked neck, normal/broiler as well as dwarf strain. The dwarf strain clustered separately into one group and the naked neck and normal strains clustered together in the last group. The separate clustering of the dwarf from the rest of Tswana chicken strains suggests significant its genetic uniqueness and very close genetic similarities between the normal and naked neck strains. The clustering pattern was confirmed by less genetic differentiation (0.013) and less genetic distances (0.013) between the naked neck and normal strains of Tswana chicken than between the two strains (0.040, 0.041) and the dwarf strain of Tswana chicken.

Further investigations on sequence polymorphisms in the promoter, 5´untranslated regions (UTR) and partial exon regions of chicken HSP-70 gene were carried out in the normal (n= 24), naked neck (n= 22) and dwarf (n=12) strains of indigenous Tswana chickens relative to the commercial broiler chicken (n=20). Genomic DNA extracted from whole blood samples of the three strains of indigenous Tswana chicken and the commercial broiler, were amplified using PCR and sequenced using Big Dye Cycle Sequencing Kit. Multiple sequence alignments of the partial sequences of chicken HSP-70 gene in indigenous Tswana chickens and the commercial broilers revealed two SNPs in the 5´UTR (A303G and G309A) and another two SNPs (G427 and A628G) in the partial exon sequence of chicken HSP-70 gene. The SNP G427A was unique to the normal strain and the other three SNPs were common to all the four

chicken strains studied. The identified four SNPs associated with individual chickens resulting in a total of seven different haplotypes in the studied chicken populations.

Keywords: Botswana, Free range, Genetic diversity, HSP-70 gene, Indigenous Tswana chicken, Phenotypic traits, SNPs.

CONTRIBUTIONS TO KNOWLEDGE

1. Chapter III: Literature on phenotypic characterization of qualitative traits of indigenous Tswana chicken is very limited. Chapter III provided baseline data on the frequency of different phenotypes of various qualitative traits in different strains of indigenous Tswana chicken.
2. Chapter IV: Phenotypic data on the performance of indigenous Tswana chicken in different quantitative traits under their natural production environment (free range or free running system) was non-existent. Chapter IV provided data on the performance of different strains of indigenous Tswana chicken in various quantitative traits under their natural production environment.
3. Chapter V: This is the first study to report genetic relationships and clustering patterns among indigenous Tswana chickens. This is also the first study to ever report the separate clustering of the dwarf from the rest of Tswana chicken strains as well as very close genetic similarities between the normal and naked neck strains.
4. Chapter VI: The first study to document SNPs in the promoter and 5´UTR regions (regulatory regions) of Tswana chicken HSP-70 gene involved in adaptation to thermal stress in chickens. The importance of chicken HSP-70 gene cannot be over emphasized in the era of global warming and climate change.

FORMAT OF THESIS

This thesis adheres to the "Guidelines for Thesis Preparation (50.8 Thesis regulation), of 50.0 General Regulations for the Degrees of Masters of Philosophy (MPhil) and Doctor of Philosophy (PhD), in which regulation No:50.8.2.5 states that "The Thesis may be submitted in the format specified in the Graduate students handbook or as a collection of papers published in peer-reviewed journals or book, provided the overall Thesis forms a logical and linked development of ideas." The thesis is therefore in the form of a collection of manuscripts submitted to scientific journals.

The thesis therefore includes:

1. Table of contents
2. General abstract
3. An introduction which clearly states the rationale and objectives of the research.
4. A comprehensive review of literature.
5. Study 1: Phenotypic characterization (qualitative traits) of various strains of indigenous Tswana chickens in Kweneng and Southern districts of Botswana
6. Study 2: Phenotypic characterization of Tswana chickens based on quantitative traits in Kweneng and Southern Districts, Botswana
7. Study 3: Genetic Diversity and Population Structure of Three Strains of Indigenous Tswana Chickens and Commercial Broiler Using Single Nucleotide Polymorphic (SNP) Markers
8. Study 4: Genetic Polymorphism in the Promoter and 5´ UTR of HSP-70 Gene in Three Strains of Indigenous Tswana Chickens and Commercial Broilers
9. Appendix 1. A survey questionnaire on phenotypic characterisation of indigenous Tswana chickens.
10. Appendix 2 Published articles locations.

CONTRIBUTIONS OF AUTHORS

The four co-authored manuscripts submitted for publication are included in the thesis.

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CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

The poultry sector continues to grow and industrialize in many parts of the world. Urbanization, greater purchasing power and an increasing population have been active drivers of growth in the poultry industry (FAO, 2023). Production of poultry has experienced rapid changes since the nineteen forties when modernised intensive production methods were introduced together with new breeds, improved biosecurity, and preventive health measures (Permin & Pedersen, 2000). The livestock sector in general is undergoing drastic changes as large-scale production expands in response to surging demand for meat, milk, and eggs (FAO, 2007). Poultry is now by far the largest livestock species worldwide (FAO, 2000a), accounting for more than 30 % of all animal protein consumption (Permin & Pedersen, 2000).

Indigenous chickens are widely distributed in the rural areas of tropical and sub-tropical countries where they are kept by most of the rural communities. Indigenous chickens in Africa are in general hardy, adapted to local environments, survive on little or no inputs and adjust to fluctuations in feed availability and quality. Flock composition is dominated by chickens which comprise about 98 % (Gueye, 2003) of the total poultry numbers (chickens, ducks, and turkeys) kept in Africa. Botswana's poultry industry plays a major role in contributing towards household food security as well as improving the standard of living of people living in rural areas through poverty alleviation and creation of employment opportunities (Moreki, 2013). Statistics Botswana (2020) estimated the Indigenous Tswana chicken population to be approximately 1.02 million, while Thutwa et al., (2012) observed that indigenous Tswana chickens are widely distributed in every part of the country with households projected to be keeping an average of 14 chickens.

Strains found within the indigenous Tswana chicken population include the dwarf, rumpless, frizzled, naked-neck and normal with the most common strain being the normal (Badubi et al., 2006). Some strains such as the rumpless, dwarf and frizzled exist at relatively low proportions within the Tswana chicken population and might be at risk of endangered even before they are characterised and/or conserved (Kgwatalala et al., 2012) owing to the high rate of genetic erosion resulting from chicken diseases, particularly Newcastle disease, predation, inclement weather, and indiscriminate crossbreeding with exotic chicken genetic resources. This risk is consistent with the FAO report (FAO, 1999), that indicated that indigenous animal genetic resources in developing countries in general, are being eroded through unplanned introduction of exotic genetic resources, before appropriate characterization, utilisation and conservation programmes of indigenous animal genetic resources are put in place.

Characterization informs both breeding, sustainable utilisation, and conservation programmes. Characterization of indigenous animal genetic resources should therefore precede any conservation and genetic improvement programmes (Rege and Lipner, 1992). Characterization of indigenous Tswana chicken genetic resources should involve both *in situ* (on farm) and *ex situ* (on station) phenotypic and genetic characterization to establish the diversity in both qualitative and quantitative traits in different strains of Tswana chicken found in the general population. According to Toro et al., (2006; Groeneveldt et al., 2010) molecular markers have played a leading role in the characterisation of genetic diversity and provide a quicker, economical, and more reliable assays of estimating diversity in the absence of quality phenotypic measures.

There is very little information on qualitative and quantitative characteristics of indigenous chickens in Botswana at farm level (*in situ* characterization). From the few published in situ characterization studies, some qualitative traits such as head shape, earlobe colour, shank colour and skin colour were not investigated. To the best of my knowledge, there are no documented studies on *in situ* characterization

of quantitative parameters like chest circumference, wingspan, shank circumference, neck length, comb height, comb width, wattle height and wattle width in different strains of Tswana chicken Thutwa et al., (2012) and Kgwatalala et al., (2012) reported on the growth performance of different strains of indigenous Tswana chickens under intensive management system however, the two studies excluded the rumpless and frizzled strains of Twana chicken. To date no studies have been carried on genetic characterization of different strains of indigenous Tswana chicken both *in situ* and *ex situ*. The purpose of the current study was therefore to bridge the gaps on both phenotypic (qualitative and quantitative traits) and genetic characterization of different strains of Tswana chicken outlined above.

1.2 Rationale

Poultry genetic resources are found everywhere in developing countries, but information on their characterization, inventory and monitoring of trends is very limited. Indigenous Tswana chickens are well adapted to local climatic conditions and are tolerant to heat, diseases and parasites (Moreki, 2010). Bettridge et al., (2018) reported that characterization provides data on present and potential future uses of indigenous chicken populations and establishes their current state. Phenotypic (qualitative and quantitative traits) and genetic characterization studies provide baseline data that informs sustainable utilization, conservation, and genetic improvement programs. From the review of the literature, a limited studies on both in situ and ex situ characterization of indigenous Tswana chicken have been carried out to date. Those characterization studies also covered a limited number of both qualitative and quantitative traits and left out some traits. Qualitative traits such as comb type, head shape, earlobe colour, shank colour, eye colour, and skin colour of indigenous Tswana chicken remain undocumented and so are quantitative traits such as chest circumference, wingspan, shank circumference, neck length, comb height, wattle height and wattle width. No studies to date have been carried out on the genetic characterization of Tswana chicken using modern molecular techniques. It therefore remains unclear

whether the strains of Tswana chicken (naked neck, normal, dwarf, frizzled and rumples) are indeed strains of one breed or they represent distinct genetic groups or breeds. In this era of global warming and climate change it is also important to evaluate the heat resilience of indigenous Tswana chicken and their potential to adapt to the expected high environmental temperatures by assessing the degree of variability in the heat tolerance genes such as heat shock protein-70 gene.

With the current limited data on both phenotypic (quantitative and qualitative traits) and genetic characterization of Tswana chicken, the current study is therefore aimed at closing the identified research gaps above. It is hoped that the current study will generate data or information that can be used to inform conservation and genetic improvements programs. The data generated will also be critical in establishing the national data bases and the base line data on indigenous Tswana chicken genetic resources for inventory and future monitoring of population trends.

The genetic characterization part would establish the degree of genetic diversity in different strains of Tswana chicken as well as the levels of inbreeding which could inform breeding management of Tswana chicken. The genetic characterization will also elucidate the degree of relatedness of different strains of Tswana chickens and inform sustainable utilization and conservation programs. The degree of diversity in the heat shock Protein-70 will attest to the heat tolerance and potential ability of Tswana chicken to withstand the expected increases in environmental temperatures resulting from global warming and climate change.

1.3 Hypothesis

Ho: There are no phenotypic and genetic differences within and between different strains of indigenous Tswana chickens.

Ha: There are significant phenotypic and genetic differences within and between different strains of indigenous Tswana chickens.

1.4 Overall Objective

The overall objective the study is to:

Evaluate phenotypic and genetic variability of different strains of indigenous Tswana chickens found in Kweneng and Southern districts of Botswana.

1.5 Specific Objectives

The specific objectives of this study were to:

1. Document phenotypic differences in qualitative traits and establish the frequencies of the different phenotypic classes in different strains of Tswana chicken.
2. Document phenotypic differences in quantitative traits and estimate mean performance of various quantitative traits in different strains of Tswana chicken.
3. Estimate genetic diversity parameters and establish genetic relationships between different strains of Tswana chicken,
4. Establish SNPs that occur in the promoter and 5ÚTR regions of chicken HSP-70 gene in different strains of Tswana chicken.

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Production Characteristics and Ownership of Indigenous Chickens

Indigenous chickens in Botswana are widely distributed across various agro-ecological zones and are reared under traditional scavenging management system. They are an important avian resource kept as a source of animal protein and income to many of the rural population. Statistics Botswana (2020) estimated the population of indigenous Tswana chickens to be approximately 1.1 million, representing 4.42% of the total chicken population in the country. The remaining 95.57% consists of commercial and exotic chicken breeds. Indigenous Tswana chickens which are reared mostly by resource-poor farmers are widely distributed across the country making them very important despite their low numbers compared to commercial chickens (Statistics Botswana, 2020). Indigenous poultry fits perfectly in the lifestyle and resource base of resource-poor farmers because they require minimal labour and low capital investment compared to several farming businesses (Tadelle and Ogle, 1996a). Generally, the rearing of chickens requires small area of land. Women own most family chicken flocks, and the revenue generated from chicken production belongs to them (Pederson et al., 2001). Indigenous chicken production thus, contributes towards financial independence of women and women empowerment of the largely patriarchial African societies. Seeberg (2002) reported that 92% of the evaluated women in Bangladesh used the income from sale of eggs and meat in their children` school activities. Furthermore, the author reported that improved indigenous chicken production had escalated farmer`s livelihood and empowerment of women. Similarly, Gale (1992) observed that the participation of women in the upgrading programmes for indigenous chickens contributes to human development both by raising accessibility to rural women to income, knowledge and by strengthening production efficiency.

2.2 Management Systems

The different chicken management systems in the tropics can be divided into extensive, semi-intensive and intensive systems. The most common indigenous chicken production systems in Africa are the free-range and backyard systems with 80% of the chicken populations kept in these systems (Guèye, 2003; Halima et al., 2007). Female-headed households are at the forefront of indigenous chicken production. According to Kgwatalala et al. (2012), indigenous Tswana chickens are usually kept in small flocks (2 to 20 chickens) of diverse ages under the traditional scavenging management system with basic supplementary feeding, housing, and health care. These chickens adequately cope with difficult environmental conditions including high disease incidence, poor nutrition, and high temperatures (FAO, 1998a, b).

2.2.1 Housing

Indigenous chickens in Botswana as in other developing countries are seldom housed to provide shelter to protect them from predators and inclement weather. Most of the time, indigenous chickens are housed not except possibly at night (Moreki, 2006). The author observed that when chicken shelters are provided, they are constructed using readily available local materials such as old tins, bricks, iron sheets, plastic bags, and thatching grass. Usually, shelters are located at the back of owners' houses/huts. Although, all family members participate in constructing shelters, it seems that men and boys are in the forefront. In the absence of shelters, chickens sleep on tree branches, piles of bricks/blocks, bush fences, old vehicles, walls, under roof overhangs or on top of huts rendering them vulnerable to predation, inclement weather, and theft (Moreki, 2003). Predation risks are high all the time since most Indigenous chicken keepers confine birds at night but allow them to free range during the day. Lack of housing results in eggs laid in the bush fence to be eaten by predators such as snakes, mongooses, and dogs (Moreki, 2003).

2.2.2 Feeds and Feeding

Previous study by Badubi et al. (2006), reported that indigenous Tswana chickens mostly rely on scavenging, with only 57.1% of the farmers feeding their chickens once a day, 41.7% twice a day and 1.1% never. The unbalanced feed supplements such as kitchen waste (3.4%), maize (70.1%), sorghum (10.3%), millet (2.4%), sorghum bran and maize bran (3.5%) and sunflower (2.2%) were preferably used in feeding indigenous Tswana chickens by most farmers (91.9%) (Badubi et al., 2006). The feed supplementation was either done as a mixture or individual feedstuff. Aganga et al. (2003) observed that feeding whole grain maize to indigenous Tswana chickens has been practised in Botswana for over 50 years. However, maize grain has high total digestible nutrient content of 70 -80% but proportionally little protein content.

2.2.3 Watering

Water is supplied to the birds routinely and occasionally. However, some farmers in developing countries usually underestimate the importance of water even though it's equally important as the feed (Dorji and Gyeltshen, 2012). The study by Atsbeha (2013) reported that male and female headed households in central zone of Tigray, Ethiopia provided water for chickens with simple earthen pot placed on the ground at any corner of the chicken house. The main sources of the water were shallow wells, spring water and hand pumped water. Atsbeha (2013) further stated that the watering troughs were always open and simply placed on the ground, possibility of contamination of water with manure and other dirt materials could be high. This might be a cause for the development of bacterial disease and other internal parasites that may affect the reproduction and productivity of the chickens (Atsbeha, 2013). Contamination can also occur at the well or pond sources if not kept clean and sanitary (Atsbeha, 2013).

2.2.4 Health Management

The chicken sustainability and productivity are mainly affected by mortality (Kugonza et al 2008). Indigenous chicken mortalities are caused by factors such as poor nutrition, lack of proper shelter and inadequate health control (Simainga et al 2011; Ali 2012). Dorji and Gyeltshen, (2012) reported that there are various causes of mortality, and the main sources were predators, diseases and unknown factors for all the ages of birds. In African countries, the loss of chickens due to predation was also rated highest (Simainga et al 2011; Ali 2012). It is noted that the farmers do not have necessary knowledge to identify the diseases and any infections encountered, they normally treat them with ethnoveterinary medicine (75%), practice isolation (17%) and both ethnoveterinary and conventional drugs (1%) (Dorji and Gyeltshen, 2012). Chicken movements through gifts and battering system could also be a way of spreading diseases in the rural areas (Petrus et al., 2011). Ectoparasites such as tampans, lice were also mentioned by farmers in Namibian indigenous chicken survey besides diseases, with higher infestation rates occurring during the rainy seasons (Petrus et al., 2011). Moreki et al. (2010) reported that parasite control in indigenous chickens was mainly by traditional remedies such as cold water with washing powder, dips, Blue Death, wood ash and chemical dusts such as Karbadust (carbryl) were predominantly.

2.2.5 Marketing

The indigenous Tswana chickens are usually marketed live or as processed products. As breeding is uncontrolled, birds breed all year round resulting in them being sold throughout the year (Moreki et al., 2016). The authors reported that income from chicken sales is used to buy school uniforms for children and pay their school fees, as well as, buying livestock feeds during dry periods when there is low or no pasture for cattle and small stock (sheep and goats). Similarly, Alders and Pym (2009) reported that chicken products in South Asia (Thailand) were used for children's education and starting the process of

aggregating asset for later use by families. In another study, Gabanakgosi et al. (2013) reported that chicken returns were used to buy goats or sheep in Botswana. Neighbours, friends, and passers-by were frequent purchasers of indigenous Tswana chickens. The study by Moreki et al. (2016) showed that the cockerels were the first to be sold at a price ranging from 60-160 Botswana Pula (BWP) depending on body size followed by female adults (50-100 BWP) and growers (40-60 BWP).

2.3 Phenotypic Characterization

Indigenous chicken strains in Botswana are diverse without breed standards and characteristics. They show some variations in qualitative and quantitative features, and this implies that there is reasonable existence of phenotypic diversity among and within the strains of indigenous chickens which is critical for their genetic development (Markos et al., 2020). Benitez (2002) reported that the genetic differences prevailing within and between Indigenous chicken population determines their conservation, usage, and prospective sustainable development. It is not easy to arrange for a good breeding programme for chicken strains or breeds which were not phenotypically and genetically characterised satisfactorily (Mwacharo et al., 2006). Research on the variability and design of comprehensive breeding programmes are dependent on the characterization of indigenous chicken breeds or strains in their production environment (Markos et al., 2020).

Few strains of the Indigenous Tswana chicken population including normal, dwarf, naked neck, frizzled, and rumpless phenotypes were reported by Moreki (1997) and Badubi et al. (2006). However, the naked neck, rumpless, dwarf and frizzled strains exist at proportionality low numbers within the Indigenous Tswana chicken population and are at risk of extinction if efforts are not made to conserve them (Kgwatalala et al., 2012).

2.3.1 Qualitative Traits

Qualitative traits such as plumage, skin, comb, shank and earlobe colours, body shapes, head shapes, comb size and types of indigenous chickens have been studied in several countries such as Eritrea, Ethiopia, Nigeria, Pakistan, Sri Lanka, Philippines, and Indonesia (Table 1). The most common plumage colour was red among indigenous chickens of North Ethiopia (Sekela Woreda) (Markos et al., 2020, Nigeria (Shuaibu et al., 2020). In Botswana, Badubi et al. (2006) reported the gold plumage as the most dominant trait among indigenous Tswana chickens. According to Liyanage et al. (2015), the occurrence of various varieties of plumage colours might be a result of segregation of alleles from random mating among chickens possessing various plumage patterns.

Plumage colour has become an important component in breeding programmes as it may determine both market demand and supply chains of indigenous breeds in developing countries (Melesse and Negesse, 2011; Emebet et al., 2013; and Al-Qamashoui et al., 2014). According to Khadidja et al. (2014), the maintenance of plumage colour variations in indigenous chickens is an indication of the presence of several genes and alleles at the plumage colour locus. Uncontrolled crossbreeding over many decades between chickens with various plumage colours within the Indigenous chicken population gave rise to other plumage colour combinations particularly those found in small proportions.

Indigenous chickens evaluated in Africa mostly had single comb type except those of Southern zone of Tigray and Western zone of Tigray in Ethiopia where the rose comb type predominated with 66.25% and 53.3%, respectively. This agrees with the findings of Shuaibi et al. (2020) who reported the predominance of single comb type in the tropics as its presence reduces body heat by 40%, which is advantageous for tropical poultry production (Duguma, 2006). The different comb types found in indigenous chickens in

some African countries are shown in Table 1. Badubi et al. (2006) reported single (90.4%, rose (4.9%), walnut (1.3%) and pea (1.0%) comb types within Indigenous Tswana chicken populations.

Previous studies have reported plain head shape as the most dominant head shape accounting for 57.92% in the southern zone of Tigray in Ethiopia (Nigussie et al., 2015) and 92.42% in Nigeria (Rotimi et al., 2016). Crested head shape was observed in indigenous chicken of North Ethiopia (55.8%) (Western zone of Tigray) (Markos et al., 2020). Additionally, the snakehead shape was reported in the South-Western Ethiopia (Tadele et al., 2018). In other studies (Bekele et al. (2015), Moreda et al. (2014) and Getu et al. (2014)) also observed snakehead shape in North Gondar zone, Ethiopia. Markos et al. (2020) also reported a higher frequency of indigenous chicken with spur than those without spurs in Ethiopia.

Variations were also observed in the colour of earlobes of indigenous chickens across Africa. A mixture of white and red earlobes was found at a frequency of 45.2% in Eritrea, 54.55% in southern zone of Tigray in Ethiopia, 70.1% in western zone of Tigray in North Ethiopia and 56.1% in east west Samar, Philippines. In some localities, earlobes were either solid red or solid white. According to Nishida et al. (2000), the differences in earlobes colour could be attributable to variations in ancestral lineages and mutations that occurred several years ago. Variations in earlobe colour resulted from hybridization between subspecies of *Gallus gallus*, mostly *G.g. gallus* that carries white earlobes and *G.g spadiceus* and *G.g. jabouillei*, that possessed red earlobes. Genetic characterization of native chickens of the Philippines indicated that 17.6% of the characterised native chickens clustered closer to *G.g. spadiceus* species, which is commonly known to have red earlobes, and 11.7% closer to a clade of Rhode Island Red or a commercial layer line. The high frequency of red with white earlobes among native chickens in

the Philippines might be due to crossbreeding between indigenous native chickens and commercial lines (Godinez et al., 2020).

Several studies across Africa indicated that the shanks of indigenous chickens are white, yellow, a combination of yellow and white, and black and in most countries white shanks predominated (Table 1). Markos et al. (2020) found that native chickens of Bangladesh had predominantly white (35%) and yellow (31%) shanks. Dana et al. (2010) and Shuaibu et al. (2020) reported predominantly yellow shanks in indigenous chickens of Nigeria. Similarly, Assefa and Melesse (2018) reported predominantly yellow shanks (52.1%) in Masha district of Ethiopia. According to Melesse and Negesse (2011), shank colour is influenced by pigments in the dermis and epidermis. For instance, when there is black pigment in dermis and yellow in epidermis, shanks have a greenish colour and in the absence of both pigments, the shanks are white (Melesse and Negesse, 2011).

Black shank colour has been reported in the native chickens of Pakistan and Indonesia (Asmara et al., 2019; Bibi et al., 2021). According to Markos et al. (2020), the diverse shank colours occur because of the combinations of pigment regulating genes responsible for colour determination. Additionally, personal preferences and natural selection also influence the shank colours of indigenous chickens in different localities.

The eye colour of indigenous chickens observed in different localities are red, orange, black and brown and black (Table 1). Markos et al. (2020) reported predominantly orange (73.4%) and brown (16.3%) eye colours in native Tanzanian chicken populations. In another study, Dahloum et al. (2016) found that indigenous chickens of Algeria had predominantly orange (81.7%), yellow (10.37%) and dark brown

(7.92%) eye colours. According to Eskindir et al. (2013), variations in eye colour are influenced by carotenoid pigments and blood supply to the structures within the eye.

A study by Markos et al. (2020) reported that variations in skin colour among indigenous chickens were due to variations in feedstuffs available to chickens in the different agro-ecological regions. According to Melesse and Negesse (2011), white skin colour is the result of the absence of carotenoid pigments while the yellow skin colour is the result of the presence of carotenoid pigments (Xanthophylls) that are consumed through feeds and deposited under the skin. The skin colour variations of indigenous chickens might also have some implications on the origin of various native chicken groups as literature revealed that yellow skin colour was inherited from the grey jungle fowl (*G. g sonneratti)* and Ceylon jungle fowl (*G. g. lafayetti*), which crossbred with red jungle fowl (*Gallus gallus*) (Cabarles et al., 2012).

Table 1: Summary distribution (%) of qualitative traits of Indigenous chicken from African and Asian countries

Research area (Localities)	Plumage colour	Comb type	Head shape	Earlobe colour	Shank colour	Eye colour	Skin colour	Sources
Central Botswana	Gold (23)	Single (90.4)	-	-	-	-	-	Badubi et al., 2006
Eritrea (Gash-Barka region)	Brownish (24.4)	Single (56)	Plain (73)	White, red (45.2)	White (27.7)	Red (37.6)	-	Habteslasie et al., 2019.
Ethiopia (southern zone of Tigray)	Red (24.17)	Rose (66.25)	Plain (57.92)	White, red (54.55)	Yellow (68.33)	-	White (77.9)	Nigussie et al., 2015.
North Ethiopia (Western Tigray)	Red (51.2)	Rose (53.3)	Crest (55.8)	White, red (70.1)	White (41.8)	Red (56.5)	White (99)	Markos et al., 2020.
Nigeria (Bauchi State)	Red (36.75)	Single (94.5)	-	White (43.75)	Yellow (25.5)	Orange (81.50)	-	Shuaibu et al., 2020.
Nigeria (Gwer-West, Benue State)	Brown (29.01)	Single (88.49)	Plain (92.42)	White (79.37)	White (41.16)	-	-	Rotimi et al., 2016.
Nigeria (Bekwarra)	Black (39.43)	Single (88.49)	-	-	-	Black (44.72)	White (75.85	Daikwo et al., 2018.
Sri Lanka (Ampara)	Lacing (44.9)	Single (60)	-	Red (65.3)	White (80)	Brown, black (40)	White (73.5)	Sornamuky et al., 2021.
Pakistan (Chhajjian,haripur)	Multi-colour (65.7)	Single (92.5)	-	Red (98.1)	Black (39.9)	-	White yellow (66.7)	Bibi et al., 2021.
Philippines (East, West Samar)	Lacing (68.3)	-	-	White, Red (56.1)	Yellow white (52.1)	-	-	Godinez et al., 2020.
Indonesia (West Java)	Black (51.9)	Single (100)	-	-	Black (62.5)	Red (40.6)	White (100)	Asmara et al., 2019.

2.3.2 Quantitative Traits

Several studies carried out on indigenous chickens under different management conditions indicated some variations in quantitative traits of indigenous chickens in different localities such as Bangladesh (Bhuiyan et al., 2005); Botswana (Badubi et al.,2006), Tanzania (Msoffe et al., 2001) and Zimbabwe (Mcainsh et al., 2004). Table 2 showed linear body measurements of quantitative traits such as shank length (cm), shank circumference (cm), wingspan (cm), chest circumference (cm), body length (cm), neck length (cm), comb length (cm), comb height (cm), wattle length (cm), wattle width (cm), beak length (cm), and body weight (g) for indigenous chickens in various localities. The mean body weight of indigenous chickens in North Thailand (1.82±1.7 kg) and Uganda (1.90±0.64 kg) are generally higher than body weights of indigenous chickens from other localities studied. According to Table 2, chickens from Northwest Ethiopia were generally lighter (1.05±0.8 kg) compared to other localities. The average body weight of indigenous chickens ranged from 1.50 to 2.27 kg for Tanzanian local chickens (Msoffe et al., 2001) and South African indigenous chicken (van Marle-Koster and Casey, 2001; Alabi et al., 2012) compared to lighter indigenous chickens reported in Northwest Ethiopia (0.64±0.4 -1.7±0.7 kg) (Halima et al., 2007) and Nigeria (1.05±0.01 – 1.32±0.02 kg) (Daikwo et al., 2011). The variations in body weight of indigenous chickens from different countries might be due to the differences in their genetic backgrounds, differences in age at maturity, differences in environment, and feeding practices. Both physical environment and management regimes usually affect quantitative traits (Buranawit et al., 2016).

Indigenous chickens are characterised as either light or medium-sized or heavy breeds which survive in natural habitats (Khan, 2008). These breeds are characterized by mosaic plumage colour patterns, slower growth rates, low egg production, and broodiness. They are found all over the place as scavenging flocks. Adult male weighs 2 to 3 kg in the medium-sized /heavy breed category, whereas in the light breeds, females weigh 0.9 to 1.5 kg (Khan, 2008). Different strains of indigenous Tswana

chickens generally exhibit variations in live weights; naked neck strain weigh 1.97±0.55 – 2.7±0.5 kg; normal strain 1.87±0.9 – 2.3±0.7 kg and dwarf strain 1.6±0.5 – 1.86±0.47 kg (Kgwatalala et al., 2012). Furthermore, males of the naked neck, normal and dwarf strains of indigenous Tswana chickens are generally heavier than their female counterparts from 14 weeks of age onwards (Kgwatalala et al., 2018).

Chest circumference is a very important trait in genetic studies of chickens because it is highly correlated with body weight and is used to predict body weight of chickens (Ige 2014; Faruque et al., 2010). The mean chest circumference measurements of indigenous chickens of Ethiopia (Assefa and Melesse, 2018) were lower those of indigenous chicken of Nigeria (Shuaibu et al., 2020), and North Thailand (Buranawit et al., 2016) respectively (Table 2).

The wingspan linear measurements of indigenous chickens were generally higher in Southwest Ethiopia (47.6±2.6), Northwest Algeria (44.45±0.2) and Bauchi State of Nigeria (43.19±1.11) and lower in indigenous chickens of Northwest Ethiopia (34.37±1.5). The observed variations in wingspan might be due to the differences in chicken genotypes, feed availability and other environmental factors (Assefa and Melesse, 2018). The wingspan of indigenous chickens from other countries has not been documented and therefore, information on wingspan of indigenous chickens from several African countries may not be available. Furthermore, Nigerian indigenous chickens had the highest body lengths (41.27±0.87 cm), followed by Southwest Ethiopian indigenous chickens (37.8±1.75 cm) and indigenous chickens of North Thailand and Central district of Botswana while Ugandan indigenous chicken had the lowest body lengths (18.20±0.26, 19.95±3.4 and 22.60±6.0 cm, respectively) (Buranawit et al., 2016; Badubi et al., 2006; Beyihayo et al., 2022).

The shank length of indigenous chickens is regarded as a good indicator of skeletal development of a bird, which is also related to the amount of meat a chicken can carry (Melesse et al., 2013).

Variations in shank length exists between indigenous chickens from different countries (Table 2). Indigenous chickens of Nigeria and Bangladesh had the longest shanks whereas indigenous chicken of Southwestern Ethiopia and Central district of Botswana had the shortest shanks (Assefa and Melesse, 2018; Badubi et al., 2006). Higher shank length for indigenous chickens reared in different countries or localities may be related to their body weight (Assefa and Melesse, 2018). Faruque et al. (2010) noted a strong positive correlation between shank length and body weight in intensively reared local chickens of Bangladesh. Higher phenotypic and genetic correlations between body weight and shank length were also reported in indigenous chickens of Ghana (Osei-Amponsah et al., 2013). Renema et al. (2007) also reported that the ratio of shank length to chest width in indigenous chickens reflects the degree of fleshing and that it is normally higher in heavier birds.

Several studies indicate that indigenous chickens from different countries had similar shank circumference except for indigenous chicken of North-western Ethiopia that had relatively thinner shanks (2.82±0.60cm) (Table 2). According to Tadele et al. (2018), relatively higher values of shank circumference in indigenous chickens may indicate suitability for meat production rather than for egg production.

Some quantitative traits such as neck length, comb length, comb height, wattle length and beak length remain undocumented in indigenous chickens across Africa and Asia. Similar neck lengths were reported between indigenous chickens of Southwestern Ethiopia (16.2.5±1.3cm) and Bangladesh (16.29±4.3cm) (Assefa and Melesse, 2018 and Azmal et al., 2006). Previous studies have shown that the comb lengths vary between indigenous chickens of Central Botswana (4.3±2.5cm), Southwestern Ethiopia (4.1±1.1cm), Bangladesh (3.68±0.9cm) and North-western Algeria (2.77±0.5cm) (Badubi et al., 2006; Assefa and Melesse, 2018; Azmal et al., 2006 and Dahloum et al., 2016). Similar comb height and wattle length were also found between indigenous chickens of Southwestern Ethiopia and Bangladesh and between native chickens of Southwestern Ethiopia and North-western Algeria (Table

2). According to Ige et al. (2012), larger combs and wattles are crucial morphological traits that allow better heat dissipation in tropical hot conditions. The comb and wattles normally have a huge role in sensible heat losses (Nigussie et al., 2015). Assefa and Melesse (2018) reported that the naked neck strain of indigenous chicken possesses higher comb and wattle dimensions than other strains of indigenous chickens, which explains their better tolerance to heat stress.

Table 2: Average body weight (kg) and linear body measurements (cm) variations of Indigenous chicken populations kept under different areas of study.

Traits/ localities	Central Botswana	Southwest Ethiopia	Bangladesh	Northwest Algeria	North Thailand	Uganda	Nigeria (Bauchi State)
Body weight (kg)	1.81±0.6	1.55±0.2	1.75±0.75	1.58±1.7	1.82±0.05	1.9±0.64	1.67±0.26
Chest (cm) circumference	-	28.1±1.8	-	-	32.95±0.37	-	31.49±1.04
Wingspan (cm)	-	47.6±2.6	-	44.45±0.2	-	-	43.19±1.11
Body length (cm)	19.95±3.4	37.8±1.75	28.91±3.4	29.65±0.08	18.20±0.26	22.60±6	41.27±0.87
Shank length (cm)	8.1±2.1	8.5±1.3	10.69±2.5	9.33±0.05	9.62±0.14	9.49±1.68	10.19±0.42
Shank (cm) circumference	-	4.25±0.5	4.93±1.12	4.23±0.25	-	4.32±0.88	-
Neck length (cm)	-	16.25±1.3	16.29±4.3	-	-	-	-
Comb length (cm)	4.36±2.5	4.1±1.1	3.68±0.9	2.77±0.5	-	-	-
Comb height (cm)	-	2.2±0.65	2.94±1.42	-	-	-	-
Wattle length (cm)	-	2.7±0.85	-	2.45±0.25	-	-	-
Wattle width (cm)	-	2.35±0.8	-	-	-	-	-
Beak length (cm)	2.85±0.4	-	3.14±0.58	2.41±0.14	-	-	-
References	Badubi et al., (2006)	Assefa and Melesse, 2018.	Azmal et al., 2006.	Dahloum et al., 2016	Buranawit et al., 2016	Beyihayo et al., 2022	Shuaibu et al., 2020.

2.4 Sexual Dimorphism in Quantitative Traits

A summary of live body weights (kg) and other linear body measurements (cm) of both male and female indigenous chickens studied at various places is shown in Table 3. Morphometric traits showed sexual dimorphism in favour of male indigenous chickens except for breast width, which was 6.7% higher in females than males (Dahloum et al., 2016). Getu et al. (2014) in North Gonder Zone of Ethiopia reported body weight for male and female chickens to be 1.63 kg and 1.37 kg, respectively. In another study, Kgwatalala et al. (2012) found live weights of indigenous Tswana chickens to be 2.0±0.8 kg for male and 1.6±0.4 for females. Live weight and linear body measurement traits of indigenous adult chickens in southwestern Ethiopia indicated average live weights of 2.1±0.02 kg for males and 1.4±0.0 g for females (Balcha et al., 2022), which are generally higher than live weights reported by Nhara et al. (2020) (males =1.6±0.8 kg; females =1.3±0.9 kg in indigenous chickens of Rushinga district, Zimbabwe). Azmal et al. 2006) in Bangladesh found that strains of indigenous chickens of Bangladesh had live weights of 2.0±0.9 kg for males and 1.5±0.5 kg) for females. Sexual dimorphism in live weights was also observed in the study by Tadele et al. (2018) who argued that the weight differences between male and female chickens could be due to their differences in growth rates because of the actions of the hormones.

A study by Balcha et al. (2022) found that male and female indigenous chickens in Southwest Ethiopia had chest circumferences of 29±0.18 cm and 24.8±0.03 cm, respectively. Similar observations were made by Azmal et al. (2006) and Nhara et al. (2020) in Bangladesh and Zimbabwe. According to Eskindir et al. (2013), chest circumference can be used as an accurate estimate of body weight as long as variations exist in genotype, feed availability and other environmental factors.

In general, indigenous chickens of Botswana appear to be smaller in size compared to other African countries. Yakubu et al. (2009) and Ajayi et al. (2012) observed that body length is the most crucial contributor to difference in body weight in normal-feathered Nigerian indigenous chickens.

Badubi et al. (2006) reported that the shank lengths for Indigenous Tswana chicken males and females were 8.9±3.3 cm and 7.3±0.9 cm, respectively. These findings are consistent with Balcha et al. (2022) who reported shank lengths of 8.2±0.08 cm for males and 7.2±0.01 cm for females in indigenous chickens of Southwest Ethiopia. However, the shank lengths of indigenous chickens of Zimbabwe (9.5±0.6 cm for males and 7.6±1.3 cm for females) and Bangladesh (11.6±2.5 cm for males and 9.8±2.4 cm for females) were higher than those of Botswana and Ethiopian indigenous chickens (Nhara et al., 2020; Azmal et al., 2006).

The average neck lengths of indigenous chickens in Rushinga district, Zimbabwe (18±1.3 cm for males, 12.4±1.3 cm for females) and Bangladesh (17.7±4.5 cm for males, 14.7±2.4 cm for females) were higher than those reported in Southwest Ethiopia (12.9±0.1 cm for males, 9.4±0.05 cm for females) (Table 3). According to Melesse and Negesse (2014), neck and shank lengths can also be related with birds' active walking potential to travel long distance in search of feed.

The comb lengths of indigenous chickens of Zimbabwe (3.8±1.7 cm for males, 1.6±1.9 cm for females) are lower than those of their counterparts in Botswana (5.6±2.0 cm for males, 3.12±0.9 cm for females), Ethiopia (5.4±0.2 cm for males, 2.4±0.02 cm for females) and Bangladesh (6.99±1.8 cm for males, 3.72±1.3 cm for females). Sexual dimorphism was also common with comb and wattle lengths because these traits are highly associated with sexual selection (+93.1 and +58.7% in males and females respectively) (Dahloum et al., 2016). According to (Nesheim et al. (1979), the size of combs and wattles are related with gonad development and secretion of sex hormones.

Table 3: Summary least squares means and standard errors of live body weight (kg) and other linear body measurements (cm) of both male and female indigenous chickens studied at various places in different countries.

Traits/ localities	Central Botswana		Southwest Ethiopia		Rushinga District, Zimbabwe		Bangladesh	
	Male	Female	Male	Female	Male	Female	Male	Female
Body weight (kg)	2.02±0.8	1.6±0.4	2.1±0.02	1.4±0.0	1.6±.0.8	1.3±0.9	2.0±0.9	1.5±0.5
Chest (cm) circumference	-	-	29±0.18	24.8±0.03	29±1.2	22±1.7	29.1±4.7	25.8±4.1
Wingspan (cm)	-	-	42.2±0.2	38.4±0.01	49±0.5	45±0.1	-	-
Body length (cm)	20.7±4.1	19.2±2.7	42±0.19	37.7±0.06	45±0.4	31±1.7	19.9±3.5	17.9±3.1
Shank length (cm)	8.9±3.3	7.3±0.9	8.2±0.08	7.2±0.01	9.5±0.6	7.6±1.3	11.6±2.5	9.8±2.4
Shank (cm) circumference	-	-	4.7±0.03	3.5±0.03	-	-	5.21±1.2	4.6±1.04
Neck length (cm)	-	-	12.9±0.1	9.4±0.05	18±1.3	12.4±1.8	17.7±4.5	14.7±2.4
Comb length (cm)	5.6±2	3.12±0.9	5.4±0.2	2.4±0.02	3.8±1.7	1.6±1.9	6.99±1.8	3.72±1.3
Comb height (cm)	-	-	4.5±0.2	1.6±0.02	3.0±0.9	2.1±0.8	3.89±1.7	1.99±1.1
Wattle length (cm)	-	-	5.1±0.2	2.1±0.02	3.2±0.4	1.8±0.2	-	-
Wattle width (cm)	-	-	4.7±0.14	2.2±0.02	-	-	-	-
Beak length (cm)	3.0±0.4	2.7±0.4	2.3±0.05	2.2±0.01	-	-	3.32±0.6	2.95±1.8
References	Badubi et al., 2006		Balcha et al., 2022		Nhara et al., 2020		Azmal et al., 2006	

2.5 Genetic Characterisation

Indigenous chickens are exposed to selection for improvement in production traits and lack of planned mating exposes them to genetic dilution and loss of genetic variation which may lead to eventual extinction (Shrestha, 2005; Scherf et al., 2006). Several factors such as mutation, migration, genetic drift, natural selection, artificial selection, and random genetic drift influence genetic differences between populations (Hofmeyr et al., 1998). Man has forced the gathering of genetic variations between and within breeds and populations by selecting for favourable traits when breeding domesticated animals. Reliable information on genetic variations between individuals, populations and breeds is crucial in setting up effective conservation and utilisation programmes (Weigend and Romanov, 2001; Msofe et al., 2004). Evaluation of genetic diversity within and among populations is a crucial step for making decisions on genetic conservation and utilisation strategy. This genetic diversity is evaluated using common techniques such as phenotypic characters and molecular markers (Weigend and Romanov, 2001; Msofe et al., 2004).

2.6 Genetic Diversity of Indigenous Chickens

The first genome sequence draft with its analysis was published in 2004 for single female red jungle fowl (Eriksson et al., 2008). The chicken genome has a diploid chromosome number with 10 pairs of macro chromosomes, one pair of the sex chromosomes and 28 micro chromosomes, totalling 39 pairs. The size of the genome is estimated to be 1.2×10^9 base pairs and is approximately a third of most mammalian genomes (Groenen et al., 2000). The macro chromosomes have a higher purine content, gene density and recombination rate compared to the micro chromosomes (International Chicken Genome Sequencing Consortium (ICGSC), 2004).

2.7 Parameters of Genetic Diversity

Some genomic studies on indigenous chickens based on a limited number of microsatellite loci in the last decade reported genetic variations between different ecotypes (Shahbazi et al., 2007; Dehghanzadeh et al., 2009; Mohammadabadi et al., 2010). Limited studies have been conducted to understand the genetic variability of various indigenous chickens at the whole genome sequence level (Sohrabi et al., 2018; Kharrati-Koopaee et al., 2019). The details about population structure and genetic diversity among indigenous chicken ecotypes is crucial for genetic improvement, understanding of environmental adaptation, as well as for conservation and sustainable management and utilisation programmes (Psifidi et al., 2016). In the Iranian study, it was revealed that indigenous chickens compared to commercial lines harbour higher levels of genetic diversity, which could be because of inherent traditional breeding practices of natural and random mating of indigenous chickens (Mpenda et al., 2019) and minimal selection pressure on production traits. The authors found that the estimated observed heterozygosity (H_o) values ranged between 0.181 and 0.211 in indigenous Iranian chicken ecotypes and between 0.168 and 0.192 in the two commercial lines. The observed heterozygosity values of Iranian chicken ecotypes were similar to those of indigenous chicken ecotypes o Thailand and Jordan (Maw et al., 2015; Mekchay et al., 2014) but lower than the observed heterozygosity values of Indian indigenous chickens (Berima et al., 2013; Mwambene et al., 2019; Jayashankar et al., 2015).

Zhang et al. (2020) carried out a genome-wide population genetic analysis of the commercial, indigenous, game, and wild chickens' populations using 60K SNP microarray and found the highest genetic diversity or genetic variation in Indigenous chicken populations of China and the Red Jungle fowl (Wild chickens) compared to game chickens and commercial chicken lines. In a related study, Setiato et al. (2017) found no significant difference in levels of genetic diversity between indigenous chickens of China and the Red

Jungle fowl possibly because of loss of genetic diversity in the wild chicken population due to their declining numbers or population bottlenecks from the random genetic drift because of excessive egg harvesting and hunting. In the same study, the lowest genetic diversity was observed in layer chickens compared to broiler chickens, indicating substantial loss of genetic variation resulting from intensive selection and standardized production (Setiato et al. 2017). This clearly indicated higher genetic variability in indigenous chickens compared to commercial breeds probably due to natural and random mating procedures and less intensity of selection in production traits. Intensive selection for meat production has resulted in loss of diversity and increased uniformity in commercial broiler lines.

Strillacci et al. (2018) assessed genetic diversity within the indigenous chicken populations of Mexico by sampling chickens from 25 States using the 600K SNP chip and found moderately high values H_o (0,319) and H_e (0.348) and low Fis value (0,084), indicating substantial genetic variation in indigenous chickens and low levels of inbreeding. Previous study by Strillacci (2017) reported that H_o varied between 0.21 and 0.34 while the H_e varied between 0.17 and 0.32 in Mexican native chickens. Similarly, Johansson and Nelson (2015) reported Fis values of -0.09 and 0.17 in two Mexican chicken populations, clearly indicating low levels of inbreeding and higher genetic variation in Indigenous chicken populations. Strillacci et al. (2018) also showed that most of the genetic variation occurred within the population compared to between populations. Mtileni et al. (2011) studied the genetic diversity of conservation (small and closed population) and field (large and open population) flocks of the Venda and Ovambo indigenous chickens of South Africa using the haplotype diversity of the D-loop region of the chicken mitochondrial genome and found lower haplotype diversity in the Venda and Ovambo conservation flocks than in their respective field populations. Lower genetic diversity in the conservation flocks than in the village populations indicates that conservation flocks represent a limited sample of the

village gene pool while a further reduction in diversity occur because of inbreeding and random loss of some alleles resulting from drift in small populations. Khanyile et al. (2015) studied the genetic diversity of Southern African village chickens and conservation flocks from South Africa using the 60 K SNP chip and found that indigenous chickens from Malawi, Zimbabwe, and South Africa had the same H_o value of 0.62 and H_e values of 0.68, 0.66 and 0.65, respectively, indicating higher levels of genetic diversity and similar diversity levels. The clustering patterns of the indigenous chickens of Southern Africa followed a geographical gradient in which the South African chickens were minimally related to the Malawian chickens and more closely related to Zimbabwean indigenous chickens (Khanyile et al., 2015). Higher levels of variation between indigenous chickens from Malawi and South Africa could be interpreted by geographical distance from each other, lack of gene flow among the two countries, and independent evolutionary forces between the two populations. Indigenous chickens of South Africa and Zimbabwe had higher variation within population as shown by their boundless clusters than the conservation flocks of South Africa. The overall linkage disequilibrium (LD) values among populations indicated significant variations among populations with higher LD obtained for the conservation flocks and low LD in the Indigenous chicken populations kept by smallholder farmers (Khanyile et al., 2015). The authors explained that the differences in LD between conservation flocks and field chicken populations could be due to the differences in population histories and the effects of various evolutionary forces such as selection, genetic drift, and mutations. Evolutionary forces (selection. migration, random genetic drift) are responsible for the differences in genetic diversity between and within populations.

2.8 Heat Shock Protein 70 (HSP-70) Gene

The heat shock protein (HSP) family comprises of greatly conserved stress protein referred as Heat Shock Protein 70 (HSP-70), which is expressed in response to heat stress (Banergee et al., 2014; Manjari et al., 2015). HSP-70 gene plays an important part in environmental stress adaptation and adjustment in various livestock species (Banergee et al., 2014). Heat stroke factors (HSFs) are produced in response to heat exposure that activates a considerable number of genes related to heat tolerance in chickens (Yunis and Cahaner, 1999; Gaviol et al., 2008). The chicken HSP-70 gene has been known as the candidate gene for heat stress and several studies have proven that this gene is associated with adaptation to thermal stress (Najafi et al., 2018). The chicken HSP-70 is responsible for controlling the refolding of proteins properly, in such a way that the cells are protected from damage influenced by heat stress (Tkáčová, and Angelovičová, 2012).

In chickens, *Gallus gallus,* the HSP-70 gene is found in the 5th chromosome and comprises of promoter, 5`UTR, one exon with a coding region of 1905 bp, and the 3` UTR (Morimoto et al., 1986) as shown in Figure 1. According to GenBank: AY143693.1, the entire chicken HSP-70 gene comprises of 2,594 bp and encodes a protein of 653 amino acids (Junprung et al., 2019). The promoter comprises of 210 bp, the 5´UTR comprises 112 bp while 3´UTR comprises 309 bp (Aryani et al., 2019). The HSP-70 gene promoter region exhibited some recognisable elements which are responsible for controlling heat shock-induced transcription, as well as, providing the area of interactivity with positive triggering transcription factors which influence expression during heat shock (Morimoto et al., 1986). In addition to coding regions, the untranslated regions play a crucial role in the regulation of gene expression since they play a significant role in terms of stability and expression levels. Nonetheless, the 5' UTR may influence the

expression level of the transcript, while the 3' UTR is understood to be involved in mRNA stability (Basiricó et al., 2011; Sodhi et al., 2013).

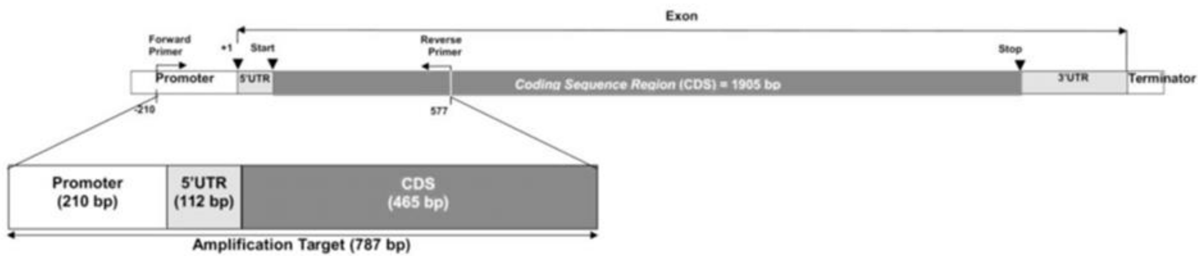

Figure 1:Schematic presentation of HSP70 gene in chicken showing promoter region and 5`UTR. Adapted from Aryani et al. (2019).

The control of translation initiation is mainly under the responsibility of 5′UTRs and gene expression can therefore be influenced by SNPs in the regulatory regions (Araujo et al. 2012; Haimov et al. 2015). According to Najafi et al. (2018), a total of 35 SNPs has been found in chicken HSP-70 with 25 SNPs occurring in the protein-coding region and the rest in the regulatory regions (Promoter, 5`UTR, and 3`UTR). Most of the research work on chicken HSP-70 gene polymorphism concentrated on the exon or protein coding region because of its direct effect on the amino acids sequence of the resulting protein (alleles of a gene) (Gan et al. 2015; Najafi et al. 2018) while only a few studies investigated polymorphisms in regulatory regions of the chicken HSP-70 gene. Polymorphisms in the regulatory region of the gene are, however, very important because they influence gene expression (Öner et al., 2017). According to Silver and Noble (2011), pre-transcriptional activation of the HSP-70 gene has been thoroughly reviewed while its downstream regulation by 5´UTR and 3´UTR has received less attention.

2.8.1 Expression and Features of Heat Shock Regulatory Elements

HSP-70 gene expression is basically controlled at the transcription level by the binding of a transcriptional activator, the heat shock factor protein (HSF) to a highly preserved DNA sequence referred to as the heat shock elements (HSE) (Schiaffonati et al., 1994). Any rise in temperature in mammalian cells result in the conversion of HSF from an inactive form that cannot bind to DNA to an active form that can bind the HSE (Schiaffonati et al., 1994). The HSP-70 family of stress proteins has important functions for cellular life because of their amplitude to act as molecular chaperones in the synthesis, translocation, folding and assembly of proteins (Flynn et al., 1989). An escalated amount of HSP-70 in cells undergoing stress seems to be required to safeguard these metabolic pathways and decrease cellular damage. HSP-70 has been indicated to revive unfolded and aggregated proteins (Flynn et al., 1989) and stimulate the degradation of proteins denatured beyond repair (Flynn et al., 1989).

Maak et al. (2003) stated that there is an individual difference in heat shock responses in relation to DNA polymorphisms in the HSP-70 gene in birds. Genetic adjustment led to differences in gene products and their expression levels. These genetic developments could be one factor contributing to phenotypic variations between different species of animals (De La Rosa et al., 1998). Various research works have shown that mutations in gene sequences modify gene expression, morphology, and physiology (Deeb and Cahaner, 2001; Iwamoto et al., 2008). For example, modifications in the AT content of the promoter influences the expression of HSP-70, which plays an important role in regulatory evolution (Chen et al., 2011). This indicates that the changes in the HSP-70 gene sequence could contribute to the evolution of this gene.

2.8.2 Promoter Region

Aryani et al. (2019) reported that the promoter region of the chicken HSP-70 gene exhibited consensus-motif sequences which comprised of a TATA box, Heat Shock Elements 1 and 2 (HSE 1 and HSE 2), Specificity Protein 1 (SP 1), and CAAT box. The essential elementary recognition element in the promoter region of the chicken HSP-70 gene is a TATA box that comprised of the sequence 5′-TATAAA-3′ (Aryani et al., 2019). An additional important element in the promoter region of chicken HSP-70 gene was Heat Shock Elements (HSE), composed of HSE 1 with a sequence of 5′-CTGGCAGGTTCCAG-3′, and the HSE 2 with a sequence of 5′-CCTTAGCGTTCTGGC-3′ (Aryani et al., 2019). It has been noted that these two HSEs overlapped at certain positions. Another recognition elements established in the promoter region of chicken HSP70 gene was SP 1 (a type of GC box), with the sequence 5′-GGGCGG-3′. Moreover, there were two CAAT box elements with complementary nucleotide sequences of 5’-ATTG-3’ (Aryani et al., 2019).

The regulation of the transcription process through the binding of different transcription factors which distinguishes specific DNA motifs such as TATA box, HSE and SP1 is regulated by the promoter region of chicken HSP-70 gene which is upstream (5`-flanking) of protein coding sequence (Mazzi et al., 2003). In eukaryotic cells, the TATA box (Goldberg-Hogness box) is normally based around 30 nucleotides upstream of the initiation of transcription inside the promoter region and is responsible for guiding RNA polymerase II during transcription (Mazzi et al., 2003). A specific DNA sequence (HSE) plays a major role in activating HSP-70 gene transcription (Pelham, 1982). The HSEs serve as the binding sites for heat shock [transcription] factors (HSFs) (Pelham, 1982). Archana et al. (2017) stated that the HSP-70 gene is recognised as an excellent biological marker for heat stress in animals.

The introduction of HSP-70 gene expression appears when stress activates and stimulates transcription factors, such as HSF, to attach to HSE. The attachment of HSF to HSE permits the transcription of the HSP-70 gene by RNA polymerase II (Akerfelt et al., 2010). Vertebrates have four transcription factors for the heat shock gene: namely HSF1, HSF2, HSF3, and HSF4. However, Aves are reported to have three HSFs, with HSF1 and HSF2 being homologous to transcription factors counterparts in mammals, whereas HSF3 is a definitive transcription factor to Aves (Fujimoto and Nakai, 2010). In chickens, the expression of the HSF3 transcription factor is slightly balanced throughout the development of different tissues. Consequently, HSF1 and HSF3 are probably associated with heat stress response.

2.8.3 5` Untranslated Regions (5ÙTR)

A 5′UTR is upstream of the protein-coding sequence in many proteins and plays a critical role in regulating gene expression (Araujo et al., 2012; Leppek et al., 2018). Aryani et al. (2019) reported that the alignment and sequencing of the 5`UTR of HSP-70 gene in Indonesian chickens revealed two deletions and one transition. The deletions of two A bases were noticed in different positions and additionally, one transition A to G was determined at location +44. These nucleotide variations, observed at positions +32, +44, and +97 in the 5′UTR resulted in two haplotypes in Indonesian chicken populations. The first haplotype was found in KUB (n= 48), Walik (n= 3), and Kate Walik chickens (n= 3), while the second haplotype was only observed in KUB (n= 2) and Walik chickens (n= 3).

The HSP-70 gene does not normally have introns in most of the organisms; it is absolutely preserved, and in most of the avian species, the protein-coding regions have similar lengths i.e., 1,905 bp (Morimoto et al., 1986; Mazzi et al.,2003; Xia et al., 2013; Zhang et al., 2015). The entire coding-region sequences of HSP70 gene are found at NCBI for several avian species, including chicken (*G. gallus*, with accession numbers of J02579, AY143691, AY143692, and AY143693), guinea fowl (*Numida meleagris*), with

accession number of AB096696), Japanese quail (*Coturnix japonica*, with accession number of AB259847), duck (*Anas platyrhynchos*, with accession number of EU678246), and goose (*Anser cygnoides*, with accession number EU680475). The coding region of avian HSP-70 gene usually starts with the standard start codon of ATG, and ends with the stop codon of TAA, and codes for 633 amino acids (Aryani et al., 2019).

2.8.4 Polymorphism in HSP-70 Gene

Association studies have indicated that genetic polymorphisms of this gene have an exceptional relationship with heat stress in chickens (Mazzi et al., 2002) and eventually make some changes to mRNA affluence of this gene (Zhen et al., 2006). Additionally, it was observed that the modifications in AT content in the promoter region of the HSP-70 gene can have an important effect on the expression level of the HSP70 gene, as well as the regulatory evolution of this gene (Chen et al., 2011). In a related study, Chen et al. (2016) reported that genetic variation in the non-coding region (C69A>G SNP) resulted in the development of a Myeloid zinc finger 1 (MZF1) binding site, which controls transcriptional regulation of numerous genes and enhance the expression level of HSP-70 gene resulting in an increase in heat stress tolerance in chickens.

Chen et al. (2007) and Graze et al. (2009) reported several mutations in the HSP-70 gene sequence in *Drosophila melanogaster*, which change the gene expression and eventually modify its morphology and physiology that could relate to other organisms including birds. Nevertheless, SNPs in this gene may add to the binding of the peptide substrate to HSP-70 or the stimulation of HSP-70 (Favatier et al., 1997). The average SNP density in the chicken genome was revealed to be 5/1000 (Wong et al., 2004), in which these SNPs are found in both coding and non-coding regions of the gene (Figure 2), and a few of them

change the amino acid sequence while the rest are associated with splicing and ultimately controlling gene expression (Kimchi-Sarfaty et al., 2007). Research to date has indicated 35 single-nucleotide polymorphisms (SNPs) and two deletions in HSP70 gene (Najafi et al., 2018). Out of the 25 SNPs in the coding region, 17, 7 and 1 SNPs were found in the ATP enzyme active region, in the polypeptide combining region, and in C-terminal region, respectively (Najafi et al., 2018).

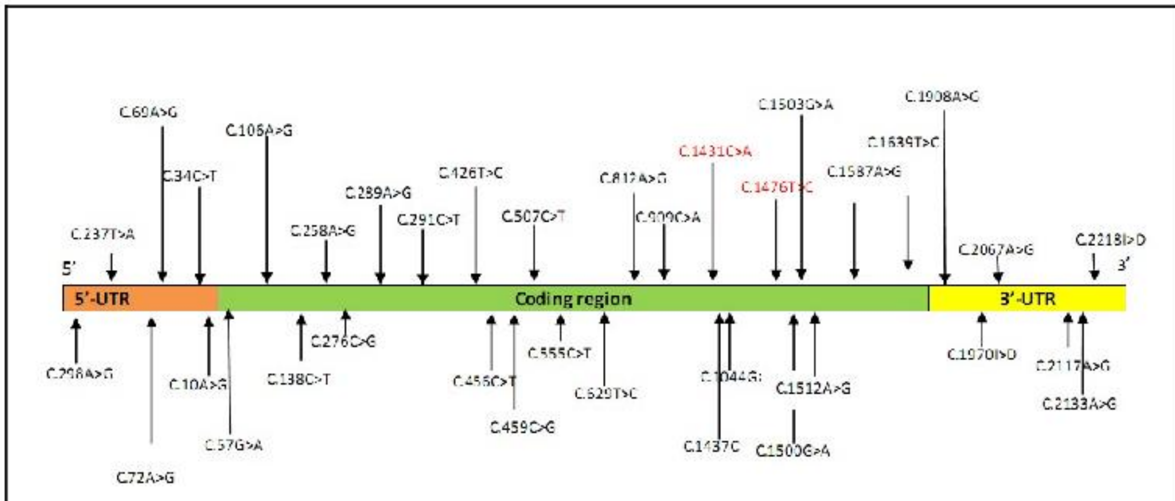

Figure 2 : Location of various SNPs within HSP-70 gene in chickens as identified by Najafi et al., (2018).

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CHAPTER 3

3.0 QUALITATIVE TRAITS OF VARIOUS STRAINS OF INDIGENOUS TSWANA CHICKENS IN KWENENG AND SOUTHERN DISTRICTS OF BOTSWANA.

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ABSTRACT

The aim of this study was to identify and describe qualitative traits of indigenous Tswana chicken populations in Kweneng and Southern districts of Botswana. The qualitative traits involved in the study included tail colour, breast colour, back colour, neck colour, comb type, shank colour, earlobe colour and head shape. Data were subjected to frequency and cross tabulation procedures of descriptive statistics in Statistical Package for Social Sciences (SPSS) to compute frequencies of occurrence of each qualitative trait. The five strains of indigenous Tswana chickens under scavenging management system showed distinct physical variations for most of the qualitative traits. Black was the most predominant tail colour across the strains (51.6%) followed by brown (27.9%). The frequency of brown breast colour and brown back colour were significantly higher in those respective regions. Brown and black were the predominant neck colours across the strains. The single comb type (81.7%), featherless shank (65.4%), red ear lobes (67.6%) and grey shank colour (32.9%) were the most predominant phenotypes across the strains. Plain and crested head shapes occurred at similar frequencies of 56.4 and 43.6%, respectively, in Tswana chickens in Southern part of Botswana.

Keywords: Botswana, Morphological characterization, Phenotypic variation, Qualitative traits, Tswana chickens.

3.1 INTRODUCTION

In Africa, rural households have kept indigenous chickens for many years on free running or scavenging management (Ndidde et al., 2014). Indigenous chickens of Botswana are known as Tswana chickens and are the most widely spread domestic animal, which almost every rural family owns. Indigenous Tswana chicken contributes enormously to supply of meat and eggs to the rural communities of Botswana (Badubi et al., 2006). According to Badubi et al., (2006) on average, households keep flocks of between 5 and 30 chickens of mixed ages and sex with very few households keeping over 50 chickens.

Indigenous chickens have different morphological identities, carrying genes which have adaptive values to their environment and local diseases (Aklilu et al., 2013). Local chicken populations are often described and grouped according to geographical location or phenotypic characteristics, while their classification into breeds or types is limited (Manyelo et al., 2020). They also exhibit great variation in performance in various qualitative and quantitative traits of economic importance (Faruque et al., 2010). Indigenous Tswana chicken exhibits numerous observable attributes including plumage, shank and earlobe colour, comb type, head shape and other qualitative traits. The possible existence of several genetically distinct subpopulations within a large population has called for the need to identify and define the subpopulations to determine genes which might be in danger of becoming extinct and therefore need conservation (Guni and Katule, 2013).

However, genetic resources identification and phenotypic characterization of different strains of Tswana chickens have not been done. Therefore, the objective of this study was to identify and describe the phenotypic variations (qualitative traits) of Tswana chicken populations in Southern and Kweneng districts of Botswana.

3.2 MATERIALS AND METHODS

3.2.1 Location of Study Area

The study was carried out in Kweneng and Southern districts of Botswana from January to June 2014. Six remote villages were selected from each district (Figure 3) and within a district; villages were selected such that there was uniformity in the chicken production system. Large villages and villages near towns were avoided due to their high populations of exotic chicken breeds and to minimize the influence of urban-affiliated farming systems on typical rural village-based traditional free running system (Desta et al., 2013). A total of 98 households within each district comprising of six villages each, rearing only indigenous Tswana chicken participated in the study. Households with exotic chickens or with a history of keeping exotic chicken breeds and those near such households did not participate in the study to ascertain the genetic purity of indigenous Tswana chicken participating in the study. This, however, limited the number of households and the total number of chickens of various strains that participated in the study.

3.2.2 Data Collection

A total of 618 indigenous Tswana chickens, comprising 246 normal feathered (54 males and 192 females), 123 naked neck (18 males and 105 females), 129 dwarf (45 males and 84 females), 57 rumples (27 males and 30 females) and 63 frizzled (18 males and 45 females) chickens, kept under traditional free running management system were used in the study (Figures 2 to 6). There were generally more females than males of various strains of Tswana chicken per household because of the inherent breeding system, hence more females than males participated in the study. Some households selected against naked neck, dwarf, rumples and frizzled chickens, which results in low frequency of such strains in the general Tswana chicken population, hence their lower sample size compared to the normal-feathered

strain. Rumples and frizzled strains did not exist at all in some selected villages. The chickens used were approximately six months of age or older as per the information provided by the owners. Qualitative morphological traits such as plumage colour, shank colour, comb type, earlobe colour, spur colour and head shape were obtained by visual observation following FAO recommended descriptors for chicken genetic resources (FAO, 2011).

3.2.3 Statistical analysis.

The qualitative variables were analysed using descriptive statistics and compared as percentages using the Statistical Package for Social Science (SPSS, 2013; version 22.0). T-test analysis was carried out to find out the differences in frequency distributions among different phenotypic classes with respect to each qualitative trait using SAS (2012).

Figure 3: Map of Botswana showing Southern and Kweneng districts in blue and orange colours

3.3 RESULTS AND DISCUSSION

Phenotypic diversity: The morphology of Tswana chickens indicated five clear phenotypic groups: Normal (Figure 4), Naked neck (Figure 5), Frizzled (Figure 7), Dwarf (Figure 6) and Rumpless (Figure 8). Normal strain does not have any special feature, but it is characterized by different plumage colours occurring because of separation of alleles from random mating between birds of variable colour patterns (Liyanage et al., 2015) (Figure 4). The Naked neck strain is easily identifiable among other strains of Tswana chickens because of absence of feathers in their neck region. The Dwarf strain is easily

distinguishable from other strains by their short legs. The Dwarf strain is also known as creeper fowl in some areas because the shorter shank length contributes to the shorter legs (Banerjee, 2012). Machete et al. (2017) reported shank length of 8.35 and 5.60 cm for female Normal and Dwarf strains of Tswana chicken, respectively. Frizzled strain of Tswana chicken is characterized by curled feathers throughout the body caused by feather related gene mutation (Liyanage et al., 2015). The Rumpless strain of Tswana chicken is characterized by the absence of tail feathers in both males and females. Of all the five strains of Tswana chicken the Normal strain was by far the most popular (39.81%), followed by Dwarf and Naked Neck at frequencies of 20.87 and 19.90%, respectively, in the study. Frizzled and Rumpless strains of Tswana chicken were the least popular at frequencies of 10.19 and 9.22%, respectively, in the Southern part of Botswana.

Figure 5: Normal strain

Figure 4: Naked neck strain

Figure 6: Dwarf strain

Figure 7: Frizzled strain

Figure 8: Rumpless strain

The variations in plumage colour in different regions of Tswana chicken body are shown in Table 4. The qualitative traits involved in the study included tail colour, breast colour, back colour and neck colour. Black was the most predominant tail colour across the strains (51.6%) followed by brown (27.9%), grey (15.7%), white (2.8%) and lastly khaki (2.0%). There were significant differences in the frequencies of black, brown and grey tail colours, while the white and khaki tail colours occurred at similar and significantly lower frequencies than black, brown and grey tail colours. Brown and black were the most

predominant breast colours across the strains at frequencies of 54.4 and 36.3%, respectively. The frequency of brown breast colour was significantly higher than that of black breast colour. White, grey and khaki breast colours occurred at similar and significantly lower frequencies than brown and black breast colours. Brown and black were by far the most predominant back colours across the strains at frequencies of 56.7 and 29.6%, respectively. The frequency of brown back colour was significantly higher than that of black back colour. White, grey and khaki back colours occurred at similar and significantly lower frequencies than brown and black back colours. Brown and black were the predominant neck colours across the strains and there were no significant differences in their frequencies (49.0 vs 36.7%). Plumage diversity, including the main phenotypes, was higher in both studied districts. Eskindir et al. (2013) stated that the plumage colour diversity is maintained as indications of random mating and many genes controlling the trait with respect to plumage colour. In the general population of Tswana chicken in the Southern part of Botswana, white and grey neck colour occurred at similar and significantly lower frequencies than brown and black neck colour.

Table 4: Frequency (%) of plumage colour variations of Tswana chickens in the Southern part of Botswana

	Strain					
Trait	**Normal**	**Naked Neck**	**Frizzled**	**Rumpless**	**Dwarf**	**Overall mean**
Tail colour						
Black	60.5	46.7	63.6	40	47.4	51.6[a]
Brown	23.3	26.7	18.2	40	31.6	27.9[b]
Grey	11.6	26.7	9.1	10	21.1	15.7[c]
White	4.7	0	9.1	0	0	2.8[d]
Khaki	0	0	0	10	0	2.0[d]
Breast colour						
Brown	44.2	53.3	36.4	80	57.9	54.4[a]
Black	41.9	33.3	54.5	20	31.6	36.3[b]
Grey	7.0	13.3	0	0	5.3	5.1[c]
White	4.7	0	9.1	0	0	2.8[c]
Khaki	2.3	0	0	0	5.3	1.5[c]
Back colour						
Brown	46.5	60.0	54.5	70	52.6	56.7[a]
Black	37.2	33.3	36.4	20	21.1	29.6[b]
White	4.7	0	9.1	10	15.8	7.9[c]
Grey	9.3	6.7	0	0	5.3	4.3[c]
Khaki	2.3	0	0	0	5.3	1.5[c]
Neck colour						
Brown	22	9	5	6	7	49.0[a]
Black	15	4.7	5	3	9	36.7[a]
White	4	1	1.2	1	2	9.2[b]
Grey	3.1	1	0	0	1	5.1[b]

a,b,c,d Means with different superscripts within trait differed significantly (P<0.05).

The single comb type was by far the most frequent comb type across the strains (81.7%) and occurred at significant higher frequency than walnut (12.9%), pea (2.9%) and rose (2.5%) comb type (Table 5). Walnut, pea and rose comb types occurred at similar frequencies in Tswana chicken in the Southern part of Botswana. The predominance of single comb type found in the current study agrees with that observed by Moreda et al. (2014) in Ethiopian indigenous chickens, and Liyanage et al. (2015) in indigenous chickens of Sri Lanka. Banerjee (2012) also reported the predominance of single comb type in indigenous chickens of India. The single comb type also dominated in several indigenous chicken populations from different countries (Cabarles et al., 2012; Egahi et al., 2010; El-Safty, 2012; Apuno et al., 2011). According to Duguma (2006), the presence of single comb helps to reduce body heat by 40% and it is therefore advantageous in tropical conditions. The single comb type might therefore play a crucial thermoregulatory role under Botswana`s hot and dry climatic conditions.

Plain and crested head shapes occurred at similar frequencies of 56.4 and 43.6%, respectively, in Tswana chickens in the Southern part of Botswana. Head shape is one the vital morphological features that can be used to separate variations between breeds or strains of indigenous chickens. All the strains of Tswana chickens had a higher frequency of plain head shape compared to crested head shape. Our results are consistent with those of Moreda et al. (2014) who observed 72.8% plain head shape and 27.2% crested head shape in indigenous chickens of South and Southwest parts of Ethiopia. Addis et al. (2013) also reported similar results in indigenous chickens of North Gondor zone of Ethiopia.

Red ear lobes were by far the most frequent (67.6%) and were significantly higher than red-black, red-yellow and red-white ear lobes, which occurred at similar frequencies of 11.8, 11.1 and 9.6%, respectively (figure 7). Variations in ear lobe colour in Tswana chickens is consistent with Faruque et al. (2010) and Moreda et al. (2014), who found similar results in indigenous chickens of Bangladesh and Ethiopia, respectively. The predominance of red earlobes in Tswana chickens is consistent with Moreda

et al. (2014) and Liyanage et al. (2015), who reported similar results in indigenous chickens of Ethiopia and Sri Lanka, respectively. To the contrary, Cabarles et al. (2012) reported predominance of red, white (57.41%) earlobes over red earlobes (37.53%) in indigenous chickens of Philippines. Duguma (2006) reported the predominance of white earlobes (67%) over red (18.6%) and red, white (17.9%) earlobes in Ethiopian indigenous chickens. According to Cabarles et al. (2012) the differences in earlobe colour are the results of adaptability to local conditions.

Table 5: Frequency (%) of qualitative morphological traits of the head region of Tswana chicken in the southern part of Botswana

			Strain			
Trait	**Normal**	**Naked Neck**	**Frizzled**	**Rumpless**	**Dwarf**	**Overall mean**
Comb types						
Single	74.4	86.7	72.7	80	94.7	81.7[a]
Walnut	23.3	13.3	18.2	10	0	12.9[b]
Pea	0	0	9.1	0	5.3	2.9[b]
Rose	2.3	0	0	10	0	2.5[b]
Head shape						
Plain	65.1	60.0	54.5	50	52.6	56.4[a]
Crest	34.9	40.0	45.5	50	47.4	43.6[a]
Earlobes colour						
Red	78	66.7	54.6	75	63.5	67.6[a]
Black	2.3	16.4	27.3	0	12.8	11.8[b]
Yellow	9.3	3.40	9.1	15	18.4	11.1[b]
White	10.4	13.40	9.10	10	5.3	9.6[b]

[a,b] Means with different superscripts within trait differed significantly ($P<0.05$).

The featherless shank occurred at significantly higher frequency (65.4%) than the feathered shank, which occurred at a frequency of 34.6%. Badubi et al. (2006) also reported a high frequency (77.8%) of featherless shanks compared to feathered shanks (22.2%) in indigenous Tswana chickens. The current results are also in agreement with those of Moreda et al. (2014), who reported a higher frequency (98.48%) of featherless shanks relative to feathered shanks (1.56%) in indigenous chickens of Ethiopia. A significantly higher percentage of Tswana chickens had spur on their shanks (94.7%) as compared to 5.3% that did not have spur on their shanks.

Grey was the most predominant shank colour (32.9%) followed by blue (24.3%), khaki (24.26%), yellow (11.8%) and lastly green (6.7%) (Table 6**Error! Reference source not found.**). A significant difference occurred only between the frequency of grey and green shank colours. Variations in shank colour, including yellow (32.48%), white (33.73%), brown (11.4%) and black (7.75%), were also reported by Moreda et al. (2014) in Ethiopian indigenous chickens. Contrary to our findings, Guni and Katule (2013) and Moreda et al. (2014) observed predominantly yellow and white shanks in indigenous chickens of Tanzania and Ethiopia, respectively. Variations in shank colour are due to variations in the production of carotenoid, dermal melanin and epidermal melanin controlled by W+ and W; Id and id+; and E and e+ genes, respectively (Petrus, 2011). Some studies have indicated that combinations of pigment controlling genes responsible of colour determination seemed to influence the occurrence of different types of shank colour (Guni and Katule, 2013) in indigenous chickens.

Table 6: Frequency (%) of qualitative morphological traits of the leg region of Tswana chicken in the Southern part of Botswana

.			Strain			
Trait	**Normal**	**Naked Neck**	**Frizzled**	**Rumpless**	**Dwarf**	**Overall mean**
Feathers on shank						
Feathered	32.6	26.7	36.4	30	47.4	34.6[a]
Featherless	67.4	73.3	63.6	70	52.6	65.4[b]
Spur						
Present	90.7	93.3	100	100	89.5	94.7[a]
Absent	9.3	6.7	0	0	10.5	5.3[b]
Shank colour						
Grey	2.3	46.7	27.3	20	68.4	32.9[a]
Blue	55.8	13.3	27.3	20	5.3	24.3[ab]
Khaki	34.9	13.3	27.3	30	15.8	24.26[ab]
Yellow	7.0	13.3	18.2	10	10.5	11.8[ab]
Green	0	13.3	0	20	0	6.7[b]

a,b Means with different superscripts within trait differed significantly (P<0.05).

3.4 CONCLUSION

Indigenous Tswana chickens are multi-coloured but brown colour was predominant in colour in breast, back and neck regions whereas black colour was most predominant in the tail region. The single comb type and red ear lobes were by far the most frequent qualitative traits across the strains. However, in some strains such as Normal, Naked neck and Dwarf, the absence of spurs on the shank were observed particularly in females and young individuals. There is generally considerable diversity in various qualitative traits in different strains of Tswana chickens. High responses to selection can therefore be expected owing to the variations in various qualitative traits in different strains of Tswana chickens. Further research is required to genetically characterize the different strains of Tswana chickens for conservation purposes and better management approaches.

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3.6 CONNECTING STATEMENT I

Chapter III described various qualitative traits in different strains of indigenous Tswana chicken under their natural production environment. Complete Phenotypic characterization should include both qualitative and quantitative traits. The next chapter therefore describes various quantitative traits in different strains of indigenous Tswana chicken.

CHAPTER 4

4.0 PHENOTYPIC CHARACTERISATION OF TSWANA CHICKENS BASED ON QUANTITATIVE TRAITS IN KWENENG AND SOUTHERN DISTRICTS, BOTSWANA.

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ABSTRACT

The objective of this study was to characterize both sexes of the naked neck, dwarf, rumpless, normal-feathered and frizzled strains of Tswana chickens in the Kweneng and Southern districts of Botswana using quantitative traits. A total of eight (8) quantitative morphological traits such as shank length, shank circumference, spur length, comb length, wattle length, wing length and wingspan were measured using flexible measuring tape, and live body weight was measured using a Spring-Dial Hoist weighing scale.

Data were analyzed using mixed model's procedures of SAS and the model included fixed effects of strain and sex and their interaction. Normal-feathered males had significantly higher shank length (9.94±0.23 versus 8.35±0.20), shank circumference (0.99±0.02 versus 0.84±0.02) wing length (20.61±0.51 versus 18.60±0.48), wingspan (41.22±1.03 versus 37.19±0.96), comb length (6.30±0.30 versus 3.48±0.26) and wattle length (3.44±0.16 versus 2.40±0.14) than their female counterparts. Among males, there were no significant strain differences in spur length, wing length, wingspan, comb length, wattle length and live weight. Normal-feathered males had the highest live weight and rumpless males had the lowest live weight.

Normal-feathered, naked- neck, frizzled and rumpless females had similar shank length and shank circumference which were all significantly higher than those of their dwarf counterparts. Only naked neck and normal-feathered females had significantly higher wingspan and wing length than dwarf females. Finally, it was noted from this study that various strains of Tswana chickens had the same quantitative traits except for shank length and shank circumference which were significantly

shorter/smaller in dwarf strain compared to the other four strains. This research work will also play an important role by provision of current information on quantitative traits of Tswana chicken strains.

Key words: *morphological measurements, quantitative traits, Tswana chickens*

4.1 INTRODUCTION

In Botswana, indigenous chickens are referred to as Tswana chickens. They are classified into five strains: dwarf, rumpless, frizzled, naked neck and normal with the most common strain being the normal (Badubi et al 2006). The terms backyard, local, traditional, village, scavenging or family chickens are used synonymously to refer to indigenous chickens. Tswana chickens are usually produced under the extensive farming systems where the birds mostly scavenge for feeds, picking food items such as food scraps and insects around the households with little or no supplementation and uncontrolled breeding (Aganga et al 2000; Moreki, 2000; Badubi et al 2006). Indigenous chickens are subjected to challenging selection pressure due to the unsuitable management conditions under which they are reared and represent an important reservoir of genetic variation that is supposed to be conserved (Guèye, 1998). Indigenous chickens are underestimated because of their poor performance under traditional free running system (Getu et al 2014). Most village farmers, however, prefer family chickens as they can survive better in local environment with available limited feed resources (Cabarles, 2013). These types of birds need minimal management to produce eggs and meat basically for household consumption and local markets (Magpantay et al 2006).

Indigenous chickens can be recognized as gene reservoir, especially for those genes that have adaptive values in the tropical conditions Aklilu et al (2013). In Botswana, family chickens are the most widespread animals where almost every rural family owns chickens, which contribute greatly to the supply of eggs and meat (Badubi et al 2006). It is believed that in remote areas, genetic originality may still be found (Daikwo et al 2011). The purpose of this study was therefore to evaluate various quantitative traits in the naked neck, normal, frizzled, rumples and dwarf strains of Tswana chicken in selected villages of Kweneng and Southern districts of Botswana.

MATERIALS AND METHODS

4.1.1 Study site

The study was carried out in Kweneng and Southern districts of Botswana. The capital village of southern district (Kanye) and is located at -24.9667 [latitude in decimal degrees], 25.3327 [longitude in decimal degrees] at an average elevation of 1406 meters above sea level. The sub-capital village of Southern district (Goodhope) lies at a latitude of -25.4852400 and longitude of 25.4475400 and has an elevation of 1259 meters above sea level. The capital village of Kweneng district (Molepolole) is located at -24.4066 [latitude in decimal degrees], 25.4951 [longitude] in decimal degrees at an average elevation of 1146 meters above sea level.

Six remote villages were selected from each district and within a district; villages were selected such that there was uniformity in the chicken production system. Large villages near towns and large villages were avoided due to their high populations of exotic chicken breeds and to minimize the influence of urban-affiliated farming systems on typical rural village-based traditional free running system (Desta et al 2013). A total of 89 households within each district comprising of six villages each, rearing only indigenous Tswana chicken participated in the study. Households with exotic chickens or with a history of keeping exotic chicken breeds and those near such households did not participate in the study to ascertain the genetic purity of indigenous Tswana chicken participating in the study. This, however, limited the number of households that participated in the study and the total number of chickens of various strains that participated in the study.

4.1.2 Data Collection

A total of 618 indigenous Tswana chicken comprising 246 normal-feathered (54 males and 192 females), 123 naked neck (18 males and 105 females), 129 dwarf (45 males and 84 females), 57 rumpless (27 males and 30 females) and 63 frizzled (18 males and 45 females) chicken, kept under traditional free running management system were used in the study (Figure 2-Figure 6). There were generally more females than males of various strains of Tswana chicken per household because of the inherent breeding system hence more females than males participated in the study. Some households selected against naked neck, dwarf, rumpless and frizzled chickens which results in the low frequency of such strains in the general Tswana chicken population hence their lower sample size compared to the normal-feathered strain. Rumpless and frizzled strains did not exist at all in some selected villages. The chickens used were approximately six months of age or older as per information provided by the owners. Quantitative morphologic traits such as shank length, shank circumference, spur length, comb length, wattle length, wing length, wingspan and body weight were measured in males and females of various strains of Tswana chicken in accordance with FAO (2011). A Spring-Dial Hoist weighing scale was used to measure live body weight (kg), whereas shank length, spur length, comb length, wattle length, wing length and wingspan were measured using a flexible Tailor's tape. Shank circumference was measured using a vernier caliper (Starrett tools model, Range: 30 cm, USA).

4.1.3 Statistical Analysis

Quantitative data for the various traits measured were analyzed using PROC MIXED procedures of SAS 9.2 2008 (SAS Institute, year of publication). The model included fixed effects of sex (male or female), strain (naked neck, normal, dwarf, rumples and frizzled), the interaction between strain and sex, and the random effect of household. Means were separated using pairwise t-tests with Scheffe's adjustment to account for unequal sampling units per strain and were determined to be significantly different at $P<0.05$.

The model being:

$Y_{ijk} = \mu + S_i + X_j + (S_i * X_j) + e_{ijk}$

Where

Y_{ijk} = mean body weight,

μ = overall mean,

S_i = Effect of the ith strain (Normal, naked neck, frizzled, rumpless and dwarf),

X_j = Effect of the jth sex (male and female),

$(S_i * X_j)$ = interaction between strain and sex

e_{ijk} = random effect of household.

4.2 RESULTS AND DISCUSSION

There were no significant differences between males and females of naked neck, frizzled, rumpless and dwarf strains of Tswana chickens in shank length, shank circumference, wingspan, wing length and wattle length under extensive management system (Table 7). Normal males however, had significantly higher values for shank length, shank circumference, wingspan, wing length, comb length and wattle length than their female counterparts. The shank length, wingspan and wattle length values found for the various strains of Tswana chickens in this study are in line with the shank lengths, wingspan and wattle length reported for indigenous chickens of Eastern Amahara, regions of Ethiopia reported by Getu et al (2014). The wingspan values in both males and females of various strains of Tswana chickens are lower than wingspan values reported for both males and females of indigenous chickens of South-Western Ethiopia reported by Bekele et al (2015). The Shank length of both males and females of various strains of Tswana chickens are however longer than those of indigenous male and female Nigerian chickens reported by Daikwo et al (2011). The values for shank length in both males and females of Tswana naked neck, normal and dwarf strains found in the current study are lower than the values reported by Kgwatalala et al (2012) in the three strains at 20 weeks of age under intensive management system. Similar shank length between males and females of the dwarf strain and significantly higher shank length in normal males than females is consistent with Kgwatalala et al (2012). Similar shank length between naked neck males and females is however contrary to Kgwatalala et al (2012) who found significantly higher shank length in Tswana naked neck males than females (2705.78 ± 91.42 versus 1976.55 ±100.14 mm) at 20 weeks of age.

Generally, males of the normal, naked neck, frizzled, rumpless and dwarf strains of Tswana chickens had higher values of shank length, shank circumference, wingspan, wing length and wattle length than their

female counterparts. Higher values in shank length, wingspan, wing length and wattle length in males than females of various strains of Tswana chickens is consistent with Aklilu et al (2013); Bekele et al (2015) and Getu et al (2014) in indigenous Ethiopian chickens. Higher wing length in naked neck males than females found in this study is consistent with Alabi et al (2012) who reported significantly higher wing length in South-African naked neck males than females (21.50 ±0.210 versus 17.20 ±0.18 cm). Higher wing length, shank circumference and shank length in males than females of the normal, frizzled, rumpless and dwarf strains of Tswana chickens is consistent with Alabi et al (2012) who reported a similar pattern in three indigenous chicken breeds of South Africa.

Dwarf males had significantly higher comb length than their female counterparts. There were no significant sex differences in spur length and live weight in all the five strains of Tswana chickens, but generally males of all the five strains had higher spur length and live weight values than their female counterparts. Generally, the body weights of different strains of Tswana chickens found in the current study are higher than those reported for other indigenous chickens in South Africa (Alabi et al 2012), in Nigeria (Yakubu and Salako 2009) and in Ethiopia (Bekele et al 2015). Higher live weight values in males than females in all the five strains of Tswana chickens is consistent with Kgwatalala et al (2012) who found a similar pattern in the naked neck, normal, and dwarf strains of Tswana chickens at 20 weeks of age under intensive management system. Njenga (2005) also reported higher mature live weights in naked neck, normal and dwarf males than females in Kenyan indigenous chickens. Higher body weight and linear body measurements in males than females of all the five strains of Tswana chickens confirm the well documented sexual dimorphism in poultry species. According to Baeza et al (2001) sexual Dimorphism in body weight and linear body measurements is attributable to differences in hormonal profiles between the sexes which ultimately lead to differential growth rates.

Table 7: Quantitative traits (cm) of males and females of various Tswana chickens under extensive management systems in the Southern part of Botswana

	Normal		Naked neck		Frizzled		Rumpless		Dwarf	
Traits	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Shank length	9.94^{a} ± 0.23	8.35^{b} ±0.20	10.67^{a} ± 0.92	8.33^{a} ± 0.26	9.62^{a} ± 0.65	8.51^{a} ± 0.32	9.46^{a} ± 0.46	7.84^{a} ± 0.36	6.30^{a} ± 0.42	5.60^{a} ± 0.25
Shank circumference	0.99^{a} ± 0.02	0.84^{b} ± 0.02	1.07^{a} ± 0.09	0.83^{a} ± 0.03	0.96^{a} ± 0.07	0.85^{a} ± 0.03	0.95^{a} ± 0.05	0.78^{a} ± 0.04	0.63^{a} ± 0.04	0.56^{a} ± 0.02
Spur length	1.19 ± 0.17	0.35 ± 0.74	0.54± 0.74	0.20 ± 0.20	1.77 ± 0.52	0.19 ± 0.25	1.66 ± 0.37	0.19 ± 0.28	0.85 ± 0.33	0.21 ± 0.18
Wingspan	41.22^{a} ± 1.03	37.19^{b} ± 0.96	44.66^{a} ± 3.14	37.38^{a} ± 1.10	36.29^{a} ± 2.30	35.35^{a} ± 1.28	38.35^{a} ± 1.71	36.54^{a} ± 1.39	35.42^{a} ± 1.57	32.45^{a} ± 1.09
wing length	20.61^{a} ± 0.51	18.60^{b} ± 0.48	22.33^{a} ± 1.57	18.69^{a} ± 0.55	18.14^{a} ± 1.15	17.68^{a}± 0.64	19.18^{a} ± 0.85	18.27^{a} ± 0.70	17.71^{a} ± 0.79	16.23^{a} ± 0.55
Comb length	6.30^{a} ± 0.30	3.48^{b} ± 0.26	5.52^{a} ± 1.30	3.95^{a} ± 0.35	8.04^{a} ± 0.92	3.86^{a} ± 0.44	5.53^{a} ± 0.65	4.21^{a} ± 0.49	6.95^{a} ± 0.58	3.30^{b} ± 0.33
Wattle length	3.44^{a} ± 0.16	2.40^{b} ± 0.14	3.78^{a} ± 0.70	2.66^{a}± 0.19	3.58^{a} ± 0.49	2.41^{a} ± 0.24	2.71^{a} ± 0.35	2.39^{a} ± 0.27	3.37^{a} ± 0.31	2.17^{a} ± 0.18
Live Weight (kg)	2.56 ± 0.11	2.02 ± 0.10	2.50 ± 0.43	1.91 ± 0.13	2.28 ± 0.31	1.78 ± 0.16	1.93 ± 0.22	1.93 ± 0.17	2.30 ± 0.20	1.75 ± 0.12

^{ab}*Means with different superscripts within strain for a particular trait were significantly different (P < 0.05)*

There were no significant differences (P>0.05) in shank length and shank circumference among male normal, naked neck, frizzled and rumpless strains of Tswana chickens (Table 8). However, males of the four strains had significantly higher shank length and shank circumference than dwarf males. Similar shank length between the naked neck, frizzled and normal strains found in this study is consistent with Fayeye et al (2006) who reported shank length of 9.7 cm, 10.2cm and 9.7 cm in the three strains, respectively, in Nigerian indigenous chicken. Similar shank length between Tswana naked neck and normal males and significantly higher shank length in the naked neck and normal males than dwarf males found in this study are consistent with Kgwatalala et al (2012) under intensive management system. Among males of the five strains of Tswana chickens the naked neck had the highest shank length and shank circumference followed by normal, frizzled, rumpless and lastly dwarfs strain. The highest shank length in naked neck males compared to the other four strains is consistent with Getu et al (2014) who found the highest shank length in naked neck males compared to Gasgie and gugut types of Ethiopian indigenous chicken. Higher shank length and shank circumference in naked neck than frizzled males found in the current study is consistent with Ige et al (2012) in Nigerian frizzled feather and naked neck chickens. There were no significant differences in spur length, wingspan, wing length, comb length, wattle length and live weight among males of the five strains of Tswana chickens (Table 8). Similar wing length among frizzled, naked neck and normal males found in this study is consistent with Adekoya et al (2013) who reported wing lengths of 17.027 ±4.089, 15.791 ±2.074 and 16.036 ±2.214cm in the three strains, respectively, of Nigerian indigenous chickens. Fayeye et al (2006) also reported similar wing length between naked neck (17.5 cm), frizzled feather (17.8 cm) and normal (17.7 cm) strains of Nigerian indigenous chicken.

Among males of the five strains of indigenous Tswana chickens, the naked neck had the highest wattle length and the rumpless had the lowest. The longest wattle length in naked neck males compared to the other strains found in the current study is consistent with Faruque et al (2010) in

Bangladeshi chicken and Getu et al (2014) in Ethiopian chicken. Among males of the five strains of Tswana chickens, normal had the highest live weight, followed by naked neck, frizzled, dwarf and lastly rumpless. Adekoya et al (2013) also found the highest body weight of 2.079 ±0.575 kg in the normal strain followed by naked neck with 0.905 ±0.259 kg and lastly frizzled feather chicken with live weight of 0.904 ±0.327 kg. Ige et al (2012) also reported higher live weight in naked neck than frizzled feather chicken (1.69 ±0.27 versus 1.44 ± 0.34 kg). Similar live weight between naked neck and normal strains of Tswana chicken is however, contrary to Kgwatalala et al (2012) who found significantly higher live weight in naked neck males than normal males (2705.78 ±91.42 versus 22.70.19 ± 69.10 g) of indigenous Tswana chicken at 20 weeks of age under intensive management system. The discrepancy could be due to the differences in the production environment between intensive and extensive management systems, particularly in the feeding. Unfavorable production environment might have prevented the growth potential of naked neck chickens under extensive management system. Higher body weight in normal males than dwarf males of Tswana chicken is however consistent with Kgwatalala et al (2012) who reported body weight of 2270.19 ± 69.10 and 1969.47±95.48 g in the two strains, respectively, at 20 weeks of age under intensive management system.

Table 8: Quantitative traits (cm) of males of normal, naked neck, frizzled, rumples and dwarf strains of Tswana chickens under extensive management system.

Traits	Normal	Naked neck	Frizzled	Rumpless	Dwarf
Shank length	$9.94^{a} \pm 0.23$	$10.67^{a} \pm 0.92$	$9.62^{a} \pm 0.65$	$9.46^{a} \pm 0.46$	$6.30^{b} \pm 0.42$
Shank circumference	$0.99^{a} \pm 0.02$	$1.07^{a} \pm 0.09$	$0.96^{a} \pm 0.07$	$0.95^{a} \pm 0.05$	$0.63^{b} \pm 0.04$
Spur length	1.19 ± 0.17	0.54 ± 0.74	1.77 ± 0.52	1.66 ± 0.37	0.85 ± 0.33
Wingspan	41.22 ± 1.03	44.66 ± 3.14	36.29 ± 2.30	38.35 ± 1.71	35.42 ± 1.57
wing length	20.61 ± 0.51	22.33 ± 1.57	18.14 ± 1.15	19.18 ± 0.85	17.71 ± 0.79
Comb length	6.30 ± 0.30	5.52 ± 1.30	8.04 ± 0.92	5.53 ± 0.65	6.95 ± 0.58
Wattle length	3.44 ± 0.16	3.78 ± 0.70	3.58 ± 0.49	2.71 ± 0.35	3.37 ± 0.31
Live Weight (kg)	2.56± 0.11	2.50 ± 0.43	2.28± 0.31	1.93 ± 0.22	2.30± 0.20

[ab] *Means with different superscripts within a row differ significantly (P < 0.05)*

There were no significant differences in shank length and shank circumference among females of normal, naked neck, frizzled and rumple strains (Table 9). The four strains however had significantly higher shank length and shank circumference than the dwarf strain. Similar shank length between normal, naked neck and frizzled strains of indigenous Tswana chickens found in this study is consistent with Liyanage et al (2015) who found similar shank length of 127, 129 and 127mm, respectively, in the three strains of Sri Lankan indigenous chicken. Significantly higher shank length in normal than dwarf Tswana chicken is consistent with Yeasmin and Howlinder (1998) who also reported significantly higher shank length in normal than dwarf Deshi hens of Bangladesh (7.7 ± 0.15 versus 5.5 ± 0.17 cm). Kgwatalala et al (2012) also reported similar shank lengths between female naked neck and normal Tswana chickens (11.26 ± 0.33 and 10.84 ± 0.18 cm, respectively) and significantly higher shank length in the two strains than female dwarf Tswana chickens at 20 weeks of age under intensive management system. Similar shank circumference between female normal, naked neck and frizzled Tswana chickens found in the current study is consistent with Liyanage et al

(2015) who reported shank circumference of 65, 66, and 67 mm in the three strains, respectively, in Sri Lankan village chickens.

There were no significant differences (P>0.05) in wingspan and wing length among females of normal, naked neck, frizzled and rumples strains, and among frizzled, rumples and dwarf strains of indigenous Tswana chickens. However, females of the normal and naked neck strains had significantly higher wingspan and wing length than females of dwarf strain. Similar wing length between female normal, frizzled and naked neck Tswana chicken is consistent with Liyanage et al (2015) who found wing length of 148, 131 and 149 cm in three strains of Sri Lankan village chickens, respectively. Among the five strains of Tswana chickens (figures 9-13) naked neck females had the highest wingspan and wing length followed by normal, rumples, frizzled and lastly dwarfs females. There were no significant differences among females of the five strains of Tswana chickens in spur length, comb length, wattle length and live weight. Similar live weight between female normal, naked neck and dwarf strains of Tswana chickens found in this study is consistent with Kgwatalala et al (2012) who reported non- significant differences in body weight in the three strains at 20 weeks of age under intensive management system. Liyanage et al (2015) also reported non-significant difference in body weight between normal, naked neck and frizzled female Sri Lankan village chicken. Dakpogan et al (2012) reported similar body weights between naked neck and frizzled Benin hens (994.4 ± 42.9 and 1065.1 ± 42 g, respectively) which were significantly higher than those of normal (839.4 ± 42.7 g) and dwarf hens (651.4 ± 43 g) at sexual maturity under intensive management system. Similarly, Isidahomen et al (2012) found significantly higher slaughter weight in naked neck (2084.00 ± 108.43 g) than frizzled (1974.10 ± 94.16 g) and normal hens (1693.00 ± 71.34 g) of Nigerian indigenous chickens under intensive management system. Females of the normal strain of indigenous Tswana chicken had the highest body weight followed by rumpless, naked neck, frizzled and lastly the dwarf strain.

Compared to the normal, naked neck, and frizzled strains of Tswana chickens, dwarf males and females had the lowest shank length, shank circumference, wingspan, wing length and live weight and this could be because of dwarfism gene that has been reported to result in up to 30 and 40 % reduction in body weight in females and males, respectively, compared to normal strain (FAO, 2010). From the current study it is clearly demonstrated that dwarfism gene not only affects body weight but body dimensions as well. The highest body weight in the normal strain (both males and females) compared with the naked neck and frizzled strains found in this study is contrary to the general belief that reduced feathering genes (naked neck and frizzled genes) lead to improved body weight and dimensions due to their improved heat tolerance which consequently positively affects appetite (Islam and Nishibori, 2009). The favourable effects of naked–neck and frizzled genes on growth performance might only be realized under the favourable production environment experienced under intensive management system and not under free range management system where balanced diets and feed availability are limited.

Table 9: Quantitative traits (cm) of females of normal. naked neck, frizzled, rumples and dwarf strains of Tswana chicken under extensive management system.

Traits	Normal	Naked neck	Frizzled	Rumpless	Dwarf
Shank length	$8.35^{a} \pm 0.20$	$8.33^{a} \pm 0.26$	$8.51^{a} \pm 0.32$	$7.84^{a} \pm 0.36$	$5.60^{b} \pm 0.25$
Shank circumference	$0.84^{a} \pm 0.02$	$0.83^{a} \pm 0.03$	$0.85^{a} \pm 0.03$	$0.78^{a} \pm 0.04$	$0.56^{b} \pm 0.02$
Spur length	0.35 ± 0.74	0.20 ± 0.20	0.19 ± 0.25	0.19 ± 0.28	0.21 ± 0.18
Wingspan	$37.19^{a} \pm 0.96$	$37.38^{a} \pm 1.10$	$35.35^{ab} \pm 1.28$	$36.54^{ab} \pm 1.39$	$32.45^{b} \pm 1.09$
wing length	$18.60^{a} \pm 0.48$	$18.69^{a} \pm 0.55$	$17.68^{ab} \pm 0.64$	$18.27^{ab} \pm 0.70$	$16.23^{b} \pm 0.55$
Comb length	3.48 ± 0.26	3.95 ± 0.35	3.86 ± 0.44	4.21 ± 0.49	3.30 ± 0.33
Wattle length	2.40 ± 0.14	2.66 ± 0.19	2.41 ± 0.24	2.39± 0.27	2.17 ± 0.18
Live Weight(kg)	2.02 ± 0.10	1.91 ± 0.13	1.78± 0.16	1.93 ± 0.17	1.75± 0.12

^{abc} *Means with different superscript within a row differed significantly ($P < 0.05$)*

Figure 9: A typical dominating spotted blck colour (naked neck hen) at Malwelwe village

Figure 10: A typical brown chicken with minor white (normal strain hen) at Sojwe village

Figure 12: A black chicken (frizzled hen) at Salajwe village

Figure 11: A typical brown mixed colour (Rumpless hen) at Maboane village

Figure 13: A brown mixed white colour (Dwarf hen) at Sojwe village

4.3 CONCLUSION

This paper contributes to the documentation of quantitative traits of Tswana chicken strains found in the Southern part of Botswana. The study indicated that body weights and dimensions of various morphological quantitative traits were generally higher in males than females in all the five strains of Tswana chicken. Different strains of Tswana chickens had similar quantitative traits except for shank length and shank circumference which were significantly smaller in the dwarf strain compared to the other four strains.

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4.5 CONNECTING STATEMENT II

Chapter IV described various quantitative traits in different strains of indigenous Tswana chicken under their natural production environment. Complete characterization of indigenous Tswana chicken should include both phenotypic and genetic characterization. The next chapter therefore seeks to establish genetic diversity parameters and genetic relationships between different strains of indigenous Tswana chicken using SNPs array technology.

CHAPTER 5

5.0 GENETIC DIVERSITY AND POPULATION STRUCTURE OF THREE STRAINS OF INDIGENOUS TSWANA CHICKENS AND COMMERCIAL BROILER USING SINGLE NUCLEOTIDE POLYMORMOPHIC (SNP) MARKERS

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ABSTRACT

The Tswana chicken is native to Botswana and comprises strains such as the naked neck, normal, dwarf, frizzled and rumples. The origins of the different strains of Tswana chicken remain unknown and it is not yet clear if the different strains represent distinct breeds within the large Tswana chicken population. Genetic characterization of different strains of Tswana chickens using SNP arrays can elucidate their genetic relationships and ascertain if the strains represent distinct breeds of Tswana chicken population. The aim of this study was therefore, to investigate population structure and diversity and to estimate genetic distances/identity between the naked neck, normal and dwarf strains of Tswana chickens. A total of 96 chickens [normal strain (n=39), naked neck strain (n=32), dwarf strain (n=13) and commercial broiler (n=12)] were used in the study. SNP genotyping was carried out using the Illumina chicken iselect SNP 60Bead chip using the Infinium assay compatible with the Illumina HiScan SQ genotyping platform. The observed heterozygosity (H_o) values were 0.610±0.012, 0.611±0.014, 0.613±0.0006 for normal, naked neck and dwarf strains of Tswana chickens respectively and averaged 0.611±0.016 across the three strains of Tswana chickens compared to Ho of 0.347±0.023 in commercial broiler chicken. The expected heterozygosity (H_e) values were 0.613±0.00012, 0.614±0.00013, 0.608±0.00021 for normal, naked neck and dwarf strains of Tswana chickens respectively and averaged 0.612±0.00015 across the three strains of Tswana chickens compared to H_e of 0.577±0.00022 in commercial broiler chicken. Principal component analysis (PCA) was used to get an insight into the population structure of indigenous Tswana chickens. The first two principal components revealed a set of three clusters. The normal strain of Tswana chicken and commercial broiler clustered together in one group. The dwarf strain clustered separately in one group and the naked neck and normal strains clustered together in the last group. The separate clustering of the dwarf strain from the rest of Tswana chicken strains suggests significant genetic uniqueness of the dwarf strain and very close genetic similarities between the normal and naked neck strains. The clustering pattern was confirmed by less genetic differentiation and less

genetic distances between the naked neck and normal strains of Tswana chicken than between the two strains and the dwarf strain of Tswana chicken.

Keywords: Genetic distances, Genetic diversity, Indigenous Tswana chickens, Population structure, SNPs.

5.1 INTRODUCTION

Chickens have more distinct use and benefits to household in different developing countries (Padhi, 2016). Indigenous Tswana chickens are one of the most important livestock species which provide most of the protein in the form eggs and meat and improve the rural economy of subsistence farmers through sales of eggs as well as live birds. The chicken products (meat and eggs) are preferred by many people in rural areas due to their taste, leanness, palatability, and appropriateness for exceptional dishes (Crawford., 1992; Igbal et al., 2009 and Juturasitha et al., 2008). Indigenous Tswana chickens contribute to food security in the rural areas and also generate emergency cash income for women since indigenous Tswana chickens are mostly owned by women. The Tswana chickens play a significant role in the sociocultural life of the rural population. Indigenous chickens also have roles in traditional ceremonies and other customs as gift payments (Mtileni et al., 2009). Nonetheless, growth rate of indigenous Tswana chickens is relatively low as compared to the commercial broiler due to poor nutritional support, poor housing, poor health care and lack of selection for growth potential under the scavenging management system (Kgwatalala et al., 2012).

Generally, indigenous chickens are kept in small flocks (2 to 20 chickens) of varied ages under traditional scavenging management system with basic supplementary feeding, housing, and healthcare (Kgwatalala et al., 2012). They possess important positive characteristics such as hardiness, the ability to tolerate the harsh environmental condition and poor husbandry practices (climate, handling, watering, and feeding) without much loss in production (Dessie et al., 2011). Indigenous chickens grow slowly and normally require up to 12 months to reach slaughter age (Riise et al., 2005) and age at first lay is approximately 7 months (Sonaiya and Swan., 2004). Desta (2015) reported a mating ratio 1 cock to 2 hens for indigenous chickens' population in Ethiopia; but the recommended mating ratio is 1 cock to 5-10 hens (Sonaiya and Swan., 2004).

Indigenous Tswana chicken population comprises several strains/ecotypes such as normal, naked neck, dwarf, frizzled and rumples strains (Moreki., 1997) and Badubi et al., 2006). The dwarf frizzled and rumpless strains are found at a relatively low frequency within the indigenous Tswana chicken population and the normal strain is by far the most common strain (Machete et al., 2021). Machete et al., (2017) evaluated quantitative traits in naked neck, normal, dwarf, rumples and frizzled strains of Tswana chickens under traditional free running management system and found similar quantitative traits except for shank length and shank circumference in the dwarf strain compared to the other four strains. Kgwatalala et al., (2012) reported similar body weights between naked neck and normal strains of Tswana chicken and significantly lower body weights in the dwarf strain from 4 to 14 weeks of age under an intensive management system. Similarly, Kgwatalala et al., (2013) reported similar preslaughter live weight, carcass weight, dressing percentage and primal cuts weights between naked neck and normal strain of Tswana chickens and significantly lower pre-slaughter live weight, carcass weight, dressing percentage and primal cuts weights in the dwarf strain. It is generally accepted that performance in various traits of economic importance is function of both the genotype and the environment. Do the similarities in performance between the naked neck and normal strains of Tswana chickens outlined above reflect the underlying genetic similarities? Is the deviation in performance of the dwarf strain compared to both the naked neck and normal strains of Tswana chicken a reflection of its genetic distinctness? To date, no genetic characterization studies have been carried out on different strains of Tswana chickens and it is still unclear if the strains represent distinct breeds. The objectives of the study were therefore to investigate population structure and diversity and to estimate genetic distances/identity between the naked neck, normal and dwarf strains of Tswana chickens.

5.2 MATERIALS AND METHODS

5.2.1 Study Population

A total of 96 mixed indigenous Tswana chickens were used in the study. The indigenous chickens from the Southern part of Botswana were represented by normal strain (n=39), naked neck strain (n=32), dwarf strain (n=13) of Tswana chickens kept under traditional free running management system and commercial broiler (n=12). The chickens used were approximately six months of age or older as per the information provided by the owners. Information on sampling locations and number of samples per sampling location is available from Table 10.

Table 10: Locations where indigenous Tswana chickens were sampled and number of samples per location in Kweneng and Southern districts of Botswana.

Sampling location	District	No. of samples per location		
		Normal	**Naked neck**	**Dwarf**
Sojwe	Kweneng	8	4	7
Kaudwane	Kweneng	2	4	0
Maboane	Kweneng	4	3	2
Malwelwe	Kweneng	4	4	2
Kweneng	Kweneng	3	0	1
Keng	Southern	5	3	0
Seherelela	Southern	4	4	1
Thankane	Southern	2	3	0
Lerolwane	Southern	3	4	0
Magotshwane	Southern	4	3	0
TOTAL		39	32	13

5.2.2 Collection of Blood Samples

Blood samples were collected from the medial metatarsal vein located on the leg of a chicken better suited for puncture using a 23-gauge, 1-in needle. The alternative site for blood collection was the brachial vein on the wings and for puncture, feathers in this area were plucked for smooth insertion of needle on the veins of interest. All blood in vacutainer tubes containing EDTA and kept under cool environment of ice packs until they reach the laboratory where blood samples were kept at -20°C until DNA extraction.

5.2.3 DNA Extraction

24 µl of NucleoMag® B-Beads and 360 µl MB2 Buffer were then added to the square- well Block and mixed by pipetting up and down, shaking for 5 minutes at room temperature. Magnetic beads were then separated against the wells by placing the square-well block on the NucleoMag SEP magnetic separator for at least 2 minutes. The supernatant was then removed from the wells and discarded by pipetting. The square-well block was then removed from the NucleoMag SEP magnetic separator and 600µl of MB3 buffer was added to each of the wells, accompanied by shaking to completely resuspend the beads. Magnetic beads were again separated against the wells by placing the square-well block on the NucleoMag SEP magnetic separator for at least 2 minutes. The supernatant was again removed and discarded by pipetting.

The square-well block was removed again from NucleoMag SEP magnetic separator. 600µl of MB4 buffer was then added to each of the wells and the beads were resuspended by shaking for 5 minutes. Magnetic beads were again separated by placing the square-well block on the NucleoMag SEP magnetic separator for at least 2 minutes and supernatant was removed and discarded by pipetting. 900 µl of MB5 buffer was then added to each of the wells while the beads were still attracted to magnets. After an incubation period of 50 seconds, the supernatant was aspirated and discarded. The square -well block was then removed from the NucleoMag SEP magnetic separator. 50 µl of DNA elution buffer was then added to each of the wells and shaking for 10 minutes at 56^0C to resuspend the beads. Magnetic beads were again separated by placing the square-well block on the NucleoMag SEP magnetic separator for at least 2 minutes. The supernatant containing purified genomic DNA was then transferred to the elution plate for SNP genotyping.

5.2.4 SNP Genotyping and Data Preparation

SNP genotyping was carried out at Agricultural Research Council- Biotechnology Platform in Pretoria according to the protocols described by (Khanyile et al., 2015). Briefly, SNP genotyping was carried out using the Illumina chicken iselect SNP 60Bead chip using the Infinium assay compatible with the Illumina HiScan SQ genotyping platform. This Infinium assay is designed to analyse a large number of SNPs at many loci concurrently through multiplexing (Illumina Inc. 2018). SNP calling was done using Illumina Genome Studio v2.0. The genotype input file was converted into a PLINK (v1.07) (Purcell et al., 2007) input file using a plug-in compatible with Genome Studio program. SNP quality control was done in a series of steps depending on population genetic parameters estimated.

5.1.5 Population Genetic Parameters

An original data set consisting of all four populations of Tswana chickens (naked neck, Normal and Dwarf populations) was filtered for SNPs that had minor allele frequency (MAF) ≤0.02 and this resulted in a total sample of 82 chickens across the three populations. There were 54 293 SNPs available to estimate observed and expected heterozygosity indices (Ho and He) and the inbreeding co-efficient of each population. PLINK (v1.07) software (Illumina Inc. 2018) was used to estimate observed and expected heterozygosity, inbreeding coefficient and minor allele frequency distribution per population using the comprehensive data set before pruning for MAF. Bins were set for minor allele frequencies of 0-0.05, 0.05-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 and 0.4-0.5 and the proportion of SNPs per bin was calculated by dividing the number of markers per bin by the total number of markers included in the MAF estimation according to Khanyile et al., (2015).

5.2.5 Population Structure

A complete SNP data set with all four populations was filtered to remove SNPs that were on sex chromosomes or had their positions unmapped. Markers with missing data > 5 %; that had a MAF ≤ 2% or were monomorphic were removed from the complete data set. SNPs that were in high linkage disequilibrium at a threshold of LD≥0.2were also filtered out of the complete data set. Individuals with missing genotypes of more than 5% and those that were closely related, as inferred by a kinship estimator ≥0.45 were also excluded from the analysis.

A principal component analysis (PCA) was then performed to establish relationships among different strains of Tswana chickens and the commercial broiler line using the Golden helix SNP variation suit (SVS) version 8.1(Golden Helix INC. 2014). Furthermore, the Admixture 1.23 software (Alexander et al., 2009) was used to estimate the most probable number of ancestral populations based on the SNP genotype data as described by Khayile et al. (2015). Admixture was run from K=2 to K=4 and the optimal number of clusters (K-value) was determined as that which had the lowest cross validation error (CV error).

5.2.6 Population Differentiation and Genetic Distances

Pairwise identity by state (IBS) distances between all four chicken populations (naked neck, normal and dwarf strains of Tswana chicken and the commercial broiler) were calculated using PLINK v1.9. Genetic distances between the four populations were evaluated based on Nie's (1987) unbiased. genetic distance using the R-package (Pembleton et al., 2013). To evaluate pair-wise genetic differentiation, the fixation index F*st* Weir and Cockerham., 1984) was calculated for all pairs of chicken populations.

5.2.7 Linkage Disequilibrium

Complete SNP data for the individual populations were filtered to remove SNPs on sex chromosomes or those were not mapped, those with MAF $\leq$ 5%, those that deviated from Hardy-Weinberg equilibrium (HWE) ($P \leq 0.001$) and individual chickens with missing genotypes (> 5%) and those with very close kinship (IBD $\geq$0.45) using PLINK (v1.07) [19]. After all quality control measures 46084 out of 48054 in the normal strain, 45080 out of 46909 in the naked neck strain, 38781 out of 42804 in the dwarf strain of Tswana chickens, and 36401 out of 39739 in the commercial broiler were available for the estimation of linkage disequilibrium. The resultant individual population data sets after quality control measures were used in the estimation of linkage disequilibrium and associated measures.

Pairwise r^2 estimation was used to measure LD between pairs of SNPs within a chromosome and population using PLINK (v1.07) program [19] for SNPs on chromosomes 1-28 that had passed quality control tests detailed above. According to Lu et al., (2012) the r^2 measure, defined as the squared correlation coefficient of alleles at two loci was chosen because it is independent of allele frequency. Briefly, its calculation, considers two loci, A and B, each locus having two alleles (denoted *A1, A2; B1, B2*, respectively) (Qanbari et al., 2010). The frequencies of the haplotypes will then be denoted as *F11, F12, F21*, and *F22* for haplotypes *A1B1, A1B2* and *A2B2*, respectively and as *FA1*, FA2, *FB1 and FB2* for *A1, A2*, *B1*and *B2 alleles*, respectively. From this, r^2 according to Khanyile et al. (2015) were then calculated as shown in formula No.1 below:

$$r^2 = (f11f22-f12f21)^2 / FA1FA2FB1FB2$$

Formula No.1.

PLINK by default only reports r^2- values above 0.2 and to allow reporting of all r^2-values observed in the populations, the -r^2-*window-ld0* option was used. An additional option, -r^2- *window-snp 5000 -kb 10000 described by [17] Khanyile et al.* allowed for estimation of r^2 for SNP marker pairs separated by at most 5000 SNPs and within a 10 MB SNP interval.

5.2.8 Effective Population Size

The effective population size trends were estimated using the procedure described by Khanyile et al. (2015). Briefly, the relationship between *Ne*, recombination frequency and expected LD (r^2) was determined using the equation from Corbin et al., (2010) shown in formula No.2:

$$E[r^2_{adj}] = (\alpha + 4N_e c)^{-1}$$

Formula No.2.

Where α =1 when assuming no mutations and 2 if mutation was considered, $r^2_{adj} = r^2 - 1/_{2n}$, c was the recombination rate, and n was the chromosomal sample size. The effective population size Ne, as $1/2c$ generations, was estimated from the adjusted r^2_{adj} values related to a given genetic distance d in Morgans, assuming $c = d$ (Qanbari et al., 2010). For each pair of SNPs on each chromosome, recombination rate was estimated by converting physical marker interval length xi (MB) to the corresponding genetic length ci using the formula: $c_i = \acute{\tilde{o}}_i x_i$, where $\acute{\tilde{o}}_i$ is the average ratio of Morgans per kilo base pair on chromosome I, which was taken from physical lengths of the chicken genome v74 (Ensembl et al., 2013). The genetic length of chromosomes was adopted from Hillier et al., (2005). The r^2 – values range from 0 and 1, whereby a zero value indicates uncorrelated SNPs while a value of one reflects SNPs that are perfectly correlated (Qanbari et al., 2010). The trends in effective population sizes for each of the defined subpopulations were then estimated by setting bins at 10, 20, 40, 60, 100, 200, 500, 1000, 2000 and 5000 kb. The bins were designed to cover the genome in tens, hundreds, thousands and hundred thousand base pairs.

5.3 RESULTS AND DISCUSSION

5.3.1 Basic Population Genetic Parameters

The observed heterozygosity (H_o) values were 0.610±0.012, 0.611±0.014, 0.613±0.0006 for normal, naked neck and dwarf strains of Tswana chickens respectively. There were no significant differences in H_o between the three strains of Tswana chickens. The H_o averaged 0.611±0.016 across the three strains of Tswana chickens compared to H_o of 0.347±0.023 in commercial broiler chicken. The expected heterozygosity (H_e) values were 0.613±0.00012, 0.614±0.00013, 0.608±0.00021 for normal, naked neck and dwarf strains of Tswana chickens respectively. There were no significant differences in H_e between the three strains of Tswana chickens. The H_e averaged 0.612±0.00015 across the three strains of Tswana chickens compared to H_e of 0.577±0.00022 in commercial broiler chicken.

The H_o and H_e values for the three strains of indigenous Tswana chickens are similar with the H_o and H_e values found in indigenous chickens of Malawi, Zimbabwe and South Africa as reported by Khanyile et al., (2015). Higher molecular diversity in indigenous Tswana chickens compared to commercial broiler chickens is consistent with Al-Atiyat and Abudabos, (2014) who reported higher gene diversity in indigenous chickens of Jordan than in Ross broiler chickens (H_e of 0.54 vs 0.09). Higher genetic diversity in indigenous Tswana chickens than commercial broiler chickens might be due to inherent traditional breeding practices of natural and random mating of indigenous chickens. Indigenous Tswana chickens are also not subjected to intensive selection in various traits of economic importance which tends to promote diversity than uniformity. Lower genetic diversity in commercial broiler compared to indigenous Tswana chickens might be due to artificial selection for traits of economic importance such as meat production (Al-Atiyat and Abudabos, 2014).

In the normal and naked neck strains of Tswana chickens H_o was less than H_e while in the dwarf strain H_o was greater than H_e is indicating that the dwarf strain was significantly outbreed while the

other two strains of Tswana chickens were inbred. In the commercial broiler chicken H_o was also less than H_e also indicating that the broiler chicken was inbred. The inbreeding coefficients (Fis) were positive for the normal, naked neck and commercial broiler and negative in the dwarf strain of Tswana chicken. Of all the four strains only the dwarf strain was therefore outbred. The commercial broiler had the highest levels of inbreeding compared to indigenous Tswana chickens. The three strains of indigenous Tswana chickens generally had lower inbreeding coefficients than indigenous chickens of Malawi, Zimbabwe and South Africa reported by Khanyile et al., (2015). Higher levels of inbreeding in commercial broiler chickens than in indigenous Tswana chickens were expected as inbreeding is part of the breed development process. Intensive selection during development of commercial broiler chickens reduced diversity and increased uniformity partially as result of inbreeding.

The minor allele frequency (MAF) was also presented in **Error! Reference source not found.** for each population from generated data set since it provides information to differentiate between common and rare variants in the population. The analysis of SNP markers indicated an average minor allele frequency (MAF) of 0.266, 0.266, 0.241 and 0.259 in the normal, naked neck, dwarf and broiler strains, respectively.

Table 11:Percentage of polymorphic markers within population diversity and inbreeding coefficient of different chicken populations in Kweneng and Southern districts of Botswana

Population	N	Tested markers	Polymorphic SNPs(%)	H_o	H_e	Fis	Average MAF
Normal	39	48054	95.9 (46084)	0.610 ±0.012	0.613 ±0.00012	0.010	0.266 ±0.0006
Naked neck	32	46909	96.1 (45080)	0.611 ±0.014	0.614 ±0.00013	0.007	0.266 ±0.0006
Dwarf	13	42804	90.6 (38781)	0.613 ±0.023	0.608 ±0.00021	-0.010	0.241 ±0.0006
Broiler	12	39739	91.6 (36401)	0.347 ±0.023	0.577 ±0.00022	0.545	0.259 ±0.0007

5.3.2 Population Structure using Principal Component and Admixture Analysis.

5.3.2.1 Population Structure

Principal component analysis (PCA) was used to get an insight into the population structure of indigenous Tswana chickens. The first two principal components revealed a set of three clusters. The normal strain of Tswana chicken and commercial broiler chicken clustered together in one group. Among the three strains of Tswana chickens, the dwarf strain clustered separately in one group and the naked neck and normal strains clustered together in the last group. The separate clustering of the dwarf strain from the rest of Tswana chicken strains suggests significant genetic uniqueness of the dwarf strain and very close genetic similarities between the normal and naked neck strains. For conservation purposes the dwarf strain of Tswana chickens needs to be included in the conservation program as a separate strain while the naked neck and normal can be conserved as either one of the two. The genetic uniqueness of the dwarf strain of Tswana chicken relative to the normal and naked neck strains implies possible different centre of Asian origin for the dwarf strain and common centre of origin for naked neck and normal strains of Tswana chickens.

The first principal component (PC) distinguishes, the commercial broiler from the rest of indigenous Tswana chicken strains while the second PC distinguishes the dwarf strain from other two strains of indigenous Tswana chickens. The commercial broiler chicken clustered more closely with the normal

strain of Tswana chickens than any other strains of indigenous Tswana chickens. The clustering together of commercial broiler and normal strain of Tswana chicken suggests high genetic similarities between the two probably because of their common centre of origin. According to Al-Atiyat and Abudabos., (2014) Asian indigenous chickens contributed to strains and lines used in the development of commercial broiler chickens and molecular genetic information suggests possible Asian centres of origin for African domestic chickens (Mwacharo et al., 2013). Another explanation is possible cross breeding between normal strain of Tswana chickens and recently introduced chicken breeds of Asian origin under our free-range management system (Figure 5.1).

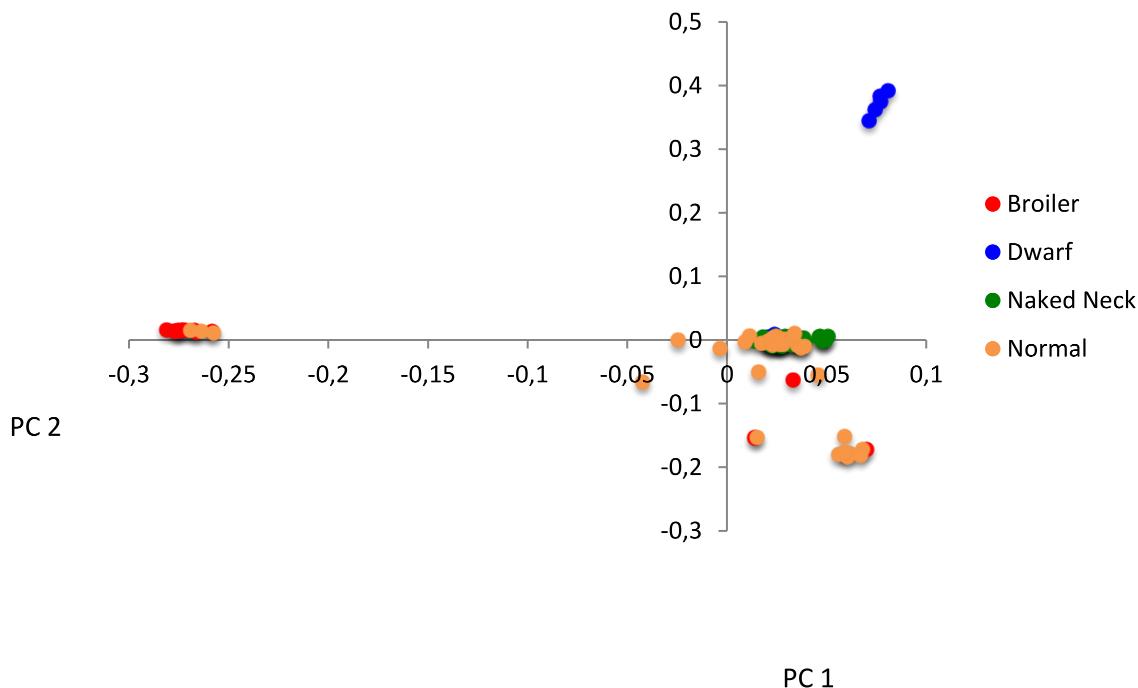

Figure 14: The genetic relationship between Tswana chicken strains sampled in Kweneng and Southern districts of Botswana using Principal Component Analysis (PCA)

5.2.2.2 Admixture Analysis

The graphic results of the clustering analysis for K= 2 to 4 are illustrated in (Figure 5 2). Based on the visual inspection and admixture plot, the results indicate that the most likely partition was for K=3 populations. The change in prediction error against K (Figure 5.3) indicates minimal improvement in model fitness between K=3 and K=4, suggesting that K=3 describes the cluster number that best describes the populations under study,

At K=2 the commercial broiler chicken separates from indigenous Tswana chickens while at K=3 the dwarf strain of Tswana chickens separates from other strains (normal and naked neck) of Tswana chickens. From K=2 analysis, it is apparent that the commercial broiler is not homogenous and contains significant admixture from the normal strain of Tswana chickens. At k=3 the dwarf strain separates from other strains of Tswana chickens but displayed significant admixture or introgression from normal and naked neck strains of Tswana chickens probably because of interbreeding between the strains under traditional free running management system. Still at K=3 naked neck and normal strains of Tswana cluster together as one distinct group.

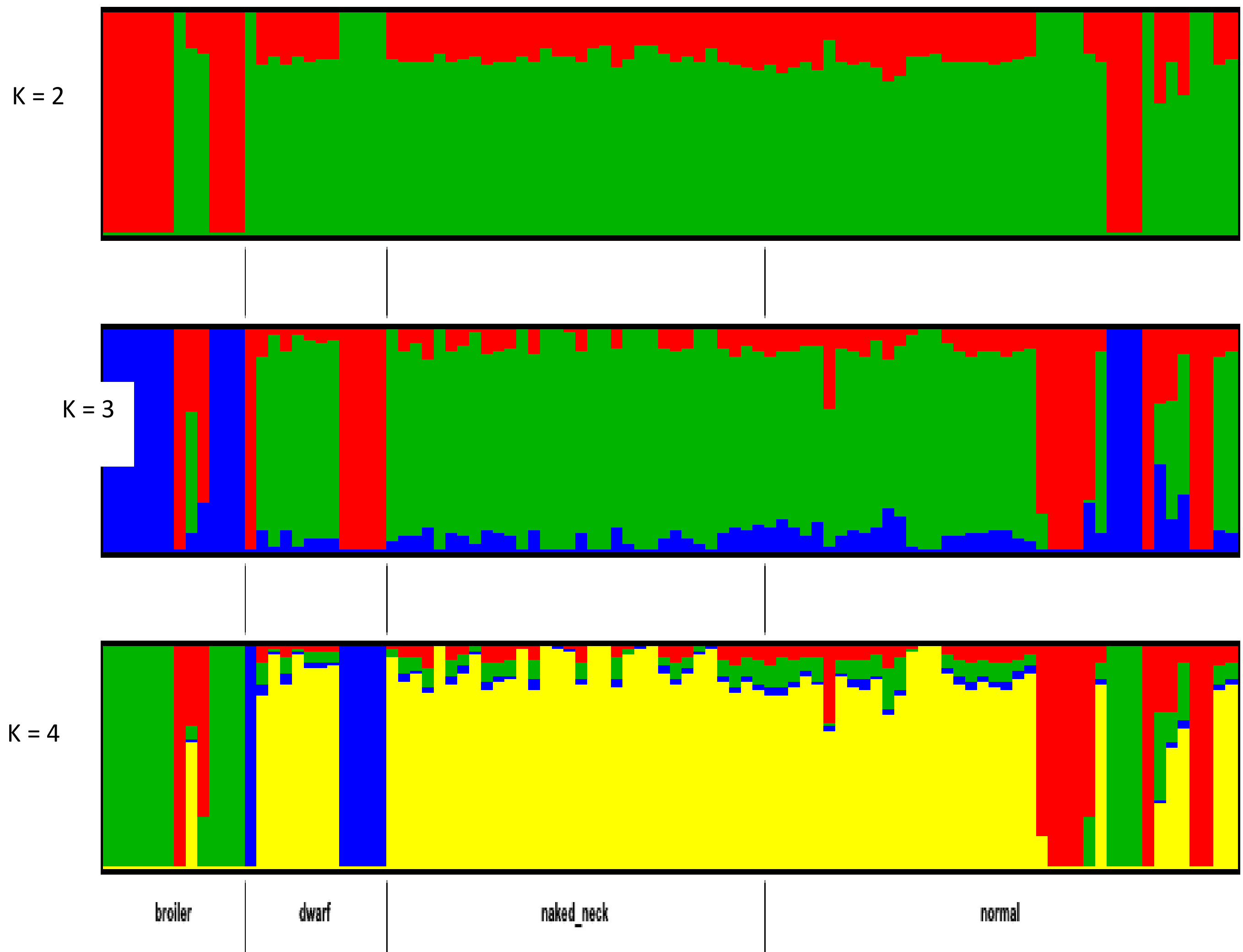

Figure 15: Population structures of Normal, Naked neck, Dwarf and Broiler chickens in Kweneng and Southern districts of Botswana based on admixture analysis.

The lowest cross validation error was observed at K= 3, which represented the number of ancestors in indigenous Tswana chicken strains and the commercial broiler strain (**Figure 16**).

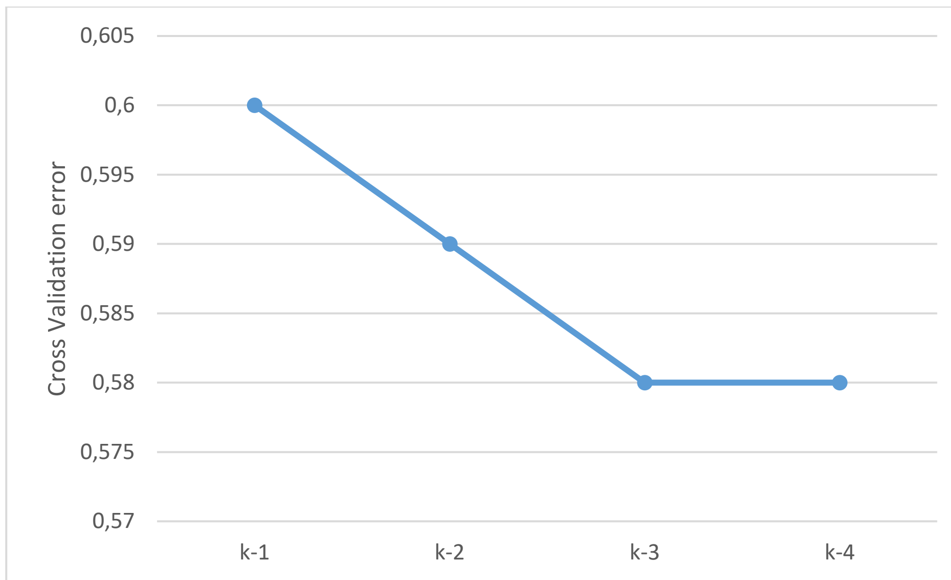

Figure 16: Optimal number of clusters according to cross-validation error

5.3.2.2 Population differentiation (F_{ST})

Pairwise population differentiation (F_{ST}) was calculated from filtered SNPs to investigate population differentiation among different strains of Tswana chickens. F_{ST} values are shown in Table 12 and varied from 0.013 to 0.084. According to Hussein et al., (2015) it is generally recognised that F_{ST} values ranging from 0 to 0.05 indicate low genetic differentiation; those ranging between 0.05 and 0.15 medium differentiation; those between 0.15 and 0.25 indicate big differentiation and those values above 0.25 indicate very big genetic differentiation. There was low genetic differentiation between the normal strain of Tswana chicken and the commercial broiler chicken (0.031). There was, however, moderate genetic differentiation between the dwarf strain of Tswana chicken and commercial broiler chicken and between the naked neck strain of Tswana chicken and the commercial broiler chicken (0.084, 0.054 respectively).

Low genetic differentiation occurred between dwarf and naked neck (0.040), dwarf and normal (0.040), and between normal and naked neck (0.013) strains of Tswana chickens. Pairwise genetic distances between different strains of Tswana chickens and commercial broiler are highly correlated with genetic differentiation values. Generally, the higher the genetic differentiation between strains the higher the genetic distances. The low genetic distances occurred between normal and naked neck strains of Tswana chickens (0.013) and between normal strain of Tswana chicken and commercial broiler chicken (0.032). Moderate genetic distance occurred between naked neck and dwarf (0.042) and between normal and dwarf (0.043) strains of Tswana chickens. High genetic distances occurred between naked neck and commercial broiler (0.057) and between dwarf and commercial broiler (0.092). Generally, less genetic differentiation and fewer genetic distances occurred between the three strains of indigenous Tswana chickens as compared to between the commercial broiler and the three strains of Tswana chickens. The less genetic differentiation and genetic distances between the three strains of Tswana chickens support the hypothesis of closer evolutionary history of common origin of the three strains of Tswana chickens. Generally higher genetic differentiation and genetic distance between strains of indigenous Tswana chickens and the commercial broiler chickens might be since indigenous Tswana chickens are not intensively selected while commercial broilers are heavily selected during their developmental process.

Table 12: Pairwise genetic differentiation (F_{ST}) values (below diagonal) and genetic distance (above diagonal) between four (4) chicken populations in Kweneng and Southern districts of Botswana

Population	Broiler	Dwarf	Naked neck	Normal
Broiler	0	0,092	0,057	0,032
Dwarf	0,084	0	0,042	0,043
Naked neck	0,054	0,040	0	0,013
Normal	0,031	0,041	0,013	0

5.3.2.3 Linkage Disequilibrium (LD) Estimates and the Effect of Strain

A summary of r^2 values for the 28 chicken autosomal chromosomes in the three strains of Tswana chickens and commercial broiler chicken are shown in Table 5.4. Overall population LD over all chromosomes ranged from 0.067 to 0.241 in indigenous Tswana chickens and from 0.342 to 0.407 in commercial broiler chicken and averaged 0.128±0.056 and 0.375±0.0152 in indigenous Tswana chickens and commercial broiler chicken, respectively. The highest LD values were found in chromosome 16 in the normal and naked neck strains of Tswana chickens and in chromosome 23 in the dwarf strain of Tswana chicken. Higher LD values on chromosome 16 for naked neck and normal strains of Tswana chickens are consistent with Khanyile et al., (2015) who also reported higher LD values in chromosome 16 for both village chickens and conservation flocks. The lowest LD values were found in chromosome 23 in the naked neck and normal strains of Tswana chickens and in chromosome 25 in the dwarf strain of Tswana chicken. Low LD values on chromosome 25 in the dwarf strain of Tswana chicken are consistent with Khanyile et al., (2015) who also reported low LD values on chromosome 25 for both village and conservation flocks. Chromosome 17 had high LD in the commercial broiler strain while chromosome 16 had low LD. Consistent with Khanyile et al., (2015) the current study also indicates that evolutionary forces affecting LD act differently on different chromosomes and different strains. Commercial broiler chicken had higher LD compared to the three strains of indigenous Tswana chickens probably because of the effects of artificial selection for higher meat yield. On the other hand, natural selection could be a major evolutionary force in the three strains of Tswana raised under free running management systems with minimal artificial selection (Khanyile et al., (2015). There was no significant difference in LD between the normal and naked neck strains of Tswana chickens. However, the two strains of Tswana chickens had significantly lower LD than dwarf strain of Tswana chicken. Of the four chicken strains, the commercial broiler chicken had significantly higher LD compared to the three strains of Tswana chickens.

Higher LD in commercial broiler compared to the three strains of Tswana chickens is consistent with Khanyile et al., (2015) who found significantly higher LD in conservation flocks compared village chicken populations kept by small holder farmers. Differences in LD between commercial broiler and the three strains of Tswana chickens could be due to their different evolutionary histories under the influence of random genetic drift, selection, and mutations (Khanyile et al., 2015). The dwarf strain of Tswana had higher LD across the 28 autosomal chromosomes compared to normal and naked neck strains of Tswana chickens. Higher LD in the dwarf strain compared to the naked neck and normal strains of Tswana chickens is consistent with its low diversity as measured by both observed and expected heterozygosity (H_o and H_e) and its low effective size (Table 13). Lower effective population size in the dwarf compared to the normal and naked neck strains of Tswana chickens are consistent with the actual population of dwarf chicken in the general population of Tswana chickens. The general population of Tswana chickens comprises mostly the normal strain followed by naked neck strain and lastly the dwarf strain of Tswana chickens (Machete et al., 2021). Across the four strains under investigation the normal strain had the lowest LD (0.087) and the commercial broiler had the highest LD (0.375).

Table 13: Linkage disequilibrium (LD) of three indigenous Tswana chickens and commercial broilers in Kweneng and Southern districts of Botswana

Chromosome No.	Normal	Naked neck	Dwarf	Broiler
1	0.089±0.137	0.093±0.138	0.209±0.225	0.395±0.331
2	0.090±0.144	0.095±0.144	0.202±0.221	0.368±0.319
3	0.089±0.131	0.097±0.136	0.194±0.213	0.371±0.320
4	0.089±0.138	0.096±0.142	0.220±0.229	0.368±0.314
5	0.084±0.129	0.084±0.128	0.188±0.210	0.366±0.317
6	0.085±0.125	0.092±0.129	0.219±0.235	0.375±0.322
7	0.084±0.132	0.087±0.133	0.219±0.230	0.374±0.320
8	0.114±0.185	0.107±0.166	0.204±0.240	0.391±0.334
9	0.078±0.119	0.083±0.119	0.215±0.221	0.363±0.320
10	0.077±0.115	0.086±0.119	0.199±0.217	0.381±0.320
11	0.098±0.155	0.104±0.160	0.222±0.237	0.399±0.331
12	0.083±0.123	0.091±0.128	0.199±0.216	0.373±0.321
13	0.088±0.135	0.090±0.132	0.213±0.231	0.384±0.321
14	0.089±0.141	0.096±0.141	0.192±0.215	0.373±0.321
15	0.105±0.153	0.099±0.144	0.208±0.229	0.374±0.334
16	0.133±0.165	0.146±0.159	0.206±0.244	0.342±0.294
17	0.089±0.143	0.100±0.154	0.216±0.231	0.407±0.334
18	0.077±0.113	0.083±0.116	0.195±0.208	0.363±0.312
19	0.079±0.125	0.085±0.130	0.184±0.205	0.361±0.316
20	0.095±0.144	0.102±0.145	0.200±0.222	0.371±0.318
21	0.086±0.130	0.088±0.131	0.230±0.236	0.378±0.319
+22	0.087±0.141	0.082±0.134	0.183±0.205	0.410±0.335
23	0.067±0.102	0.073±0.108	0.241±0.240	0.361±0.317
24	0.081±0.132	0.089±0.138	0.200±0.221	0.366±0.312
25	0.069±0.104	0.074±0.108	0.172±0.198	0.358±0.308
26	0.074±0.107	0.081±0.113	0.196±0.212	0.391±0.329
27	0.082±0.130	0.085±0.127	0.194±0.216	0.370±0.333
28	0.074±0.122	0.090±0.136	0.199±0.222	0.371±0.320
Overall means	0.087±0.002[a]	0.092±0.002[a]	0.204±0.002[b]	0.375±0.002[c]

5.3.2.4 Trends in Effective Population Size (N_e)

Plots of estimated effective population size (N_e) at *t*-generations ago for various strains of Tswana chickens and commercial broiler chickens are shown in Figure 17. The adjusted Linkage Disequilibrium (LD) based estimates of N_e indicated low effective population sizes of 123, 738 and 748 for dwarf, naked neck, and normal strains of Tswana chickens, respectively, 19 generations ago. Higher Ne of 613, 2640, 2650 was found for the dwarf, naked neck, and normal strains of Tswana chickens respectively, 120 generations ago. The effective population size (N_e) was even higher with

values of 3768, 7477 and 7891 for the three strains of Tswana chickens, respectively, 982 generations ago. The commercial broiler had N_e of 147 859 and 5994 at 19, 120 and 982 generations ago, respectively. The graphs illustrate a steady decrease in N_e from over 980 to below 20 generations ago in both indigenous Tswana chickens and commercial broiler chickens. The naked neck and normal strains of indigenous Tswana chickens showed similar patterns of decreasing N_e values but had higher N_e values than the dwarf strain. In comparison with the three strains of indigenous Tswana chickens, the commercial broiler chicken had higher N_e values at all generations than the dwarf strain. The LD patterns are consistent with effective population size and diversity patterns in the commercial broiler and the three strains of Tswana chickens. Generally, higher LD patterns are associated with low effective population sizes and lower diversity in the populations.

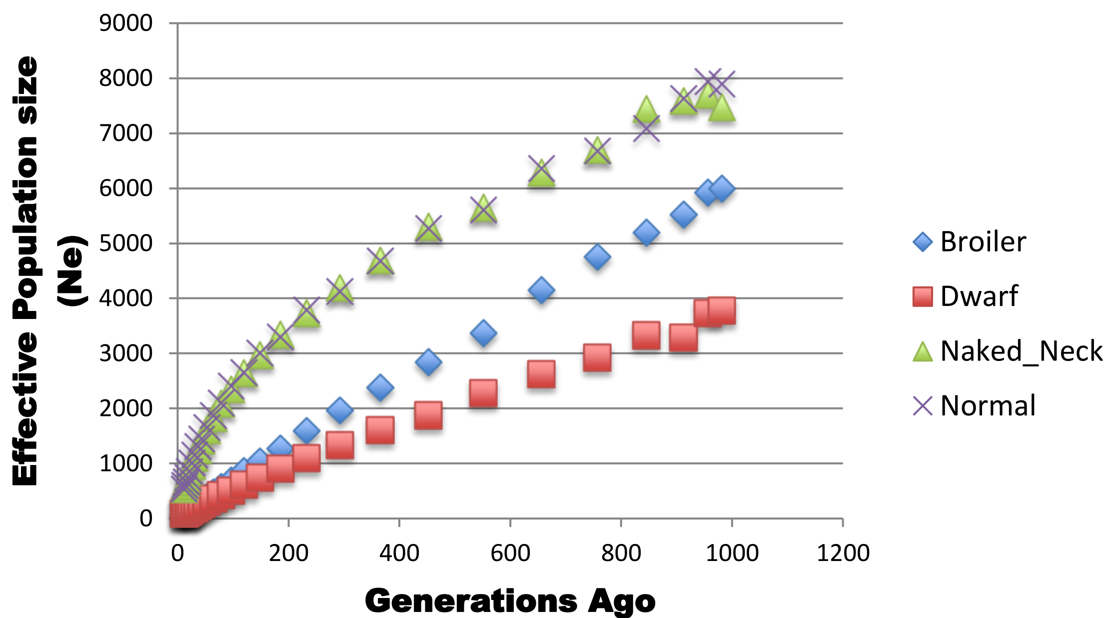

Figure 17: The effective population size of Normal, Naked Neck, Dwarf strains of Tswana chicken and Broiler breeds in Kweneng and Southern districts of Botswana from 982 to 12 generations ago

5.4 CONCLUSION

The naked neck, normal and dwarf strains of Tswana chicken had similar, moderate genetic diversity measures (Observed and expected heterozygosity which were significantly higher than those of the modern commercial broiler chicken. The principal component analysis (PCA) which was used to get an insight into the population structure of indigenous Tswana chickens and the commercial broiler revealed a set of three clusters or wild ancestors. The commercial broiler clustered separately in one group. The dwarf strain clustered separately in another group and the naked neck and normal strains clustered together in the last group. The clustering pattern was confirmed by less genetic differentiation and less genetic distances between the naked neck and normal strains of Tswana chicken than between the two strains and the dwarf strain of Tswana chicken. Linkage disequilibrium patterns across chromosomes were also similar between the naked neck and normal strains of Tswana chicken and different from those of the dwarf strain of Tswana chicken. The separate clustering of the dwarf strain from the rest of Tswana chicken strains and different linkage disequilibrium in the dwarf than in the other two strains of Tswana chicken suggests significant genetic uniqueness of the dwarf strain and very close genetic similarities between the normal and naked neck strains.

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5.6 CONNECTING STATEMENT III

Chapter V established genetic relationships between the different strains of indigenous Tswana chicken using an array of SNPs scattered all over the chicken genome. The next chapter concentrated on the identification of SNPs in the Promoter and 5`UTR region of chicken HSP-70 gene in different strains of indigenous Tswana chicken. The chicken HSP-70 gene was chosen because of its increasing importance to adaptation to heat stress in the era of global warming and climate change.

CHAPTER 6

6.0 GENETIC POLYMORPHISM IN THE PROMOTER AND 5´ UTR OF HSP-70 GENE IN THREE STRAINS OF INDIGENOUS TSWANA CHICKENS AND COMMERCIAL BROILER CHICKENS.

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ABSTRACT

The study was conducted to investigate sequence polymorphisms in the promoter, 5´untranslated regions (UTR) and partial exon regions of chicken HSP-70 gene in the normal (n= 24), naked neck (n= 22) and dwarf (n=12) strains of indigenous Tswana chickens relative to the commercial broiler chicken (n=20). Genomic DNA extracted from whole blood of the three strains of indigenous Tswana chicken and the commercial broiler, amplified using PCR and sequenced using Big Dye Cycle Sequencing Kit. The PCR amplicons comprised a 210 bp promoter region, a 112 bp 5´UTR and a 463 bp partial exon of chicken HSP-70 gene. Multiple sequence alignments of the partial sequences of chicken HSP-70 gene in indigenous Tswana chickens and the commercial broilers revealed no polymorphisms in the promoter region, two SNPs in the 5´UTR (A303G and G309A) and another two SNPs (G427 and A628G) in the partial exon sequence of chicken HSP-70 gene. The SNP G427A was unique to the normal strain and the other three SNPs were common to all the four chicken strains studied. The identified four SNPs linked up in individual chickens resulting in a total of seven different haplotypes in the studied four chicken populations. A total of seven different haplotypes were found in indigenous Tswana chickens and only two haplotypes were found in the commercial broilers. More nucleotide (4SNPs vs 3 SNPs) and more haplotype diversity (7 haplotypes vs 2) were thus found in indigenous Tswana chickens compared to the commercial broilers in the partial sequence of HSP-70 gene.

Keywords: HSP-70 gene; Indigenous Tswana chicken; polymorphism; Sequencing; SNPs.

6.1 INTRODUCTION

Chickens do not have sweat glands and thus, body heat dissipation to the environment is not easy and, in most cases, chickens are prone to heat stress (Afsal et al.2021), (Aryani et al. 2019), (Tamzil. 2014). When ambient temperature goes beyond the comfort zone of >28^{0}C, then heat stress can crop up anytime in both egg laying and broiler chickens (Aryani et al. 2019). In chickens, heat stress cause high temperatures that can affect growth, egg quality (Oberheitmann. 2013), and cause decreased feed intake, eggshell quality, decreased egg production, reduced body weight gains and even death in domestic chickens during summertime (Aryani et al. 2019), (Cahaner et al. 2008), (Melesse et al.2011). Cellular mechanisms used to alleviate heat stress in animals may include changes in the functions of cells, including transcription, translation, and protein synthesis (Negri et al. 2013). One of the adaptive mechanisms to increased environmental temperatures in chickens include the production of heat shock proteins (HSPs) which play an important role in the protection and repair of cells and tissues exposed to high environmental temperatures (Chen et al. 2016). HSP-70 is one the heat shock proteins produced by animals in response to extreme heat conditions and it plays an important role in heat tolerance (Zuiderway et al. 2012).

In chickens, the HSP-70 protein is encoded by HSP-70 gene which is found in chicken autosome 5 and comprises a single exon, the upstream Promoter and 5` UTR and downstream 3` UTR (Morimoto et al. 1986). According to GenBank: AY143693.1, the entire chicken HSP-70 gene comprises of 2594 bp and encodes a protein of 653 amino acids (Junprung et al.2019). The promoter comprises of 210 bp, the 5´UTR comprises 112 bp and the 3´UTR comprises 309 bp. According to Najafi et al. (2018), a total of 35 SNPs has been found in chicken HSP-70 with 25 SNPs occurring in the protein coding region and the rest in the regulatory regions (Promoter, 5`UTR and 3`UTR). Most of the studies on chicken HSP-70 gene polymorphism concentrated on the exon or protein-coding region because of

its direct effect on the amino acids sequence of the resulting protein (alleles of a gene) (Gan et al. 2015), (Najafi et al. 2018). Only a few studies investigated polymorphisms in regulatory regions of chicken HSP-70 gene. Polymorphisms in the regulatory region of gene are however very important because they influence gene expression (Öner et al. 2017). According to Silver and Noble. (2011) pre-transcriptional activation of HSP-70 gene has been thoroughly reviewed while its downstream regulation by 5´UTR and 3´UTR has received less attention. The 5´UTR regions of genes are useful in controlling mRNA expression levels and stability (Öner et al. 2017), (Basirico et al. 2011), (Sodhi et al. 2013). The control of translation initiation is mainly under the responsibility of 5′UTRs and gene expression can therefore be influenced by SNPs in the regulatory regions (Araujo et al. 2012), (Haimov et al. 2015). Indigenous chickens are known to be more thermotolerant to heat stress compared to exotic chickens (Duangjinda et al.2017). We hypothesised that the differences in thermotolerance between indigenous Tswana chickens and exotic chickens could, therefore be due to sequence variations in the promoter and 5´UTR regions of the chicken HSP-70 gene.

The objectives of the current study were to investigate polymorphism in the Promoter and 5` UTR regions of chicken HSP-70 gene in normal, naked neck and dwarf strains of Tswana chickens as compared to the commercial broiler.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Animals

Blood samples were collected from a total of 24 normal, 22 naked neck, and 12 dwarf strains of indigenous Tswana chickens. The sampling locations of different strains of Tswana chickens (Table 14). Additional 20 blood samples were collected from Ross broiler chickens at Notwane farm located in Gaborone. The blood samples were collected from the medial metatarsal vein located on the leg of a chicken better suited for puncture using a 23-gauge, 1-in needle. The alternative site for blood collection was the brachial vein on the wings. All blood samples were collected into vacutainer tubes containing EDTA and kept on ice in the field and later stored in the freezer at -20ºC until DNA extraction.

Table 14: Locations where indigenous Tswana Chickens were sampled and number of samples per location in Kweneng and Southern districts of Botswana.

Sampling location	District	Number of samples per location		
		Normal	**Naked neck**	**Dwarf**
Sojwe	Kweneng	4	3	5
Kaudwane	Kweneng	1	1	0
Maboane	Kweneng	2	2	2
Malwelwe	Kweneng	3	3	2
Kweneng	Kweneng	2	2	1
Keng	Southern	3	1	0
Seherelela	Southern	3	2	1
Thankane	Southern	2	4	0
Lerolwane	Southern	2	3	0
Magotshwane	Southern	2	1	1
TOTAL		24	22	12

6.2.2 DNA Isolation

Total genomic DNA was extracted from avian blood using QIAGEN Multiplex PCR Kit, California, USA following the manufacturer`s protocol. Briefly 200µl of digestion buffer and 20µl of proteinase K were added to 5µl of whole blood. The mixture was vortexed for 15 minutes and then incubated in

a water bath at 50^0C for 10 minutes. Two hundred microliters (200µl) of cold 100% ethanol was then added to each sample and mixed by vortexing for 15 seconds. The resulting mixture was transferred into a spin column placed inside a collecting tube and centrifuged at 8000 x g for 1minute. Tubes containing the flow through were discarded and the spin column were then transferred to new collecting tubes. In the next step, 500µl of the first washing buffer was then added to each spin column and centrifuged at 1000 x g for 1 minute. Again, the collection tubes containing flow through were discarded and the spin columns were transferred to new collection tubes. Afterward, 500µl of second washing buffer were added to the spin column and then centrifuged at 14000 x g for 3 minutes. Following centrifugation, spin columns were transferred to clean microcentrifuge tubes. Then 150µl of elution buffer was added to the spin columns and incubated at room temperature for 5 minutes. Following the incubation, the microcentrifuge tubes holding the spin columns were centrifuged at 8000 x g for 1 minute to elute the DNA.

6.2.3 DNA Quantification

The quantity and quality of extracted DNA was established using a Nano Drop 2000/2000c Spectrophotometer. DNA quality was established by measuring absorbance at 260/280.

6.2.4 PCR amplification and DNA Sequencing

PCR amplifications were performed with a programmable thermal cycler, PTC-100^{TM} (MJ-Research, Inc., Watertown, MA, USA) in a final reaction volume of 50 ng of genomic DNA, 0.2 mM dNTPs, 1.5 mM, $MgCl_2$, 0.6 µM of each primer and 1.5 units *Taq* DNA polymerase. After an initial denaturation step of 94^{0}C for 3 minutes, the reaction mixture was subjected to the following cycling conditions: 34 cycles at 94^{0}C for 45seconds, 54^{0}C for 30 seconds, and 72^{0}C for 1minute 30 seconds, plus a final extension step of 72^{0}C for 10 minutes. Amplification was confirmed by running the PCR products on 2 % agarose gels and visualizing them under ultraviolent rays. PCR products were then shipped to Inqaba Biotec-Africa`s Genomics Company, Pretoria, South Africa for sequencing. At Inqaba Biotec PCR amplicons were purified using The PureLink PCR purification kit of Thermo Fisher Scientific, South Africa following the manufacturer’s protocol (Vallone et al., 2008). DNA sequences were generated using ABI V3.1 Big dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and the thermocycler GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). Sequences were generated with both forward and reverse primers (Table 15) and read on ABI3500XL DNA analyzer (Applied Biosystems, Foster City, USA).

Table 15: Primers used on PCR amplification of chicken 5`UTR of HSP-70 gene.

Primer’s name	Sequence	Position on reference sequence
HSP-70-F1	5’ GAGTGGCGCAGCGTAGAAAG ‘3	18
HSP-70-F2	5’ GATTGGTCCTTAGCGTTCTGGC ‘3	208
HSP-70-R1	5’ TTCCTCTTGGTCAGTCAGCC ‘3	382
HSP-70-R3b	5’ CTGGGAGTCGTTGAAGTAAGCG ‘3	856

The letters F and R in primer names refer to their orientation (forward and reverse, respectively). Adopted from (Mazzi et al. 2003).

6.2.5 Sequence Analysis

Chromatographs developed from sequencing were processed using ApE, A Plasmid Editor version 2.0.53, (Jorgensen.biology.utah.edu) to verify the sequences and SNPs in the amplified regions of chicken HSP-70 gene. Furthermore, samples and reference sequences were aligned using the Clustal W multiple sequence alignment program (Simmonds, 2012) to establish the presence of SNPs in the amplified regions (Brocehieri et al. 2008). Nucleotide substitutions at the identified SNP loci were identified from chromatographs generated by the program Chromas Lite (https://technelysium.com.au/wp/). Translation of DNA sequences into protein sequences was done online using the Nucleotide Amino Acid Derived Visualization (NADV) (Abascal et al., 2010).

6.2.6 Statistical Analysis

Potential functional motifs in the sequenced promoter region of chicken HSP-70 gene in the three strains of indigenous Tswana chicken and commercial broiler were identified using Proscan software (http.//www-bimass.cit.nih.gov/molbio/proscan/). Allele and genotype frequencies at the identified SNP loci were calculated using Gene Pop program (v 1.2) (Lachance. 2008), (Raymond and Rousset. 1995). Haplotypes in the partial sequence of chicken HSP-70 gene were determined by clustal X (v 1.81) and MEGA (v 4.0) (Tamura et al. 2007). Observed heterozygosity (H_o) and expected heterozygosity (H_e) were estimated using FSTAT (v.2.9.3.2) (Goudet. 2002).

6.3 RESULTS AND DISCUSSION

6.3.1 Amplified Regions of Chicken HSP-70 Gene

The two primer pairs used in the sequencing of chicken HSP-70 gene in the study resulted in a 200 bp promoter region, 112 bp 5´ UTR and 453 bp fragment of the chickens HSP-70 gene coding region. The study thus sequenced a 775 bp fragment of the chickens HSP-70 gene in different strains of indigenous Tswana chickens and the commercial broiler chickens.

6.3.2 Functional Motifs in the Partial Sequence of Chicken HSP-70 Gene

Functional motifs found in the regulatory regions (Promoter and 5´ UTR) of chicken HSP-70 gene in indigenous Tswana chickens and commercial broiler chickens included CAAT box, specificity protein 1(SP1), heat shock element (HSE2) and heat shock element 1(HSE1) and a TATA box (Figure 6.1). All these motifs were previously reported by Aryani et al. (2019) in four different strains of indigenous Indonesian chickens. According to Morimoto et al. (1986) the chicken HSP-70 gene shares 73% and 80% sequence homology with *Drosophila* and human HSP-70 genes respectively, including common features such as TATA box, CAAT box, SP1, HSE1, and HSE2 in the Promoter region. Heat shock elements in the promoter region are a common feature of both mammalian and plant HSP-70 gene. According to Zhao et al. (2020) sequence variations in heat shock elements, the position of the heat shock elements within the promoter and the molecular architecture of the heat shock elements may be responsible for the varying affinity in the Heat shock factors-Heat shock elements interaction which ultimately influence transcription rate of HSP-70 gene and consequently the magnitude of the heat shock response.

6.3.3 SNPs in the Partial Sequence of Chicken HSP-70 Gene

SNPs found in the partial sequence of chicken HSP-70 gene in normal, naked neck and dwarf strains of indigenous Tswana chickens (Table 16) and depicted in Figure 18, Figure 19, Figure 20, Figure 21, and Figure 22. There were no SNPs in the promoter region of chicken HSP-70 gene in normal, naked neck and dwarf strains of indigenous Tswana chickens as well as in the commercial broiler chickens. According to (Öner et al. 2017), SNPs in the promoter region of cattle may have negative effect on pregnancy, calving rate, spermatogenesis, and embryonic mortality. The monomorphism of the promoter region of HSP-70 gene in different strains of indigenous Tswana chickens and the commercial broiler may, therefore, be meant to guard against the afore-mentioned negative effects of mutations in the Promoter of chicken HSP-70 gene. Two SNPs (A303G, G309A) were found in the 5´UTR and while the other two SNPs (G427A, A628G) were found in the partial exon sequence of chicken HSP-70 gene. Gan et al. (2015) found 6 SNPs and 24 SNPS in the 5´UTR and coding region of chicken HSP-70 gene respectively, in Chinese indigenous chickens. More variation has been reported in the 5´UTR of bovine HSP-70 gene with a total of 43 SNPs (Öner et al. 2017). The A303G SNP in the 5´UTR was found in the three strains of indigenous Tswana chickens and the commercial broiler chickens while the G309A SNP was found only in normal and dwarf strains of Tswana chickens. An adenine nucleotide deletion at the 63rd nucleotide (A63 del mutation) was only found in a single chicken of the dwarf strain within what appears like a CAAT box,

According to (Öner et al. 2017) untranslated regions of genes play an important role in terms of gene expression levels and mRNA stability. The 5´UTR controls expression levels of the transcript (Öner et al. 2017); (Silver and Noble.2011) and variations or mutations in the 5´UTR may therefore directly influence the HSP-70 protein expression levels which may result in phenotypic differences in performance. SNP G427A was unique to the normal strain of indigenous Tswana chickens and SNP A628G was found in the three strains of indigenous Tswana chickens and the commercial broiler

chickens. More SNPs were thus found in the partial sequence of chicken HSP-70 in indigenous Tswana chickens than in commercial broiler chickens which is consistent with (Öner et al. 2017) who reported more variability in 5´UTR and 3´UTR regions of bovine HSP-70 gene among native Turkish cattle breeds than the exotic Holstein breeds.

Table 16: SNPs and the locations in HSP-70 gene sequence in three strains of indigenous Tswana chickens and commercial broiler chickens.

Strain	Nucleotide position**	Type	Change	Amino acid identity
Normal, Naked neck, Dwarf and Broiler	5´UTR, 303	Transition	a → g	
Normal and Dwarf	5´UTR, 309	Transition	g → a	
Normal	Exon, 427	Transition	g → a	Same, gt**g** → gt**a** (valine → valine)
Normal, Naked neck, Dwarf and Broiler	Exon, 628	Transition	a → g	Same, tc**a** → tc**g** (serine → serine)

*Altered nucleotides and amino acids are shown in bold

**Nucleotides position is based on GenBank No. AY143693.1

All the mutations found in the current study were transitional exchanges and the two SNPs in the coding region were silent (conservative) mutations that do not result in amino acids substitutions in the resulting protein. More transitional mutations (A/G or T/C) and lack of transversional mutations (A/C or T/G) in chicken HSP-70 gene is consistent with Vignal et al. (2002) who reported more transitional mutations over transversion mutations in the study of human and mammalian genomes. According to Lamolle et al. (2006) silent mutations in the coding regions of most genes introduce genetic diversity while maintaining protein integrity and functionality.

```
Ref:TGGCGCAGCGTAGAAAGCGAGACGGATCGAGAAAAAACAGGAAGAAGCCCGATCTGGCTG
Nak:------------------------------CTRGAAACAGGGAGA-GCCCGATCTGGCTG
Dwa:--------------------------------AAAAMRGGGAGGAAGCCCGATCTGGCTG

    CAAT box
Ref:CAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTACGCTTCTGATTGGGCAGGAGG
Nak:CAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTACGCTTCTGATTGGGCAGGAGG
Dwa:CA-TCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTTCCCTTCTGATTGGGCAGGAGG
A63 del
        SP 1
Ref:CAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGATTGGTCAACTGCGGCAGTCGGGT
Nak:CAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGATTGGTCAACTGCGGCAGTCGGGT
Dwa:CAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGATTGGTCAACTGCGGCAGTCGGGT

          CAAT     HSE2 / HSE1                            TATA box
Ref:GTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAAGAAGGCTAAGCGGACTATAAAG
Nak:GTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAAGAAGGCTAAGCGGACTATAAAG
Dwa:GTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAAGAAGGCTAAGCGGACTATAAAG
```

Ref=reference sequence, Nak=naked neck; Dwa=dwarf

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Ref:AGGGCGCGAGCGGCGCCGTAACGGCAGATCGCGCCGCAGACAGCAGCGAGAGCGGGCGGA
Nak:AGGGCGCGAGCGGCGCCGTAACGGCAGATCGCGCCGCAGACAGCAGCGAGAGCGGGCGGA
Dwa:AGGGCGCGAGCGGCGCCGTAACGGCAGATCGCGCCGCAGACAGCAGCGAGAGCGGGCGGA


Ref:GGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGAGTGGCTGACTGACCAGAGGA
Nak:GGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGAGTGGCTGACTGACCAAGAGG
Dwa:GGRGACGTRACTGCGAGCGAGCAAGTGACTGGCGGAGCGAGTGGCTGACTGACCAGAGGA
    ************************************************************
    A303G  G309A
```

Ref=reference sequence, Nak=naked neck; Da=dwarf

Figure 18: Functional motifs and SNP positions in the Promoter and 5´UTR of indigenous Tswana chicken HSP-70 gene.

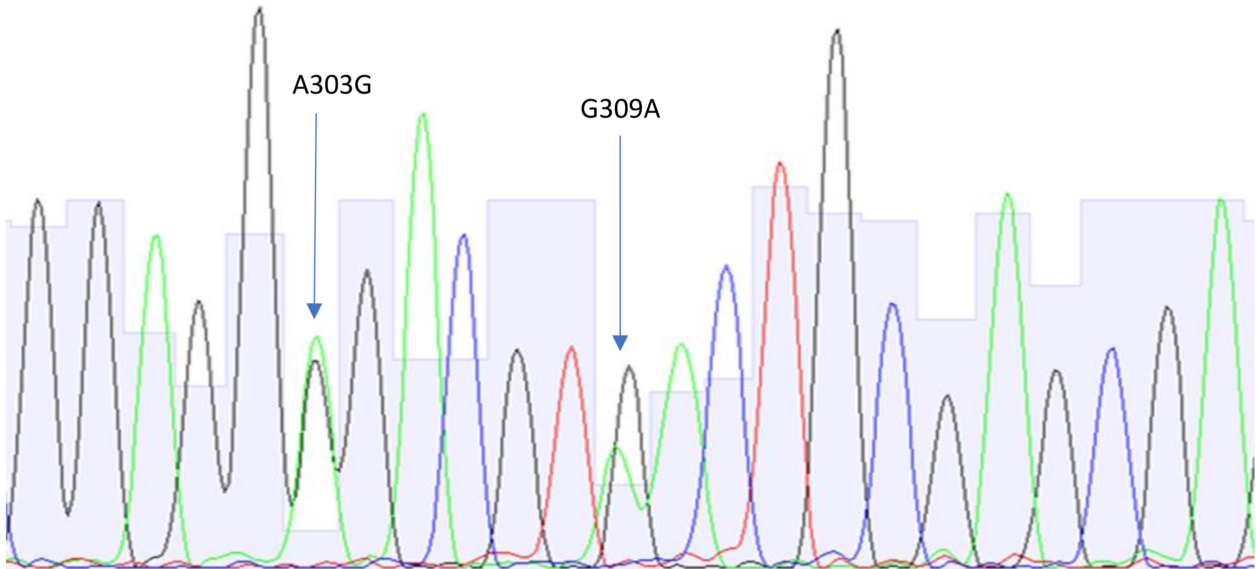
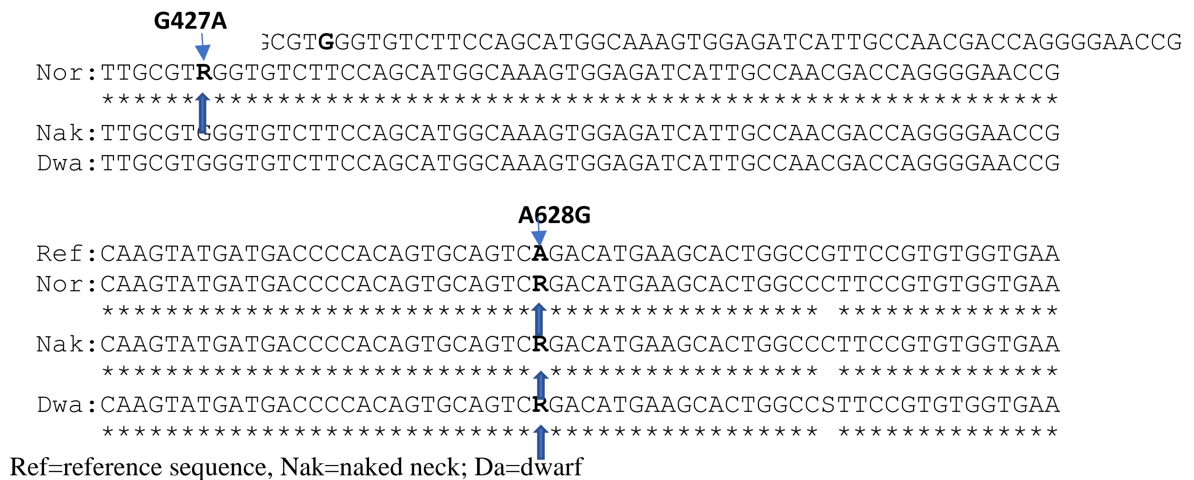


Figure 19: Sequence chromatograph showing SNPs in the 5´UTR of indigenous Tswana chicken HSP-70 gene



Ref=reference sequence, Nak=naked neck; Da=dwarf

Figure 20: SNP positions in the Partial exon sequence of indigenous Tswana chicken HSP-70 gene

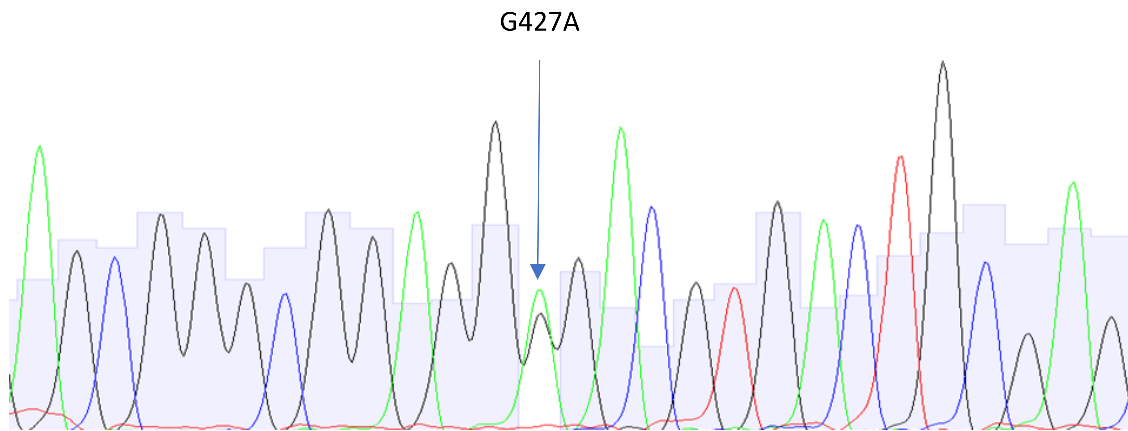


Figure 21: Sequence chromatograph showing the SNP in the partial exon sequence of indigenous Tswana chicken HSP-70 gene.

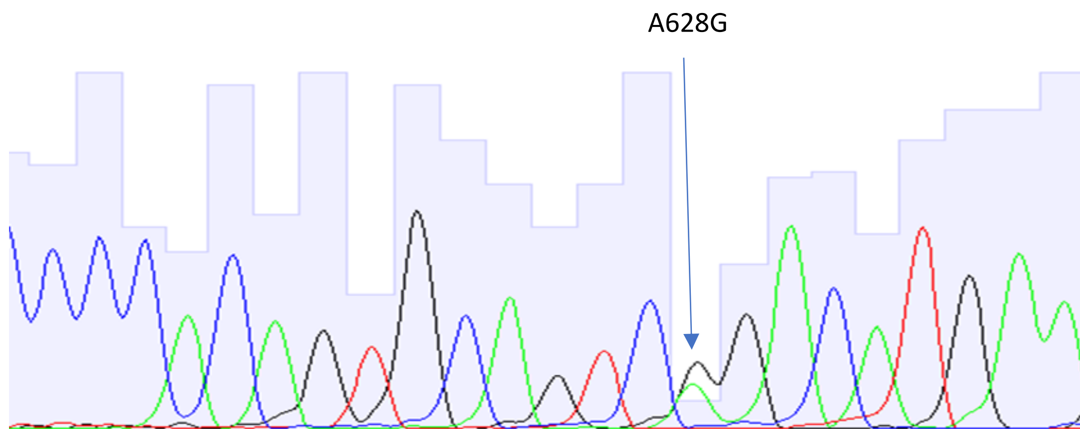


Figure 22: Sequence chromatograph showing the SNP in the partial exon sequence of indigenous Tswana chicken HSP-70 gene.

6.3.4 Allele and Genotype Frequencies at the 5´UTR of Chicken HSP70 Gene

Allele and genotype frequencies of the identified SNPs at 5´UTR of the chicken HSP-70 gene in different strains of Tswana chickens and the commercial broilers (Table 17). The A- allele at A303G locus was the most frequent in the normal, naked neck and dwarf strains of indigenous Tswana chickens and the commercial broiler chickens. The G-allele was the least frequent at A303G locus in all the four strains of chickens under the study suggesting that the A-allele was the wild type. At the G309 locus, the G-allele occurred at the highest frequency in all the four strains of investigated chickens. (Table 17). The A-allele at G309A locus occurred at low frequencies in the normal and

dwarf strains of indigenous Tswana chickens and was completely absent in the naked neck strain of indigenous Tswana chickens and the commercial broiler chickens suggesting that the A-allele is likely a new variant resulting from very recent mutation.

Allele and genotype frequencies of the identified SNPs in the partial sequence of the coding region of chicken HSP-70 gene in different strains of Tswana chickens and the commercial broiler (Table 17). The A- allele at G427A locus was the only one found in the normal strain of indigenous Tswana chickens and was completely absent in naked neck, dwarf and the commercial broiler chickens. The frequency of G-allele was higher than that of the A-allele at A628G in the normal strain of indigenous Tswana chickens. The A-allele occurred at a higher frequency than the G-allele in normal and dwarf strains of indigenous Tswana of chickens. The A-and G-alleles at A628G locus occurred at equal frequencies in commercial Ross broiler and the A-allele was completely absent in the naked neck strain of indigenous Tswana chickens. The naked neck strain was monomorphic for the G- allele at A628G locus resulting in 100% frequency of the G-allele in the naked neck population of indigenous Tswana chickens.

Table 17: Genotype and allele frequencies across the strains of indigenous Tswana chickens and commercial broiler.

		Allele	frequency		Genotype frequencies			
Strains	**Loci**	**G**	**A**	**GG**	**GA**	**AA**	**H_o**	**H_e**
Normal	A303G	0.42	0.58	2 (0.08)	16 (0.67)	6 (0.25)	0.67	0.49
	G309A	0.96	0.04	22 (0.92)	2 (0.08)	0	0.08	0.08
	G427A	0.87	0.13	20 (0.83)	2 (0.08)	2 (0.08)	0.08	0.23
	A628G	0.37	0.63	0	18(0.75)	6 (0.25)	0.75	0.47
Nakedneck	A303G	0.23	0.77	0	10 (0.46)	12 (0.55)	0.46	0.35
	G309A	1.00	0	22 (1.00)	0	0	0	0.0
	A628G	1.00	0	22(1.00)	0	0	0	0.0
Dwarf	A303G	0.14	0.86	0	4 (0.29)	10 (0.71)	0.29	0.24
	G309A	0.93	0.07	12 (0.86)	2 (0.14)	0	0.14	0.13
	A628G	0.43	0.57	0	12 (0.86)	2 (0.14)	0.86	0.49
Broiler	A303G	0.05	0.95	0	2 (0.10)	18 (0.90)	0.10	0.10
	G309A	1.00	0	20 (1.00)	0	0	0	0.0
	A628G	0.50	0.50	0	20 (1.00)	0	0	0.5

Genotype frequencies at A303G and G309A loci in the 5´UTR of the chicken HSP-70 in different strains of indigenous Tswana chickens and the commercial broiler chicken (Table 17). The heterozygous GA genotype at the A303G locus of chicken HSP-70 gene was the most frequent (0.67) followed by homozygous AA genotype, and lastly the GG genotype in the normal strain of indigenous Tswana chicken. Homozygous AA genotype at A303G locus was however, the most frequent followed by heterozygous GA in the naked neck and dwarf strains of indigenous Tswana chickens and the commercial broiler. The homozygous GG genotype at A303G locus was completely absent in sampled population of naked neck and dwarf strains of indigenous Tswana chickens and commercial broiler. The GG genotype at G309A locus was the most frequent, followed by the GA heterozygotes and lack of AA homozygotes in the sampled population of the normal strain of

indigenous chickens. The naked neck strain of indigenous Tswana chickens and the commercial broilers were monomorphic at G309A locus resulting in 100% homozygous GG genotype at that locus. The GG genotype at G309A locus occurred at the highest frequency, followed by GA heterozygotes and lack of homozygous AA genotype in the dwarf strain of indigenous Tswana chickens. The small number of individuals of different strains of indigenous Tswana chickens and the commercial broilers sequenced in the current study might have contributed to absenteeism of some alleles and genotypes (Kgwatalala et al. 2012). The authors observed that selection (both artificial and natural) also has the potential to change both allele and genotype frequencies in the population (Buffalo and Coop. 2020).

The homozygous GG genotype at the G427A locus of chicken HSP-70 was the most frequent (0.83) and only found in the normal strain of indigenous Tswana chicken. Homozygous AA and heterozygous GA genotypes at G427A locus in the normal strain of indigenous chickens occurred at a similar frequency of 0.08. The GG genotype at A628G locus was the only genotype in naked neck strain of indigenous Tswana chicken and was completely absent in the dwarf strains of Tswana chickens and the commercial broiler chicken. The heterozygous GA genotype at A628G locus occurred at a relatively higher frequency than the homozygous AA genotype in the dwarf strain of indigenous Tswana chicken and the commercial Ross broiler. All commercial broiler chickens were in fact heterozygous GA at A628G locus.

Genetic diversity measures (observed heterozygosity [H_o] and expected heterozygosity [H_e]) at different SNP loci are presented in Table 4. The highest genetic diversity (measured by H_o) was found at the A628G locus in the normal and dwarf strains and at A303G locus in the normal strain of indigenous Tswana chicken. Moderate genetic diversity was only found at A303G locus in the naked neck strain of indigenous Tswana chicken. Low genetic diversity was found at G309A locus in the normal and dwarf strains of Tswana chickens and at G427A locus and at A628G locus in the normal and dwarf strains of Tswana chickens, respectively. There was no genetic diversity at G309A and

A628G loci in the naked neck strain of Tswana chicken and commercial broiler. H_o was generally higher than H_e at A303G locus all the four populations under study at A628G in the normal strain, and at G309A and A628G loci in the dwarf strain indicating an excess of heterozygous individuals in the general population and possibly lack of inbreeding at those loci.

6.3.5 Haplotypes Found in the Partial Sequence of Chicken HSP70 Gene.

The SNPs reported in Table 16 linked up in individual chickens to produce haplotypes in the partial sequence of the HSP-70 gene shown in Table 18. The haplotypes are characterized by nucleotides at SNP loci 303, 309, 427, and 628 respectively, with SNP loci numbering according to GenBank: AY143693.1. Seven unique haplotypes were found in the four-chicken population under the study. The H1 haplotype was the most frequent and common to naked neck, normal and dwarf strains of Tswana chickens and the commercial broilers. The H2 haplotype was found in the normal, naked neck and dwarf strains of Tswana chickens and was completely absent in the commercial broilers. The H3, H4, and H5 haplotypes were unique to the normal strain of indigenous Tswana chickens and the H6 haplotype was found only in the naked neck and dwarf strains of indigenous Tswana chickens. The H7 haplotype was found only in the dwarf strain of indigenous Tswana chickens. More haplotype diversity at the HSP-70 locus was thus found in indigenous Tswana chickens compared to the commercial broiler chickens. This is not surprising as indigenous Tswana chickens have not been subjected to any form of artificial selection while the commercial broilers were subjected to intense selection during development, hence the low genetic diversity in commercial broilers. The high diversity at the HSP-70 locus in indigenous Tswana chickens might also explain their heat tolerance compared to the commercial broilers.

Table 18: Haplotypes and their frequencies (5´UTR and coding region of chicken HSP-70 gene) of indigenous Tswana chicken strains and commercial broiler chickens

Strains	Nucleotide's combinations	Haplotypes	Number	Frequencies
Normal	$A_{303}G_{309}G_{427}A_{628}$	H1	28	0.58
	$G_{303}G_{309}G_{427}G_{628}$	H2	14	0.29
	$G_{303}G_{309}A_{427}G_{628}$	H3	2	0.04
	$G_{303}G_{309}A_{427}A_{628}$	H4	2	0.04
	$G_{303}A_{309}A_{427}G_{628}$	H5	2	0.04
Naked neck	$A_{303}G_{309}G_{427}A_{628}$	H1	24	0.55
	$A_{303}G_{309}G_{427}G_{628}$	H6	10	0.23
	$G_{303}G_{309}G_{427}G_{628}$	H2	10	0.23
Dwarf	$A_{303}G_{309}G_{427}A_{628}$	H1	14	0.50
	$A_{303}G_{309}G_{427}G_{628}$	H6	10	0.36
	$G_{303}G_{309}G_{427}G_{628}$	H2	2	0.07
	$G_{303}A_{309}G_{427}G_{628}$	H7	2	0.07
Broiler	$A_{303}G_{309}G_{427}A_{628}$	H1	20	0.50
	$A_{303}G_{309}G_{427}G_{628}$	H6	20	0.50

6.4 CONCLUSION

There were no SNPs in the promoter region of chicken HSP-70 gene. Two SNPs (A303G and G309A) were found in the 5´UTR (A303G and G309A) and another two SNPs (G427 and A628G) were found in the partial exon sequence of the chicken HSP-70 gene. The SNP (G427A) was unique to the normal strain of indigenous Tswana chicken and the other three SNPs were common to all the four chicken strains studied. The identified four SNPs linked up in individual chickens resulting in a total of seven (7) different haplotypes in the studied four chicken populations. A total of seven different haplotypes were found in indigenous Tswana chickens and only two haplotypes were found in the commercial broilers.

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CHAPTER 7

7. GENERAL DISCUSSION

The current study sought to document qualitative and quantitative attributes of Tswana chicken under their natural production environment in the Southern part of Botswana. The main objective being to assess the variation in both qualitative and quantitative traits and DNA sequence variations in different strains of Tswana chicken to determine their diversity status and their potential for genetic improvement and to respond to the anticipated changes in their natural environment as a result of global warming and climate change and the ever-changing consumer preferences and taste.

The current study revealed considerable variation in both qualitative and quantitative attributes and molecular variation in the chicken HSP-70 gene in different strains of Tswana chicken compared to the commercial lines. Similar variations in both qualitative and quantitative traits (linear body measurements) have also been reported in indigenous chickens of Ethiopia, Nigeria, Algeria, Uganda, (Assefa and Melesse, 2018; Shuaibu et al., 2020; Dahloum et al., 2016; Beyihayo et al., 2022) and other Asian countries such as Thailand and Bangladesh (Buranawit et al., 2016; Azmal et al., 2006). The current diversity status of indigenous Tswana chicken clearly demonstrates minimal or no intentional selection for qualitative and quantitative traits. In addition, the results indicate minimal inbreeding and random mating in the general Tswana chicken population. Indigenous Tswana chicken farmers have a tendency of keeping very few breeding males and introducing new breeding males (population migrations) from other flocks and this explains the low inbreeding and maintaining diversity within the Tswana chicken population. The high diversity status of Tswana chicken attests to the minimal effects of population bottlenecks resulting from the occasional outbreaks of Newcastle disease.

Variation (both phenotypic and genetic variation) is the material for selection which is employed in genetic improvement programs to improve both appearance and productivity. The diversity in

qualitative traits in the Tswana chicken population presents an opportunity to select Tswana chickens to meet the varying preferences of the Botswana poultry farmers. Although qualitative traits are of no economic importance, they have the potential to influence the direction of selection as qualitative trait preference may influence the demand of product. The variation in quantitative traits found in the Tswana chicken population presents an opportunity for within breed selection to bring about improvements in traits of economic importance as opposed to crossbreeding which may result in faster improvements at the expense of fitness traits and erosion and eventual extinction of indigenous animal genetic resources(ref). The phenotypic and genetic diversity within the Tswana chicken population also presents the elasticity to respond to the changing environment because of global warming and climate change and offers an opportunity to reverse the direction of selection in response to the changing consumer tastes as the consumer becomes even more health conscious.

Genetic characterization of three (3) strains of Indigenous Tswana chicken using the 60K chicken SNP array confirmed moderate and higher levels of genetic diversity in the naked neck, normal and dwarf strains of Tswana chickens compared to the commercial broiler line. Moderate levels of genetic diversity imply that responses to selection could be realized in breeding programs you are now repeating unnecessary SNP data further revealed very close genetic similarities between the naked neck and normal strains of Tswana chicken (similar genetic backgrounds and just differences in physical attributes) and the genetic uniqueness of the dwarf strain of Tswana chicken. The genetic differences between the dwarf and the other two strains might imply differences in ancestries and lineages and possible different routes of introduction into the countries. In fact, the naked neck and normal strains of Tswana chicken correctly qualify to be classified as strains of one breed (Tswana breed) while the dwarf might be classified as a completely different breed. The dwarf strain occurs at a relatively low frequency within the Tswana chicken population and owing to its genetic uniqueness, efforts should be made as a matter of urgency towards its conservation and multiplication.

While the SNP study was aimed at global assessment of genetic variation in the three strains (Naked neck, normal and dwarf) of indigenous Tswana chicken the last study sought to assess variation in HSP-70 gene which plays a major role in the regulation of thermotolerance in chicken. The current study revealed more DNA polymorphisms (SNPs and INDEL) in the promoter and 5' UTR region of the chicken HSP-70 gene which translated to more haplotype diversity in indigenous Tswana chicken compared to the commercial broiler line. The higher haplotype diversity in indigenous Tswana chicken compared to the commercial broiler line could be the basis for better heat tolerance in Tswana chicken compared to the commercial broiler line and represents the potential ability of Tswana chicken to better adapt to the ever-increasing environmental temperature resulting from global warming and climate change.

6.6 CONCLUSION

Phenotypic variability could be the main distinguishing characteristics of indigenous Tswana chicken populations in the Kweneng and Southern districts of Botswana. Variations in linear body measurement traits were observed indicating the existence of genetic differences in major performance traits, which makes selection between indigenous populations a viable option to improve the genetic potentials of local chicken populations. The naked neck, normal and dwarf strains of Tswana chicken had similar, moderate genetic diversity measures (observed and expected heterozygosity which was significantly higher than those of the modern commercial broiler chicken). The principal component analysis (PCA) which was used to get an insight into the population structure of indigenous Tswana chickens and the commercial broiler revealed a set of three clusters or wild ancestors.

6.7 RECOMMENDATIONS

Based on the findings of the current study, it would be beneficial if phenotypic and genetic characterisation of indigenous Tswana chickens could cover the remaining districts of the country. It is also recommended that the genetic characterisation be expanded to include all the five strains (normal, naked neck, dwarf, frizzled and rumples frizzled) of indigenous Tswana chicken. Given the moderate genetic variation of indigenous Tswana chicken, it is recommended that within breed selection be employed to improve Tswana chicken in various traits of economic importance such as meat and egg production. Special attention should also be given to the dwarf strain of Tswana chickens in conservation programmes given its genetic uniqueness compared to the naked neck and normal strains.

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APPENDIX 1. SURVEY QUESTIONAIRE

Date:

Respondent's name:

District:

Village/Location/Area:

Farm type: Commercial____Communal___Institutional_________

SECTION A-PERSONAL CHARACTERISTICS

1. Gender of respondent (record without asking) 1-❒ Male 2-❒ Female

2. Gender of household head 1-❒ Male 2-❒ Female

3. Household head's marital status 1-❒ Married 2-❒ Single 3-❒ Divorced 4-❒ Widowed 5-❒ Separated.

4. Age of household head (If unknown, judge by probing e.g., asking historical events) 1-❒ 20 – 40 2-❒ 41 – 60 3-❒ ≥61and above

5. Education level attained.
   1-❒ None 2-❒ Primary 3-❒ Secondary 4-❒ Tertiary 5-❒ Vocational (Goat production, Beef production, Poultry management, Bee keeping , Seeds conservation)

6. Economic status of the family 1-❒ Poor 2-❒ Medium 3-❒ Rich

7. Total number of animals (birds) kept by a farmer.
   ____

8. How long have chickens been kept in the household? 1-❒ 0-5 years 2-❒ 6-10 years 3-❒ 11-15 years 4-❒ ≥16 years

9. Which chicken strain(s) are you keeping?
   1-❒ Dwarf 2-❒ Naked neck 3-❒ Frizzled 4-❒ Normal 5-❒ Rumpless

10. Where do you obtain the replacement stock? 1-❒ (Inherited Relatives, Neighbours, friends) 2-❒ other farmers 3-❒ LIMID programme 4-❒ Mafisa payment 5-❒ Exchange with males

11. Which one of the following do you spend more money on? 1-❒ Purchase of birds 2-❒ Purchase of feeds 3-❒ Purchase of veterinary products

12. Where do you obtain funds to finance your family poultry farming? 1-❒ salary 2-❒ other piece jobs/drought relief 3-❒ Son`s/daughter`s support 4-❒ Home business (Home brew sales, farm sales, hawker) 5-❒ Old age funds

13. How much time per day do you and your family spend on checking the birds?
1-❒ 0-1 hour 2-❒ 2-3 hours 3-❒ more than 3 hours

14. Are you happy with the productivity of chickens? 1-❒ Yes 2-❒ No

15. Do you think an improvement of Tswana chickens is needed? 1-❒ Yes 2-❒ No

16. What type of management system do you use to raise your poultry?
1-❒ Extensive 2-❒ Semi-intensive 3-❒ Intensive

17. Where do birds sleep at night?
1-tree branch 2-perches (Ground , Sekereme) 3-❒ Unused materials (old, corrugated sheets, bricks, old donkey cart) 4-❒ Improved local chicken house

18. How often do you remove chicken faecal contents or litter?
1-❒ Daily2-❒ Once in three months (Fortnightly, Once a week, once a month, twice a month)3-❒ Once in six months 4-❒ Never 5-❒ Annually

19. Who is responsible for removing chicken manure from sleeping area? 1-❒ man 2-❒ woman 3-❒ children (boys, girls) 4- other relatives

21. Do you provide supplementary feeding to your birds? 1-❒ Yes 2-❒ No

22. If yes, what type of feed materials do you give to your chickens?
1-❒ Mixed chicken feeds (yellow maize, sorghum, Sunflower) 2-❒ family grocery (rice, samp) 3-❒ Bran 4-❒ White maize 5-❒ Marotse

23. Do you produce your own feeds? 1-❒ yes 2-❒ no.

24. If answer to question 23 above is no, where do you buy your feeds for chickens?
1-❒ other farmers 2-❒ Supermarket (choppies) 3-❒ Marketing board
4-❒ Cooperatives

25. How often do you feed your birds in a day? 1-❒ Once 2-❒ Twice
3-❒ Thrice or more 4-❒ none

26. How do you feed your chickens? 1-❒ Put feed in containers 2-❒ broadcasting

27. If you do not feed, what could be the reasons for not providing supplementary feeds?
1-❒ Unavailable 2-❒ Expensive 3-❒ Lack of cash 4-❒ No time to feed

28. Do you provide your birds with water? 1-❒ Yes 2-❒ No

29. Who is responsible for providing your chickens with water? 1-❒ man 2-❒ woman 3-❒ children 4-❒ herd-boy

30. If you supply water for chickens, where do you obtain it?
1-❒ Borehole 2-❒ Rainwater 3-❒ River 4-❒ Tap water

31. What types of containers do you use to supply water for your birds? 1-❐ cut tyre 2-❐ old dish and galvanised bath 3-❐ Containers lids (rubbish bin lid, Clay pot lid, Paint bucket lid) 4- prepared pit

32. How often do you wash the water containers? 1-❐ Daily 2-❐ Fortnightly (Once a week, twice a week) 3-❐ Once a month 4-❐ Never

SECTION B-PRODUCTIVITY

33. What is the productivity of your chickens in the following table? *

Strains of chickens	Age at sexual maturity(month) Hen	Cock	Number of clutches?		Average No of eggs per clutch	Average No. of days per clutch		No. of chicks hatched per clutch	No. of chicks surviving to adulthood
Dwarf									
Neckedneck									
Frizzled									
Normal									
Rumpless									

35. What is the main method of incubation in your farm? 1-❐ Natural (hen sitting) 2-❐ incubators

36. At what age (months) do your birds reach slaughter weight? 1-❐Time (slaughter age) 2-❐ depends on size 3-❐ depends on relish demand 4-❐ depends on number of males

CULLING OF CHICKENS

37. Do you cull your birds with purpose at any time? 1-❐ Yes 2-❐ No

38. For what purpose do you cull your birds? 1-❐ Consumption 2-❐ Sale 3- ❐ Gifts

39. What factors determine the culling process of birds?
1-❐ Increased number of birds 2-❐ lack of feeds
3-❐ cash and consumption 4-❐ lack of proper housing

40. Are there any production constraints to Tswana chickens' production and productivity?
1-❐ Yes 2-❐ No

41. If your answer to question 37 is yes, state challenges to Tswana chicken production?
1-❐ Diseases 2-❐ Parasites 3-❐ Predators (small wild animals, dogs, cats) 4-❐ Thieves 5-❐ Others (harsh environment, lack of feeds, lack of proper housing, poor management, overgrown feathers, lack of males, neighbourhood jealously)

SECTION C-HEALTH AND DISEASE CONTROL

42. Have you ever experienced serious fowl disease outbreaks in your farm?
    1-❐ Yes 2-❐ No
43. What actions do you take when birds fall sick?
    1-❐ Treat them myself 2-❐ Call in veterinary personnel 3-❐ Kill and consume
    4- Burry dead ones immediately

44. Name the most common diseases you have come across in your flock?
    1-❐ Newcastle 2-❐ fowl-pox 3-❐ Respiratory infection 4-❐ Botulism

45. What is the most susceptible age to diseases from all the strains kept?
    1-❐ Adults 2-❐ growers 3-❐ young chicks 4-❐ all ages

46. If you do treatment yourself, what kind drugs or vaccines do you use to administer to your birds? 1-❐ terramycin soluble powder

47. Where do you obtain or buy chicken drugs and vaccines? _
    1-❐ LAC 2-❐ borrow from neighbours 3-❐ Vet clinic shops

48. Have you ever used traditional remedies to treat your birds? 1-❐ Yes 2-❐ No

49. Give the names of traditional remedies used for treatment of infections.
    1-❐ Indigenous trees (mokgwapa, Sekaname, Monepenepe, Mogalakane) 2-❐ Potassium permanganate & Brake fluid 3-❐ Tobacco leaves 4-❐ Onions

50. Which parasites affect your chickens?
    1-❐ Tampans 2-❐ lice 3-❐ mites 4-❐ fowl fleas

51. What do you use to control parasites on your birds?
    1-❐ wood ash 2-❐ karba dust 3-❐ Foreign chemicals (blue death, cattle dip, paraffin, used engine oil) 4-❐ warm water and clothes washing power

SECTION D-MARKETING

52. Where do you sell your chicken and chicken products?
    1-❐ Individual farmers 2-❐ Schools 3-❐ Catering services 4-❐ Supermarkets

53. What are some of the challenges concerning chicken marketing in your farming experience?
    1-❐ Price refusal 2-❐ unavailable buyers @ right time 3-❐ Lower prices

54. How often do sell your chickens?
    1-❐Whenever chickens available and depends on size 2-❐ Never
    3-❐ Seasonally and annually 4-❐ depends on buyers' availability

55. Give reasons for selling your chickens?
    1-❐ Cash and home consumption 2-❐ to reduce number of chickens
    3-❐ affordable feeding 4-❐ to satisfy consumers

56. What are average unit prices of any of the following chicken products in the table below?

Type of Products	Unit Price
Eggs	
chicks	
Growers (pullets & cockerels)	
Adult male	
Adult female	

SECTION E-EXTENSION SERVICES

57. Do you receive any technical support on chicken production from extension officers?
1-❒ Yes 2-❒ No

58. What type of support are you receiving?
1-❒ Advice medications 2-❒ Never reported 3-❒ vaccinations

59. How often do you receive the support from extension officers?
1-❒ Once in week 2-❒ Never 3-❒ Once in a month 4-❒ whenever called for

60. Are you satisfied with poultry extension services? 1-❒ Yes 2-❒ No

61. If your answer to question 56 above is no, what do you think can be done?
1-❒Try to visit us 2-❒ don`t know 3-❒ self-help due to distance 4-❒ inform government top officials

62. In case, you heard about improved poultry practices, what is your source of information on improved poultry production?

1-❒ Extension officer 2-❒ Relatives/ Neighbours 3-❒ Media (Radio, Television, Newspaper) 4-❒ Kgotla meeting

APPENDIX 2. PUBLISHED ARTICLES locations.

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Full Length Research Paper
Phenotypic characterization (qualitative traits) of various strains of indigenous Tswana chickens in Kweneng and Southern districts of Botswana.

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Genetic Diversity and Population Structure of Three Strains of Indigenous Tswana Chickens and Commercial Broiler Using Single Nucleotide Polymormophic (SNP) Markers.

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Genetic Polymorphism in the Promoter and 5´ UTR of HSP-70 Gene in Three Strains of Indigenous Tswana Chickens and Commercial Broilers.

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