

**CHARACTERIZATION AND DEVELOPMENT OF SELECTION INDICES FOR
GENETIC IMPROVEMENT OF UGANDAN INDIGENOUS CHICKENS**

BEYIHAYO GEOFFREY AKIIKI

REG. NO: 19/U/3189/PhD/PS

BSC. AGRIC-ANIMAL OPTION; MSC. ANIMAL SCIENCE

(MAKERERE UNIVERSITY)

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JULY, 2025

DECLARATION

I, **BEYIHAYO Geoffrey Akiiki**, hereby declare that this dissertation is entirely my original work and has not been submitted to any institution of higher learning for the award of a degree.

Signature: 

Date th16-07-2025

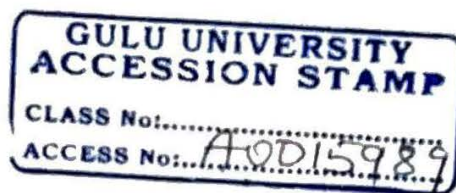


APPROVAL

This dissertation was submitted with our approval as the supervisors to the Directorate of Research and Graduate Training of Gulu University.

1) Associate Professor Donald Rugira Kugonza (PhD)

Makerere University.



A handwritten signature in dark ink, appearing to be 'K. Kugonza'.

16-07-2025

Signature.....

Date

2) Associate Professor Elly Kurobuza Ndyomugenyi (PhD)

Gulu University

A handwritten signature in dark ink, appearing to be 'Elly Kurobuza Ndyomugenyi'.

Signature.....

Date ...22/07/2025

3) Associate Professor Richard Echodu (PhD)

Gulu University

A handwritten signature in dark ink, appearing to be 'Richard Echodu'.

Signature.....

Date ...22nd - July 2025

DEDICATION

This dissertation is dedicated to my parents (Eribankya S. Muhonge Adyeeri and Birungi Josephine Amooti), my wife (Aturihaihi Christine Amooti) and children (Beyeza I. Abwooli; Baleka P. Ateenyi; Kyahurwaboona E. Amooti and Besisira J. Apuuli).

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ABBREVIATIONS AND ACRONYMS

AnGR	Animal Genetic Resources
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
GA	Genetic Advance
h^2	Broad Sense Heritability
IC	Indigenous Chickens
ISAG	International Society for Animal Genetics
mtDNA	mitochondrial Deoxyribonucleic Acid
NARO	National Agricultural Research Organization
RPE	Percent Relative Efficiency
SAARI	Serere Agricultural and Animal Research Institute
SDG	Sustainable Development Goals
USD	United States Dollar

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DEFINITION OF KEY TERMS

Ecotype: This is a population or group of chicken adapted to local ecological conditions, exhibit phenotypic differences within the group and are capable of interbreeding with other ecotypes.

Genetic correlation: Is the proportion of variance that two traits share due to genetic causes.

Heritability: The proportion of phenotypic variability attributed to genetic factors. Also described as the proportion of parent variance that is passed onto the offspring. The values of heritability range from zero to one.

Indigenous Chicken: This refers to localized, adapted, hardy and multi-purpose chickens associated with scavenging.

Morphometric measurements: This is the quantitative analysis of body dimensions (including shape, size and weight) used as indicators chicken productivity and phenotype.

Phenotypic correlation: Is the sum of genetic and environmental components of variation.

Selection index: Is a single value resulting from the summation of weighted trait scores and is used to assess candidate animals based on multiple traits. The traits are weighted according to their economic importance.

Traits: Is a character or feature of interest when breeding/selecting individual chickens for example; egg number, clutch size and body weight.

ABSTRACT

Indigenous chicken (IC) farming is constrained by their innate slow growth rate and low egg production. To date, Ugandan IC have not been appropriately characterized, and low-cost breeding tools are lacking for their genetic improvement. Therefore, this study assessed the phenotypic and genetic diversity, estimated genetic parameters, and designed selection indices for the genetic improvement of growth and egg production traits of IC. Phenotypic diversity was assessed based on morphometric measurements of 576 IC sampled across the 9 agro-ecological zones of Uganda. To assess genetic diversity, whole blood was collected from the same IC for DNA extraction and subsequent genotyping based on 15 microsatellites. To estimate genetic parameters and construct selection indices, measurements of body weight, shank length and reproductive traits of hens corresponding to three consecutive generations were recorded. Phenotypically, Ugandan IC ecotypes were not unique from each other. Genetically, Ugandan IC ecotypes belong to two major population clusters and were characterized by large genetic admixture. The first cluster, population one, comprises the Teso, Nsoga, and Gisu ecotypes, while the Madi, Nganda, Nyoro, Lango, Acholi, and Ankole chickens belong to the second cluster, population two. Weight of hens at four weeks of age (W4) and weight at on-set of lay (WOL) were components of the top-ranked selection indices in the equal (w_1) and broad sense heritability (wh^2) weight categories. Selection index $I_{17} = 3.25W_4 - 176.59SL_4 + 0.84W_{12} + 22.12SL_{12} + 0.38WOL + 21.89EW + 2.51EN$, with the highest genetic advance, is recommended for genetic improvement of growth and egg production traits. Incorporating more traits in the index increases the value of genetic advance, the number of generations, and years required to achieve the breeding objectives. In conclusion, shank length at 8 and 12 weeks of age is a phenotypic marker of higher body weight at first egg and egg yield, respectively. It is recommended that genetic parameters of qualitative traits such as broodiness be estimated and consequently incorporated in the selection indices.

DISSERTATION STRUCTURE

This dissertation has been developed based on paper format as guided by the graduate handbook (Page 66). Chapter One consists of the introduction to the study, review of literature, statement of the problem, objectives, hypotheses, and the significance of the study.

Chapter Two assessed the diversity of nine IC ecotypes based on quantitative phenotypic traits. Specifically, the nine IC ecotypes were compared based on live-body weight, and linear body measurements. Consequently, the diversity among IC ecotypes was established based on Principal Component Analysis of morphometric measurements, egg geometry, and live weight estimation models. The study provided a comprehensive insight into the relationship among chicken ecotypes. Upon establishing the relationship among IC ecotypes, genetic and phenotypic parameters were estimated to accommodate all the ecotypes in the Chapter Four. A genetic diversity study was conducted in Chapter Three to confirm the results and conclusions drawn from the phenotypic study. Out of this Chapter, a manuscript titled “In-situ morphological characterization of indigenous chicken ecotypes in Uganda” was produced and published in the journal *Ecological Genetics and Genomics (Elsevier)*.

Chapter Three assessed the diversity of ICs at a genetic level. Specific sequences within genomic DNA were amplified using a set of 15 microsatellites recommended by the International Society for Animal Genetics (ISAG) and the Food and Agriculture Organization of the United Nations (FAO). The results of the genetic analysis were used to assess the morphological similarity and clustering of IC ecotypes proposed in Chapter Two. In addition, Analysis of Molecular Variance (AMOVA) was performed and variance within and across ecotypes estimated. Genetic distance across ecotypes, microsatellite polymorphism, and loci diversity were estimated. Population structure analysis was performed using a phylogenetic tree, factorial analysis, and by the construction of the dendrogram. In conclusion, Ugandan IC

ecotypes were divided into two clusters based on genetic relatedness. The results of Chapter Two and three guided the selection of superior chickens across ecotypes. The grouping of IC as superior or control flocks was then used to estimate the genetic and phenotypic parameters associated with IC in Chapter Four.

In Chapter Four, genetic and phenotypic parameters associated with IC were estimated. Heritability, variance, genetic and phenotypic correlation of productive and reproductive traits of IC hens under a supplementary scavenging system were estimated. Phenotypic markers of the desired productive and reproductive traits were also identified. The genetic and phenotypic parameter estimates serve as raw materials when designing alternative selection indices for use in breed improvement programs aimed at the genetic improvement of multiple traits. On the basis of the genetic and phenotypic parameters estimated in the current Chapter, alternative selection indices were constructed in the next Chapter. A journal article titled “Genetic and phenotypic parameter estimates for selection within Ugandan indigenous chickens” was published in the journal *Tropical Animal Health and Production* (Springer).

Chapter Five presents alternative selection indices for indigenous chickens based on growth and egg production traits. Alternative selection indices were constructed based on either equal or heritability as weight factors. In addition, the number of generations and years of breeding required to achieve at least 24 percent genetic improvement were estimated. The alternative indices provide breeders with a range of options they can select from to meet specific objectives. A manuscript titled “Alternative selection indices for simultaneous genetic improvement of multiple traits within indigenous chickens” was developed, and an abstract was published in a conference proceeding.

Finally, the conclusions and recommendations are presented in Chapter Six. This Chapter highlights the main findings of the study, innovations, limitations, and contributions of the study, and a take home-message.

CHAPTER ONE

INTRODUCTION

1.0 Background

In Africa generally and Uganda in particular, rural smallholder poultry farming is dominated by indigenous chickens (ICs), which account for 90% of the chicken population (FAO, 2014). Considering the harsh effects of climate change, ICs adapt well and can overcome the challenges associated with water scarcity, increasing temperature, declining feed resources, high incidence of diseases and parasites (Assan *et al.*, 2017). As they scavenge, ICs play a pivotal role in nutrient utilization and recycling, thus enhancing the performance of mixed livestock-crop farming systems (Wong *et al.*, 2017). Apart from income, ICs are kept in mixed farming systems as insurance against shortfalls in productivity of crops or other enterprises (Ssewanyana, 2008; Natukunda *et al.*, 2011). Nutrition-wise, consumers prefer indigenous chicken meat, which is tasty, less fatty and has tough muscles compared to exotic types especially when making dishes on special days or celebrations (Magala *et al.*, 2012). Consumers tend to avoid exotic chicken meat as they associate them with drug residues (Ssewanyana *et al.*, 2001; Kyarisiima *et al.*, 2011). Eggs from IC have a yellow yolk and are more tasty than exotic eggs, thus driving a high consumer preference (Ssewanyana *et al.*, 2008; Yussif *et al.*, 2023). As such, IC and their eggs cost two to three times more than their exotic counterparts.

The reasons for keeping chicken have been evolving from the time of their domestication to the current uses in different regions of the world. Archaeological evidence suggests the introduction and existence of chickens at Kibiro in Uganda between 1780 to 1880 AD (MacDonald, 1992). At the time of introduction in Africa, IC served mainly social and cultural purposes including religious obligations (MacDonald & Edwards, 1993). However, with the

increasing population and money economy, IC are increasingly being reared for income generation (Lubandi *et al.*, 2018). Hence, IC can contribute to both household and national development.

At the household level, the reasons for rearing IC are largely similar across the regions of Uganda. Indigenous chickens are a source of protein, food security, and improved nutrition directly through the consumption of eggs and meat or indirectly through sales to acquire cash to access a diversity of foods (Lubandi *et al.*, 2018; Ssewanyana *et al.*, 2008). Ssewanyana *et al.* (2008) reported that 33% and 36% of the households rearing chickens directly consume eggs and chicken meat respectively. Alternatively, households sell their chickens and eggs and use the cash to meet family expenses like medication, food, clothing, and education among others (Ssewanyana *et al.*, 2008). In an effort to widen their income base, some households exchange chickens for other livestock like goats (Ampaire and Rothschild, 2010). As such, rearing IC is one avenue of accumulating the much-needed capital for vulnerable women and youth to invest in other large income-generating businesses (Rajkumar, 2021). Other uses of IC include presenting them to friends or relatives as gifts, and fulfilling cultural and religious obligations (Natukunda *et al.*, 2011; Ampaire & Rothschild, 2010; Ssewanyana *et al.*, 2008). Scavenging chickens also improve the productivity and profitability of crop-livestock systems by modifying the household ecology by providing manure, eliminating pests, and nutrient recycling (Wong *et al.*, 2017).

At the national level, rearing IC is one pathway towards attainment of the Sustainable Development Goals (SDG), starting with poverty eradication (SDG one), ending hunger (SDG two), guaranteeing healthy lives and promoting well-being for all at all ages (SDG three) (Wong *et al.*, 2017). Indirectly, IC increase access to quality education (SDG four) and promotes gender equality (SDG five) among the smallholder households in Uganda (Huck, 2023; Wong *et al.*, 2017). As of 2015, the IC population was estimated at 40 million producing

65,000 tons of meat and 0.9 billion eggs valued at USD 87.7 and 36 million respectively (MAAIF & UBOS, 2018). Therefore, a streamlined and well-designed IC sub-sector can contribute significantly to Uganda's Vision 2040 that was launched in 2013 (National Planning Authority, 2013).

On the breeding aspect, IC present a diverse genetic resource and possess specific traits for future breeding programs. This is because, IC have acquired genetic mutations that enable them to fit well and remain productive in their environment despite the challenges associated with climate change including water scarcity, increased ambient temperatures, declining feed resources, and high incidence of diseases, and parasites (Assan *et al.*, 2017; Fleming *et al.*, 2016). Their innate broodiness and mothering ability make IC the most suitable, economical and sustainable source of replacement or stock multiplication in most rural areas of Uganda, where incubators are absent. The highest percentage of eggs (45%) are reserved for chick production using natural incubation, while sales and consumption account for 20% and 33% respectively (Ssewanyana *et al.*, 2008; Kayitesi, 2015). Compared to the exotic breeds, rural resource-constrained households prefer to rear scavenging chickens for their known hardiness, excellent scavenging and resistance to common diseases and parasites (Ssewanyana *et al.*, 2008). As such, IC are a reserve of important adaptive and economic traits and provide a fallback position for developing new breeds.

1.2 Production and reproductive potential of indigenous chickens

Several studies have been undertaken to establish the productive and reproductive potential of Ugandan ICs. The productive and reproductive performance of IC sampled from Western, Central, Eastern and Northern districts of Uganda are summarized in Table 1.1. The performance of ICs varies across different regions of Uganda (Kugonza *et al.*, 2008; Ssewanyana *et al.*, 2008; Mukungu, 2019). These differences in performance are associated with variations in available feed resources, which is a result of the farming system practiced

and the overall climate of the agro-ecological zone (Mwacharo *et al.*, 2013). The differences in performance is also an indication of adaptability to the local environment and the significant variation that presents genetic resources for breed improvement (Ssewanyana *et al.*, 2008; Fleming *et al.*, 2016).

Variation in the performance of ICs within regions has also been reported (Dessie *et al.*, 2011; Ssewanyana, 2004). For example, a study on cockerels and pullets raised in the Northern districts of Gulu and Kiryandongo reported variation in age at sexual maturity. In the same study, hens in Gulu performed better than those in Kiryandongo in terms of eggs hatched (9.8 vs 8.7), number of chicks weaned (6.7 vs 5.8), except for eggs laid per clutch, where hens in Kiryandongo performed better (12.3 vs 13.9) (Nakkazi *et al.*, 2014). However, the difference in the performance of IC within and among regions were largely estimated by farmers during surveys (Ssewanyana *et al.*, 2008; Kugonza *et al.*, 2008). As such, the validity of performance estimates depends on the memory and experience of the chicken farmer as households do not keep records.

Table 1.1: Performance of indigenous chicken across the regions of Uganda

Variables	East ¹	East ²	Central ¹	Central ⁴	West ¹	North ³	Overall means
Average weight of adult cocks (kg)	2.0	-	2.4	1.7	1.7	-	2.1
Average weight of adult hens (kg)	1.4	-	1.5	1.3	1.2	-	1.4
Age at first egg (months)	6.9	6.3	7.9	7.9	7.6	-	7.2
Age to sexual maturity-pullets	-	6.5	-	8.0	-	6.1	6.3
Age to sexual maturity-Cockerels	-	5.5	-	7.9	-	6.2	5.9
Egg production/hen/clutch	14	13	14	9	15	13	13.8
Hatchability (%)	86.6	90	90.3	91.2	82.3	82.5	86.5
Number of clutches per year	2.3	3.5	2.2	3.7	2	3.2	2.6
Clutch Interval (months)	2.2	2.5	3.0	-	3.5	-	2.8
Hatch-weaning period (months)	2.7	-	2.8	1.2	3.4	-	2.9
Average number of chicks weaned	6	-	8	-	4	6	6

Source: ¹Ssewanyana *et al.* (2008); ²Kugonza *et al.* (2008); ³Nakkazi *et al.* (2014); ⁴Mukungu, 2019

More reliable performance indicators of IC have been collected from households supported to keep records of their flock (Nakkazi *et al.*, 2014; Kayitesi, 2015; Mukungu, 2019). Unlike the survey method, detailed performance indicators like feed conversion efficiency, daily weight gain, carcass yield, and dressing percentage have been reported from on-farm records. In a multi-location study, the performance of ICs in two districts of Gulu and Kiryandongo was studied over a period of 20 weeks on supported host farms (Kayitesi, 2015). On these supported farms, chickens raised in Gulu attained higher final live weight and growth rate of 1.5kg and 13.6g/day compared to 1.2kg and 10.8 g/day respectively for chickens raised in Kiryandongo. In addition, the same author also reported an increase of 57% in live weight when ICs were raised under the intensive system, implying that the environment, in form of nutrition, largely limits performance.

Whereas ICs to dominate the poultry sector (MAAIF & UBOS, 2018), their low genetic potential is responsible for the inadequate chicken meat and eggs available for consumption or

marketing (Natukunda *et al.*, 2011). At the same age, IC tend to weigh 55 percent less compared to exotic breeds (FAO, 2010). In addition, several challenges limit the population of IC and their by-products available on the market. Disease outbreaks, predators, poor nutrition, housing, and difficulty in accessing veterinary and extension services are major constraints to scavenging chicken productivity (Wong *et al.*, 2017). Irrespective of the harsh conditions and constraints, IC provide a substantive amount of meat and eggs (Mahendra, 2016). Therefore, the variation in performance and resilience of ICs provide a basis for their genetic improvement through selection.

1.3 Origin and distribution of indigenous chickens in Africa

The origin of domesticated multi-purpose chickens (*Gallus gallus domesticus*) in Africa can be traced to their wild relatives, the red junglefowls (*Gallus gallus*) with their cradle land in South Asia, Southwest China, and Southeast Asia (Fumihito *et al.*, 1996; Miao *et al.*, 2012). This is confirmed by a recent global mitochondrial DNA profile analysis which established a common haplogroup (haplogroup D) in the maternal lineage among chickens in Africa, South Asia, Southeast Asia and East Asia (Miao *et al.*, 2012). The close relationship between IC and red junglefowls in Asia is also linked to the fact that both have 21% haplotypes in common (Sawai *et al.*, 2010). Domestication of the chicken from the wild junglefowl is estimated to have occurred $58,000 \pm 16,000$ years ago (Sawai *et al.*, 2010).

In their cradleland, in the early years following domestication, chickens were important for non-food benefits including games like cock fights (Zuener, 1963). Currently, IC contribute to the income of rural mixed farmers through the sale of live chicken and or eggs, providing quality animal protein in addition to being a vital resource for the emancipation of women in developing African countries (Mahendra, 2016; Lubandi *et al.*, 2018; Yussif *et al.*, 2023).

1.4 Chicken breeds and ecotypes in Uganda

The chicken industry is characterized by indigenous and exotic breeds and their crosses. Indigenous chicken ecotypes include the Nyoro, Nganda, Nkedi, Nsoga, and Teso among others, and are largely named according to their region of origin in Uganda (Ssewanyana, 2004). The population of ICs increased by 27 percent corresponding to 41.7 million in a period of ten years starting from 2008 to 2017 (UBOS, 2018).

Exotic breeds of chicken are largely the specialized broiler or layer type and include Rhode Island Red, the Hubbard, Arbor Acres, Hybro, Bovans Brown, Bovine Goldline, and Australops among others (Ssewanyana, 2004). The population of exotic chickens increased by 28.1 percent corresponding to 5.9 million in a period of ten years ranging from 2008 to 2017 (UBOS, 2018).

In the last 10 years, dual-purpose birds became common in Uganda with most of them originating from Asia (DID, 2019). Currently, the Kuroilers are the dominant dual-purpose hybrid from the Kegg Farm breeders in India with a high feed efficiency and laying potential compared to IC (Semahoro *et al.*, 2018). Other dual-purpose chickens are Rainbow Rooster, Kenbro, and Sasso mainly from Kenchic and Kukuchick breeders in Kenya (DID, 2019). However, dual-purpose hybrids never become broody and farmers rely on the use of artificial incubators or IC and ducks to hatch the eggs (Lubandi *et al.*, 2018).

1.5 Indigenous chicken breeding programs in Uganda

1.5.1 Overview

Genetic and non-genetic approaches aimed at improving the productivity and profitability of ICs are on-going in Uganda. A sustainable chicken improvement program requires adequate investment in proper husbandry practices, elimination of diseases, supplementation and genetic improvement (Natukunda *et al.*, 2011; Mahendra, 2016). Genetic improvement can be achieved

through selection within the indigenous breeds, crossbreeding with imported commercial lines, or a combination of both selection and crossbreeding (Rajkumar, 2021; Mahendra, 2016). Although crossbreeding with imported breeds yields rapid genetic improvement compared to selection, its effects are not permanently fixed in the generations produced thereafter (Sorensen & Ssewanyana, 2003). Selection within IC populations, on the other hand, preserves the distinctive traits associated with multipurpose indigenous breeds (Ssewanyana, 2004). Possession of multiple traits by IC facilitates their compatibility with the resource-constrained village farming systems (Adoligbe *et al.*, 2020; Addisu *et al.*, 2014; Padhi, 2016). Therefore, genetic improvement programs should aim at preserving the majority of such traits (Dana & Waaij, 2010).

Presently, Uganda lacks a clear, streamlined breeding program for the genetic improvement and conservation of ICs (Dessie *et al.*, 2011). In comparison, commercial egg and meat chicken breeds have been developed in Asia and Europe over the years and are adequately genetically characterized (Liu *et al.*, 2019; Yuan *et al.*, 2015). However, in Africa, no systematic selection has been done and IC largely remain multipurpose (FAO, 2010). Uncontrolled breeding still occurring in many rural areas of developing countries has enabled IC to remain dominant compared to highly specialized breeds developed in Europe and Asia (Tixier-Boichard *et al.*, 2011). At the national and household level, breeding is uncontrolled and usually associated with inbreeding, and this is exacerbated by the lack of records by farmers, which makes the selection of replacement stock difficult (Dana *et al.*, 2010). Repeated importation of exotic breeds into the country to improve the productivity of IC through crossbreeding has drastically reduced indigenous chicken genetic resources and loss of their desirable traits (Kejela, 2020). Currently, crossbreeding with Kuroilers, a multipurpose chicken imported from India, improves body weight (Semahoro *et al.*, 2018) but eliminates or reduces the trait of broodiness from IC (Romanov *et al.*, 2002). Unfortunately, most chicken farmers have no access to

incubators and artificial brooders hence the need to keep the more efficient brooding mother hens to hatch and nurse their chicks (Ssewanyana, 2008; Roy *et al.*, 2004). Commonly, homegrown chicks are a source of replacement stock and a method of flock expansion (Ssewanyana, 2008). Therefore, Assan *et al.* (2017) suggested the development of well-adapted chicken by selecting from the existing populations, a strategy more compatible with the existing flock multiplication system. As such, the SAARI and Rakai chicken breeding programs were developed to exploit the genetic and adaptive potential of existing IC.

1.5.2 Serere Agricultural and Animal Research Institute Chicken Breeding Program

In a collaborative breeding program, Uganda imported exotic cocks of the Arbor Acres, Hybro, and Bovans Brown breeds to improve the growth and egg production potential of ICs. The implementation of the breeding program and performance of the offspring were reported by Sorensen & Ssewanyana (2003) and Ssewanyana *et al.* (1998). Briefly, the breeding program was stationed and coordinated at Serere Agricultural and Animal Research Institute (SAARI), a zonal institute of the National Agricultural Research Organization (NARO). As such, the chickens produced from the program were widely referred to as SAARI chicken. The breeding program used the approach of mating the exotic cocks with superior indigenous hens. The objective of the crossing was to take advantage of IC (adaptive traits) and exotic genes (fast growth and high egg production potential). The performance of the SAARI chicken was evaluated and compared to IC both on-station and on-farm. On-station, crossing with exotic breeds resulted in more than doubling the live-body weight and egg size of F1 and F2 progenies (Ssewanyana *et al.*, 1998). Sorensen & Ssewanyana (2003) reported the on-farm performance of SAARI chicken with 25% and 50% Bovans Brown genes compared to IC. As expected, the SAARI chickens with 50% Bovans Brown genes expressed a maximum of 29 % higher body weight at four months compared to chicken groups having 25 % Bovans Brown genes and IC. The higher weight gain of SAARI chickens with 50 % Bovans Brown genes

enabled their marketing at one month earlier compared to IC. However, at the age of seven months, the crosses and IC had similar weights. Unfortunately, the reproductive potential of the SAARI chickens was either inadequately investigated or is lacking.

1.5.3 The Rakai Chicken Model

The Rakai chicken model was a community-based breeding program implemented in the then-greater Rakai district of Uganda at the height of the AIDS scourge. The objective of the project was to improve the welfare of the largely orphan-headed households through improving the productivity of their IC. This breeding program was fully described by Roothaert *et al.* (2011). The farmers participated in identifying the traits for improvement, and the program largely utilized local extension agents. The Rakai chicken model utilized a three-breed cross involving IC, Bovans Brown, and a broiler cock. The breeding was based on indigenous chicken hens progressively selected for egg production. The selected elite IC hens were then crossbred with Bovans Brown cocks to produce the first filial generation, F_1 referred to as Rakai₁ (R_1), which were good layers compared to the IC. The F_1 offspring were then crossbred with broiler cocks to produce the second filial generation, F_2 referred to as Rakai₂ (R_2). Finally, the F_2 offspring were backcrossed with the selected IC cocks to produce F_3 referred to as Rakai₃ (R_3). As such, the chickens produced from the breeding program were referred to as Rakai chickens. In the third filial generation, F_3 offspring grew faster, laid more eggs, retained the flavor of IC, were disease resistant, and were easy to multiply and market. Participating households with flocks of 40 chickens on average were capable of earning a net income of US\$1,050 per annum. Unfortunately, the reproductive performance of the Rakai chickens was also not fully reported. Similar to SAARI chickens, the Rakai chicken cannot be traced among the Ugandan chicken populations.

1.6 Phenotypic and genetic selection of replacement breeding stock

The major source of replacement stock in households rearing IC is selecting within the offspring raised (Zemelak *et al.*, 2018; Ssewanyana, 2008). In the face of uncontrolled mating and lack of performance records, farmers use their experience to select chickens with desirable traits as breeding stock (Addisu *et al.*, 2014). Body weight, weight at first egg, age at first egg, egg productivity, and disease resistance are economic traits highly desired in breeding chicken (Mahaole *et al.*, 2023; Kebede & Tadesse, 2023; Fekede & Tadesse, 2021; Fitsum, 2017; Kayitesi, 2015; Addisu *et al.*, 2014; Okeno *et al.*, 2011). IC also possess several innate traits, including morphometric attributes, mothering ability, broodiness, and hatchability (Zemelak *et al.*, 2018). At the household level, chicken farmers use phenotypic markers as criteria for selecting replacement stock, and it is common for IC owners to use shank length to select for large body sizes in cocks (Fekede & Tadesse, 2021; Kayitesi, 2015). In Northern Uganda, most households prioritize body size, body length, growth rate, and uniform feathering in that order as the criterion for selecting breeding cocks (Kayitesi, 2015). On the other side, households rank size, egg productivity, mothering ability, and hatchability as critical traits in selecting breeding hens (Kayitesi, 2015).

In Kenya, chicken farmers prioritize the number of eggs laid per clutch, followed by body size, growth rate, hatchability, mothering ability, broodiness, disease resistance, egg size, and plumage color in that order when selecting breeding hens (Okeno *et al.*, 2011). On the other hand, when selecting breeding cocks, body size ranked first followed by growth rate, disease resistance, plumage color, and lastly hostility (Okeno *et al.*, 2011).

In Ethiopia, chicken owners rank egg size as the most important followed by plumage color, broodiness, hatchability, and lastly, mothering ability when selecting replacement hens. In selecting breeding cocks, chicken owners ranked egg production potential of its parent mother

hen, followed by comb type, plumage color, disease resistance, and lastly their growth rate (Fitsum, 2017).

The criteria for selecting replacement stock are based on qualitative and quantitative phenotypic traits observed in the chicken flock (Fitsum, 2017; Kayitesi, 2015; Addisu *et al.*, 2014; Okeno *et al.*, 2011). The selection criteria can be sex specific or applicable to both cocks and hens (Mahaole *et al.*, 2023; Fekede & Tadesse, 2021). Despite the use of different phenotypic traits as criteria for selecting breeding stock, heritability and genetic parameters of such traits have not been comprehensively studied or estimated (Ssewanyana, 2004). In addition, the use of phenotypic markers is unreliable, and a slow process, as selection is delayed until a time when such markers can be identified on an individual chicken (Vieira *et al.*, 2020).

1.7 Characterization of Indigenous Chickens

1.7.1 Overview

Indigenous chickens could be characterized phenotypically or genotypically. Indigenous chickens in Uganda were previously characterized based on flock structure per homestead, purpose, and their productive or reproductive performance (Ssewanyana *et al.*, 2008). Most recently, Ugandan IC were phenotypically characterized based on feather coverage or type (normal, naked neck, and frizzled), skin and leg color, comb type, and shape or size of earlobes (Yussif *et al.*, 2023). Unfortunately, the characterization of IC previously done in Uganda has limited value for estimating genetic parameters and the development of selection indices. Semakula *et al.* (2011) quantitatively characterized IC by determining the morphometric values of chickens within the Lake Victoria crescent of Uganda. The morphometric measurements were used by the same author, Semakula *et al.* (2011), to develop a live weight estimator model in an attempt to increase the economic value of IC. To date, Ugandan IC have not been fully characterized genetically. Partial genetic analysis on IC sampled from Nganda (from districts

of Masaka and Luwero) and Nsoga (Kamuli district) ecotypes revealed admixture, crossing over, and that all ecotypes had a common ancestry (Fleming *et al.*, 2016). Therefore, the characterization previously done in Uganda does not provide the required information and data for the derivation of genetic parameter estimates and the development of selection indices specific to IC.

1.7.2 Genetic parameter estimates of indigenous chicken traits

The initial critical stage before embarking on the genetic and productivity improvement of ICs is to characterize them through establishing the genetic parameters of traits to be selected (FAO, 2007). This is because successful inheritance and conservation of economic and non-economic traits among offspring is a property of genetic parameters and heritability (Rotimi *et al.*, 2016). Information from studying genetic parameters of IC guides the setting of breeding objectives, selection indices, timing of selection, and marker traits applicable in selection programs (Magothe *et al.*, 2010). Heritability, genetic and phenotypic correlations among traits are important genetic parameters used to characterize IC (Chomchuen *et al.* 2022; Sanda *et al.*, 2022; Singh *et al.* 2018).

1.7.2.1 Heritability of traits

Heritability is one of the genetic parameters that determines genetic gain, and its values are critical and inform the design and evaluation of chicken breeding programs (Ghazikhani *et al.*, 2007). Specifically, moderate to high heritability estimates are critical for successful breeding programs (Niknafs *et al.*, 2012). As such, heritability estimates of productive and reproductive traits determine the breeding approaches and, therefore, the success of the breeding program (Niknafs *et al.*, 2012).

The high heritability and genetic association between body weight and egg production among IC in Ethiopia at 16 weeks of age allowed indirect selection of hens for egg production based

on live weight at an early age (Nigussie *et al.*, 2011). Selection of IC across the ecological zones of Kenya for improved egg and body weight was possibly based on the high heritability values of body weight at 12 weeks and its high positive correlation with subsequent body weights up to 26 weeks of age (Magothe *et al.*, 2010). However, heritability estimates of a trait are specific to a particular environment for a given population and cannot be replicated for selection in another population (Chomchuen *et al.* 2022; Sanda *et al.* 2022; Gwaza *et al.* 2016; Dana *et al.*, 2011; Magothe *et al.* 2007). Apart from varying across populations and environments, heritability also varies with changing age and physiological status of the individual chickens being investigated (Nigussie *et al.*, 2011; Singh *et al.*, 2018). The variation in heritability of traits with age, therefore, requires timing of selection at a stage when heritability is at its peak. Indigenous chickens possess several innate traits whose genetic parameters have not been estimated, for example, heritability estimates of clutch size (eggs per clutch).

1.7.2.2 Genetic and phenotypic correlations

Genetic and phenotypic correlations determine a combination of traits that can be co-selected in a multi-trait breeding program. A moderate to high genetic correlation among traits is required to achieve a high breeding value (Niknafs *et al.*, 2012). Indigenous chickens are multi-purpose, hence, genetic improvement programs should aim at preserving most of the desired traits. The association between traits indicates the underlying gene-gene interaction. Egg production and body weight are examples of traits negatively correlated (Zonuz *et al.*, 2013; Niknafs *et al.*, 2012). Also, the gene-environmental or gene-gene interactions determine the expression of a trait and genetic gain in a selection program.

1.8 Selection indices and animal breeding

Unlike the specialized egg-type and meat-type chicken breeds, IC are multipurpose in nature and possess several traits (Mahaole *et al.*, 2023). Therefore, the superiority of an individual chicken is assessed based on multiple traits. As such, breeding programs targeting IC should evaluate the phenotypic and genetic superiority of individual chickens considering multiple traits (Fekede & Tadesse, 2021). The most appropriate breeding approach is one that considers multiple traits simultaneously at each stage of selection (Kebede & Tadesse, 2023). Over the years, geneticists have worked with statisticians to develop tools to aid the selection of superior replacement farm animals. Among them is the selection index or total score approach modified by Hazel for sheep (Hazel & Lush, 1942), beef cattle (Hazel, L.N. 1951) and pigs (Hazel & Kline, 1951). This selection index method is suitable for the simultaneous selection and improvement of multiple traits at a single generation. This approach has been widely applied in animal and crop breeding programs to simultaneously improve multiple traits (Goyani, 2022; Stephens *et al.*, 2012; Synthetic & Kaimook, 2022; Ziadi *et al.*, 2021). The selection index approach assesses candidate animals based on a single value resulting from the summation of economically weighted trait scores (Hazel *et al.*, 1994). Traits (economic traits) are weighted depending on their contribution to the market value of the individual animal. That is, a larger coefficient is attached to the economic trait that contributes more to the market value of the animal in the selection index (Lwelamira & Kifaro, 2010). Selection index as compared to tandem and independent culling level methods is more efficient and results in a multiple trait genetic improvement (Hazel & Lush, 1942). In addition, the selection index method has the potential to improve genetically antagonistic but economically important traits (Tseveenjav *et al.*, 2020).

Lwelamira & Kifaro (2010) suggested alternative selection indices for the simultaneous genetic improvement of disease resistance, body weight at 16 weeks of age, age at first egg, egg

number, and weight in the Tanzanian medium chicken ecotype. The same authors (Lwelamira & Kifaro, 2010) also suggested three selection indices targeting improved disease resistance, body weight at 16 weeks of age, and egg number in the Kuchi ecotype of Tanzania. Apart from chicken, selection indices were recently constructed for the genetic improvement of fleece weight, fiber diameter, and length among Mongolian Cashmere goats (Tseveenjav *et al.*, 2020). In the field of crop production, Goyan (2021) constructed two sets of selection indices for Indian beans corresponding to equal and heritability as weight factors. In addition, Goyan (2021) also estimated the expected genetic gain and percent relative efficiency (PRE) for all possible selection indices.

Selection indices are specific to animal breeds, production, and marketing systems. That is, indices constructed for a specific breed for a particular production environment cannot be replicated elsewhere without modification (Goyani, 2022; Lwelamira & Kifaro, 2010). The selection index method as a breeding tool targeting genetic improvement of Ugandan ICs is either absent or underdeveloped. Therefore, characterization of Ugandan ICs, estimation of their genetic parameters, and consequently construction of specific selection indices is overdue.

1.9 Statement of the Problem

Indigenous chicken farming in Uganda is constrained by their innate slow growth rate and low egg production, which are important economic traits in the chicken sector (Kebede & Tadesse, 2023; (Fekede & Tadesse, 2021; Sorensen & Ssewanyana, 2003). Consequently, IC contribute less towards household income and Uganda's Vision 2040 (FAO, 2022; National Planning Authority, 2007). Until recently, many developing countries, and Uganda in particular, lacked a streamlined breeding program for the sustainable use and conservation of ICs (FAO, 2007). As a result, Uganda's IC genetic resources have not been adequately characterized; as such, genetic parameters based on growth and egg production traits have not

been estimated. In addition, low-cost selection tools to improve growth and egg production traits of multi-purpose IC have not been developed.

1.10 General Objective

The overall objective was to characterize and develop selection indices for the genetic improvement of growth and egg production traits of Ugandan IC ecotypes.

1.10.1 Specific Objectives

The specific objectives were:

- i. To assess ecotype diversity of Ugandan ICs based on phenotypic traits.
- ii. To assess the genetic diversity of Ugandan IC ecotypes.
- iii. To estimate heritability, genetic, and phenotypic correlations of growth and egg production traits of Ugandan IC.
- iv. To develop selection indices for improved growth and egg production traits for Ugandan IC.

1.11 Hypotheses

- i. Indigenous chicken ecotypes are phenotypically unique from each other.
- ii. Ugandan IC ecotypes are not genetically identical.
- iii. The growth and egg production traits of IC ecotypes possess specific genetic parameters.
- iv. Localized selection indices can yield significant improvement in growth and egg production traits of the multipurpose IC.

1.12 Summary of research problem, gap, and corresponding objectives

The research problem, research gap, and the derived specific objectives are summarized in Table 1.2.

Table 1.2: Illustration of association among the research problem, gap, and objectives

Research Problem	Research gap	Research Objective
Innate slow growth and low egg production potential of IC	IC is not sufficiently characterized phenotypically	Assess phenotypic diversity of Ugandan IC ecotypes
	IC is not characterized genotypically	Assess genetic diversity of Ugandan IC ecotypes
	Genetic parameters of IC not known (trait correlations & heritability)	Estimate the genetic parameters of IC
	Advanced molecular/gene-based breeding is not a reality in the near future	Design low-cost breeding tools (selection indices)

1.13 Significance of the study

The Global Plan of Action highlights the need for the characterization and establishment of diversity as the first step towards the genetic improvement of Animal Genetic Resources, AnGR (FAO, 2007). Morphological assessment, genetic diversity studies, and estimation of genetic parameters are part of this characterization and inform decision-making when developing breeding programs (FAO, 2011). Specifically, phenotypic and genotypic characterization is the basis for the sustainable use, improvement, and conservation of IC (FAO, 2011; Ssewanyana, 2004). Improvements in feeding, management, and health alone are temporary and inadequate to realize the full potential of ICs (Magala *et al.*, 2012; Nakkazi *et al.*, 2014). As such, the inclusion of the breeding component is imperative to achieving increased productivity. This research will estimate genetic parameters for Uganda's ICs which in turn forms the foundation for the construction of selection indices (Ndung'u *et al.*, 2020; Faruque *et al.*, 2017). Construction of appropriate selection indices will increase accuracy,

simplify but most importantly improve and retain multiple traits associated with IC at each generation. Application of the selection index approach requires basic training and simple infrastructure hence lowering the cost of breeding programs compared to molecular based breeding tools. With increased productivity, the rearing of IC will become more sustainable and profitable. As a result of improved productivity, increased protein intake, income, and conservation of IC will be realized.

1.14 Ethical considerations

The study was approved by Gulu University Research Ethics Committee (GUREC-2020-18). In summary, the investigator sent letters to Chief Accounting Officers (CAO) introducing the research teams and the purpose of their visits in the respective districts two weeks in advance. The CAO would in turn inform the sub county chiefs to support the investigator. The investigator was required to present the informed consent form to the household heads before starting on any activity. Upon agreeing to participate, any engagement with the farmer followed procedures to minimize spread of Covid 19. The benefits and risks associated with participating in the study were made clear to farmers. The participating households were assured of confidentiality of their biodata and any other information they submitted. A premium price of 1000 Uganda Shillings was paid per fresh fertile egg to only farmers willing to sell to the investigator. However, a bar of soap was given to all households visited in appreciation to the time rendered to the team (See Appendix 5). The research protocol was also cleared and registered with the Gulu University Research Ethics Committee (GUREC) and Uganda National Council for Science and Technology, UNCST, NO. A154ES (See Appendix 3).

CHAPTER TWO

Phenotypic diversity among indigenous chicken ecotypes in Uganda

Abstract

Genetic improvement of indigenous chickens (ICs) in Uganda is hindered by limited knowledge of the various chicken ecotypes. In a cross-sectional study, the phenotypic diversity among nine IC ecotypes was assessed. A total of 576 ICs (288 cocks and 288 hens) were sampled from 288 households across the nine agro-ecological zones of Uganda. Linear body measurements and live-body weight of IC were collected using a field guide developed by the African Union Inter-African Bureau for Animal Resources (AU-IBAR). In addition, 200 fertile IC eggs were purchased from each agro-ecological zone for the determination of weight and geometry parameters (egg length, breadth, and volume). Overall, live-body weight (LWT), shank length (SL), drumstick length (DL), thorax circumference (TC), and body length (BL) of IC varied significantly ($P < 0.05$) across ecotypes except, tarsus circumference (TAC) of cocks. Cocks and hens on average weighed 2.2 ± 0.53 and 1.6 ± 0.58 kg, respectively. On average, SL, DL, TC, BL, and TAC were 9.8 ± 1.23 , 4.9 ± 2.50 , 16.2 ± 1.63 , 38.4 ± 5.96 , 24.7 ± 4.93 cm for cocks and 7.8 ± 1.04 , 3.9 ± 0.53 , 13.2 ± 1.42 , 33.8 ± 5.29 , 21.3 ± 4.37 cm for hens, respectively. Body weight and all linear morphological traits except TAC were significantly ($P < 0.05$) influenced by ecotype. Egg weight, breadth, and volume varied significantly ($P < 0.05$) across ecotypes except egg length. The traits and models for estimating live weight varied across ecotypes. However, principal component analysis based on quantitative traits did not identify any distinct chicken ecotypes. The current study, therefore concludes that ICs in Uganda cannot be clustered based on phenotypic traits. The observed variation in quantitative traits across ecotypes is ecologically induced. The difference in live weight estimation models

further emphasizes the ecologically induced morphological variations across ecotypes. Therefore, genetic improvement can be achieved through the selection of phenotypically superior individual chickens from across all ecotypes.

Key words: Diversity, Ecological, indigenous chicken, Morphology, Quantitative traits

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2.1 Introduction

In Uganda, IC account for 87.7% of the 47.6 million total chicken population and produces 80% of the 907 million eggs in the country (MAAIF and UBOS, 2018). IC are predominantly reared by subsistence, resource-constrained farmers under the scavenging system. Occasionally, chicken are supplemented with local cereal grains, and households own a flock size of 28 chickens on average (Wong *et al.*, 2017; Lubandi *et al.*, 2018). Indigenous chickens continue to support the social and economic well-being of many rural households through the provision of meat, income, and are part of many other social functions (Ssewannyana *et al.*, 2008; Lubandi *et al.*, 2018). Indigenous chickens play a pivotal role in nutrient utilization and recycling through scavenging and thus enhance the performance of livestock-crop mixed farming systems (Wong *et al.*, 2017). In light of the harsh effects of climate change, IC adapt well and overcome the challenges of water scarcity, declining feed resources, increased temperature, and high incidence of diseases and parasites (Assan *et al.*, 2017). Irrespective of the harsh conditions and constraints, IC are more beneficial, and their profit margins are favorable, given the low inputs under village conditions compared to commercial egg or meat type chickens (Mahendra, 2016).

Despite the advantages associated with IC, their innate productive and reproductive performance has consistently remained low. In an attempt to increase their productivity, IC are at risk of genetic erosion resulting from their substitution or crossbreeding with imported breeds (Semahoro *et al.*, 2018). As such, the potential of IC in fighting hunger, poverty and the attainment of sustainable development has not been fully exploited. In an effort to exploit the full potential of IC, the Global Plan of Action for Animal Genetic Resources highlights the need for countries to characterize, establish diversity, distribution, and determination of their comparative performance as the first strategic step towards improvement of animal genetic resources (FAO, 2007).

As part of their characterization, African chickens were previously grouped into Cluster one (Northern and Central African chickens) and Cluster two (East African chickens) based on their points of origin and entry (Osman *et al.*, 2016). Traditionally, Ugandan IC have been categorized and identified based on the ecological zones of origin (Ssewanyana, 2004). Ssewanyana *et al.* (2008) further characterized IC on the basis of flock size, use, performance, and phenotype. The high levels of phenotypic diversity across IC provide an opportunity for genetic improvement through identification and selection of highly performing breeding cocks and hens (Ssewanyana *et al.*, 2008; Fleming *et al.*, 2016). The reported phenotypic diversity of Ugandan ICs is based on data collected from within the same or a few ecotypes and does not follow the ecological zoning approach of categorizing chickens (Ssewanyana, 2004; Ssewanyana *et al.*, 2008). To date, no comprehensive phenotypic and morphological studies have been carried out to characterize IC ecotypes. In addition, the use of phenotypic traits is subjective, not confirmatory, and necessitates additional data from a quantitative method of determining diversity. Examination of linear morphological traits is one quantitative approach that could be used to assess chicken diversity. Therefore, the aim of this study was to assess the phenotypic diversity of IC ecotypes based on quantitative traits.

2.2 Materials and methods

2.2.1 Study area

The study was conducted in nine agro-ecological zones of Uganda (Figure 2.1). In order to identify the IC ecotypes, the study adopted a nomenclature system suggested by Ssewanyana (2004). Therefore, agro-ecological zones and their corresponding ecotypes were: West Nile (Madi), Acholi (Acholi), Lango (Lango), Teso (Teso) and Busoga (Nsoga), Buganda (Ngada), Bunyoro (Nyoro), Gisu (Bugisu) and Ankole (Ankole). In each agro-ecological zone, two districts and two sub-counties per district with the highest IC population were selected based on the livestock census of MAAIF and UBOS (2018).



Figure 2.1: Map of Uganda showing agro-ecological zones, study area and the corresponding ecotypes

2.2.2 Sampling approach

A total of 576 IC (288 cocks and 288 hens) were sampled from 288 households across the 9 agro-ecological zones of Uganda in a cross-sectional study. Eight households were selected per sub-county. History of having kept IC for a minimum of three years and ownership of a mature breeding cock were the criteria for selecting participating households. A minimum of 4 villages along a transect drawn through the sub-county were sampled. In addition, 200 fresh fertile IC eggs (1-5 days) per agro-ecological zone were purchased from willing households. Eggs were packed in paper trays and delivered to the Gulu University poultry unit for determination of egg parameters (weight, length, breadth, and volume) and subsequent artificial incubation.

2.2.3 Data collection

One mature breeding cock and hen per household were selected for quantitative morphometric body assessment. Linear body measurements (shank/Tarsus Length = SL; Tarsus circumference = TAC, Drumstick length = DL, Thorax circumference = TC, Body length = BL; Wattle length = WL; Neck length = NL; Comb height = CH; Comb length = CL; Head length = HL; Wing span = WAPN; Wing length = WL and Beak length = BKL) and live-body weight=LWT were collected using a field guide developed by African Union Inter-African Bureau for Animal Resources (AU-IBAR, 2015) as shown in Figure 2.2. A tailor's measuring tape (cm) was used to measure the quantitative linear morphometric traits (Tyasi *et al.*, 2020). Live-body weight was measured in kilograms using a hanging digital scale (WeiHeng WH-A25, China). Egg weight was measured using an analytical weighing scale (Model: FA2004, China). Egg breadth and length were measured using a digital caliper (Model: DIN 862, Vogel Germany). Egg volume, V was calculated using the following formula (Narushin and Romanov, 2002):

$$V = 2.854LB$$

Where; L is egg length in millimeters, and B is the egg maximum breadth in millimeters.

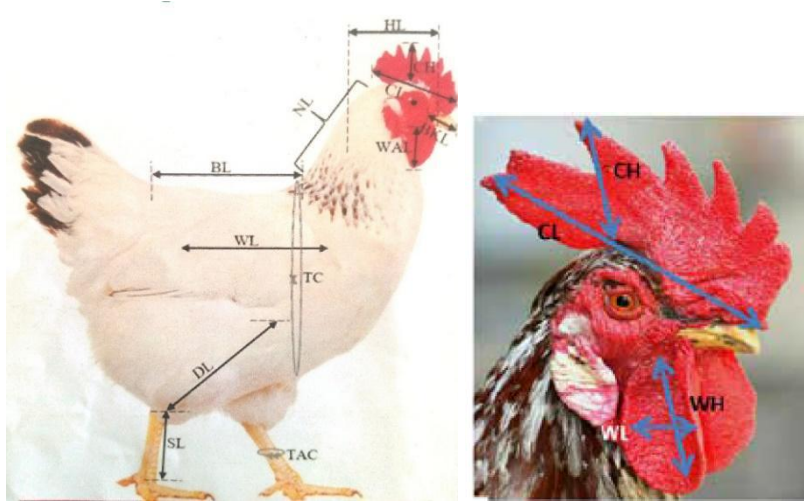


Figure 2.2: AU-IBAR Illustration of morphometric measurements

Shank/Tarsus Length (SL) = SL; Tarsus circumference = TAC, Drumstick length = DL, Thorax circumference = TC, Body length = BL; Wattle length = WL; Wattle height = WH; Neck length (NL) = NL; Comb height= CH; Comb length = CL; Head length =HL; Wing span = WAPN; Wing length=WL; Beak length = BKL and Body live weight =LWT

2.2.4 Data analysis

The effect of ecotype, sex, and their interaction on quantitative traits was assessed by the general linear model package, in R version 3.5.1 (R Core Team, 2018). Means were separated using the least significant difference at 5% significance level. The model used is shown below:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Y= traits -live-weight & linear body measurements, as variables-*i*; A=ecotype effect-*i*; B=sex effect-*j* AB= their interaction; e=residual error

Pearson's correlation coefficients between live weight and selected morphometric traits were estimated, and from the correlation matrix, the Principal Component Analysis (PCA) was performed. The first two outstanding principal components (PCA1 and PCA2) were used to identify population clusters (R Core Team, 2018). Linear morphological traits with the highest

Pearson's coefficient with live-body weight were used to derive power model equations for predicting live-body weight of cocks and hens in each ecotype (Semakula *et al.*, 2011).

2.3 Results

2.3.1 Effect of ecotype, sex, or their interaction on live-body weight and linear morphological traits of nine indigenous chicken ecotypes

The effect of ecotype and sex, or their interaction, on body weight and the five (5) linear morphological traits of nine (9) Ugandan IC Ecotypes are presented in Table 2.1. The average live-body weight-LWT of IC ecotypes was 2.2 ± 0.53 kg for cocks and 1.6 ± 0.58 kg for hens. LWT of IC varied significantly ($P < 0.05$) across ecotypes. Teso (2.6 ± 0.51 kg) and Lango (2.55 ± 0.47 kg) ecotypes produced the heaviest cocks, while Gisu (1.68 ± 0.38 kg), Ankole (1.71 ± 1.21 kg), and Teso (1.7 ± 0.40 kg) ecotypes produced the heaviest hens. Cocks (1.7 ± 0.36 kg) and hens (1.31 ± 0.23 kg) of Madi origin were the lightest ecotype. Overall, the heaviest and lightest chicken ecotypes originated from Teso and Madi, respectively.

The average shank length –SL across IC ecotypes was 9.8 ± 1.23 cm for cocks and 7.8 ± 1.04 cm for hens. Shank length varied significantly across ecotypes. Lango (11.3 ± 0.94 cm) and Acholi (10.7 ± 1.12 cm) cocks had the longest SL, Madi and Nyoro ecotype cocks had the shortest SL of 9.4 ± 0.97 and 9.3 ± 1.13 cm, respectively. Acholi (8.2 ± 1.03 cm) and Lango (8.4 ± 0.91 cm) hens had the longest SL, followed by Gisu (8.04 ± 0.67 cm), Nganda (7.6 ± 1.0 cm) and Nyoro (7.5 ± 1.12 cm) ecotypes had the shortest SL. Overall, Lango, Acholi and Teso chickens had longer ($P < 0.05$) SL compared to Nsoga, Nganda, Ankole, Madi, and Nyoro ecotypes.

On average, the tarsus circumference -TAC of IC ecotypes was 4.9 cm for cocks and 3.9 cm for hens. Unlike cocks, the tarsus circumference of hens significantly ($P < 0.05$) varied across ecotypes. Nyoro hens exhibited the largest tarsus circumference (4.3 cm), followed by Ankole

(4.2 cm) and Nganda (4.09 cm) ecotype. On the other hand, Madi hens exhibited the least tarsus circumference (3.4 cm). On average, tarsus circumference was highest in the Nsoga, Ankole and Nyoro ecotypes and lowest in Madi ecotype.

Overall, drumstick length -DL of IC ecotypes were 16.2 ± 1.63 cm for cocks and 13.2 ± 1.42 cm for hens and varied significantly across ecotypes ($P < 0.05$). DL of cocks was highest among Acholi, Gisu and Teso and lowest for Nyoro and Madi Ecotypes ($P < 0.05$). Nsoga, Gisu and Teso hens had the highest ($P < 0.05$) DL.

Generally, thorax circumference -TC of IC ecotypes was 38.4 ± 5.96 cm for cocks and 33.8 ± 5.29 cm for hens and varied significantly ($P < 0.05$) across ecotypes. The Gisu and Teso cocks and hens had larger ($P < 0.05$) TC compared to other ecotypes. Gisu and Teso had the largest TC followed by Nsoga, while Ankole ecotype had the least ($P < 0.05$) TC.

Overall, body length-BL of IC was 24.7 ± 4.93 cm for cocks and 21.3 ± 4.37 cm for hens and varied significantly ($P < 0.05$) across ecotypes. Lango, Acholi, Nganda, Ankole and Nyoro had longer bodies (23.42 ± 3.83 , 22.6 ± 6.0 , 26.0 ± 4.06 , 25.91 ± 3.99 , 25.9 ± 3.27 cm) compared to Teso and Gisu ecotypes (19.2 ± 2.75 and 18.87 ± 2.63 cm), respectively.

The effect of sex on body weight and the five (5) linear morphological traits was significant ($P < 0.05$) across all ecotypes. Cocks dominated hens in all six measured traits (LWT, SL, TAC, DL, TC, and BL). The effect of ecotype on body weight and the linear morphological traits, except TAC, was significant ($P < 0.05$) across all ecotypes. The ecotype-sex interaction was only significant ($P < 0.05$) for LWT, DL, and TC ($P < 0.01$). Shank length, tarsus circumference, and body length were not influenced ($P > 0.05$) by the ecotype-sex interaction. On average, Teso chickens were the heaviest (2.1 ± 0.64 kg), and Madi ecotype (1.5 ± 0.36 kg) produced the lightest cocks. Gisu and Teso ecotype cocks and hens had longer DL, but Madi chickens,

specifically cocks exhibited shorter DL. Gisu and Teso ecotypes had similar but large thorax circumferences for both cocks and hens, while the Ankole ecotype had the lowest TC.

Table 2 1: The effect of ecotype, sex or their interaction on body weight and linear morphological traits of nine (9) indigenous chicken ecotypes

Trait	Sex	Ecotypes										Ecotype (E)	Sex (S)	E X S	R ² _{adj}
		Acholi	Gisu	Nsoga	Nganda	Lango	Ankole	Teso	Madi	Nyoro	All				
LWT/kg	C	2.39±0.53 ^{ab}	2.44±0.46 ^{ab}	2.17±0.57 ^{bc}	2.29±0.46 ^{bc}	2.55±0.47 ^a	2.13±0.49 ^c	2.6±0.51 ^a	1.7±0.36 ^d	2.2±0.41 ^{bc}	2.2±0.53	***	***	*	0.33
	H	1.57±0.30 ^{ab}	1.68±0.38 ^a	1.57±0.27 ^{ab}	1.52±0.33 ^{ab}	1.59±0.25 ^{ab}	1.71 ±1.21 ^a	1.7±0.40 ^a	1.31±0.23 ^b	1.5±0.31 ^{ab}	1.6±0.58				
	Aver.	1.9±0.64 ^{abcd}	2.1±0.56 ^{abc}	1.9± 0.53 ^{cd}	1.9±0.55 ^{cd}	2.1±0.61 ^{ab}	1.9±0.95 ^{bcd}	2.1±0.64 ^a	1.5±0.36 ^e	1.9±0.48 ^d					
SL/cm	C	10.7±1.12 ^{ab}	10.1±0.94 ^{bc}	9.7±1.29 ^{cd}	9.6±1.11 ^{cd}	11.3±0.94 ^a	9.3±1.00 ^d	10.0±0.87 ^c	9.4 ± 0.97 ^d	9.3± 1.13 ^d	9.8±1.23	***	***	.na	0.54
	H	8.2 ± 1.03 ^a	8.04±0.67 ^{abc}	7.6 ± 0.56 ^{bc}	7.6±1.00 ^c	8.4 ± 0.91 ^a	7.59 ± 0.88 ^{bc}	8.1±1.60 ^{ab}	7.6±1.13 ^{bc}	7.5±1.12 ^c	7.8±1.04				
	Aver.	9.49±1.68 ^b	9.07±1.32 ^{bc}	8.63±1.436 ^d	8.56±1.48 ^d	9.91±1.70 ^a	8.41±1.28 ^d	9.02±1.59 ^c	8.5±1.38d	8.34±1.44 ^d					
TAC/cm	C	4.98 ± 0.76	4.67±0.64	4.47±0.57	4.88±0.69	4.83 ± 0.57	4.95 ± 0.64	4.73±0.48	4.3±0.78	4.95 ± 0.62	4.9±2.50	na	***	na	0.07
	H	3.67± 0.35 ^{cd}	3.74±0.30 ^c	3.68±0.35 ^c	4.09±0.49 ^b	3.74 ± 0.60 ^c	4.21 ± 0.47 ^{ab}	3.76±0.38 ^c	3.4±0.47 ^d	4.3 ± 0.50 ^a	3.9± 0.53				
	Aver	4.32±0.88 ^{ab}	4.21±0.69 ^{ab}	4.56±0.48 ^a	4.47±0.71 ^{ab}	4.30±0.80 ^{ab}	4.57±0.67 ^a	4.2±0.65 ^{ab}	3.9±0.78 ^b	4.6±0.64 ^a					
DL/cm	C	16.92±1.59 ^a	17.13±0.90 ^a	16.35±1.40 ^{ab}	15.9±1.81 ^{bc}	16.71 ±1.22 ^{ab}	16 ±1.71 ^{bc}	17±1.24 ^a	15.3 ±1.47 ^c	15.6±1.86 ^c	16.2±1.63	***	***	*	0.53
	H	12.9±1.31 ^{ab}	13.8±0.78 ^a	13.7±1.91 ^a	12.8±1.28 ^b	12.7 ± 1.65 ^b	13.2±1.28 ^{ab}	13.7±1.57 ^a	12.9±1.76 ^{ab}	13.2±1.51 ^{ab}	13.2±1.42				
	Aver.	14.9±2.47 ^{ab}	15.4±1.91 ^a	14.9± 1.87 ^{ab}	14.3±2.22 ^{cd}	14.8±2.48 ^{abc}	14.6±2.05 ^{bcd}	15.4±2.18 ^a	14.1±1.97 ^d	14.4±2.04 ^{cd}					
TC/cm	C	38.9±4.47 ^c	44.9±3.99 ^a	42.2± 3.72 ^b	34.2± 4.16 ^c	40.4±6.24 ^{bc}	34.5±3.51 ^c	45.9±3.20 ^a	39.5±2.95 ^c	35.3±3.77 ^c	38.4±5.96	***	***	**	0.52
	H	34.2±3.44 ^b	38.7±2.40 ^a	37.4±6.22 ^a	31.2±3.46 ^{cd}	33.6±3.80 ^{bc}	31.6±3.06 ^{cd}	38.1±7.21 ^a	35.2±2.78 ^b	31.8±3.51 ^{cd}	33.8±5.29				
	Aver	36.59±4.61 ^c	41.80±4.50 ^a	39.77±5.64 ^b	36.65±4.01 ^c	37.10±6.19 ^c	31.01±3.57 ^d	42.0±6.80 ^a	37.39±3.57 ^c	36.5± 4.03 ^c					
BL/cm	C	24.4±4.71 ^a	20.3±2.60 ^b	19.9±4.46 ^b	27.9±4.18 ^{bc}	25.1±3.88 ^a	27.9±3.80 ^{bc}	21.0±2.14 ^b	23.2±3.07 ^a	27.7±3.38 ^{bc}	24.7±4.93	***	***	na	0.50
	H	20.84±6.69 ^a	17.47±1.82 ^b	16.86±2.36 ^b	24.3±3.04 ^{bc}	21.61±2.87 ^a	24.04±3.18 ^{bc}	17.4±2.01 ^b	20.30±2.84 ^a	24.4±2.24 ^{bc}	21.3±4.37				
	Aver	22.6±6.00 ^{ab}	18.87±2.63 ^c	18.38±3.86 ^c	26.0±4.06 ^{ab}	23.42±3.83 ^a	25.91±3.99 ^{ab}	19.2±2.75 ^c	21.74±3.27 ^b	25.9±3.27 ^{ab}					

*C= Cock; H = Hen; Tarsus/shank Length = SL; Tarsus circumference =TAC, Drumstick length = DL, Thorax circumference = TC, Body length = BL; and Body live-weight =LWT; ^{na}not significant; ****p* < 0.001; ** *p* < 0.01; * *p* < 0.05; ^{a,b,c,d} Means within the same row with a different superscript are significantly different at *p* < 0.05.*

2.3.2 Egg weight, geometry, and indigenous chicken ecotype diversity

The weight and geometry of indigenous chicken eggs from selected ecologies are presented in Table 2.2. Overall, IC eggs weighed 41.13 g. Egg weights were highest ($P < 0.05$) in Teso (42.73 g) while the Madi ecotype (38.99 g) produced the lightest eggs. Egg breadth varied across ecotypes, with Teso, Nsoga, and Lango producing large eggs compared to the Madi ecotype ($P < 0.05$). All IC ecotypes produced eggs with similar volume, except the Madi (5503 mm³) ecotypes, which produced eggs with the lowest volume ($P < 0.05$). Egg length was similar across all ecotypes ($P > 0.05$).

Table 2.2: Egg weight and geometry of selected indigenous chicken ecotypes

Egg parameters	Ecotypes					All	SEM	P-Value
	Teso	Nsoga	Acholi	Lango	Madi			
Weight/g	42.73 ^a	42.61 ^a	41.65 ^a	40.23 ^b	38.99 ^b	41.13	0.23	5.83x10 ⁸
Length L/mm	52.29	52.22	52.11	52.14	51.91	52.13	0.12	0.911
Breadth/mm	38.44 ^{ab}	38.64 ^a	38.11 ^b	38.44 ^{ab}	37.11 ^c	38.17	0.08	2.23x10 ¹⁰
Volume V/mm ³	5740 ^a	5768 ^a	5672 ^a	5723 ^a	5503 ^b	5684	21.36	3.39x10 ⁴

^{a,b,c} Means within the same row with a different superscript are significantly different at $p <$

0.05.

2.3.3 Principal component analysis

The relationship among IC ecotypes based on morphological traits was visualized using Principal Component analysis, PCA. Principal Component analysis revealed the overlapping among indigenous cock ecotypes (Figure 2.3). The percentage variation explained by PCA1 and PCA2 was 40.2% and 10.7% respectively.

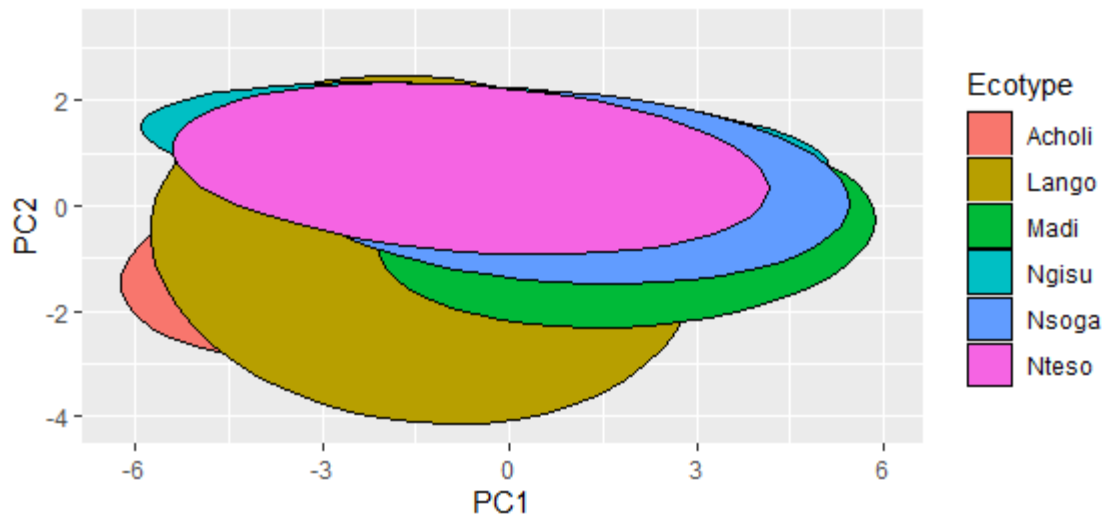


Figure 2.3: Principal component plot of six indigenous cock ecotypes

The first two outstanding principal components indicate overlapping among indigenous hen ecotypes (Figure 2.4). The percentage variation explained by PCA1 and PCA2 was 32.4% and 12.1% respectively.

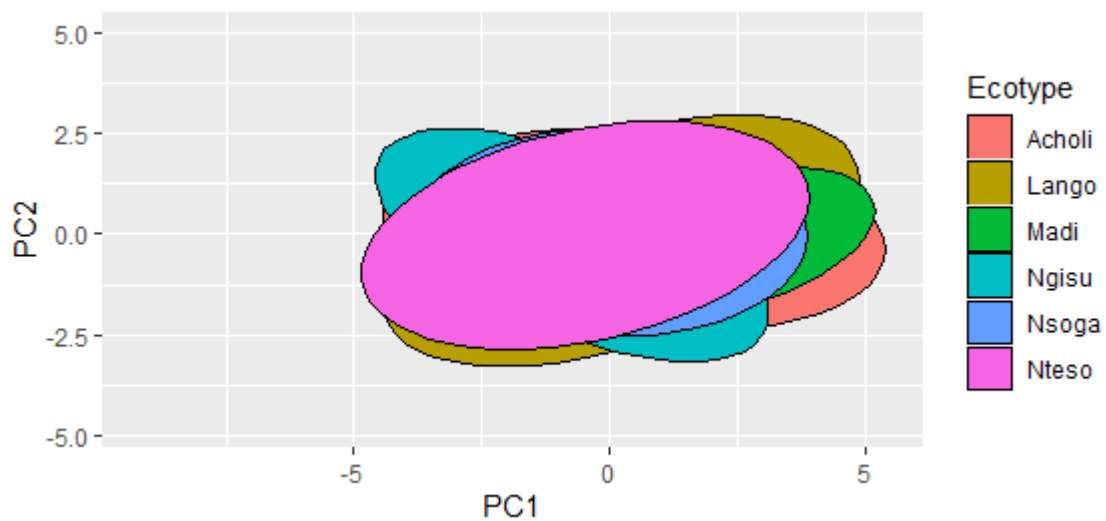


Figure 2.4: Principal component plot of six IC hen ecotypes

The first two outstanding principal components (PCA one and PCA two) indicate overlapping among Ugandan chicken ecotypes. The percentage variation explained by PCA one and PCA two was 63.3% and 10.2% respectively (Figure 2.5).

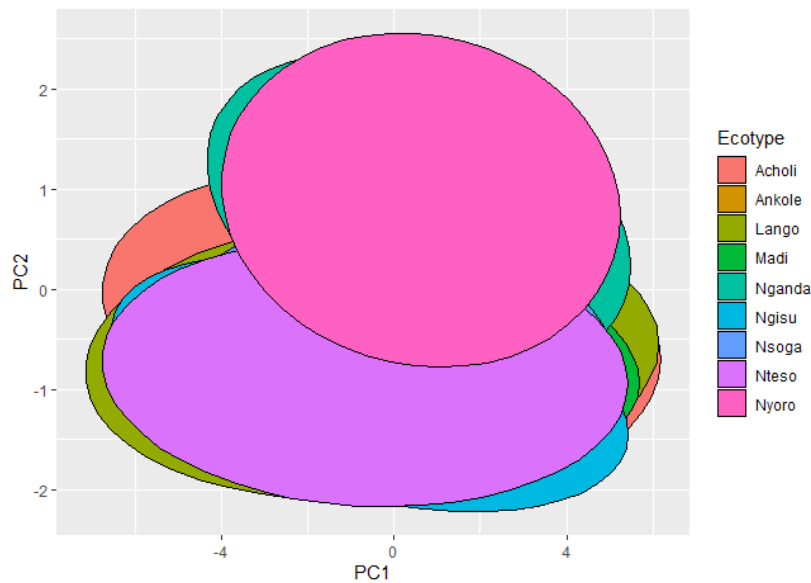


Figure 2.5: Principal component analysis of nine IC ecotypes

2.3.4 Correlation across morphological linear traits and body weight

Correlation coefficients between pairs of traits are presented in Tables 2.3; 2.4; 2.5; 2.6; 2.7; 2.8; 2.9; 2.10; 2.11; 2.12. Among all the morphological linear traits, TAC resulted in the highest Pearson's coefficient of 0.85 with body live weight for Acholi ecotype cocks. On the other hand, SL resulted in the highest Pearson's coefficient of 0.57 with body live weight of Acholi ecotype hens (Table 2.3). Negative coefficients resulted from the correlation between SL - WL; DL - WL; DL - BKL and CH - BKL.

Table 2.3: Correlation of morphological traits of Acholi ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	0.10	0.14	-0.14	-0.07	0.38	0.24	0.02	0.15
NL	0.35	1	0.30	0.25	0.53	0.54	0.47	0.50	0.24
TAC	0.50	0.33	1	0.28	0.32	0.42	0.39	0.31	0.43
SL	0.44	0.256	0.53	1	0.69	0.32	0.40	0.300	0.57
DL	0.23	0.44	0.75	0.48	1	0.31	0.28	0.47	0.40
CH	0.45	0.35	0.43	0.15	0.33	1	0.81	0.44	0.47
CL	0.74	0.36	0.66	0.55	0.47	0.78	1	0.50	0.46
BKL	0.08	0.04	0.22	0.28	-0.01	-0.012	0.04	1	0.46
BLW	0.63	0.36	0.85	0.75	0.70	0.47	0.78	0.21	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Within the Lango chicken ecotype cocks, DL resulted in the highest coefficient of 0.58 with live-body weight and closely followed by CL (0.572). The correlation between TAC and live-body weight culminated into the highest coefficient of 0.75 for Lango chicken ecotype hens (Table 2.4).

Table 2.4: Correlation of morphological traits of Lango ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	0.19	0.38	0.18	0.18	0.47	0.28	0.23	0.32
NL	0.64	1	0.35	0.42	0.27	-0.26	-0.46	0.02	0.17
TAC	0.42	0.07	1	0.66	0.69	0.10	0.10	0.61	0.75
SL	0.59	0.42	0.54	1	0.59	-0.20	-0.21	0.26	0.49
DL	0.64	0.44	0.54	0.44	1	-0.11	-0.07	0.63	0.59
CH	0.43	0.04	0.42	0.45	0.10	1	0.70	0.02	0.27
CL	0.44	-0.03	0.18	0.40	0.17	0.78	1	0.14	0.22
BKL	0.68	0.40	0.18	0.24	0.38	0.20	0.15	1	0.36
BLW	0.51	0.17	0.48	0.50	0.58	0.45	0.57	0.07	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Among the Madi chicken ecotype, the highest correlation coefficient with body weight was linked to TAC (0.80) and CH (0.66) for cocks and hens respectively (Table 2.5). A negative coefficient only occurred from the pairwise correlation of SL – BKL and DL – CH.

Table 2.5: Correlation of morphological traits of Madi ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	0.32	0.72	0.63	0.33	0.82	0.88	0.30	0.64
NL	0.21	1	0.40	0.44	0.63	0.25	0.41	0.19	0.43
TAC	0.59	0.53	1	0.78	0.36	0.65	0.70	0.20	0.52
SL	0.18	0.62	0.32	1	0.55	0.46	0.58	0.25	0.52
DL	0.23	0.27	0.37	0.36	1	0.31	0.40	0.24	0.30
CH	0.75	0.15	0.41	0.05	-0.05	1	0.86	0.31	0.66
CL	0.79	0.29	0.48	0.19	0.24	0.75	1	0.25	0.51
BKL	0.51	0.18	0.58	-0.04	0.30	0.46	0.43	1	0.52
BLW	0.67	0.47	0.80	0.28	0.49	0.45	0.62	0.70	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Among all the morphological linear traits, CL and TAC resulted in the highest Pearson's coefficients with body live weight for Gisu ecotype cocks (0.82) and hens (0.56) respectively (Table 2.6). CH followed closely with a coefficient of 0.806 with cock body weight. CH could adequately substitute CL as a phenotypic marker for body weight.

Table 2.6: Correlation of morphological traits of Gisu ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	-0.04	0.16	-0.13	0.25	0.75	0.76	0.08	0.36
NL	0.13	1	-0.13	-0.04	-0.39	-0.32	-0.16	0.15	-0.17
TAC	0.46	-0.10	1	0.42	-0.12	0.01	0.08	0.22	0.56
SL	0.22	0.53	0.53	1	0.19	-0.21	-0.16	0.66	-0.02
DL	0.61	0.25	0.69	0.52	1	0.29	0.11	0.03	-0.15
CH	0.78	0.01	0.57	0.48	0.52	1	0.87	0.03	0.34
CL	0.82	0.04	0.72	0.53	0.68	0.94	1	0.15	0.45
BKL	0.57	-0.21	0.71	0.40	0.59	0.71	0.76	1	0.39
BLW	0.71	-0.13	0.75	0.48	0.62	0.81	0.82	0.80	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Among all the morphological linear traits, TAC and BKL resulted in the highest Pearson's coefficients with body live weight for Nsoga ecotype cocks (0.73) and hens (0.60) respectively

(Table 2.7). Majority of the coefficients were positive. Negative Pearson's coefficients were associated with NL – WL, TAC – NL and CL – NL pairwise correlations.

Table 2.7: Correlation of morphological traits of Nsoga ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	-0.01	0.29	0.19	0.17	0.60	0.62	0.12	0.31
NL	0.20	1	-0.09	0.22	0.46	0.10	-0.06	0.29	0.14
TAC	0.56	0.27	1	0.40	0.18	0.26	0.27	0.36	0.57
SL	0.11	0.17	0.47	1	0.30	0.14	0.11	0.26	0.50
DL	0.27	0.26	0.51	0.46	1	0.33	0.223	0.34	0.40
CH	0.18	0.04	0.13	0.20	0.06	1	0.70	0.42	0.49
CL	0.81	0.22	0.53	0.05	0.17	0.27	1	0.21	0.29
BKL	0.31	0.38	0.62	0.50	0.52	0.21	0.20	1	0.60
BLW	0.67	0.48	0.73	0.43	0.61	0.21	0.61	0.65	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Comb length, CL and TAC generated the highest correlation coefficients with body weight among the Teso cocks (0.81) and hens (0.66) respectively (Table 2.8). Wattle length, WL resulted in a coefficient (0.80) close to the value obtained between CL and cock body weight. Majority of the linear morphological traits generated positive coefficients except CH- DL, BKL- CH, BKL-CL and BLW-CH which resulted in negative values for Teso ecotype hens. All morphometric correlation coefficients associated with cocks were positive.

Table 2.8: Correlation of morphological traits of Teso ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	0.29	0.05	0.10	0.05	0.53	0.47	0.26	0.15
NL	0.25	1	0.16	0.55	0.48	0.38	0.36	0.21	0.28
TAC	0.57	0.19	1	0.30	0.26	0.05	0.15	0.62	0.66
SL	0.51	0.35	0.48	1	0.79	0.03	0.07	0.36	0.27
DL	0.42	0.28	0.38	0.74	1	-0.05	0.11	0.34	0.48
CH	0.61	0.23	0.50	0.51	0.38	1	0.83	-0.01	-0.08
CL	0.76	0.34	0.58	0.65	0.54	0.91	1	-0.01	0.03
BKL	0.33	0.42	0.50	0.57	0.64	0.30	0.37	1	0.58
BLW	0.80	0.44	0.75	0.75	0.67	0.63	0.81	0.61	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

All correlation coefficients between morphometric linear traits of Nganda cocks were positive. Comb height, CH and CL resulted in the highest correlation coefficients with body weight for the Nganda cocks (0.62) and hens (0.71) respectively (Table 2.9). Correlation of WL and DL with body weight also resulted in coefficients close to 0.617 obtained with cock CH. Tarsus/shank Length, SL resulted in only negative coefficients with WL, NL and TAC for Nganda hens.

Table 2.9: Correlation of morphological traits of Nganda ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	-0.09	0.35	-0.11	-0.04	0.32	0.50	0.41	0.43
NL	0.35	1	0.15	-0.19	0.44	-0.06	0.06	-0.24	0.23
TAC	0.38	0.01	1	-0.02	0.13	0.37	0.40	0.14	0.55
SL	0.14	0.10	0.16	1	0.13	0.06	0.00	0.13	0.12
DL	0.44	0.42	0.56	0.37	1	0.07	0.22	-0.03	0.53
CH	0.57	0.24	0.29	0.09	0.32	1	0.39	0.27	0.26
CL	0.63	0.23	0.34	0.06	0.34	0.71	1	0.33	0.71
BKL	0.26	0.25	0.18	0.18	0.42	0.19	0.33	1	0.24
BLW	0.60	0.20	0.50	0.55	0.61	0.62	0.55	0.26	1.00

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Like the Nganda cocks, all correlation coefficients between morphometric linear traits of Nyoro cocks were positive. Out of all the morphological linear traits, WL and CL generated the highest Pearson's coefficients with body weight (BWL) of Nyoro cocks (0.66) and hens (0.55), respectively (Table 2.10).

Table 2.10: Correlation of morphological traits of Nyoro ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	0.01	0.05	0.08	-0.02	-0.02	0.25	-0.41	0.25
NL	0.50	1	-0.00	-0.09	0.48	-0.43	0.18	-0.14	0.05
TAC	0.13	0.26	1	0.23	0.02	-0.11	0.13	0.03	0.29
SL	0.20	0.06	0.30	1	-0.17	0.15	0.30	0.31	0.50
DL	0.53	0.56	0.33	0.15	1	-0.09	0.08	0.01	0.21
CH	0.78	0.48	0.09	0.08	0.44	1	0.02	0.02	0.08
CL	0.90	0.54	0.15	0.12	0.54	0.88	1	0.08	0.55
BKL	0.17	0.10	0.34	0.07	0.25	0.38	0.26	1	0.19
BLW	0.66	0.29	0.52	0.45	0.46	0.52	0.59	0.38	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

All correlation coefficients between morphometric linear traits of Ankole cocks were positive except for NL, which exhibited negative values with TAC, SL, and BKL (Table 2.11). Tarsus circumference, TAC generated the largest coefficient of 0.60 with Ankole hen body weight. Likewise, tarsus circumference, TAC resulted in the highest coefficient of 0.69 with live-body weight of Ankole cocks, closely followed by 0.68 (CL) and 0.67 (WL).

Table 2.11: Correlation of morphological traits of Ankole ecotype cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	0.36	0.14	0.01	0.34	0.59	0.65	-0.13	0.55
NL	0.28	1	-0.20	-0.25	0.35	0.01	0.06	-0.21	0.09
TAC	0.36	-0.00	1	0.57	0.27	0.19	0.06	0.59	0.60
SL	0.06	-0.13	0.33	1	0.09	0.15	0.06	0.54	0.59
DL	0.44	0.43	0.24	0.31	1	0.23	0.13	0.22	0.45
CH	0.52	0.19	0.49	0.19	0.25	1	0.84	0.10	0.54
CL	0.78	0.15	0.41	0.09	0.37	0.72	1	-0.03	0.42
BKL	0.26	-0.15	0.50	0.42	0.30	0.26	0.30	1	0.32
BLW	0.67	0.11	0.69	0.39	0.53	0.57	0.68	0.61	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

Out of all the morphological linear traits, WL and TAC generated the highest Pearson's coefficients with the body weight (BWL) of indigenous cocks (0.70) and hens (0.45), respectively (Table 2.12). All the Pearson's coefficient values resulting from the correlation of morphological linear traits of cocks were positive. Considering hens, only the pair-wise correlation of NL & WL, TAC & NL, and BKL & WL resulted in negative Pearson's coefficients.

Table 2.12: Correlation of morphological traits of indigenous cocks (lower triangle) and hens (upper triangle)

	WL	NL	TAC	SL	DL	CH	CL	BKL	BLW
WL	1	-0.00	0.30	0.05	0.10	0.34	0.46	-0.00	0.34
NL	0.37	1	-0.22	0.21	0.40	0.07	0.13	0.23	0.20
TAC	0.40	0.03	1	0.21	0.19	0.11	0.24	0.07	0.45
SL	0.30	0.34	0.32	1	0.30	0.13	0.19	0.32	0.39
DL	0.46	0.46	0.42	0.43	1	0.13	0.22	0.27	0.41
CH	0.42	0.29	0.18	0.24	0.24	1	0.53	0.21	0.28
CL	0.75	0.32	0.38	0.28	0.40	0.54	1	0.16	0.43
BKL	0.34	0.43	0.21	0.378	0.41	0.33	0.29	1	0.36
BLW	0.70	0.31	0.62	0.54	0.60	0.39	0.64	0.42	1

WL = Wattle length; NL = Neck length; TAC = Tarsus circumference; SL = Tarsus/shank Length; DL = Drumstick length; CH = Comb height; CL = Comb length; BKL = Beak length; BLW = Body live weight

2.3.5 Ecotype diversity and live weight estimation models

The linear morphometric trait-based estimation models of body live weight across chicken ecotypes are presented in Table 2.12. Morphometric linear traits with the highest Pearson's coefficient with the body weight of cocks and hens in each ecotype (Tables 2.3 to 2.12) were used to construct live weight estimation models. The selected linear morphometric traits per ecotype had positive and the strongest association with live-body weight compared to others. Generally, TAC and WL were the suitable traits for estimating the body weight of Ugandan indigenous hens and cocks, respectively. The traits for estimating live weight varied across ecotypes the and sex of chickens. However, TAC was the dominant trait for estimating live weight across all ecotypes and sexes.

Table 2.13: Live weight estimation of indigenous chicken ecotypes based on linear morphometric traits

Correlation of traits and live weight				Derived power model equations			
ecotype	Sex	Trait	Pearson's coefficient ^b	Live weight (y) estimator across ecotypes ^c	R ² _{adj} ^d	SE	Significance
Acholi	C	TAC	0.85	$y = 318.99(\text{TAC})^{1.25}$	0.99	77.90	***
	H	SL	0.57	$y = 249.94(\text{SL})^{0.87}$	0.99	78.20	Na
Lango	C	DL	0.58	$y = 38.505(\text{DL})^{1.49}$	0.99	64.50	**
	H	TAC	0.75	$y = 615.14(\text{TAC})^{0.72}$	0.99	57.90	***
Madi	C	TAC	0.80	$y = 431.85(\text{TAC})^{0.94}$	0.99	57.90	***
	H	CH	0.66	$y = 1279(\text{CH})^{0.17}$	0.81	41.70	***
Gisu	C	CL	0.82	$y = 663.65(\text{CL})^{0.62}$	0.99	35.80	***
	H	TAC	0.56	$y = 217.51(\text{TAC})^{1.55}$	0.99	283.00	*
Nsoga	C	TAC	0.73	$y = 264.85(\text{TAC})^{1.39}$	0.99	97.20	***
	H	BKL	0.60	$y = 327.08(\text{BKL})^{1.28}$	0.99	123.30	***
Teso	C	CL	0.81	$y = 647.12(\text{CL})^{0.66}$	0.99	31.840	***
	H	TAC	0.66	$y = 214.8(\text{TAC})^{1.55}$	0.99	158.70	***
Nganda	C	CH	0.62	$y = 1374(\text{CH})^{0.42}$	0.98	58.60	***
	H	CL	0.71	$y = 879.78(\text{CL})^{0.49}$	0.99	33.10	***
Nyoro	C	WL	0.66	$y = 1381.3(\text{WL})^{0.35}$	0.97	44.40	***
	H	CL	0.55	$y = 912.14(\text{CL})^{0.48}$	0.99	65.50	***
Ankole	C	TAC	0.69	$y = 301.62(\text{TAC})^{1.22}$	0.99	85.60	***
	H	TAC	0.60	$y = 332.43(\text{TAC})^{1.07}$	1.00	78.50	***
General weight estimation models for Ugandan IC							
Hens		TAC	0.45	$y = 616.37(\text{TAC})^{0.68}$	0.999	125.3	*
Cocks		WL	0.69	$y = 1318.9(\text{WL})^{0.42}$	0.970	72.30	*

C = Cock; H = Hen; Tarsus circumference = TAC; Tarsus/shank Length = SL; Drumstick length = DL, Wattle length = WL; Comb length = CL; Comb height = CH; Beak length = BKL; ^bPair-wise Pearson's correlation coefficient, traits with the highest coefficient with live weight (y) were selected; ^cPower model equations based on a trait with the highest Pearson's coefficient; ^dAdjusted R-squared values to evaluate the power model equations; SE = Standard Error; ^{na}not significant; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

2.4 Discussion

2.4.1 Live-body weight and morphometric traits across ecotypes

The average live-body weights of 2.2 ± 0.53 and 1.6 ± 0.58 kg in the current study are similar to values of 2.3 and 1.6 kg for indigenous cocks and hens, respectively reported by Semakula *et al.* (2011). Previously, Ssewanyana *et al.* (2008) reported slightly lower values of 2.1 and 1.4

kg for indigenous cocks and hens, respectively. Semakula *et al.* (2011) reported consistently higher values of body length, drumstick length, and thorax circumference in a study involving only Nganda chicken. Live weight and morphometric traits of IC are influenced by the nature or type of the agro-ecological zone occupied. In the current study, ecological zones significantly affected live-body weight and morphometric traits of IC except tarsus circumference. Recently, Abiyu (2019) reported significant agro-ecological effects on body weight and linear body traits except back length. Significant ecological effects on live-body weight and morphometric traits have also been reported in IC ecotypes of Algeria (Dahloum *et al.*, 2016) and Ethiopia (Assefa & Melesse, 2018; Negassa *et al.*, 2014). Negassa *et al.* (2014) reported significant variations in body weight, wingspan, comb length, beak length, breast angle, and keel bone length of IC sampled from three agro-ecological zones (Highland, Midland and Lowland). In a recent study, chickens reared in highlands were heavier at maturity, and their survivability was higher than their counterparts in lowland ecologies. However, their egg production traits were not influenced by agroecologies (Guni *et al.*, 2021). Mulugeta *et al.* (2020) reported better performance of different breeds reared in highlands compared to lowland ecologies. Similarly, Alem (2014) and Shishay *et al.* (2015) reported low hatchabilities and survival rates in lowlands. However, body weight and egg production traits were not affected by agro ecologies. On the contrary, Ajayi *et al.* (2020) reported higher live weight gain and more chances of survival for dual-purpose breeds reared in lowland agro ecologies.

The effect of ecology on body weight and morphometric traits is probably influenced by the variety of scavengeable feed resources, which are largely influenced by the climate, vegetation and farming systems practiced in the different zones (Kinderen, 2020; Muchadeyi *et al.*, 2007). Dahloum *et al.* (2016) attributed variations in live-body weight of chicken across ecologies to genetic and environmental factors. Precisely, the variation in body weight and linear

morphometric traits observed among ICs was attributed to the breed and ecology or their interaction (Kassa *et al.*, 2021). Origin of chicken ecotypes could be another source of variation in body weight. The Madi ecotype, West of the River Nile, which most likely belongs to Cluster one of African chickens, is the lightest of all the ecotypes and this can be attributed to its origin (Osman *et al.*, 2016). The Madi ecotype is likely to have originated from the Indus valley of Asia and entered Uganda along the River Nile to Nubia and finally into Uganda (MacDonald & Edwards, 1993).

2.4.2 Egg weight and geometry across ecotypes

The Madi ecotype consistently produced eggs with the least weight, volume and breadth amongst all ecotypes. The weight of Madi chicken eggs corresponds to the low weight chickens from the same ecology. Egg weight and geometry have been reported to influence hatching weight and post-hatch performance. Ewonetu (2017) reported higher hatching weights, body weight gain, feed intake and survival rates with increasing egg size. Apart from predicting hatchability and chick weight, egg geometry has an application in comparison of chicken morphology and diversity across ecologies (Narushin, 2005). In the current study, the variation in egg size could be an indicator of genetic variability especially between Madi and other ecotypes.

2.4.3 Principal Component Analysis

Principal component analysis considering individual sex did not identify distinct cock or hen ecotypes. Overlapping of ecotypes based on the first two principal components is evidence of absence of distinct chicken ecotypes. The variations observed across the chicken ecotypes are probably ecologically induced. As a result of human activities including trade and migration, the variation across chicken clusters reported by Osman *et al.* (2016) have reduced due to planned or unplanned cross-breeding. The absence of distinct chicken ecotypes is as a result of

social, economic and cultural interactions among communities across the different ecologies (Okoth *et al.*, 2017). Cross-breeding and ecological effects are responsible for the creation of new sub-groups and phenotypic diversity among chicken ecotypes reported by Ssewanyana *et al.* (2008) and Fleming *et al.* (2016). Okoth *et al.* (2017) grouped eight Kenyan IC into two distinct populations using principal coordinates analysis-PCA, where PCA1 and PCA2 contributed 27.28% and 8.97% respectively. Previously, Lyimo *et al.* (2013) had grouped Tanzanian IC into three distinct populations with PCA1 and PCA2 explaining 89.9 and 5.13% of the variation respectively.

2.4.4 Ecotype diversity and live weight estimation models

The traits and models for estimating live-body weight varied across ecotypes and sex of chicken. Similarly, Negassa *et al.* (2014) reported varying traits and models for estimating live weight of chicken ecotypes across three agro-ecological zones of Ethiopia. In the present study, comb height and length were the best estimators of live weight for cocks and hens respectively for the Nganda chicken ecotype. However, Semakula *et al.* (2011) reported thorax circumference as the best predictor of live weight for mature Nganda chicken ecotype. Negassa *et al.* (2014) demonstrated that though chicken live-body weight was generally predictable by breast angle, body length and shank circumference were specific to hens and cocks respectively. Recently, Abiyu (2019) also indicated that body length is the best estimator of live weight among hens. Tabassum *et al.* (2014) and Assefa & Melesse (2018) reported the highest correlation coefficient of 0.70 and 0.64 between body weight and thorax circumference among Bangladesh and Ethiopian IC respectively. It is a common occurrence to have different studies presenting different traits as the best live weight estimators for one or all sexes. The prediction of live-body weight based on regression models provides an alternative to selecting superior individual chickens based on highly correlated linear traits (Dahloum *et al.*, 2016; Assefa & Melesse, 2018; Abiyu, 2019). Compared to simple and multiple regression, power

model equations have been reported to be the most reliable in predicting live weight of chickens (Semakula *et al.*, 2011). Live-body weight is an indicator of the general performance of IC and therefore an important tool when selecting breeding stock. In addition, prediction models reduce the challenges of determining live weight (Negassa *et al.*, 2014), increase income (Semakula *et al.*, 2011) and a potential tool of assessing diversity across chicken ecotypes.

2.4.5 Ecotype-ecology interaction

In the current study, linear morphometric traits for estimating live weight varied across ecotypes and sex of chickens. However, tarsus circumference was the dominant trait across ecotypes for estimating live weight of either cocks or hens. This is because tarsus circumference did not vary significantly across ecotypes as compared to other traits. In a statistical modeling study involving indigenous female chicken, the values of body weight and length of linear body traits except back length were significantly influenced by the agro-ecologies (low land, Midland and Highlands) they occupy thus confirming ecotype-ecology interaction (Abiyu, 2019).

Therefore, in the present study, apart from estimating body weight, the diversity in the weight estimation models is an indicator of wide morphological and existence of genetic variation within and across the chicken ecotypes. The observed morphological variations across ecotypes provides an opportunity for genetic improvement of IC through selection (Abiyu, 2019).

2.5 Conclusion

The study revealed that all linear morphometric traits and body live weight were influenced by ecotype except TAC. This further explains the dominance of TAC in body weight estimation models across all ecotypes. The study also confirmed that all morphometric traits and body weight are influenced by the sex of chickens across all ecotypes.

On the basis of Principal Component Analysis, the study presented the nine ecotypes as one large group of IC characterized by within population variations. That is, the study provided no evidence of distinct IC ecotypes on the basis of live weight and morphological traits. Thus, the observed variation in body weight and the morphological traits across ecotypes are ecologically induced. The difference in weight estimation models further emphasizes the ecologically induced morphological variations across ecotypes. Despite the similarity across ecotypes, the Madi chicken weighs less and correspondingly produces eggs with the least weight, volume and breadth.

Therefore, based on the evidence provided by the study, it's recommended to consider all the IC ecotypes as one group. Thus, genetic improvement can be achieved through selection of superior individual chickens across all ecotypes.

CHAPTER THREE

Genetic diversity of Ugandan indigenous chicken ecotypes

Abstract

Genetic characterization of the targeted animal population constitutes the initial stages towards designing a sustainable and impactful improvement program. In this study, the genetic diversity of nine (9) IC ecotypes was assessed based on 15 microsatellites. Genomic DNA was extracted from whole blood drawn from the wing vein of the same cocks and hens sampled in Chapter Two. Whole blood was collected from 200 chickens spread across the nine ecotypes, namely; Madi, Acholi, Lango, Teso, and Nsoga, Nganda, Gisu, Nyoro, and Ankole. Genomic DNA was extracted using the QiAGen kit following the manufacturers recommended procedures. Amplification of the target sequence fragments in the genomic DNA was performed by PCR using the 15 microsatellites recommended by the International Society for Animal Genetics and FAO (ISAG-FAO). The allele size/bands were visualized under UV light and scored manually to create genetic data. Data was imported into the GenAlEx6.5 software to compute the genetic diversity, variance, and polymorphism. Genetic distance among chicken ecotypes was illustrated using a phylogenetic tree, factorial analysis and dendrogram constructed and visualized using the DARwin 6.0.21 software. The genetic structure of Ugandan IC ecotypes was inferred from genotypic data based on the Bayesian approach and performed in STRUCTURE. The output of STRUCTURE was then uploaded into the StructureSelector web server to obtain the optimal K-value (Delta K). Analysis of molecular variance estimated 33 and 67 percent variation among and within ecotypes respectively. The Teso ecotype maintained a large genetic distance with other ecotypes except the Gisu and Nsoga chickens. The shortest genetic distance was between the Lango and Acholi ecotypes. Average loci polymorphism of microsatellites was $61.48 \pm 6.46\%$. The lowest and highest number of effective alleles were

1.00±0.00 and 1.54±0.12 corresponding to ADL0278 and MCW0081 respectively. In addition, MCW0081 exhibited the highest diversity of 0.31. The Nyoro (1.38±0.08) and Gisu (1.33±0.08) ecotypes were associated with the highest number of effective alleles while the Acholi and Ankole had the lowest. The lowest and highest unbiased diversity occurred in Ankole (0.13±0.05) and Nyoro (0.25±0.04) ecotypes respectively. On the basis of the phylogenetic tree, factorial analysis and dendrogram structure analysis, Ugandan IC ecotypes can be grouped into two populations. Population one comprises the Teso, Nsoga and Gisu ecotype while the Madi, Nganda, Nyoro, Lango, Acholi and Ankole chicken belong to population two. The results in the current study therefore confirm the genetic diversity and admixture within Ugandan IC ecotypes. The two chicken populations revealed in the current study need to be conserved and additional genetic studies are recommended to identify the distinct genes they possess. In conclusion, genetic improvement can be achieved by selecting superior cocks and hens within or across the two chicken populations.

Key words: Ecotype, diversity, microsatellites, indigenous chickens, selection, clusters, variance

3.1 Introduction

In an effort to trace the diversity and origin of IC, archaeological, historical and molecular approaches have been used to determine their entry points and distribution routes in Africa (Sawai *et al*, 2010; Mwacharo *et al.*, 2013). All approaches concur with the fact that repeated introduction of chickens in Africa from multiple sites in Asia occurred at different points in time via both water and land (Mwacharo *et al.*, 2013). The descendants of chickens in East Africa and Uganda in particular are believed to have entered Africa through North Africa, around Egypt and dispersed Southwards along the Nile Valley to Nubia (Fuller *et al.*, 2011). These chickens are thought to have originated from the Indus valley of Asia (MacDonald & Edwards, 1993). The second point of entry is the East African coast as a result of the Indian

Ocean trade and just like other crops and livestock, chickens were introduced in Africa (Fuller *et al.*, 2011). These points of entry were further used by Osman *et al.* (2016) to categorize African chickens into Cluster one (Northern and Central African chickens) and Cluster two (East African chickens). Overtime, chickens were dispersed in-wards through different nonlinear tracks into the interior of Africa (Mwacharo *et al.*, 2013). In East Africa and Uganda in particular, archaeological evidence traces the presence of chickens at Kibiro between 1780 to 1880 AD (MacDonald, 1992).

Indigenous chickens are multi-purpose in nature thereby providing several products and services to the keepers. In the earlier days of their introduction in Africa, ICs supported mainly socio-cultural functions and were either slaughtered on special gatherings or days. Currently, IC keepers are shifting from socio-cultural to commercial oriented production to meet the increasing demand for livestock products in the form of eggs and meat (Natukunda *et al.*, 2011; Lubandi *et al.*, 2018). In an attempt to improve the performance of IC to meet the animal-protein demand, the Global Plan of Action for Animal Genetic Resources (AnGR) encourages countries to first establish their genetic diversity among others (FAO, 2007). In response, countries have over time carried out genetic characterization of their IC genetic resources. A genetic characterization study in South Africa revealed that IC in the villages were genetically similar and formed one population cluster when genotyped using 29 microsatellites (Mtileni *et al.*, 2011). Kenyan IC were also genotyped using 18 microsatellites and the results indicated a high within population variance (96%) and upon conducting a structure analysis, chickens were finally pooled into two groups (Okumu *et al.*, 2017). On the basis of 28 microsatellites, Rwandan IC were clustered into four ecotypes characterized by high levels (92%) of within population variance (Habimana *et al.*, 2020). Recently, in Burkina Faso, IC were subdivided into three major populations characterized by high levels of genetic admixture and a large within population variance (77%) on the basis of 20 microsatellite markers (Yacouba *et al.*,

2022). Up to-date, chicken ecotypes in Uganda are identified, characterized and named after the regions they occupy, for example Teso, Nsoga, Nyoro, Nkedi chickens from Teso, Busoga, Bunyoro and Bukedi respectively (Ssewannyana, 2004). Apart from the limited partial phenotypic studies, Ugandan IC ecotypes have not been genetically characterized. Ssewannyana. (2004) highlighted the need to first characterize indigenous animal genetic resources as a strong pillar to designing strategic, efficient and sustainable breed improvement programs. Therefore, the aim of this study was to characterize through assessing the genetic diversity and possible population structuring of Ugandan IC ecotypes based on 15 polymorphic microsatellite markers.

3.2 Materials and Methods

3.2.1 Study area

The study area has been extensively described in Chapter One. Briefly, whole blood was collected from nine chicken ecotypes corresponding to agro-ecological zones of Uganda namely, West Nile, Acholi, Lango, Teso, and Busoga, Buganda, Bugisu, Bunyoro, and Ankole. Chicken ecotypes were inferred based on the agro-ecological zones of Uganda. In each agro-ecological zone, two districts were selected. In each selected district, two sub-counties were sampled. The population of IC was the criteria for selecting the districts and sub-counties. Hence, districts and sub-counties with the highest IC population based on the livestock census of MAAIF and UBOS (2018) were selected.

3.2.2 Ethical consideration

The study was approved by Gulu University Research Ethics Committee (GUREC-2020-18). The research protocol was also registered with the Uganda National Council for Science and Technology (UNCST) for final clearance (NO. A154ES) as described in the previous Chapter.

3.2.3 Chicken population

In total, whole blood was collected from 580 chickens consisting of an equal number of cocks and hens from selected farmers across the 9 (nine) agro-ecological zones of Uganda (Figure 2.1).

3.2.4 Chicken blood collection

Whole blood was drawn from the wing vein of a cock and hen identified in each of the selected households as described in Chapter Two. Upon extraction, blood was transferred into cryotubes containing EDTA or alcohol as a preservative and labeled. The preserved blood samples were packed in cool boxes sandwiched in charged ice packs and transported by road transport to the Multi-Functional Laboratory, Gulu University. Transportation was organized in such a way that samples do not spend more than 24 hours in transit. In the laboratory, samples were kept at -20°C. Sample processing and DNA extraction commenced once all the blood samples were delivered in the laboratory.

3.2.5 DNA extraction

Genomic DNA was extracted using the QiAGen kit (Cat. No./ID:51104) supplied by QIAGEN, Germany. Initially, 20 µL of protease, 200 µL of whole blood and 200 µL of buffer were added into a 1.5 mL microcentrifuge tube followed by vortexing for 15 seconds. The mixture was then incubated in a water bath at 56°C for 10 minutes. After incubation, the mixture was briefly centrifuged to get rid of drops from the inside of the lid. Ethanol (200 µL) was added and mixed with the incubated sample by vortexing. The mixture was then transferred into the QIAamp mini spin column and centrifuged at 8000 rpm for one minute. At the end of centrifugation, the QIAamp mini spin column was placed in a clean 2 mL collection tube and the tube containing the filtrate discarded. The QIAamp mini spin column was placed in a 2mL collection tube and 500 µL of buffer, centrifuged at 8000 rpm for one minute. Again, the tube containing the filtrate

was discarded. Another 500 µL of buffer was added, centrifuged at 14000 rpm for three minutes, the spin column placed in a new 2 mL collection tube and the filtrate discarded. The spin column and collection tubes were again centrifuged at 14000 rpm for one minute. The spin column was then placed in clean 1.5 mL microcentrifuge tubes and the filtrate discarded. Finally, 200 µL of elution buffer were added and incubated at room temperature for one minute, centrifuged at 8000 rpm for one minute. The mini spin column was discarded and finally genomic DNA in microcentrifuge tubes stored at -20°C.

The presence and quality of genomic DNA was assessed using 1% agarose gel electrophoresis and visualized using UV light. After the quality assessment, 200 genomic DNA samples out of the 580 collected were selected for PCR assay. The 200 genomic samples consisted of samples from each of the nine ecotypes under study (Table 3.1).

Table 3.1: Origin of selected and amplified genomic DNA

Ecotype	Districts	District Code	No. of Samples	Sub-Total
Madi	Maracha	D03	15	30
	Yumbe	E03	15	
Lango	Lira	I05	14	29
	APAC	H06	15	
Acholi	Omoro	H04	13	13
Gisu	Kapchorwa	KO7	8	14
	Mbale	K08	6	
Teso	Kumi	JO7	4	16
	Serere	L07	7	
	Amuria	L06	1	
Nganda	Nakaseke	G08	13	34
	Nakasongola	G07	13	
	Mubende	F09	12	
Nyoro	Kibaale	E08	13	31
	Hoima	E07	14	
	Kiryandongo	G06	4	
Ankole	Kiruhura	D11	6	15
	Rukungiri	B12	5	
	Kabale	B13	4	
Nsoga	Kamuli	I08	7	18
	Iganga	L09	7	
	Jinja	I09	4	
Total				200

3.2.6 PCR amplification

Amplification of sequence fragments by PCR in the extracted genomic DNA was performed using 15 microsatellite markers (Macrogen Europe B.V, Netherlands). The 15 markers were selected from a list of thirty highly polymorphic markers recommended by ISAG-FAO (FAO, 2011). The sequences and associated characteristics are shown in Table 3.2 (also see Appendix 6). The PCR reactions were performed in a total reaction volume of 25 μ l. The total reaction volume comprised of 12.5 μ l of OneTaq[®] 2X Master Mix with standard buffer (Inqaba Biotec East Africa Ltd, Uganda Branch), 9.5 μ l of nuclease free water, 0.5 μ l (100 pmol/ μ l) of forward primer, 0.5 μ l (100 pmol/ μ l) reverse primer (Macrogen Europe B.V, Netherlands), and finally, 2 μ l of DNA template. Amplification of sequences was performed using a thermocycler

(SimpliAmp™, Singapore) after optimizing their corresponding PCR conditions in Table 3.2. PCR Amplicons were separated by electrophoresis at 120 V, 400mA through a 1.5% agarose-TBE gel, for 45 minutes depending on the expected band size. The fragment sizes (See Appendix 4) were visualized under UV light and scored manually on the basis of band size.

Table 3.2: Sequences and characteristics of microsatellite markers used for PCR amplification

Oligo Name	Chromosome	Forward primer 5` - Oligo Seq - 3`	Reverse primer 5` - Oligo Seq - 3`	Annealing temperature (°C)	Allele size (Base-pairs)
MCW0111	1	GCTCCATGTGAAGTGGTTTA	ATGTCCACTTGTCAATGATG	60	96-120
ADL0268	1	CTCCACCCCTCTCAGAACTA	CAACTTCCCATCTACCTACT	60	102-116
MCW0034	2	TGCACGCACTTACATACTTAGAGA	TGTCCTTCCAATTACATTCATGGG	60	212-246
MCW0037	3	ACCGGTGCCATCAATTACCTATTA	GAAAGCTCACATGACACTGCGAAA	74	154-160
LEI0094	4	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	60	247-287
MCW0014	6	TATTGGCTCTAGGAACTGTC	GAAATGAAGGTAAGACTAGC	58	164-182
MCW0183	7	ATCCCAGTGTGCGAGTATCCGA	TGAGATTTACTGGAGCCTGCC	58	296-326
ADL0278	8	CCAGCAGTCTACCTTCCTAT	TGTCATCCAAGAACAGTGTG	60	114-126
MCW0067	10	GCACTACTGTGTGCTGCAGTTT	GAGATGTAGTTGCCACATTCCGAC	60	176-186
MCW0123	14	CCACTAGAAAAGAACATCCTC	GGCTGATGTAAGAAGGGATGA	60	76-100
MCW0330	17	TGGACCTCATCAGTCTGACAG	AATGTTCTCATAGAGTTCCTGC	60	256-300
MCW0069	E60C04W23	GCACTCGAGAAAACCTCCTGCG	ATTGCTTCAGCAAGCATGGGAGGA	60	158-176
MCW0295	4	ATCACTACAGAACACCCTCTC	TATGTATGCACGCAGATATCC	60	88-106
MCW0081	5	GTTGCTGAGAGCCTGGTGCAG	CCTGTATGTGGAATTACTTCTC	60	112-135
MCW0222	3	GCAGTTACATTGAAATGATTCC	TTCTCAAAACACCTAGAAGAC	60	220-226

3.2.7 Statistical analysis

Genetic variation and relationship

The scored fragment sizes were entered into Microsoft Excel data templates compatible with the analytical software tools. Genetic diversity, distribution of genetic variation among or within ecotypes, allelic parameters and microsatellite polymorphism were analyzed using GenAlEx6.5 tool (Genetic analysis in Excel version 6). A phylogenetic tree, factorial analysis and dendrogram illustrating the genetic distance among the chicken ecotypes was constructed and visualized using Dissimilarity Analysis and Representation for windows software (DARwin 6.0.21) based on Nei's genetic distance and neighbor joining commands.

Cluster analysis and genetic admixture

The genetic structure and possible assignment of the nine Ugandan IC ecotypes into clusters was inferred from a multi-locus genotypic data based on the Bayesian approach and performed in STRUCTURE software, version 2.3.4 (Pritchard, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2003) and coded by Pritchard, Falush & Hubisz, (2012). The analysis utilized the admixture model, and the parameter settings; 192 individuals, 15 loci, 10 Burn-in periods and 10000 replications. The tested values of K (number of clusters) ranged from two to nine. The output of the STRUCTURE software were input into a zipped folder and uploaded into the StructureSelector web server (Chinese Academy of Sciences, China) developed by Li & Liu, (2018) to obtain the optimal K (Delta K). The value of Delta K was calculated using the method described by Evanno *et al.* (2005).

3.3 Results

3.3.1 Analysis of Molecular Variance of nine Ugandan indigenous chicken ecotypes

The genetic variance of Ugandan IC ecotypes as partitioned by Analysis of Molecular Variance (AMOVA) is presented in Table 3.3. The highest genetic variation of 0.121 out of 0.181, corresponding to 67 percent was distributed within IC ecotypes. On the other side, genetic variation among IC ecotypes was 0.060 out of 0.181 corresponding to 33 percent. Therefore, chickens within the same ecotype exhibit high levels of genetic variation compared to across ecotypes. The low genetic variation across ecotypes is an indicator of high chances of genetic similarity across ecotypes.

Table 3.3: Analysis of molecular Variance of nine Ugandan indigenous chicken ecotypes

Source	Df	SS	MS	Est. Var.	%
Among ecotypes	8	10.988	1.373	0.060	33%
Within ecotypes	186	22.530	0.121	0.121	67%
Total	194	33.518		0.181	100%

3.3.2 Genetic distance among ecotypes

The genetic distance across Ugandan IC ecotypes based on Nei's genetic distance is presented in Table 3.4. The largest genetic distance occurred between Teso and other ecotypes except Gisu and Nsoga. The largest genetic distance was between Teso on one side and Ankole (0.618), Acholi (0.570), Madi (0.549), Lango (0.499) and Nganda (0.423) ecotypes on the other side. However, the Nsoga (0.113) and Gisu (0.088) exhibited close genetic proximity to the Teso ecotype. In addition, the Gisu and Nsoga ecotypes exhibited a short genetic distance (0.023) between them but maintained a large distance ranging from 0.227 and 0.352 with other ecotypes. Acholi and Lango ecotypes maintained the same genetic distance of 0.060 from the Ankole chicken. Acholi and Lango ecotypes exhibited the least genetic distance (0.014) between them.

Table 3.4: Pairwise population matrix of Nei's genetic distance among Ugandan IC ecotypes

Acholi	Ankole	Lango	Madi	Nganda	Gisu	Nsoga	Teso	
0.06	0.00							Ankole
0.01	0.07	0.00						Lango
0.02	0.03	0.03	0.00					Madi
0.04	0.09	0.04	0.03	0.00				Nganda
0.31	0.34	0.26	0.31	0.23	0.00			Gisu
0.30	0.35	0.26	0.30	0.22	0.02	0.000		Nsoga
0.57	0.62	0.50	0.55	0.42	0.09	0.113	0.00	Teso
0.02	0.06	0.02	0.02	0.02	0.20	0.190	0.40	Nyoro

3.3.3: Percentage of Polymorphic Loci

The percentage loci polymorphism based on Ugandan IC ecotypes is summarized in Table 3.5. The average percentage of loci polymorphism was 61.48 ± 6.46 percent. The largest polymorphism occurred in the Nyoro, Madi and Nganda ecotypes corresponding to 86.67, 80.00, 80.00 percent respectively. The Ankole ecotype exhibited the lowest loci polymorphism of 33.33 percent.

Table 3.5: Microsatellite Polymorphism in Ugandan IC ecotypes

Population	Polymorphism (%)
Acholi	53.33
Ankole	33.33
Lango	73.33
Madi	80.00
Nganda	80.00
Gisu	60.00
Nsoga	40.00
Teso	46.67
Nyoro	86.67
Average	61.48
SE	6.46

3.3.4: Allelic parameter estimates and diversity indicators

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh) of Ugandan IC ecotypes is detailed in Tables 3.6 to 3.14. In the Acholi, Ankole and Lango ecotypes,

MCW0081 provided more molecular information and resulted in the highest unbiased diversity values of 0.513, 0.556 and 0.497 respectively. Microsatellite MCW0069 resulted in the highest Unbiased Diversity of 0.508 among the Madi ecotype. In the Nganda ecotype, LEI0094 was characterized by high Unbiased Diversity value of 0.491, while ADL0278, MCW0067 and ADL0268 did not indicate any diversity. In the Gisu ecotype, MCW0111 and LEI0094 were equally informative and exhibited the highest Unbiased Diversity of 0.527. Microsatellites MCW0081, MCW0295, MCW0330, ADL0278, MCW0014 and MCW0067 did not exhibit any diversity in the Gisu ecotype. Three microsatellites, MCW0222, MCW0111 and LEI0094 were equally informative and resulted in the highest diversity index of 0.536 in the Nsoga ecotype. Microsatellite MCW0067 produced the highest Unbiased Diversity value of 0.513 in the Teso ecotype. In the Nyoro ecotype, microsatellite MCW0081 exhibited the highest diversity value of 0.508, while ADL0278 and MCW0037 were not informative.

Table 3.6: Allelic parameter estimates of Acholi ecotype

Microsatellite	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.846	0.846	0.154	2.000	1.352	0.429	0.260	0.282
MCW0123	0.846	0.846	0.154	2.000	1.352	0.429	0.260	0.282
MCW0111	0.923	0.923	0.077	2.000	1.166	0.271	0.142	0.154
MCW0034	0.923	0.923	0.077	2.000	1.166	0.271	0.142	0.154
MCW0081	0.615	0.615	0.385	2.000	1.899	0.666	0.473	0.513
MCW0295	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0069	0.923	0.923	0.077	2.000	1.166	0.271	0.142	0.154
MCW0330	0.769	0.769	0.231	2.000	1.550	0.540	0.355	0.385
MCW0183	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
LEI0094	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0268	0.923	0.923	0.077	2.000	1.166	0.271	0.142	0.154
MCW0037	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0014	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index-I, Diversity-h and Unbiased Diversity-uh

Table 3.7: Allelic parameter estimates of Ankole ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0123	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0111	0.778	0.778	0.222	2.000	1.528	0.530	0.346	0.389
MCW0034	0.889	0.889	0.111	2.000	1.246	0.349	0.198	0.222
MCW0081	0.444	0.444	0.556	2.000	1.976	0.687	0.494	0.556
MCW0295	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0069	0.111	0.111	0.889	2.000	1.246	0.349	0.198	0.222
MCW0330	0.667	0.667	0.333	2.000	1.800	0.637	0.444	0.500
MCW0183	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
LEI0094	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0268	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0037	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0014	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index-I, Diversity-h and Unbiased Diversity-uh

Table 3.8: Allelic parameter estimates of Lango ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0034	0.900	0.900	0.100	2.000	1.220	0.325	0.180	0.186
MCW0081	0.400	0.400	0.600	2.000	1.923	0.673	0.480	0.497
MCW0295	0.967	0.967	0.033	2.000	1.069	0.146	0.064	0.067
MCW0069	0.933	0.933	0.067	2.000	1.142	0.245	0.124	0.129
MCW0330	0.633	0.633	0.367	2.000	1.867	0.657	0.464	0.480
MCW0183	0.967	0.967	0.033	2.000	1.069	0.146	0.064	0.067
LEI0094	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0268	0.633	0.633	0.367	2.000	1.867	0.657	0.464	0.480
MCW0037	0.967	0.967	0.033	2.000	1.069	0.146	0.064	0.067
MCW0014	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

Table 3.9: Allelic parameter estimates of Madi ecotype

Microsatellite	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.867	0.867	0.133	2.000	1.301	0.393	0.231	0.239
MCW0123	0.833	0.833	0.167		1.385	0.451	0.278	0.287
MCW0111	0.967	0.967	0.033	2.000	1.069	0.146	0.064	0.067
MCW0034	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0081	0.367	0.367	0.633	2.000	1.867	0.657	0.464	0.480
MCW0295	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0069	0.567	0.567	0.433	2.000	1.965	0.684	0.491	0.508
MCW0330	0.800	0.800	0.200	2.000	1.471	0.500	0.320	0.331
MCW0183	0.900	0.900	0.100	2.000	1.220	0.325	0.180	0.186
LEI0094	0.933	0.933	0.067	2.000	1.142	0.245	0.124	0.129
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	0.867	0.867	0.133	2.000	1.301	0.393	0.231	0.239
ADL0268	0.933	0.933	0.067	2.000	1.142	0.245	0.124	0.129
MCW0037	0.933	0.933	0.067	2.000	1.142	0.245	0.124	0.129
MCW0014	0.967	0.967	0.033	2.000	1.069	0.146	0.064	0.067

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

Table 3.10: Allelic parameter estimates of Nganda ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.214	0.214	0.786	2.000	1.508	0.520	0.337	0.363
MCW0123	0.786	0.786	0.214	2.000	1.508	0.520	0.337	0.363
MCW0111	0.429	0.429	0.571	2.000	1.960	0.683	0.490	0.527
MCW0034	0.143	0.143	0.857	2.000	1.324	0.410	0.245	0.264
MCW0081	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0295	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0069	0.857	0.857	0.143	2.000	1.324	0.410	0.245	0.264
MCW0330	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0183	0.143	0.143	0.857	2.000	1.324	0.410	0.245	0.264
LEI0094	0.429	0.429	0.571	2.000	1.960	0.683	0.490	0.527
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0268	0.786	0.786	0.214	2.000	1.508	0.520	0.337	0.363
MCW0037	0.786	0.786	0.214	2.000	1.508	0.520	0.337	0.363
MCW0014	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000

Table 3.10: Allelic parameter estimates of Nganda ecotype

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

Table 3.11: Allelic parameter estimates of Gisu ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.947	0.947	0.053	2.000	1.111	0.206	0.100	0.102
MCW0123	0.895	0.895	0.105	2.000	1.232	0.336	0.188	0.193
MCW0111	0.974	0.974	0.026	2.000	1.054	0.122	0.051	0.053
MCW0034	0.895	0.895	0.105	2.000	1.232	0.336	0.188	0.193
MCW0081	0.105	0.105	0.895	2.000	1.232	0.336	0.188	0.193
MCW0295	0.974	0.974	0.026	2.000	1.054	0.122	0.051	0.053
MCW0069	0.974	0.974	0.026	2.000	1.054	0.122	0.051	0.053
MCW0330	0.763	0.763	0.237	2.000	1.566	0.547	0.361	0.371
MCW0183	0.816	0.816	0.184	2.000	1.430	0.478	0.301	0.309
LEI0094	0.605	0.605	0.395	2.000	1.915	0.671	0.478	0.491
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
ADL0268	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0037	0.895	0.895	0.105	2.000	1.232	0.336	0.188	0.193
MCW0014	0.974	0.974	0.026	2.000	1.054	0.122	0.051	0.053

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

Table 3.12: Allelic parameter estimates of Nsoga ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.825	0.825	0.175	2.000	1.406	0.464	0.289	0.296
MCW0123	0.900	0.900	0.100	2.000	1.220	0.325	0.180	0.185
MCW0111	0.875	0.875	0.125	2.000	1.280	0.377	0.219	0.224
MCW0034	0.800	0.800	0.200	2.000	1.471	0.500	0.320	0.328
MCW0081	0.450	0.450	0.550	2.000	1.980	0.688	0.495	0.508
MCW0295	0.925	0.925	0.075	2.000	1.161	0.266	0.139	0.142
MCW0069	0.800	0.800	0.200	2.000	1.471	0.500	0.320	0.328
MCW0330	0.600	0.600	0.400	2.000	1.923	0.673	0.480	0.492
MCW0183	0.750	0.750	0.250	2.000	1.600	0.562	0.375	0.385
LEI0094	0.725	0.725	0.275	2.000	1.663	0.588	0.399	0.409
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	0.875	0.875	0.125	2.000	1.280	0.377	0.219	0.224
ADL0268	0.925	0.925	0.075	2.000	1.161	0.266	0.139	0.142
MCW0037	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0014	0.975	0.975	0.025	2.000	1.051	0.117	0.049	0.050

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

Table 3.13: Allelic parameter estimates of Nyoro ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.375	0.375	0.625	2.000	1.882	0.662	0.469	0.536
MCW0123	0.875	0.875	0.125	2.000	1.280	0.377	0.219	0.250
MCW0111	0.625	0.625	0.375	2.000	1.882	0.662	0.469	0.536
MCW0034	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0081	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0295	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0069	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0330	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0183	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
LEI0094	0.625	0.625	0.375	2.000	1.882	0.662	0.469	0.536
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	0.750	0.750	0.250	2.000	1.600	0.562	0.375	0.429
ADL0268	0.875	0.875	0.125	2.000	1.280	0.377	0.219	0.250
MCW0037	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0014	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

Table 3.14: Allelic parameter estimates of Teso ecotype

Microsatellites	Band Freq.	p	q	Na	Ne	I	h	uh
MCW0222	0.308	0.308	0.692	2.000	1.742	0.617	0.426	0.462
MCW0123	0.846	0.846	0.154	2.000	1.352	0.429	0.260	0.282
MCW0111	0.077	0.077	0.923	2.000	1.166	0.271	0.142	0.154
MCW0034	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0081	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0295	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0069	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0330	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
MCW0183	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
LEI0094	0.154	0.154	0.846	2.000	1.352	0.429	0.260	0.282
ADL0278	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000
MCW0067	0.385	0.385	0.615	2.000	1.899	0.666	0.473	0.513
ADL0268	0.846	0.846	0.154	2.000	1.352	0.429	0.260	0.282
MCW0037	0.154	0.154	0.846	2.000	1.352	0.429	0.260	0.282
MCW0014	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000

Band Frequency, Allele Frequency (p & q), Number of Alleles (Na), Number of Effective Alleles (Ne), Information Index (I), Diversity (h) and Unbiased Diversity (uh)

The number of alleles (Na & Ne), Shannon's Information Index (I) and Diversity (h & uh) of Ugandan IC ecotypes over the loci are presented in Table 3.15. The Nyoro (1.38±0.08) and Gisu (1.33±0.08) ecotypes had the highest number of effective alleles. The Acholi and Ankole ecotypes had the same and lowest number (1.19) of effective alleles. The Nyoro (0.38±0.06) ecotype had the highest Shannon's Information Index followed by Gisu ecotypes (0.31±0.07) and lowest among the Ankole ecotypes. The unbiased diversity ranged from 0.13±0.05 in Ankole to the highest diversity value of 0.25±0.04 within the Nyoro ecotype.

Table 3.15: Average allelic parameter estimates of ecotypes

Ecotype	N	Na	Ne	I	h	Uh
Acholi	13.0±0.00	1.53±0.13	1.19±0.06	0.21±0.06	0.13±0.04	0.14±0.04
Ankole	9.00±0.00	1.33±0.13	1.19±0.08	0.17±0.07	0.11±0.05	0.13±0.05
Lango	30.0±0.00	1.73±0.12	1.27±0.09	0.26±0.07	0.17±0.05	0.17±0.05
Madi	30.00±0.00	1.80±0.11	1.27±0.08	0.30±0.06	0.18±0.04	0.19±0.04
Nganda	38.00±0.00	1.80±0.11	1.21±0.07	0.25±0.05	0.15±0.04	0.15±0.04
Gisu	14.00±0.00	1.47±0.19	1.33±0.08	0.31±0.07	0.20±0.05	0.22±0.05
Nsoga	8.00±0.00	1.13±0.22	1.25±0.10	0.22±0.08	0.15±0.05	0.17±0.06
Teso	13.00±0.00	1.20±0.22	1.21±0.08	0.22±0.07	0.14±0.04	0.15±0.05
Nyoro	40.00±0.00	1.87±0.09	1.38±0.08	0.38±0.06	0.24±0.04	0.25±0.04

Band Frequency, Allele Frequency (p & q), Samples Size, Number of Alleles-Na, Number of Effective Alleles-Ne, Information Index-I, Diversity-h and Unbiased Diversity-uh

The number of alleles (Na & Ne), Shannon's Information Index (I) and Diversity (h & uh) over loci for Ugandan IC ecotypes are presented in Table 3.16. The mean number alleles over the loci for the 9 ecotypes ranged from 1.00±00 to 2.00±00. The number of effective Alleles ranged from 1.00±00 to 1.54±0.12 corresponding to ADL0278 and MCW0081 respectively. The highest and lowest Shannon's Information Index was associated with MCW0081 (0.41±0.09) and ADL0278 (0.00±00) respectively. Loci MCW0081 exhibited the highest unbiased diversity of 0.31.

Table 3.16: Average allelic parameter estimates loci

		MCW 0222	MCW 0123	MCW 0111	MCW 0034	MCW 0081	MCW 0295	MCW 0069	MCW 0330	MCW 0183	LEI 0094	ADL 0278	MCW 0067	ADL 0268	MCW 0037	MCW 0014
N	Mean	21.67	21.67	21.67	21.667	21.67	21.67	21.67	21.67	21.667	21.67	21.67	21.67	21.67	21.667	21.67
	SE	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.29
Na	Mean	1.89	1.89	2.00	1.44	1.33	1.33	1.78	1.33	1.33	1.67	1.00	1.44	1.78	1.56	1.33
	SE	0.09	0.09	0.00	0.23	0.26	0.13	0.11	0.26	0.22	0.13	0.00	0.14	0.11	0.14	0.13
Ne	Mean	1.39	1.27	1.40	1.18	1.54	1.03	1.26	1.46	1.18	1.44	1.00	1.23	1.28	1.15	1.02
	SE	0.07	0.04	0.09	0.04	0.12	0.01	0.08	0.10	0.06	0.11	0.00	0.08	0.07	0.05	0.01
I	Mean	0.40	0.34	0.40	0.24	0.41	0.06	0.29	0.40	0.21	0.36	0.00	0.22	0.31	0.19	0.04
	SE	0.05	0.04	0.05	0.05	0.09	0.03	0.06	0.08	0.06	0.08	0.00	0.07	0.06	0.05	0.02
h	Mean	0.26	0.20	0.25	0.14	0.29	0.03	0.18	0.27	0.13	0.25	0.00	0.14	0.19	0.11	0.02
	SE	0.04	0.03	0.04	0.03	0.06	0.01	0.04	0.05	0.04	0.06	0.00	0.05	0.04	0.03	0.01
Uh	Mean	0.27	0.21	0.27	0.15	0.31	0.03	0.18	0.28	0.13	0.26	0.00	0.16	0.20	0.12	0.02
	SE	0.04	0.03	0.05	0.03	0.07	0.01	0.04	0.06	0.04	0.06	0.00	0.05	0.04	0.04	0.01

Band Frequency, Allele Frequency (p & q), Samples Size, No. Alleles, No. Effective Alleles, Information Index, Diversity and Unbiased Diversity
Na = No. of Different Alleles, Ne = No. of Effective Alleles, I = Shannon's Information Index, h = Diversity, uh = Unbiased Diversity

3.3.5: Clustering of IC ecotypes

The phylogenetic tree (Figure 3.4), factorial analysis (Figure 3.3), dendrogram based on neighbor-joining cluster analysis (Figure 3.4) and STRUCTURE analysis (Figure 3.7) grouped Ugandan IC into two major populations. In addition, the output at Delta K = 2 (two), produced the most optimal number of clusters for the IC ecotypes. The Teso, Nsoga and Gisu ecotypes constitute one cluster and the Madi, Nganda, Nyoro, Lango, Acholi and Ankole constitute the second cluster. However, the nine chicken ecotypes exhibited high levels of genetic admixture and gene flow among them (Figure 3.7). The high levels of genetic mixing are probably due to uncontrolled traditional mating and movement of IC across. In addition, ecotypes in the second cluster were characterized by limited geographic specificity.

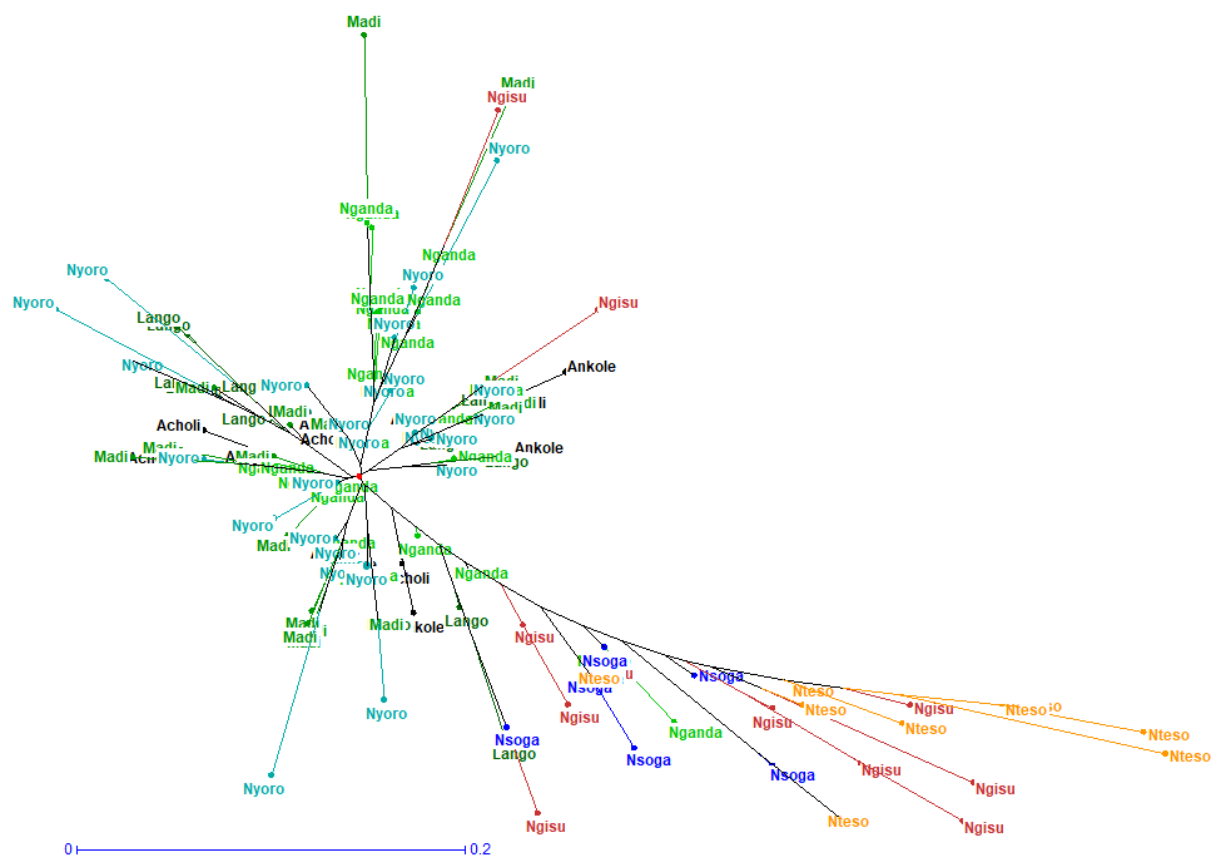


Figure 3.4: Phylogenetic tree of Ugandan IC ecotypes

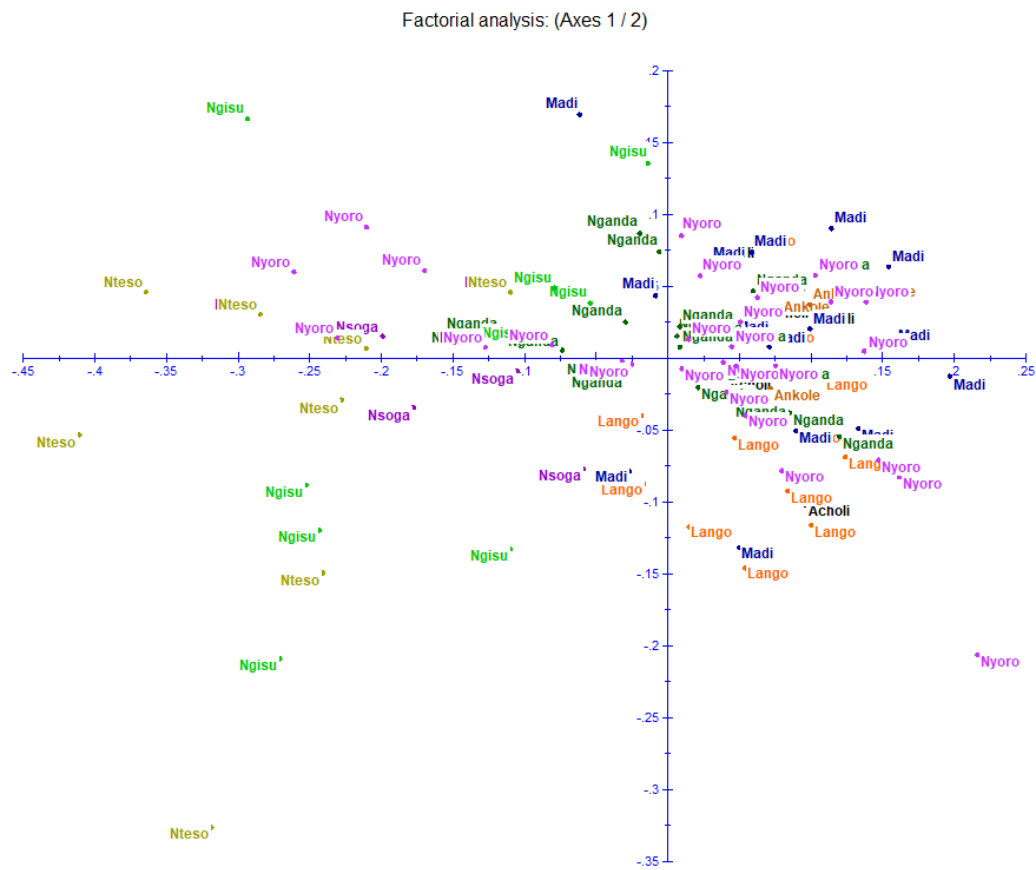


Figure 3.5: Factorial analysis of IC ecotypes

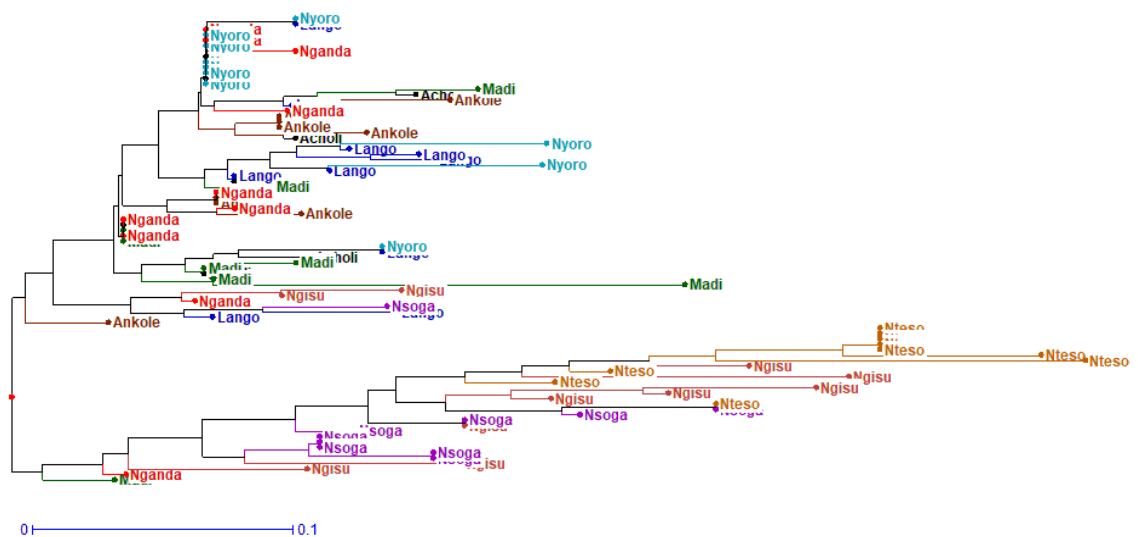


Figure 3.6: Dendrogram based on genetic distance estimates of 15 microsatellites

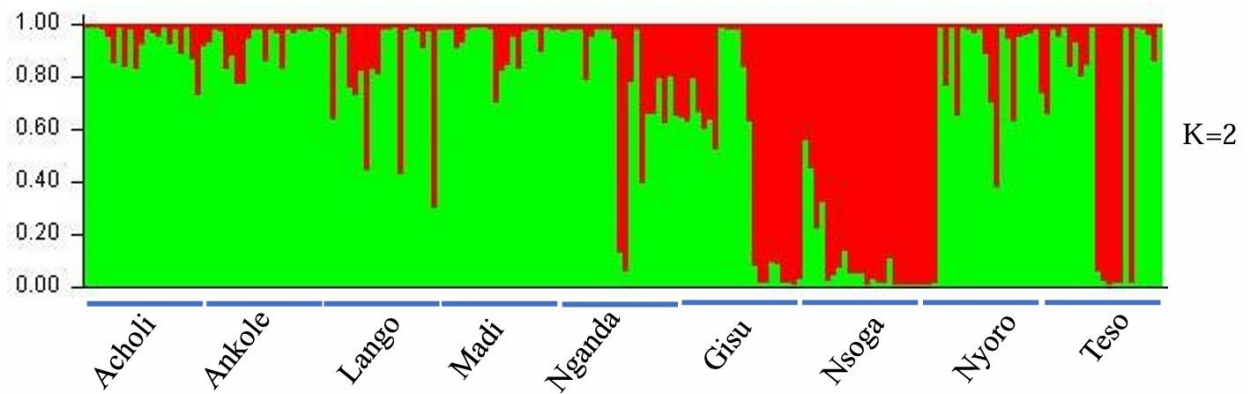


Figure 3.7: Structure clustering of nine Ugandan IC ecotypes

3.4 Discussion

3.4.1 Genetic variation and relationship among ecotypes

Analysis of molecular variance

Ugandan IC expressed high genetic variation of 67 percent within compared to across ecotypes (33 percent). Previously, comparable values of 64.8 and 35.2 percent corresponding to genetic variations within and among populations respectively were reported for African chickens characterized using mtDNA D-loop sequences (Mobegi, 2006). In a recent study, values of 77 and 23 percent corresponding to within and among ecotype variance, respectively were reported in Burkina Faso chickens (Yacouba *et al.*, 2022). However, high genetic variance within IC ecotypes corresponding to 96 and 92 percent were reported in Kenya and Rwanda chickens (Habimana *et al.*, 2020; Okumu *et al.*, 2017). The current study confirms the phenotypic variance with respect to productive and reproductive traits within and among selected Ugandan IC ecotypes (Ssewanyana *et al.*, 2008). The large percentage of within ecotype variance is also an indicator of absence of streamlined selection of IC. However, the large phenotypic and genetic variance within the IC ecotypes provide a raw material for the synthesis of superior lines (Ssewanyana *et al.*, 2008). That is, genetic improvement of IC can

be achieved by applying within ecotype selection. Superior chickens could be selected from any ecotype and recruited into a breeding program for the synthesis of a new line.

Loci polymorphism and ecotype diversity

The highest levels of loci polymorphism occurred within the Nyoro (86.67), Madi (80.00) and Nganda (80.00) chicken ecotypes. The current study estimated an average loci polymorphism of 61.48 ± 6.46 percent which is lower than 96.71 percent reported by Okumu *et al.* (2017) based on similar microsatellites for Kenyan IC ecotypes. The high polymorphism in Kenyan chickens is probably due to their wide geographical distribution and associated attainment of selection signatures. The high levels of loci polymorphism could be due to the acquisition of different forms of alleles to enable adaptation of chickens to stressful conditions in the respective ecologies (Elbeltagy *et al.*, 2019; Fleming *et al.*, 2016). The large loci polymorphism in Nganda chicken could be due to repeated introductions of chickens from other ecotypes into the Central where the Nganda ecotype is habited. The Ankole ecotype exhibited the lowest loci polymorphism of 33.33 percent and this could probably be attributed to limited chicken rearing and population in the agro-ecological zone.

In the current study, MCW0081 exhibited the highest Shannon's Information Index and diversity. In a related study, MCW0123 had the highest genetic variance among the microsatellites considered in genotyping Kenyan chickens (Okumu *et al.*, 2017). In Tanzania, MCW0016 was the most highly polymorphic loci out of the 18 microsatellite markers used in the study (Moto & Rubanza, 2020).

Genetic distance among ecotypes

In the present study, the Teso chicken maintained a large genetic distance ranging from 0.618 to 0.423 with the Acholi, Ankole, Lango, Madi and Nganda ecotypes. However, the Gisu and Nsoga chickens have a short genetic distance from the Teso ecotype and are closely related.

The Teso, Gisu and Nsoga ecotypes originate from the Eastern agro-ecological zone of Uganda, which produces over 31 percent of the IC (MAAIF & UBOS, 2018). The Teso ecotype remains distinct from others probably due to limited inward compared to outward movement of chickens from the zone. The Teso, Gisu and Nsoga ecotypes inhabit the same Eastern agro-ecological zone and are closely related due to their geographical proximity. Likewise, the Acholi and Lango ecotypes are closely genetically similar and this could be due to their geographical proximity. The short genetic distance across ecotypes indicates continuous exchange of IC genetic materials within and across the agro-ecological zones of Uganda.

Population structure analysis

Indigenous chicken ecotypes in Uganda belong to two population clusters based on the phylogenetic tree, factorial analysis, dendrogram and STRUCTURE analysis in the present study. The two chicken populations revealed in the current study could be traced and associated with the two clusters of African chickens suggested by Osman *et al.* (2016). Osman *et al.* (2016) categorized African chickens into cluster one (Northern and Central African chickens) and cluster two (East African chickens) based on the points of entry into Africa. As such, population one comprising Acholi, Ankole, Lango, Madi, Nganda and Nyoro ecotypes are more likely to be descendants of the Northern and Central African chickens (cluster one). This is because population one ecotypes occupy the entry route for cluster one chickens which stretches from North Africa, Egypt and Southwards along the Nile Valley to Nubia. On the other side, population two comprising Teso, Gisu and Nsoga ecotypes occupy the entry route of cluster two chickens, the East African chickens. Therefore, population two ecotypes are more likely to be descendants of the East African chickens, which entered Uganda via the East African coast. Generally, African chickens were previously split into six populations based on phylogenetic analysis mtDNA and corresponding to six maternal lineages (Mobegi, 2006).

In a related study, Rwandan IC were pooled into two clusters when genotyped using 28 microsatellite markers (Habimana *et al.*, 2020). Similarly, Kenyan chickens were also grouped into two clusters when genotyped using eighteen microsatellite markers (Okumu *et al.*, 2017). Using the same number of microsatellite markers, Moto & Rubanza, (2019) revealed that chickens in Central Tanzania districts belong to one cluster. In the same year, Mwambene *et al.* (2019) genotyped IC in Southern Highlands of Tanzania based on one microsatellite marker (LEI0258) and found no distinct ecotype.

STRUCTURE analysis in the present study also revealed high levels of genetic admixture across chicken populations. Previously, high levels of overlapping across the Nganda and Teso chicken ecotypes have been reported (Fleming *et al.*, 2016). The overlapping associated with IC ecotypes is probably due to the movement of IC during trade or social practices which facilitate uncontrolled inter-ecotype mating. In addition, based on SNP genotyping, IC ecotypes in Uganda share a common ancestry in Asia irrespective of their points of entry into Africa (Fleming *et al.*, 2016).

3.5 Conclusion

The genotyping of Ugandan IC based on 15 polymorphic microsatellites evidently reveals the large variance within and among ecotypes. The large variance also confirms and explains the widespread phenotypic diversity across IC reported in the previous Chapter. On the basis of the phylogenetic tree, factorial analysis, and dendrogram structural analysis, Ugandan IC ecotypes belong to two major populations. Ecotypes in population one are likely descendants of the Northern and Central African chickens, while population two are associated with East African chickens. Despite the presence of distinct populations, ecotypes exhibit high levels of genetic admixture. The high levels of genetic admixture are due to continuous movement and random mating of chickens across ecotypes. In addition, the current results disregard the recommendation made in Chapter Two of considering Ugandan IC as one population. Hence,

there is a need to conserve the two populations, and additional genetic studies are recommended to identify the distinct genes they possess. Therefore, as suggested in the previous Chapter, genetic improvement could be achieved by selecting superior cocks and hens across the two chicken populations.

CHAPTER FOUR

Heritability, genetic and phenotypic correlation estimates of growth and egg production traits for Ugandan indigenous chickens

Abstract

The high genetic variation within Ugandan IC, which provides an opportunity to select superior breeding stock for sustainable production and conservation, is under-exploited. This study aimed at estimating heritability, genetic and phenotypic correlation coefficients of productive and reproductive traits of Ugandan IC as a basis for selection. Data on traits were collected across three consecutive generations of scavenging IC. Weight (W) and shank length (SL) of chicks at hatching (HW) as well as at two (W2; SL2), four (W4; SL4), six (W6; SL6), eight (W8; SL8), and twelve (W12; SL12) weeks of growth. Body weights at the on-set of lay (WFE) were also measured. In addition, egg number (EN-60), egg weight (EW), clutch number (CLN-60), and clutch size (CLS-60) over a period of 60 days were recorded. Genetic parameters were estimated using the univariate animal model analysis with restricted maximum likelihood procedure using the variability package of R, version 4.1.1 (R Core Team, 2021). Heritability of traits ranged from 0.30 and 0.72, except SL4 (0.02), SL12 (0.14), and EN-60 (0.17). The traits EN-60 and W4 were negatively phenotypically correlated (-0.49). Body weight at first egg was positively, and genetically correlated (0.99) with SL8. Egg number was significantly, negatively, and genetically correlated (-0.96) with SL12. In conclusion, shank length is a potential phenotypic marker when selecting for live weight at the on-set of lay and egg yield. The shank length could, therefore, permit selection of superior chickens at an early age.

Keywords: Variation, Selection, Trait, Indigenous chicken, Phenotypic marker

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4.1 Introduction

Indigenous chickens (IC) continue to retain a wide range of desirable attributes and contribute to the nutritional and socio-economic well-being of rural households in Asia and Africa (Mahendra, 2016; Rajkumar, 2021). However, the contribution of IC to the well-being of rural households is limited by their innate slow growth and low egg productivity (Wong *et al.* 2017). On average, the on-set of lay is delayed up to 7.2 months, and hens lay only 36 eggs per year. On the side of cockerels, sexual maturity is delayed up to 5.9 months (Ssewanyana *et al.* 2008; Nakkazi *et al.* 2014). The high genetic variation within IC, which provides an opportunity to identify replacement stock with desired productive and reproductive traits, remains under-exploited (Ssewanyana *et al.* 2008; Magothe *et al.* 2010; Fleming *et al.* 2016; Wong *et al.*, 2017; Lubandi *et al.* 2019; Adoligbe *et al.* 2020;). The common practice of genetic improvement through cross-breeding with highly specialized breeds has accelerated loss of important adaptation, broodiness, and reproductive traits critical for survival under harsh, rural resource-constrained farming systems (Tadelle *et al.* 2000; Dana *et al.*, 2010; Semahoro *et al.* 2018). Unlike cross-breeding, selecting within the surviving ecotypes provides an opportunity to conserve the IC genetic resources and its associated multiple traits (Dana *et al.*, 2010; Osei-Amponsah *et al.* 2013; Zonuz *et al.* 2013). In addition, the unique genomic and phenotypic selection signatures acquired by IC over generations for their adaptation to harsh environments need to be conserved (Fleming *et al.* 2016; Elbeltagy *et al.* 2019).

Acquisition and expression of traits by the offspring depend on the degree of inheritance and association of the responsible genes. Apart from varying across genotypes and environments, genetic parameters change with age and physiological status of chickens (Magothe *et al.* 2007; Dana *et al.*, 2011; Gwaza *et al.* 2016; Singh *et al.* 2018; Chomchuen *et al.* 2022; Sanda *et al.* 2022). The variation in heritability of traits with age, therefore, requires timing of selection at a stage when heritability is at its peak (Adeyinka *et al.* 2006; Osei-Amponsah *et al.* 2013; Singh *et al.* 2018).

The association between traits indicates the underlying genetic interactions, and it is always important to know the effect of selecting one trait on others (Sanda *et al.* 2022). Genetic and phenotypic correlations determine a combination of traits that can be considered simultaneously in a multi-trait selection program (Magothe *et al.* 2007; Sanda *et al.* 2022). A moderate to high genetic correlation among traits is required to achieve a high breeding value (Magothe *et al.* 2007; Niknafs *et al.* 2012). Negative additive genetic correlations have been reported for egg production and live weight (Ghazikhani *et al.* 2007). Such negative genetic correlation of traits creates a puzzle for upgrading multipurpose IC reared mostly for both meat and eggs.

Genetic improvement through selection of superior replacement stock requires population-specific genetic parameter estimates of productive and reproductive traits. Genetic and phenotypic parameter estimates guide the setting of breeding objectives, stage of selection, and marker traits in a breeding program. In addition, previous genetic parameter estimation studies targeted a single ecotype from a selected province (Norris & Ngambi, 2006; Dana *et al.*, 2011). Therefore, this study aimed to estimate the genetic and phenotypic parameters of productive and reproductive traits of a mixed population of IC ecotypes under a supplemented scavenging system.

4.2 Materials and Methods

4.2.1 Acquisition of eggs and rearing of chickens

One thousand two hundred (1200) fertile IC eggs were purchased from selected rural households in six agro-ecological zones of Uganda as previously described in Chapter Two. Fertile eggs laid within a period of one to five days were purchased. Rural households rearing IC with no access to exotic chicken or eggs were selected. In addition, egg-contributing rural households were selected on the basis of having kept IC for a minimum of three years. Ownership of an indigenous mature cock by the household was also considered for higher egg fertility and hatchability. Eggs were packed in paper trays and delivered to the Gulu University Poultry Unit for artificial incubation using a commercial incubator operated by an Automatic Computer Controller (Model: XM-18, India). Seven hundred thirteen (713) chicks were hatched, wing tagged, and brooded artificially for four weeks using a commercial chick starter (20.3% crude protein, 7.5% crude fiber, 0.5% phosphorus, and 1.1% calcium) purchased from Biyinzika Enterprises Limited, Kampala, Uganda. Heat for the brooder was provided by clay pots using charcoal as a source of fuel for a period of two weeks. After the brooding period, chickens were reared under a scavenging system and supplemented with 15% and 11% crude protein diets during their growth and laying phases, respectively. Chickens were vaccinated following a schedule recommended for commercial egg-type chickens (Ugachick Poultry Breeders Limited, Uganda).

Chickens were sexed at eight weeks of age into 250 pullets and 241 cockerels. Using body weight at the on-set of lay and the number of eggs laid in two consecutive clutches as criteria, 16 out of the 68 hens evaluated were selected to become parents for the second generation (See Appendix 7). Hens weighing at least 1213 g at the on-set of lay, with the potential of laying 16 eggs or above in two consecutive clutches, were selected. Four cockerels were selected based on live-body weight at twelve weeks of age for random mating with the selected hens at each

generation. The selected cockerels weighed between 657 and 992 g at twelve weeks. The ratio of cocks to hens was maintained at 1:4 to increase the chances of fertility for each hen and variation within the population. Unselected chickens were then culled due to limited space. The collection of fertile eggs intended for hatching and raising the next generation was done at or above 32 weeks of age. Fresh, fertile eggs collected within ten days from different selected hens were pooled and incubated at once. The second generation consisted of 181 chicks at hatching. They comprised 55 cockerels and 85 hens at 8 weeks of age and were evaluated following the same protocol as in the first generation.

4.2.2 Data collection

Data on productive and reproductive traits were collected across three consecutive generations of IC of both sexes raised at the Gulu University Poultry Unit. The live weight of chicks at hatching (HW) as well as at two (W2), four (W4), six (W6), eight (W8), and twelve (W12) weeks of growth were measured using a portable electronic scale (Model: TS200, Canada). Shank lengths (SL) were also measured at two (SL2), four (SL4), six (SL6), eight (SL8), and twelve (SL12) weeks of growth using a Tailor's measuring tape as described by the African Union Inter-African Bureau for Animal Resources (AU-IBAR, 2015) field manual. Locally made trap nests were used to collect data on egg production traits. The reproductive traits, such as body weight of hens at on-set of lay (WFE), egg weight (EW), clutch number (CLN-60), clutch size (CLS-60), and number of eggs laid within 60 days (EN-60), were recorded.

4.2.3 Data analysis

Descriptive statistics of productive and reproductive traits were generated using the R software package, version 4.1.1 (R Core Team, 2021). Heritability of traits was estimated using the equation below.

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Where h^2 = broad sense heritability, σ_g^2 = genotypic variance, σ_p^2 = phenotypic variance.

Genetic analysis was performed using a univariate animal model with the restricted maximum likelihood program (Dana *et al.*, 2011).

$$y_i = Xb_i + Za_i + e_i$$

Correlations among traits were estimated by the animal model for traits i ($i=1$ and $i=2$) using the variability package of R, version 4.1.1 (R Core Team, 2021).

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

Where: y_i = vector of observations; b_i = vector of fixed effects of generation; a_i = vector of random direct genetic effects; e_i = vector of random residual effects; and X_i and Z_i are incidence matrices relating the observations to the respective fixed and direct genetic effects for trait i .

4.3 Results

4.3.1 Averages, variance components and heritability of traits

The averages, genetic variance, phenotypic variance and heritability of productive and reproductive traits of indigenous hens under a supplemented scavenging system are summarized in Table 4.1. Starting with a hatching weight (HW) of 25.35 g, chicken gained up to 1498.57 g of body weight at on-set of lay (WFE). Shank length (SL) increased from 3.38 cm at two weeks to 7.98 cm at 12 weeks of growth. On average, hens laid 19.29 eggs within a period of 60 days, spread across 1.90 clutches of 8.70 eggs each. Heritability ranged from 0.02 to 0.72. Majority of the traits had values above 0.3 which is moderate and adequate for targeted increased genetic improvement. Low heritability values were estimated for trait SL4 (0.02), SL12 (0.14) and EN-60 (0.17). Body weight at two weeks of growth (W2) was highly heritable (0.72) compared to other traits in the study.

Table 4.1: Averages, variance components and heritability of productive and reproductive traits of indigenous hens under a supplemented scavenging system

Trait	Mean $\pm$ SE	CV (%)	σ_g^2	σ_p^2	h^2
HW (g)	25.35 $\pm$ 0.72	9.10	1.66	5.35	0.31
W2 (g)	80.12 $\pm$ 3.15	19.57	176.17	245.74	0.72
W4 (g)	178.32 $\pm$ 5.70	14.73	462.07	689.96	0.67
W6 (g)	281.46 $\pm$ 10.16	11.39	304.26	1027.35	0.30
W8 (g)	409.89 $\pm$ 13.85	12.81	1412.83	2756.01	0.51
W12 (g)	623.10 $\pm$ 27.59	18.10	7388.59	12715.39	0.58
WFE (g)	1498.57 $\pm$ 60.64	16.96	38852.51	64597.21	0.60
SL2 (cm)	3.38 $\pm$ 0.06	7.23	0.03	0.06	0.57
SL4 (cm)	4.70 $\pm$ 0.11	6.44	0.00	0.09	0.02
SL6 (cm)	5.617 $\pm$ 0.09	4.93	0.02	0.08	0.30
SL8 (cm)	6.52 $\pm$ 0.08	5.22	0.07	0.12	0.64
SL12 (cm)	7.98 $\pm$ 0.12	4.28	0.02	0.12	0.14
EW (g)	37.34 $\pm$ 1.02	9.95	6.44	13.82	0.47
EN-60	19.29 $\pm$ 1.10	20.60	3.24	19.07	0.17
CLN-60	1.94 $\pm$ 0.25	1.06			
CLS-60	8.70 $\pm$ 0.53	4.73			

HW, W2, W4, W6, W8 and W12 = weight at hatching, two, four, six, eight and twelve weeks of age respectively; SL2, SL4, SL6, SL8 and SL12 = shank length at two, four, six, eight and twelve weeks of age respectively; WFE = body weight at on-set of lay; EW = egg weight; EN-60, CLN-60 and CLS-60 = egg number, clutch number and clutch size over a period of 60 days respectively; σ_g^2 = genotypic variance; σ_p^2 = phenotypic variance; h^2 = broad sense heritability.

4.3.2 Genetic and phenotypic correlation of traits

Genetic and phenotypic correlation coefficients of productive and reproductive traits of IC are summarized in Table 4.2. Most of the phenotypic correlation coefficients were positive, significant and ranging from moderate to high. The highest significant ($P < 0.001$) phenotypic correlation coefficient (0.83) was between SL2 and W2. The lowest ($P < 0.001$) and only negative phenotypic correlation coefficient (-0.49) was between EN-60 and W4. Weight at first egg (WFE) was highly correlated with W2 (0.56), SL4 (0.40), W6 (0.45), SL6 (0.70), W8 (0.52), SL8 (0.81) and SL12 (0.38). Egg weight (EW) was highly correlated with W2 (0.45),

SL6 (0.51), SL8 (0.54), and WFE (0.65). Significant genetic correlation coefficients ranged from -0.96 (SL12-EN-60) to 1.04 (HW-SL2). Weight at first egg (WFE) was highly ($P < 0.01$) and positively genetically correlated (0.99) with SL8. Egg number (EN-60) was significantly ($P < 0.01$) negatively genetically correlated with shank length (SL12).

Table 4.2: Genetic correlation (lower triangle) and phenotypic correlation (upper triangle) of productive and reproductive traits of indigenous chicken hens under a supplementary scavenging system

	HW	W2	SL2	W4	SL4	W6	SL6	W8	SL8	W12	SL12	WFE	EW	EN-60
HW	1**	0.53**	0.66**	-0.07	0.28	0.11	0.24	0.18	0.25	0.12	0.23	0.35	0.05	0.29
W2	0.86	1**	0.83**	-0.02	0.28	0.14	0.36	0.21	0.37	-0.22	0.05	0.56**	0.45*	0.12
SL2	1.04*	0.92	1**	-0.17	0.16	-0.05	0.25	0.07	0.25	-0.18	0.06	0.36	0.25	0.18
W4	-0.38	-0.13	-0.50	1**	0.33	0.49**	0.48**	0.70**	0.64**	0.33	0.03	0.37	0.12	-0.49**
SL4	0.02	1.37	0.36	2.02	1**	0.57**	0.72**	0.65**	0.56**	0.36	0.35	0.40*	0.21	0.25
W6	0.09	0.07	-0.15	1.09	1.63	1**	0.47*	0.78**	0.54**	0.56**	0.29	0.45*	-0.03	-0.22
SL6	-0.24	0.50	-0.05	0.85	1.99	0.72	1**	0.60**	0.79**	0.11	0.19	0.70**	0.51**	-0.08
W8	0.07	0.19	-0.11	0.99*	1.85	1.09	0.81	1**	0.73**	0.66**	0.37	0.52**	0.15	-0.11
SL8	0.34	0.50	0.19	0.84	2.25	1.08	0.83	1.03*	1**	0.37	0.40*	0.81**	0.54**	-0.29
W12	0.12	-0.38	-0.28	0.60	-0.10	0.80	-0.15	0.59	0.46	1**	0.61**	0.18	-0.26	-0.17
SL12	0.95*	0.30	0.45	0.62	0.38	1.10	-0.05	0.88	0.83	1.06	1**	0.38*	-0.03	-0.16
WFE	0.51	0.76	0.47	0.67	2.52	0.87	0.91	0.91	0.99*	0.17	0.64	1**	0.65**	-0.25
EW	0.27	0.84	0.44	0.51	2.61	0.56	1.10	0.64	0.74	-0.37	-0.04	0.91	1**	0.10
EN-60	0.36	0.70	0.87	-1.10	-2.14	-1.29	-0.44	-1.16	-0.71	-1.26	-0.96*	-0.26	0.11	1**

HW, W2, W4, W6, W8 and W12 = weight at hatching, two, four, six, eight and twelve weeks of age respectively; SL2, SL4, SL6, SL8 and SL12 = shank length at two, four, six, eight and twelve weeks of age respectively; WFE = body weight at on-set of lay; EN-60 and EW = egg number and weight over a period of 60 days respectively; ** $p < 0.001$; * $p < 0.01$.

4.4 Discussion

4.4.1 Phenotypic traits

The average body weight at 0, 2, 4, 8, and 12 weeks of age in the present study was higher than that of IC ecotypes in Eastern Uganda (Semahoro *et al.* 2018). In the current study, chickens were raised under a supplementary scavenging system different from the intensive system used by Semahoro *et al.* (2018). Probably, the disparity in body weight could be attributed to the management system and nutritional profile of the feeds used in the two studies. In the current study, the mean shank length of hens at eight to twelve weeks of age was higher than the values estimated for Nganda chickens within the same age range in Central Uganda (Semakula *et al.*, 2011). The body weight of hens at the on-set of lay was within the range of 1200 and 1500 g reported for adult hens in Eastern and Western ecological zones of Uganda (Ssewanyana *et al.*, 2008). The mean weight of eggs (37.34 g) was, however, lower than the value of 41.13 g recorded for eggs laid by mature scavenging hens in rural households in Chapter Two. In the current study, eggs were weighed at the on-set of lay, a period characterized by small and lightweight eggs (Anene *et al.*, 2020; Udeh, 2009). The average clutch size of 8.70 eggs per clutch in the present study was outside the range of 13 to 15 eggs reported for hens in Northern (Nakkazi *et al.* 2014), Western, and Eastern (Ssewanyana *et al.* 2008) ecological zones of Uganda. The small clutch size in the present study could be attributed to the short resting period between clutches, as hens were not allowed to incubate and brood chicks. In addition, phenotypic differences in traits could be attributed to variations in feeding regimes and chicken ecotypes used in various performance studies.

4.4.2 Heritability of traits

Heritability values of 0.72, 0.67, 0.30, and 0.51 in the current study were close to the values of 0.66, 0.55, 0.52 and 0.63 for body weight at 2, 4, 6, and 8 weeks, respectively, estimated for

local chickens in Ghana (Osei-Amponsah *et al.*, 2013). The heritability of body weight at 4 and 8 weeks of age in the current study is similar to the values estimated for Indian Ghagus chicken (Haunshi *et al.*, 2022). Like in the current study, a similar body weight heritability value of 0.55 was estimated for Fars chickens at 12 weeks of age, but lower values of 0.25 and 0.23 were reported for Iranian Azerbaijan and Esfahan ecotypes (Ghazikhani *et al.*, 2007). Likewise, body weight heritability of 0.72 and 0.67 in the current study differed from the estimates of 0.45 and 0.38 at 2 and 4 weeks of age, respectively (Chomchuen *et al.*, 2022). However, a similar heritability of 0.3 at week 6 of growth was recently reported (Chomchuen *et al.*, 2022). In related studies, Adeyinka *et al.* (2006) and Norris & Ngambi (2006) reported similar heritability values at hatching and a low value at the fourth week of growth. The decline in body weight heritability with increasing age was attributed to the corresponding reduction in maternal effect (Tongsiri *et al.*, 2019). Heritability estimates for shank length in the present study ranged from 0.02 and 0.58 and are in agreement with the values of 0.57, 0.3, and 0.58 at 2, 6, and 8 weeks of age, respectively, estimated for local chickens in Ghana (Osei-Amponsah *et al.*, 2013).

The heritability value of 0.47 for egg weight in the current study was similar to 0.46 estimated for Mazandaran chicken but varied from 0.64, 0.22, and 0.25 associated with Far, Azerbaijan, and Esfahan chicken ecotypes, respectively (Ghazikhani *et al.*, 2007). The estimated heritability of egg number (0.17) was close to 0.15 reported by (Tongsiri *et al.*, 2019) and within a range of 0.16 to 0.18 for Mazandaran and Esfahan chickens in Iran (Ghazikhani *et al.*, 2007). The heritability value of 0.17 for egg number highly varied from the average of 0.36 and 0.32 estimated for Ethiopian Horro chickens (Dana *et al.*, 2011) and Iranian Fars chickens (Ghazikhani *et al.* 2007). The variation in heritability estimates of traits reported in different studies is attributed to the use or disuse of pedigreed data (Ndung *et al.* 2020) and or number of generations considered (Haunshi *et al.*, 2022; Zonuz *et al.*, 2013). The trait of body weight

could be adequately improved by direct selection except for egg number which is associated with low heritability. Tongsiri *et al.* (2019) suggested the use of a multiple-trait selection index method for improved egg number.

4.4.3 Genetic correlation of traits

Egg number and shank length at 12 weeks of age are negatively genetically correlated. The negative genetic correlation implies that hens with short shanks at 12 weeks are more likely to lay more eggs, and the reverse is true. On the other hand, shank length at 12 weeks of age is positively genetically correlated with hatching weight. Implying that light chicks at the time of hatching are expected to have short shanks at 12 weeks of age and are more likely to lay more eggs compared to heavy chicks. This implies that hens can probably be selected for large clutch size using shank length at 12 weeks as a phenotypic marker trait.

In the current study, egg number and weight at the on-set of lay were negatively genetically correlated with a coefficient of -0.26. Likewise, Ghazikhani *et al.* (2007) & Tongsiri *et al.* (2019) reported a similar negative value of genetic correlation between egg number and weight at the on-set of lay. Additionally, Zonuz *et al.* (2013) reported negative genetic correlations of egg number and body weight at 0 (-0.04), 8 (-0.09), and 12 (-0.14) weeks of growth. The antagonistic pleiotropic relationship involving three candidate genes, namely; *CPEB3*, *MAST2* and *CACNA1H* is responsible for the negative cross-phenotypic association between body weight and egg production traits (Tarsani *et al.*, 2021). Probably, a gene-controlled biological mechanism in light hens directs abundant nutrients for egg production as compared to body maintenance. Unlike most studies, Dana *et al.* (2011) reported positive genetic correlation between egg number and body weight, excluding a negative correlation (-0.16 and -0.54) at two and six weeks of age. The positive genetic correlation could be due to errors introduced by the high mortality of 13 to 29 percent reported during the laying period. The genetic correlation coefficient between weight at the on-set of lay and egg weight was 0.908 and was higher than

0.6 reported by Tongsiri *et al.* (2019). However, negative genotypic correlation coefficients were reported for egg number against egg weight (-0.39) and weight at on-set of lay (-0.28) among local chickens in Nigeria (Oleforuh-okoleh, 2011).

4.4.4 Phenotypic correlation of traits

Weight at the on-set of lay and shank length at eight weeks of age in the current study were moderate, positively correlated, and in agreement with estimates reported for Ghagus chicken in India (Haunshi *et al.*, 2022). Therefore, heavier hens at on-set of lay can be indirectly selected using shank length at eight weeks as a marker trait. The phenotypic and genotypic correlation coefficients between shank length and live weight were positive, significant, and similar to the values of 0.86 and 0.94, respectively, reported for local chickens in Ghana (Osei-Amponsah *et al.*, 2013). The same study (Osei-Amponsah *et al.* 2013) reported high phenotypic correlation coefficients between body weight and shank length at 6 (0.93) and 12 (0.87) weeks of age. In a recent study involving Alpha and Noiler chicken genotypes, relatively lower genetic correlations of 0.59 and 0.71 were reported for body weight and shank length at 8 and 12 weeks of age, respectively (Sanda *et al.*, 2022). Genetic correlations are indicators of pleiotropic association and chromosomal linkage of genes affecting a pair of traits (Sanda *et al.*, 2022). The disparities in phenotypic and genotypic correlation coefficients reported from different studies are due to the influence of the chicken breeds, rearing environment (Chomchuen *et al.*, 2022; Sanda *et al.*, 2022).

4.5 Conclusion

Growth traits of IC were associated with moderate to high heritability values, indicating high chances of large genetic gain per generation of selection. As such, selection based on body weight at four, twelve weeks of age, and at the on-set of lay, gives more genetic progress. On the other hand, reproductive traits, specifically egg number, exhibited a low heritability value.

The low heritability of egg number makes indirect selection necessitates the use of other approaches to its genetic improvement apart from direct selection.

Genetic improvement of IC with respect to body weight at the on-set of lay, as well as egg weight, can be achieved through direct selection of superior chickens based on shank length. Shank length at 8 and 12 weeks of age is a potential phenotypic marker when selecting for both high body weight at the on-set of lay and egg yield. Chicken breeders could, for instance, undertake selection of hens for higher body weight at the on-set of lay based on shank length. This is because light hens with short shanks are more likely to lay more eggs per clutch. The negative genetic correlation between egg number and shank length is one example of the underlying gene-gene antagonistic association. In addition, the significant phenotypic correlation between body weight and egg number is indicative of the potential of selecting replacement breeding hens at an early age of four weeks.

Trait pairs including SL8-WFE, EW-WFE, SL2-W2, and SL8-W8 are positively correlated and can be considered together in a breeding program. The significant positive phenotypic correlation between shank length and body weight permits indirect selection and improvement of the latter. The nature of the association between traits reported in the study guides decision making when developing chicken breeding and improvement programs. Further studies are required to estimate the genetic and phenotypic parameters of other reproductive traits such as broodiness, hatchability, and mothering ability of indigenous hens.

CHAPTER FIVE

Alternative selection indices for improved growth and egg production traits of Ugandan indigenous chickens

Abstract

Selection index is a low-cost approach that facilitates the selection of superior animals based on multiple traits. The present study aimed at constructing selection indices for IC based on growth and egg production traits. Two consecutive generations of IC were raised at the Gulu University Poultry Unit. Hatching weight (HW) as well as weight at two (W2), four (W4), six (W6), eight (W8), and twelve (W12) weeks of growth were recorded. Shank length (SL) was also measured at two (SL2), four (SL4), six (SL6), eight (SL8), and twelve (SL12) weeks of growth. The body weight of hens at the on-set of lay (WOL), egg weight (EW), clutch number (CLN-60), clutch size (CLS-60), and the number of eggs laid within 60 days (EN-60) were recorded. Genetic parameters, including broad-sense heritability were estimated using the restricted maximum likelihood procedure embedded within the variability package of R, version 4.1.1 (R Core Team, 2021). The selection index package of R, version 4.1.1 (R Core Team, 2023), was used to estimate the coefficients (b), genetic advance yield (GA), and percent relative efficiency (PRE). Equal weight (w_1) and broad-sense heritability (wh^2) were used as weight factors to construct two sets of alternative selection indices. In the selected group, selection response was reduced by 1.9, 6.1, 1.6, and 11.5 percent for W0, W8, CLN-60, and EW but increased by 2.4, 3.5, 7.6, 6.4, and 21.6 percent for W4, W10, WMat, CLS-60, and EN-60. The weight of hens at four weeks of age (W4) was part of all the indices, consisting of two traits and more in both weight factor categories. All selection indices contained WOL. The results showed that only SL4 was associated with negative coefficients under both weight factors. In addition, the results showed that as the number of traits incorporated in the index

increases, so does GA, and this subsequently requires more generations and years to achieve the breeding objectives. The results further showed that selection index, I_{17} with the highest GA and PRE is a potential breeding tool for IC. In conclusion, the combination of traits under each selection index remains the same regardless of the weight factors.

Keywords: Weight factor, genetic gain, multipurpose chicken, generation, selection response.

The Chapter generated a published article;

Beyihayo, G.A., Kugonza, D. R., Ndyomugenyi, E. K., Echodu, R., Okot, M.W., Filomena Dos Anjos, 2023. Alternative selection indices for the simultaneous genetic improvement of multiple traits within indigenous chickens. Third Joint NARO-MAK Scientific Conference, Munyonyo, Kampala, 14-16th March 2023.

5.1 Introduction

Indigenous chickens (IC) continue to dominate many rural economies, and their total egg production exceeds that of imported breeds (UBOS, 2018; Chomchuen *et al.*, 2022). Despite their contribution to the rural economies, IC are characterized by low innate performance (Wong *et al.*, 2017) and their full production potential has not been fully exploited. Indigenous chickens are multipurpose in nature and preferred by small-scale mixed farming households compared to imported specialized breeds. Unlike the imported breeds, IC are a reservoir of innate traits like incubation, hatching, brooding, and protecting their chicks against predators. In the light of the prevalent climate change, IC genetic resources also provide a rich reservoir of genes for adaptation to harsh environments and resistance to emerging diseases (Assan *et al.*, 2017). Hence, IC provides a foundation stock for generating or regenerating chicken breeds (Fleming *et al.*, 2016; Elbeltagy *et al.*, 2019).

Accordingly, genetic improvement and retention of the multiple traits are necessary for the sustainable and profitable rearing of IC. Recent advances in genetic research are not yet locally

applicable for high-output screening due to cost and skill limitations. This then calls for the need to develop low-cost selection tools, which are locally applicable but still with high precision. The selection index or total score approach developed by Hazel (Hazel, 1943; Hazel *et al.*, 1994) and modified by Yamada *et al.* (1975) is suitable for the simultaneous selection and improvement of multiple traits at a single generation. This is because the breeding and market value of an individual animal is assessed based on multiple traits. The index method simplifies the assessment of candidate animals using a single value derived from the summation of economically weighted phenotypic scores (Hazel *et al.*, 1994). Selection index as compared to tandem and independent culling level methods is more efficient and results in a multiple-trait genetic improvement (Hazel & Lush, 1942).

The selection index approach has been widely applied in animal breeding programs to improve disease resistance, growth, egg numbers, milk yield, fertility, profits, and a few selection indices have been proposed (Lwelamira & Kifaro, 2010; Sivaraman & Kumar, 2013; Tseveenjav *et al.*, 2020; Ziadi *et al.*, 2021; Alcantara *et al.*, 2022; Chomchuen *et al.*, 2022). Selection indices targeting genetic improvement of traits, typical of multipurpose IC as a breeding tool remain underdeveloped. Therefore, the current study aimed to construct selection indices for IC breeding programs based on growth and egg production traits as well as estimate the number of generations and years required to achieve the desired genetic improvement.

5.2 Materials and methods

5.2.1 Assembling and rearing of breeding stock

The base population of IC was introduced into the Gulu University Poultry Farm using fertile eggs collected from randomly mating flocks in the nine ecological zones of Uganda as described in Chapter Two. Upon hatching, the resultant seven hundred thirteen (713) chicks were brooded artificially up to four weeks of age. At the end of the brooding period, the

chickens were allowed to scavenge during the day and were supplemented with a 15% crude protein (CP) diet during their growth phase and an 11% CP diet in the laying phase. The same diets were maintained in the subsequent generations. Chickens were sexed at eight weeks of age into 250 pullets and 241 cockerels, and this made the base population. The first generation consisted of 181 chicks at hatching, 55 cockerels, and 85 pullets at eight weeks of age. In both generations, hens were divided into two groups - the superior and control groups based on body weight at the on-set of lay and the number of eggs in two consecutive clutches. A selection intensity of 30 percent was applied across the two generations considered. The animal study protocol was approved by the Research Ethics Committee of Gulu University (GUREC-2020-18, dated 03/12/2020). The research protocol was also approved by the Uganda National Council for Science and Technology (UNCST), clearance NO. A154ES, dated 15/11/2021, as described fully in Chapter One.

5.2.2 Measurement of live-weight, shank length, and egg production

Hatching weight (HW), weight at two (W2), four (W4), six (W6), eight (W8), and twelve (W12) weeks of growth were measured using a portable electronic scale (Model: TS200, Canada). Shank length (SL) was measured at two (SL2), four (SL4), six (SL6), eight (SL8), and twelve (SL12) weeks of growth using a Tailor's measuring tape as described in a field guide provided by the African Union Inter-African Bureau for Animal Resources (AU-IBAR, 2015). Body weight of hens at on-set of lay (WOL), egg weight (EW), clutch number (CLN-60), clutch size (CLS-60), and the number of eggs laid within 60 days (EN-60) were recorded. Locally made trap nests were used to collect data on egg production traits in both generations.

5.2.3 Construction of selection indices

Construction of the selection indices was based on seven (7) traits, and these included W4, SL4, W12, SL12, WOL, EW, and EN-60. Selection indices were constructed based on the definition of the aggregate breeding value of farm animals (Hazel, 1943) as below:

$$H = a_1G_1 + a_2G_2 \dots + a_nG_n$$

Where H is the sum of all the genotypes being considered, and each genotype (G) is weighted by the economic value (a) of that particular trait, it influences up to the n^{th} trait. Hence, selection indices (I) were constructed based on multiple phenotypic traits to indirectly identify superior genotypes (Hazel & Lush, 1942; Hazel, 1943). Selection indices were constructed using a general expression $I = \mathbf{bX}$ (Hazel, 1943; Yamada *et al.*, 1975) where b is the coefficient associated with trait X . To cater for multiple traits, the index was expanded and rewritten (Hazel, 1943) as follows:

$$I = b_1X_1 + b_2X_2 \dots + b_nX_n$$

where traits range from one (1) to the last n th trait. Multiple regression coefficients (b) were estimated to cater for the phenotypic and genetic parameters (Becker, 1984) using the expression as below:

$$b = P^{-1}Gw$$

where P is a phenotypic variance-covariance matrix ($n \times n$), G is a genetic variance-covariance matrix ($n \times m$) and w , is a vector ($1 \times n$) of relative weight factors.

5.2.4 Weight factors

Equal weight (w_1) and broad-sense heritability (wh^2) were used as weight factors to construct alternative selection indices. Equal weight was used on the basis that IC are multipurpose in

nature and all traits are equally desired. In constructing the indices based on w1, the weight factors of all traits were considered equal and assigned a value of one (1). On the basis that the expression of traits is influenced by their heritability, broad-sense heritability was also used as an alternative weight factor, wh^2 .

5.2.5 Statistical Analysis

Broad sense heritability, genetic advance, genetic and phenotypic correlation coefficients were estimated using the restricted maximum likelihood procedure embedded within the variability package of R, version 4.1.1 (R Core Team, 2021) as described in Chapter Three. The selection index package of R, version 4.2.3 (R Core Team, 2023), was used to estimate the coefficients of traits (b), genetic advance yield (GA), and percent relative efficiency (PRE). In the index package of R, genotype was replaced by generations, and two treatments (superior and control) were considered. All the possible combinations of the seven traits under each weight category were derived. The index with the highest GA and PRE and ranked number one was selected from each combination level. The number of generations, q, required to achieve the desired phenotypic improvement was estimated using the equation suggested by Yamada *et al.* (1975) as below:

$$q = \frac{\sigma^I}{i_I} = \frac{\sqrt{b^I P b}}{i_I}$$

where σ^I is the standard deviation of the index and $i_I = 30$ percent was the selection intensity applied to achieve the desired genetic advance, GA = 24.3 percent per generation. The number of years t, required to realize the desired phenotypic improvement, at an average generation interval y, was calculated as $t = qy$ (Yamada *et al.*, 1975). Selection response, SR was calculated as the phenotypic gain or loss of a trait across the two generations, $SR = \underline{X}g_1 - \underline{X}g_2$ where $\underline{X}g_1$ and $\underline{X}g_2$ are mean phenotypic values of traits in generation 1 and 2 respectively.

An average generation interval (y) of 0.6 years was considered from previous studies involving ICs (Nakkazi *et al.*, 2014; Kugonza *et al.*, 2008).

5.3 Results

5.3.1 Performance and selection response of indigenous chickens

Growth, egg production, and selection response accruing from three consecutive generations of IC breeding are summarized in Table 5.1. In generation one, average weight increased from 24.46 g and 23.85 g at hatching to 1549.45 g and 1450.33 g at maturity for the superior and control group, respectively. In generation two, chicken weight increased from an average of 24 g at hatching to an average of 1348.7 g and 1240 g at on-set of lay in the superior and control group, respectively. In generation three, average hatching weight reduced to 21.82 ± 2.55 g and 23.1 ± 1.94 g in the superior and control group, respectively. Within the superior group, the total number of eggs laid within a period of 60 days increased from 15.63 ± 4.46 eggs in generation one to 33.6 ± 3.46 eggs in generation three. Likewise, the number of eggs laid within a period of 60 days increased from 14.25 ± 4.37 to 31.5 ± 3.45 eggs in the third generation within the control group.

In general, breeding resulted in positive total selection response in all body weight traits (W4, W8, W10, WOL, and WMat) except weight at hatching (W0) across the three generations in both the superior and control groups. The average hatching weight was reduced by 2.64 g and 0.75 g, corresponding to a percentage of 10.8 and 3.1 in the superior and control groups, respectively by the third generation. The largest selection response of 72.4 and 67.1 percent was realized for body weight at 10 weeks (W10) in both groups. Overall, there was an increase in body weight of chickens at the different stages of growth as a result of breeding.

Among the reproductive traits, clutch size (CLS-60) greatly increased as indicated by a large positive selection response (155.7%) in the superior group. However, the number of clutches

within a period of 60 days reduced by 23.9 percent in the same group. Among the control group, both clutch size and clutch number were associated with a positive selection response of 110 and 14.3 percent, respectively. Overall, egg weight was reduced by 2.22 g, representing a decline of six percent at the third generation of breeding among the superior group. In the same group, the number of eggs (EN-60) increased from 15.63 ± 4.46 to 19 ± 5.42 and 33.6 ± 3.46 in generations one, two, and three, respectively. The increase in egg number corresponds to a total selection response of 18 eggs at generation three. Hence, an increase in the number of eggs laid per clutch, resulted in a decline in egg weight in the superior group. On the other side, egg weight and egg number increased by 5.47g and 17.3, corresponding to 15.5 and 121.1 percent, respectively, in the control group.

Table 5.1: Performance and selection response of indigenous chicken under selection

	Traits	Generation I	Generation II		Generation III		
		Mean $\pm$ SD	Mean $\pm$ SD	SR	Mean $\pm$ SD	SR	Total SR
Superior	W0/g	24.46 $\pm$ 2.67	24 $\pm$ 2.79	-0.46 (-1.9)	21.82 $\pm$ 2.55	-2.18 (-9.1)	-2.64 (-10.8)
	W4/g	173.22 $\pm$ 27.03	177.45 $\pm$ 17.40	4.23 (2.4)	194 $\pm$ 12.10	16.55 (9.3)	20.78 (12.0)
	W8/g	407.97 $\pm$ 52.67	383.09 $\pm$ 29.48	-24.88(-6.1)	449.72 $\pm$ 31.23	66.63 (17.4)	41.75 (10.2)
	W10/g	503.45 $\pm$ 84.78	521.27 $\pm$ 41.55	17.82 (3.5)	868.18 $\pm$ 61.35	346.91 (66.6)	364.73 (72.4)
	WOL/g	-	1348.75 $\pm$ 58.53	-	1813.7 $\pm$ 51.56	464.95 (34.5)	464.95 (34.5)
	WMat/g	1549.35 $\pm$ 261.7	1667.27 $\pm$ 134.95	117.92 (7.6)	1718.6 $\pm$ 121.94	51.33 (3.1)	169.25 (10.9)
	CLS-60	9.15 $\pm$ 4.17	9.74 $\pm$ 3.43	0.59 (6.4)	23.4 $\pm$ 2.22	13.7 (140.2)	14.25 (155.7)
	CLN-60	1.84 $\pm$ 0.37	1.81 $\pm$ 0.98	-0.03 (-1.6)	1.4 $\pm$ 0.12	-0.41 (22.7)	-0.44 (-23.9)
	EW/g	37.24 $\pm$ 3.92	32.95 $\pm$ 2.04	-4.29 (-11.5)	35.02 $\pm$ 2.05	2.07 (6.3)	-2.22 (-6.0)
	EN-60	15.63 $\pm$ 4.46	19 $\pm$ 5.42	3.37 (21.6)	33.6 $\pm$ 3.46	14.6 (76.8)	18.0 (115.0)
Control	W0/g	23.85 $\pm$ 2.06	24.89 $\pm$ 3.30	1.04 (4.4)	23.1 $\pm$ 1.94	-1.79 (-7.2)	-0.75 (-3.1)
	W4/g	158.58 $\pm$ 20.10	166.44 $\pm$ 12.40	7.86 (5.0)	191 $\pm$ 13.24	24.56 (14.8)	32.42 (20.4)
	W8/g	356 $\pm$ 64.91	335.33 $\pm$ 23.58	-20.7 (-5.8)	412.55 $\pm$ 59.10	77.22 (23.0)	56.55 (15.9)
	W10/g	459.08 $\pm$ 50.93	488.22 $\pm$ 41.02	29.1 (6.3)	766.9 $\pm$ 78.24	278.68 (57.1)	307.82 (67.1)
	WOL/g	-	1240 $\pm$ 160.86	-	1587.5 $\pm$ 170.32	347.5 (28.1)	347.5 (28.0)
	WMat/g	1450.33 $\pm$ 215.1	1568.13 $\pm$ 198.0	117.8 (8.1)	1766.5 $\pm$ 191.45	198.37 (12.7)	316.17 (21.8)
	CLS-60	7.5 $\pm$ 3.85	8.42 $\pm$ 2.06	0.92 (12.3)	15.8 $\pm$ 1.56	7.3 (87.1)	8.3 (110)
	CLN-60	1.75 $\pm$ 0.45	2 $\pm$ 1.00	0.25 (14.3)	2 $\pm$ 0.54	0 (0.0)	0.25 (14.3)
	EW(g)	35.18 $\pm$ 3.8	34.25 $\pm$ 3.30	-0.93 (-2.6)	40.65 $\pm$ 2.93	6.4 (18.7)	5.47 (15.5)
	EN-60	14.25 $\pm$ 4.37	18.44 $\pm$ 3.75	4.19 (29.4)	31.5 $\pm$ 3.45	13.1 (70.8)	17.3(121.1)

W0, hatching weight; W2, weight at two weeks; W4, weight at four weeks; W6, weight at six weeks; W8, weight at eight weeks; W12, weight at twelve weeks; WOL, body weight at on-set of lay; WMat, body weight at maturity; EW, egg weight; CLN-60, number of clutches; CLS-60, clutch size; EN-60, number of eggs laid within 60 days; SD, standard deviation; Min, Minimum; Max, Maximum; SR, selection response. Numbers in parenthesis are percentages.

5.3.2 Heritability, genetic advance, and correlation of traits

Broad sense heritability, expected genetic advance, genetic and phenotypic correlations used in the construction of the selection indices are presented in Table 5.2. Heritability ranged from a minimum of 0.02 to a maximum of 0.67 for traits SL4 and W4, respectively. Likewise, expected genetic gain ranged from a minimum of 0.3% to a maximum of 20% for SL4 and W4, respectively. Egg number (EN-60) exhibited negative genetic correlation coefficients with almost all traits except egg weight. Also, egg number (EN-60) exhibited negative phenotypic correlation coefficients with almost all traits except SL4 and EW. Shank length (SL12) exhibited a significantly high positive phenotypic correlation (0.61) with W12. The association between egg number (EN-60) and W4 resulted in a significant negative phenotypic correlation. Weight at first egg (WOL) also exhibited a positive and significant phenotypic coefficient of 0.38 with SL12. The association between egg weight and WOL resulted in a strong positive phenotypic coefficient of 0.65.

Table 5.2: Broad sense heritability (h^2), expected genetic advance (GA), genetic (below diagonal), and phenotypic (above diagonal) correlations of traits

Trait	h^2	GA (%)	W4	SL4	W12	SL12	WOL	EW	EN-60
W4	0.67	20.0	1	0.33	0.33	0.031	0.37	0.12s	-0.49 **
SL4	0.02	0.3	2.02	1	0.36	0.35	0.40	0.21	0.25
W12	0.58	21.7	0.60	-0.10	1	0.61**	0.18	-0.26	-0.17
SL12	0.14	1.2	0.62	0.38	1.00	1	0.38*	-0.03	-0.16
WOL	0.60	21.0	0.67	2.51	0.17	0.63	1	0.65**	-0.24
EW	0.47	9.6	0.51	2.604	0.37	-0.03	0.91	1	0.09
EN-60	0.17	10.7	-1.10	-2.13	-1.25	-0.96*	-0.26	0.11	1

W4 & W12, body weight at four and twelve weeks of age; WOL, body weight at on-set of lay; EW, egg weight; EN-60, number of eggs laid within 60 days; SL4 & SL12, shank length at four and twelve weeks of age

5.3.3 Selection indices based on one trait

Selection indices with corresponding genetic advance (GA) and percent relative efficiency (PRE) for each group of trait combinations are presented in Tables 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, and 5.10 (See Appendix 1 & Appendix 2). Selection indices with corresponding expected

genetic gain and percent relative efficiency based on one out of seven traits under equal and broad-sense heritability are presented in Table 5.3. The coefficients of traits, GA, and PRE for selection indices under the equal weight category approximately doubled their corresponding counterparts in the broad-sense heritability as a weight category. Weight at first egg (WOL) yielded selection indices (I_{11} and I_{21}) with the highest GA and PRE in single-trait selection indices under both weight categories. On the other hand, SL4 exhibited the lowest GA and PRE under both weight categories.

5.3.4 Selection indices based on two traits

Selection indices with corresponding expected genetic gain and percent relative efficiency based on two out of seven traits under equal and broad-sense heritability as weight methods are presented in Table 5.4. A pair of traits, W4, and WOL, resulted in a selection index with the highest GA and PRE under both equal and broad sense heritability as weight categories. The selection index based on W12 and SL12 resulted in the lowest GA and PRE under both equal and broad sense heritability as weight categories. Selection indices composed of WOL were ranked higher in both weight categories. Expected genetic gain (GA) and percent relative efficiency (PRE) reduced from 36.8 and 151.5 to 13.6 and 56, respectively, under the equal weight category. Likewise, expected genetic gain (GA) and percent relative efficiency (PRE) reduced from 22.3 and 91.8 to 7.9 and 32.4, respectively, under the broad sense heritability. The coefficients of traits, GA, and PRE for selection indices under the equal weight category approximately doubled their corresponding counterparts in the broad sense heritability as weight category.

Table 5.3: Selection indices, expected genetic gain, and percent relative efficiency based on one trait

Selection Indices (I)	GA	PRE	Rank
Equal weight (w_1)			
$I_{11} = 0.60WOL$	31.5	129.72	1
0.58W12	13.5	55.5	2
0.67W4	3.6	14.9	3
0.47EW	0.35	1.4	4
0.17EN	0.15	0.63	5
0.14SL12	0.01	0.04	6
0.02SL4	0.001	0.006	7
Broad sense heritability (wh^2)			
$I_{21} = 0.36WOL$	18.9	77.8	1
0.34W12	7.8	32.2	2
0.45W4	2.40	10.0	3
0.22EW	0.17	0.69	4
0.02EN	0.03	0.11	5
0.02SL12	0.001	0.005	6
0.0005SL4	0.00	0.00	7

GA, genetic advance; PRE, percent relative efficiency of traits; W4, body weight at four weeks of age; W12, body weight at twelve weeks of age; WOL, body weight at on-set of lay; SL4, shank length at four weeks of age; SL12, shank length at twelve weeks of age; EW, egg weight; EN-60, number of eggs laid within 60 days; I_{xn} , selection index derived from x ($x = w_1$ or wh^2) and comprising of n number of traits.

5.3.5 Selection indices based on three traits

Selection indices with corresponding expected genetic gain and percent relative efficiency based on three out of seven traits under equal and broad-sense heritability as weight methods are presented in Table 5.5. Selection indices (I_{13} and I_{23}) comprising W4, W12, and WOL were ranked number one based on their high GA and PRE values in both weight categories. Weight at on-set of lay (WOL) was present in all selection indices. Selection indices composed of SL4, WOL, and EW scored the lowest GA and PRE values under both weight categories.

5.3.6 Selection indices based on four traits

Selection indices with corresponding expected genetic gain and percent relative efficiency based on four out of seven traits under equal and broad-sense heritability as weight methods are presented in Table 5.6. Selection indices (I_{14} and I_{24}) consisting of W4, W12, WOL, and

EW were ranked first in both weight categories. Two traits, W4 and WOL, appeared in all the alternative selection indices under both categories. The expected genetic gain (GA) of indices under the equal weight category doubled their corresponding values in the broad sense heritability weight category.

5.3.7 Selection indices based on five traits

Selection indices with corresponding expected genetic gain and percent relative efficiency based on five out of seven traits under equal and broad-sense heritability as weight methods are presented in Table 5.7. Selection indices (I_{15} and I_{25}) comprising W4, SL4, W12, WOL, and EW scored the highest GA, PRE and were ranked number one under both weight categories. Considering the equal weight category, GA ranged from 44.9 to 38.4. Under the broad-sense heritability, GA ranged from 27.0 to 23.2.

Table 5.4: Top ten alternative selection indices (I), expected genetic gain (GA) and percent relative efficiency (PRE) based on two traits

Selection Indices (I)	GA	PRE	Rank
Equal weight (w1)			
$I_{12} = 2.90W4 + 0.54WOL$	36.8	151.5	1
$0.56W12 + 0.60WOL$	36.2	149.1	2
$0.50WOL + 10.96EW$	32.5	133.8	3
$53.83SL4 + 0.58WOL$	31.7	130.3	4
$0.62WOL + 4.16EN$	31.6	130.2	5
$0.62WOL - 37.58SL_{12}$	316.4	130.1	6
$1.48W4 + 0.55W12$	17.2	71.0	7
$0.51W12 - 7.79EN$	14.8	61.1	8
$0.67W12 - 94.60SL4$	14.5	60.0	9
$0.63W12 - 28.45SL_{12}$	13.6	56.0	10
Broad sense heritability (wh^2)			
$I_{22} = 1.78W4 + 0.32WOL$	22.3	91.8	1
$0.33W12 + 0.36WOL$	21.6	88.9	2
$0.30WOL + 6.54EW$	19.4	80.0	3
$0.37WOL + 2.43EN$	19.0	78.2	4
$32.4SL4 + 0.35WOL$	19.0	78.1	5
$0.37WOL - 22.58SL_{12}$	18.9	78.0	6
$0.92W4 + 0.33W12$	10.3	42.4	7
$0.30W12 - 4.56EN$	8.8	36.0	8
$0.39W12 - 54.88SL4$	8.4	34.8	9
$0.37W12 - 16.5SL_{12}$	7.9	32.4	10

GA, genetic advance, PRE, percent relative efficiency of traits; W4, body weight at four weeks of age; W12, body weight at twelve weeks of age; WOL, body weight at on-set of lay; SL4, shank length at four weeks of age; SL12, shank length at twelve weeks of age; EW, egg weight; EN-60, number of eggs laid within 60 days; I_{xn}, selection index derived from x (x = w1 or wh²) and comprising of n number of traits.

Table 5.5: Top ten alternative selection indices (I), expected genetic gain (GA) and percent relative efficiency (PRE) based on three traits

Selection Indices (I)	GA	PRE	Rank
Equal weight (w1)			
$I_{13} = 4.06W4 + 0.38W12 + 0.51WOL$	43.7	179.5	1
$3.22W4 + 0.38WOL + 15.78EW$	38.3	157.5	2
$3.73W4 + 0.55WOL + 11.91EN$	37.8	155.5	3
$0.70W12 + 0.49WOL + 11.32EW$	36.9	152.0	4
$2.88W4 + 9.18SL4 + 0.53WOL$	36.8	151.6	5
$2.88W4 - 7.42SL12 + 0.54WOL$	36.8	161.6	6
$0.71W12 - 83.69SL12 + 0.63WOL$	36.5	150.3	7
$0.66W12 - 46.08SL4 + 0.62WOL$	36.3	149.6	8
$0.53W12 + 0.58WOL - 4.22EN$	36.2	14.9	9
$63.3SL4 + 0.47WOL + 11.41EW$	32.7	13.4	10
Broad sense heritability (wh²)			
$I_{23} = 2.45W4 + 0.22W12 + 0.31WOL$	26.2	108.0	1
$1.97W4 + 0.23WOL + 9.50EW$	23.1	95.3	2
$2.31W4 + 0.33WOL + 7.14EN$	23.0	94.5	3
$1.77W4 - 3.91SL12 + 0.33WOL$	22.3	91.9	4
$1.76W4 + 5.39SL4 + 0.32WOL$	22.3	91.9	5
$0.41W12 + 0.29WOL + 6.83EW$	22.0	90.5	6
$0.41W12 - 49.52SL12 + 0.38WOL$	21.7	89.5	7
$0.31W12 + 0.35WOL - 2.41EN$	21.7	89.2	8
$0.35W12 - 25.42SL4 + 0.37WOL$	21.6	89.1	9
$37.86SL4 + 0.28WOL + 6.80EW$	19.5	80.5	10

GA, genetic advance, *PRE*, percent relative efficiency of traits; *W4*, body weight at four weeks of age; *W12*, body weight at twelve weeks of age; *WOL*, body weight at on-set of lay; *SL4*, shank length at four weeks of age; *SL12*, shank length at twelve weeks of age; *EW*, egg weight; *EN-60*, number of eggs laid within 60 days; *I_{xn}*, selection index derived from *x* (*x* = *w1* or *wh²*) and comprising of *n* number of traits.

Table 5.6: Top ten alternative selection indices (I), expected genetic gain (GA) and percent relative efficiency (PRE) based on four traits

Selection Indices (I)	GA	PRE	Rank
Equal weight (w1)			
$I_{14} = 4.14W4 + 0.55W12 + 0.36WOL + 14.54EW$	44.6	18.3	1
$4.19W4 - 95.52SL4 + 0.45W12 + 0.54WOL$	43.9	18.1	2
$4.25W4 + 0.29W12 + 46.40SL12 + 0.48WOL$	43.7	179.8	3
$4.36W4 + 0.37W12 + 0.51WOL + 4.57EN$	43.5	277.2	4
$3.80W4 + 0.43WOL + 11.88EW + 9.45EN$	38.7	159.5	5
$3.37W4 + 57.87SL12 + 0.32WOL + 18.44EW$	38.4	158.2	6
$3.19W4 + 16.11SL4 + 0.37WOL + 15.85EW$	38.3	157.7	7
$4.39W4 - 121.77SL4 + 0.61WOL + 16.73EN$	38.2	157.3	8
$3.76W4 + 11.20SL12 + 0.55WOL + 12.04EN$	37.8	155.6	9
$0.70W12 + 0.42WOL + 15.13EW - 7.01EN$	37.2	153.0	10
Broad sense heritability (wh²)			
$I_{24} = 2.50W4 + 0.33W12 + 0.21WOL + 8.91EW$	26.8	110.2	1
$2.53W4 - 55.31SL4 + 0.26W12 + 0.33WOL$	26.4	108.7	2
$2.66W4 + 0.22W12 + 0.31WOL + 2.88EM$	26.3	108.2	3
$2.57W4 + 0.17W12 + 27.86SL12 + 0.29WOL$	26.3	108.2	4
$2.34W4 + 0.26WOL + 7.15EW + 5.63EN$	23.5	96.7	5
$2.06W4 + 35.89SL12 + 0.19WOL + 11.16EW$	23.2	95.7	6
$2.70W4 - 72.73SL4 + 0.36WOL + 10.01EN$	23.2	95.6	7
$1.95W4 + 9.45SL4 + 0.22WOL + 9.53EW$	23.1	95.3	8
$2.33W4 + 8.29SL12 + 0.33WOL + 7.23EN$	23.0	94.6	9
$1.75W4 + 7.09SL4 - 5.54SL12 + 0.32WOL$	22.3	91.9	10

GA, genetic advance, *PRE*, percent relative efficiency of traits; *W4*, body weight at four weeks of age; *W12*, body weight at twelve weeks of age; *WOL*, body weight at on-set of lay; *SL4*, shank length at four weeks of age; *SL12*, shank length at twelve weeks of age; *EW*, egg weight; *EN-60*, number of eggs laid within 60 days; *I_{xn}*, selection index derived from *x* (*x* = *w1* or *wh²*) and comprising of *n* number of traits.

Table 5.7: Top ten ranked selection indices, expected genetic gain and percent relative efficiency based on all possible combinations of the five traits

Selection Indices (I)	GA	PRE	Rank
Equal weight (w_1)			
$I_{15} = 4.30W_4 - 110.58SL_4 + 0.65W_{12} + 0.38WOL + 15.93EW$	44.9	184.9	1
$4.42W_4 + 0.43W_{12} + 68.54SL_{12} + 0.31WOL + 15.43EW$	44.7	183.8	2
$5.25W_4 - 200.70SL_4 + 0.51W_{12} + 0.60WOL + 12.53EN$	44.4	182.7	3
$4.22W_4 + 0.53W_{12} + 0.37WOL + 13.36EW + 1.80EN$	44.2	182.1	4
$4.46W_4 - 101.98SL_4 + 0.33W_{12} + 59.50SL_{12} + 0.51WOL$	44.0	181.3	5
$4.67W_4 + 0.24W_{12} + 63.03SL_{12} + 0.48WOL + 5.30EN$	43.6	179.5	6
$4.29W_4 - 92.59SL_4 + 0.49WOL + 9.83EW + 13.58EN$	39.0	160.4	7
$3.95W_4 + 57.7SL_{12} + 0.37WOL + 14.51EW + 9.54EN$	38.9	160.1	8
$3.37W_4 - 0.91SL_4 + 58.1SL_{12} + 0.32WOL + 18.47EW$	38.5	158.3	9
$4.72W_4 - 155.90SL_4 + 58.67SL_{12} + 0.59WOL + 18.77EN$	38.4	158.0	10
Broad sense heritability (wh^2)			
$I_{25} = 2.59W_4 - 64.82SL_4 + 0.38W_{12} + 0.23WOL + 9.72EW$	27.0	111.1	1
$2.67W_4 + 0.25W_{12} + 41.33SL_{12} + 0.19WOL + 9.45EW$	26.8	110.5	2
$3.19W_4 - 119.65SL_4 + 0.30W_{12} + 0.36WOL + 7.62EN$	26.8	110.4	3
$2.57W_4 + 0.32W_{12} + 0.22WOL + 8.26EW + 1.13EN$	26.7	110.0	4
$2.69W_4 - 59.07SL_4 + 0.19W_{12} + 35.45SL_{12} + 0.31WOL$	26.5	109.0	5
$2.85W_4 + 0.14W_{12} + 38.00SL_{12} + 0.29WOL + 3.32EN$	26.3	108.5	6
$2.64W_4 - 54.99SL_4 + 0.29WOL + 5.92EW + 8.08EN$	23.6	97.2	7
$2.44W_4 + 36.86SL_{12} + 0.22WOL + 8.83EW + 5.68EN$	23.6	97.1	8
$2.90W_4 - 94.22SL_4 + 37.03SL_{12} + 0.35WOL + 11.30EN$	23.3	96.0	9
$2.07W_4 - 1.09SL_4 + 36.18SL_{12} + 0.19WOL + 11.17EW$	23.2	95.7	10

GA, genetic advance, *PRE*, percent relative efficiency of traits; *W4*, body weight at four weeks of age; *W12*, body weight at twelve weeks of age; *WOL*, body weight at on-set of lay; *SL4*, shank length at four weeks of age; *SL12*, shank length at twelve weeks of age; *EW*, egg weight; *EN-60*, number of eggs laid within 60 days; *I_{nx}*, selection index derived from weight factor x ($x = w_1$ or wh^2) and comprising of n number of traits.

5.3.8 Selection indices based on six traits

Selection indices with corresponding expected genetic gain and percent relative efficiency based on six out of seven traits under equal and broad-sense heritability as weight methods are presented in Table 5.8. Inclusion of *W4*, *SL4*, *W12*, *WOL*, *EW*, and *EN* in a six-trait index resulted in the highest *GA* and *PRE*. expected genetic gain (*GA*) reduced from 78.3 to 41.1 under the equal weight category. Under the broad sense heritability weight category, *GA* reduced from 27.1 to 11.1.

Table 5.8: Selection indices, expected genetic gain and percent relative efficiency based on all possible combinations of the six traits

Selection Indices (I)	GA	PRE	Rank
Equal weight (w1)			
$I_{16} = 3.15W4 - 168.86SL4 + 0.87W12 + 0.39WOL + 21.92EW + 8.82EN$	78.3	442.5	1
$2.62W4 + 0.99W12 - 50.67SL12 + 0.35WOL + 21.94EW + 7.49EN$	77.8	439.9	2
$3.35W4 - 178.64SL4 + 0.55W12 + 26.89SL12 + 0.59WOL + 12.41EN$	77.2	436.6	3
$2.24W4 - 139.02SL4 + 1.19W12 - 66.62SL12 + 0.37WOL + 24.82EW$	76.5	432.6	4
$1.16W12 - 103.4SL4 - 92.70SL12 + 0.44WOL + 21.05EW + 2.51EN$	74.7	422.4	5
$0.83W4 - 159.62SL4 + 0.89W12 - 15.90SL12 + 8.74EW + 1.48EN$	48.5	274.6	6
$2.67W4 - 19.03SL4 + 30.10SL12 + 0.41WOL + 14.66EW + 7.69EN$	41.1	609	7
Broad sense heritability (wh^2)			
$I_{26} = 3.08W4 - 110.76SL4 + 0.38W12 + 0.28WOL + 7.09EW + 7.09EN$	27.1	111.8	1
$2.82W4 - 71.12SL4 + 0.29W12 + 51.90SL12 + 0.19WOL + 10.47EN$	27.1	111.6	2
$3.67W4 - 142.51SL4 + 0.17W12 + 74.7SL12 + 0.33WOL + 9.39EN$	27.0	111.3	3
$2.79W4 + 0.23W12 + 45.02SL12 + 0.19WOL + 8.60EW + 1.57EN$	26.8	110.4	4
$2.94W4 - 82.99SL4 + 59.43SL12 + 0.26WOL + 8.00EW + 9.41EN$	23.8	98.1	5
$0.48W12 - 1.42SL4 - 39.76SL12 + 0.27WOL + 8.60EW - 3.95EN$	22.3	91.8	6
$0.86W4 - 58.46SL4 + 0.37W12 + 3.52SL12 + 1.68EW - 2.31EN$	11.1	45.7	7

5.3.9 Selection indices based on the seven traits

Selection indices with corresponding expected genetic gain and percent relative efficiency based on all seven traits under equal and broad-sense heritability as weight methods are presented in Table 5.9. All seven traits were part of the selection index constructed under each weight category. Expected genetic gain (GA) under the equal weight category doubled the value obtained from broad-sense heritability. The percent relative efficiency (PRE) obtained under the equal weight category was four times more than the value obtained with broad-sense heritability as a weight category.

Table 5.9: Selection indices, expected genetic gain and percent relative efficiency based on the seven traits

Selection Indices (I)	GA	PRE	Rank
Equal weight (w1)			
$I_{17} = 5.91W4 - 225.97SL4 + 0.41W12 + 131.49SL12 + 0.42WFE + 11.9EW + 12.6EN$	78.3	442.7	1
Broad sense heritability (wh^2)			
$I_{27} = 3.58W4 - 134.43SL4 + 0.25W12 + 78.79SL12 + 0.25WOL + 7.44EW + 7.56EN$	27.4	112.8	1

GA, genetic advance, PRE, percent relative efficiency of traits; W4, body weight at four weeks of age; W12, body weight at twelve weeks of age; WOL, body weight at on-set of lay; SL4, shank length at four weeks of age; SL12, shank length at twelve weeks of age; EW, egg weight; EN-60, number of eggs laid within 60 days; I_{xn}, selection index derived from x (x = w1 or wh²) and comprising of n number of traits.

5.3.10 Summary of the top-ranked selection indices under each trait combination and weight category

The top-ranked selection indices under each trait combination and weight category are summarized in Table 5.10. Genetic advance and percent relative efficiency increased from 31.5 and 1297.2 in a single-trait index to 45.4 and 1869.9, respectively, in a seven-trait combination index under the equal weight category (w_1). Genetic advance and PRE increased from 18.9 and 778.2 for a single-trait index, to the maximum of 27.4, and 1128.4, respectively, in a seven-trait combination index, using broad sense heritability as the weight factor (wh^2). Weight of hens at four weeks of age (W4) appeared in all indices, consisting of two traits and more in both weight categories (w_1 and wh^2).

Corresponding selection indices in both weight categories had similar trait combinations. However, the coefficients under w_1 were higher than their counterparts in wh^2 . All selection indices contained body weight at the on-set of lay (WOL). Only the shank length at four weeks of age (SL4) was associated with negative coefficients under both weight factors. Genetic Advance (GA) and PRE increases with the incorporation of more traits in the selection index.

Table 5.10: Summary of the top ranked selection indices under each trait combination and weight category

Trait Combination	Selection Indices (I)	GA	PRE
	Equal weight (w1)		
One-trait	$I_{11} = 0.60WOL$	31.5	1297.2
Two-traits	$I_{12} = 2.89W4 + 0.54WOL$	36.9	1515.7
Three-traits	$I_{13} = 4.05W4 + 0.37W12 + 0.505WOL$	43.7	1795.8
Four-traits	$I_{14} = 4.13W4 + 0.55W12 + 0.35WOL + 14.54EW$	44.6	1833.1
Five-traits	$I_{15} = 4.30W4 - 110.58SL4 + 0.65W12 + 0.38WOL + 15.93EW$	44.9	1849.8
Six-traits	$I_{16} = 4.68W4 - 121.26SL4 + 0.50W12 + 86.58SL12 + 0.33WOL + 17.19EW$	45.2	1858.4
Seven-traits	$I_{17} = 5.91W4 - 225.97SL4 + 0.41W12 + 131.49SL12 + 0.42WOL + 11.9EW + 12.6EN$	45.5	1869.9
Broad sense heritability (wh^2)			
One-trait	$I_{21} = 0.360WOL$	18.9	778.2
Two-traits	$I_{22} = 1.78W4 + 0.32WOL$	22.3	918.9
Three-traits	$I_{23} = 2.45W4 + 0.22W12 + 0.31WOL$	26.2	1080.9
Four-traits	$I_{24} = 2.50W4 + 0.33W12 + 0.22WOL + 8.91EW$	26.8	1102.3
Five-traits	$I_{25} = 2.59W4 - 64.82SL4 + 0.38W12 + 0.23WOL + 9.72EW$	27.0	1111.5
Six-traits	$I_{26} = 3.08W4 - 110.76SL4 + 0.38W12 + 0.28WOL + 7.09EW + 5.78EN$	27.2	1118.5
Seven-traits	$I_{27} = 3.58W4 - 134.44SL4 + 0.25W12 + 78.79SL12 + 0.25WOL + 7.44EW + 7.56EN$	27.4	1128.4

GA, genetic advance, *PRE*, percent relative efficiency of traits; *W4*, body weight at four weeks of age; *W12*, body weight at twelve weeks of age; *WOL*, body weight at on-set of lay; *SL4*, shank length at four weeks of age; *SL12*, shank length at twelve weeks of age; *EW*, egg weight; *EN-60*, number of eggs laid within 60 days; *I_{xn}*, selection index derived from x ($x = w1$ or wh^2) and comprising of n number of traits.

5.3.11: Number of generations and years of breeding

The number of generations and years required to realize the desired phenotypic improvement under alternative selection indices is summarized in Table 5.11. In the equal weight category, the number of generations and number of years ranged from 5.1 to 7.3 and 3.1 to 4.4 respectively. Under the broad sense heritability category, the number of generations and years ranged from 3.1 to 4.4 and 1.8 to 2.7 respectively. In both weight categories, the number of generations and years required to realize the breeding objective increased with the number of traits incorporated in the index. The number of generations and years for indices under the equal weight category almost doubled the value of the corresponding estimates under the broad sense heritability category.

Table 5.11: Selection indices and corresponding estimated number of generations and years of selection

Weight factor	Index, I	The standard deviation of the index, σ^I	Number of generations, q	Number of years, t
Equal weight				
	I ₁₁	152.5	5.1	3.1
	I ₁₂	179.6	6.0	3.6
	I ₁₃	210.8	7.0	4.2
	I ₁₄	214.1	7.1	4.3
	I ₁₅	217.2	7.2	4.3
	I ₁₆	218.6	7.3	4.4
	I ₁₇	219.9	7.3	4.4
Broad-sense heritability				
	I ₂₁	91.5	3.1	1.8
	I ₂₂	107.7	3.6	2.2
	I ₂₃	128.3	4.3	2.6
	I ₂₄	131.3	4.4	2.6
	I ₂₅	130.9	4.4	2.6
	I ₂₆	131.3	4.4	2.6
	I ₂₇	133.1	4.4	2.7

I_{xn}, selection index based on weight factor x (x = w1 or wh²) comprising of n number of traits; σ^I , standard deviation of the index; q, number of generations; t, number of years of selection required to achieve the desired phenotypic improvement.

5.4 Discussion

5.4.1 Growth and egg production performance indicators

Live-body weight and egg production are the most important traits considered by farmers and breeders when selecting replacement stock (Chomchuen *et al.*, 2022; Kassa *et al.*, 2021). The average weight of hens at maturity in generation one for both the superior and control group in the current study is within the range of 1.2 to 1.5 kg reported for Ugandan IC (Nakkazi *et al.*, 2014; Ssewanyana *et al.*, 2008; Kugonza *et al.*, 2008). The average weight at maturity for the superior (1718.6 g) and control (1766.5 g) groups in the third generation of the current breeding program was above the average 1400 g, 1500 g, and 1200 g reported in the randomly mating IC ecotypes in Eastern, Central, and Western Uganda, respectively (Mukungu, 2019; Nakkazi *et al.*, 2014; Ssewanyana *et al.*, 2008; Kugonza *et al.*, 2008). In addition, the mean egg weight of superior hens was in the range of 35.02 and 40.65 g, lower than the average of 41.13g reported for eggs from selected chicken ecotypes in Chapter Two. In the current study, the number of eggs laid per clutch was in the range of 6 to 18 in the selected group compared to 9 to 15 eggs reported in previous studies in Ugandan ICs (Mukungu, 2019; Ssewanyana *et al.*, 2008).

In the current study, selection resulted in positive and negative responses in both the superior and control groups. The positive selection response for egg number at the expense of clutch number (CLN-60) and egg weight (EW) could be explained by the negative genetic correlation between the former and other traits, as indicated in Table 2. Similar negative associations between egg number and egg weight or clutch number have been reported previously (Tongsiri *et al.*, 2019; Zonuz *et al.*, 2013). The observed negative association is the manifestation of chromosomal linkage among genes controlling a pair of traits (Sanda *et al.* 2022). The negative association could also be attributed to the selection criteria, which favored hens with the highest number of eggs per clutch, irrespective of other traits. Therefore, it is highly predictable that

light chicks at hatching and eight weeks of age have the potential to lay more eggs of reduced size in limited clutch numbers.

5.4.2 Genetic parameter estimates

Genetic parameters such as heritability, genetic and phenotypic correlation have been shown to guide the choice and development of suitable multi-trait selection tools (Ndung'u *et al.*, 2020; Faruque *et al.*, 2017). The heritability of egg number was precisely equal to the 0.17 estimated for Mazandaran IC in Iran (Niknafs & Nejati-javaremi, 2012). The heritability of weight at four (0.67) weeks, twelve weeks of age (0.58), and at the on-set of lay (0.60) closely doubled the values reported in Thai IC (Chomchuen *et al.*, 2022; Tongsirir *et al.*, 2019). The heritability of body weight, W12 (0.58), and egg weight, EW (0.47), were close or equal to the values reported in Tanzanian ICs except for egg number, EN-60 (Lwelamira *et al.*, 2009). In this study, egg number was negatively correlated with all traits except shank length (SL4). Similarly, egg number was negatively genetically correlated with all traits except egg weight (EW). Comparable negative genetic correlations of egg number with other traits including body weight from hatching to 12 weeks of age, weight at sexual maturity, and on-set of lay, have been observed in Iranian (Niknafs & Nejati-javaremi, 2012) and Tanzanian IC (Lwelamira *et al.*, 2009).

5.4.3 Selection indices for simultaneous improvement of growth and egg production traits

Irrespective of the weight factor (w_1 or wh^2) used to construct the selection indices, the corresponding trait combinations were similar. In both weights, GA and RPE simultaneously increased with the number of traits in the index, and a similar trend was also witnessed by Goyani (2022). Weight at first egg (WOL) as revealed in selection index I_{11} and I_{21} , is the most appropriate trait to consider in a single-trait selection aimed at improving both egg production and body weight of hens. Recently, a similar study suggested 6 and 32 weeks of age for selection aimed at improving body weight and egg production of IC, respectively (Chomchuen

et al., 2022). The coefficients of egg production, EN for weights w_1 (12.6) and wh^2 (7.56) for the final indices I_{17} and I_{27} , respectively, are higher than 1.59, estimated for the Tanzanian medium ecotypes preferred for egg production (Lwelamira & Kifaro, 2010). The variation in coefficients of egg production is probably a result of different weight factors used and the difference in the length of the laying period considered in the two studies. Unlike in the current study, Lwelamira & Kifaro (2010) attempted to improve growth and egg production separately in two chicken ecotypes of Tanzania to overcome the negative genetic correlation effect of the two traits. In this study, selection indices I_{17} and I_{27} , comprised all seven traits and at the same time exhibited the highest GA and PRE, which caters for the simultaneous genetic improvement of growth and egg production traits.

5.4.4 Number of generations and years corresponding to alternative selection indices

In the current study, the number of generations required to increase growth and egg production ranged from 5.1 to 7.3 under the equal weight category and 3.1 to 4.4 for indices under the broad sense heritability weight category. Previously, Lwelamira & Kifaro (2010) reported an average of 5.4 generations required to increase live-body weight at sixteen weeks of age in the Kuchi chicken ecotype. However, the same author reported a high number of generations ranging from 8 to 10.6 for indices aimed at improving egg production in a medium-weight chicken ecotype of Tanzania. The high number of generations reported by Lwelamira & Kifaro (2010) could be due to the inclusion of more egg production traits in the indices, which exhibit antagonism with growth, hence requiring more years to balance out traits.

5.5 Conclusion

Egg number is inversely proportional to egg weight, number of clutches, hatching weight, and body weight at eight weeks of age. Genetic advance and percent relative efficiency increased with the incorporation of more traits into the selection index. Alternative selection indices, I_{17}

and I₂₇, with the highest GA and PRE under both weight categories, are suggested for selecting within IC for genetic improvement of growth and egg production traits. A genetic improvement of 24.3 percent with respect to growth and egg production traits could be achieved in a maximum of 7.3 generations, corresponding to 4.4 years of selection. In conclusion, genetic advance yield increases with the number of traits incorporated in the index, and this subsequently requires more generations and years to achieve the breeding objectives.

CHAPTER SIX

OVERALL CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Results

The overall objective of the study was to characterize and develop selection indices for genetic improvement of growth and egg production traits of Ugandan IC ecotypes. The objectives of the study were specifically to assess the phenotypic diversity (Objective 1) and genetic diversity (Objective 2), estimate genetic parameters (Objective 3), and design alternative selection indices for genetic improvement of growth and egg production traits of IC (Objective 4).

Ecotype had a significant effect on all the traits except tarsus circumference. The effect of sex on body weight and linear morphological traits was significant across all ecotypes. As expected, cocks dominated hens in all six traits considered. The ecotype-sex interaction was only significant for body weight, drumstick length, and thorax circumference. The Teso, Nsoga, and Acholi chickens produced heavier eggs compared to the Madi and Lango ecotypes. Eggs of the Madi chicken ecotype had the least breadth and volume. On the basis of Principal Component analysis, the overlapping of clusters is evidence for the absence of distinct chicken ecotypes.

Analysis of Molecular Variance (AMOVA) of the 9 Ugandan IC ecotypes indicated 33 and 67 percent variation corresponding to ‘among’ and ‘within’ ecotypes. A pairwise population matrix based on Nei’s genetic distance among Ugandan IC demonstrated that the largest genetic distance exists between the Teso Gisu and Nsoga on one side and Madi, Lango, Acholi, Nyoro, Nsoga, Nganda, and Ankole ecotypes on the other side. The Gisu and Nyoro ecotypes expressed the highest levels of unbiased diversity. Acholi and Ankole ecotypes were characterized by the least number of effective Alleles.

Weight at first egg had a significant positive phenotypic correlation with body weight at two weeks of age, and shank length at six and eight weeks of age. The number of eggs laid and body weight at four weeks were negatively phenotypically correlated. Weight at first egg was also significantly positively genetically correlated with shank length at eight weeks of age. The number of eggs was significantly negatively, and genetically correlated with shank length at 12 weeks of age.

In general, selection resulted in a positive selection response in live-body weight at different ages except for weight at hatching. Among the reproductive traits, clutch size increased by a positive selection response of 155.7 percent in the superior group, but the number of clutches reduced by 23.9 percent. Weight at first egg yielded selection indices with the highest GA and PRE in single-trait selection indices under both weight categories. On the other hand, shank length at four weeks of age exhibited the lowest GA and PRE. Body weight at four weeks of age and body weight at the on-set of lay produced a selection index with the highest GA and PRE under both equal and broad sense heritability categories. Selection indices comprising body weight at four, twelve, and weight at the on-set of lay were ranked number one based on their high GA and PRE values in both weight categories. Selection indices consisting of weight at four weeks of age, twelve weeks of age, weight at on-set of lay, and egg weight were ranked first in both weight categories. Selection indices comprising five traits: weight at four and twelve weeks of age, shank length at four weeks, weight at on-set of lay, and egg weight scored the highest GA, PRE, and were ranked number one under both weight categories. A combination of weight at four weeks of age, shank length at four weeks of age, weight at twelve weeks of age, weight at on-set of lay, egg weight, and egg number in a six-trait index resulted in the highest GA and PRE.

6.2 Conclusions

Phenotypically, Ugandan IC ecotypes are indistinguishable. The traits for estimating live weight varied across the ecotypes and the sex of the chickens. However, tarsus circumference is a dominant trait in weight estimation models across ecotypes. Apart from estimating body weight, the diversity in the weight estimation models is an indicator of wide morphological and consequently, genetic variation within and across chicken ecotypes.

Genetically, Ugandan IC ecotypes are not identical; they belong to two major populations and are characterized by high levels of genetic admixture. Population one consists of the Teso, Nsoga, and Gisu ecotypes. On the other hand, Population two consists of the Madi, Nganda, Nyoro, Lango, Acholi, and Ankole ecotypes.

Shank length at 8 and 12 weeks of age is potential phenotypic marker when selecting for both high body weight at the on-set of lay and egg yield, respectively. Light hens with short shanks are more likely to lay more eggs per clutch. Growth traits are associated with moderate to high heritability. On the other hand, egg production traits are associated with low heritability. At large, the number of eggs produced was genetically and phenotypically negatively correlated with most traits.

Coefficients of traits associated with equal weight (w_1) selection indices almost double their counterparts under the broad-sense heritability (wh^2). The trait, weight at first egg, appeared in all top-ranking indices consisting of two traits or more in both weight categories. Selection indices, I_{17} and I_{27} , under the equal (w_1) and broad-sense heritability (wh^2) as weight factors, respectively, are suggested for genetic improvement of growth and egg production traits since they yield the highest genetic gain and percentage relative efficiency. The two indices are presented below;

$$I_{17} = 5.91W_4 - 225.97SL_4 + 0.41W_{12} + 131.49SL_{12} + 0.42WFE + 11.9EW + 12.6EN$$

$$I_{27} = 3.58W4 - 134.43SL4 + 0.25W12 + 78.79SL12 + 0.25WOL + 7.44EW + 7.56EN$$

Incorporating more traits increases genetic gain and percentage relative efficiency, number of generations, and years required to achieve the breeding objectives.

6.3 Limitations

- First, the use of broad sense heritability as a weight factor to develop selection indices yet it is less accurate. Instead, narrow sense heritability would have been used as it is more accurate compared to broad sense heritability.
- Secondly, pedigree data was not captured as selected superior cocks and hens were allowed to mate randomly. As such, a number of parameters like inbreeding coefficients could not be captured.

6.4 Recommendations

- Weight estimation models specific to ecotypes or generally applicable to all IC have been developed but they need to be validated independently to gain wide application. Computer or phone-based applications (Apps) could be developed to ease the utilization of the weight estimation models.
- It is recommended to estimate genetic parameters and consequently construct selection indices that include qualitative traits such as broodiness, hatchability, and mothering ability. Inclusion of such qualitative traits in selection indices is one strategy for conserving them in IC.
- Training of researchers, breeders, extension workers and farmers on the choice and application of the alternative selection indices is recommended.
- I recommend the uptake of the developed selection indices by National Animal Genetic Resources Center and Data Bank (NAGRIC&DB) and their incorporation into the chicken breeding program.

6.5 Innovation/Contribution of the study

Live-weight estimation models specific to or applicable to all Ugandan ecotypes have been developed to facilitate the selection, management, and marketing of chicken. In situations or homesteads where weighing scales are absent, weight estimation models embedded in phone applications or weighing bands are an alternative.

On the basis of genetic and phenotypic correlations, farmers and breeders can now select superior layer hens and higher body weight at the on-set of lay based on shank length at 12 weeks of age and shank length at eight weeks of age respectively.

Low-cost selection tools/indices for breeding superior IC are suggested. The alternative selection indices ensure multiple trait improvement at a single selection. In addition, breeders or farmers have the option to choose an index that improves their specific traits of interest. Finally, a line of superior chickens with the potential to lay up to 30 eggs per clutch has been produced over a period of four years of the study.

6.6 Take home message

- 1) Phenotypically, ICs are indistinguishable but genetically, belong to two population clusters.
- 2) Light hens with short shanks are more likely to lay more eggs per clutch.
- 3) Weight at first egg, WFE should always be included in selection indices targeting IC

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APPENDICES

Appendix 1: Alternative selection indices (I), expected genetic gain (GA) and percent relative efficiency (PRE) based on Equal weight

Trait Combn	Selection Indices (I)	GA	PRE	Ran k
1	$I_{11} = 0.60WOL$	31.5	129.72	1
	$0.58W_{12}$	13.5	55.5	2
	$0.67W_4$	3.6	14.9	3
	$0.47EW$	0.35	1.4	4
	$0.17EN$	0.15	0.63	5
	$0.14SL_{12}$	0.01	0.04	6
	$0.02SL_4$	0.001	0.006	7
2 (Top 10)	$2.90W_4 + 0.54WOL$	36.8	151.5	1
	$0.56W_{12} + 0.60WOL$	36.2	149.1	2
	$0.50WOL + 10.96EW$	32.5	133.8	3
	$53.83SL_4 + 0.58WOL$	31.7	130.3	4
	$0.62WOL + 4.16EN$	31.6	130.2	5
	$0.62WOL - 37.58SL_{12}$	316.4	130.1	6
	$1.48W_4 + 0.55W_{12}$	17.2	71.0	7
	$0.51W_{12} - 7.79EN$	14.8	61.1	8
	$0.67W_{12} - 94.60SL_4$	14.5	60.0	9
	$0.63W_{12} - 28.45SL_{12}$	13.6	56.0	10
3 (Top 10)	$4.06W_4 + 0.38W_{12} + 0.51WOL$	43.7	179.5	1
	$3.22W_4 + 0.38WOL + 15.78EW$	38.3	157.5	2

	3.73W4 +0.55WOL +11.91EN	37.8	155.5	3
	0.70W12+0.49WOL+11.32EW	36.9	152.0	4
	2.88W4+9.18SL4 +0.53WOL	36.8	151.6	5
	2.88W4 – 7.42SL12 +0.54WOL	36.8	161.6	6
	0.71W12 – 83.69SL12 + 0.63WOL	36.5	150.3	7
	0.66W12 – 46.08SL4 + 0.62WOL	36.3	149.6	8
	0.53W12 +0.58WOL -4.22EN	36.2	14.9	9
	63.3SL4 + 0.47WOL + 11.41EW	32.7	13.4	10
4 (Top 10)	4.14W4 + 0.55W12 + 0.36WOL + 14.54EW	44.6	18.3	1
	4.19W4 – 95.52SL4 +0.45W12 + 0.54WOL	43.9	18.1	2
	4.25W4 +0.29W12 + 46.40SL12 + 0.48WOL	43.7	179.8	3
	4.36W4 +0.37W12+ 0.51WOL +4.57EN	43.5	277.2	4
	3.80W4 +0.43WOL+11.88EW+9.45EN	38.7	159.5	5
	3.37W4 + 57.87SL12 + 0.32WOL +18.44EW	38.4	158.2	6
	3.19W4+16.11SL4+0.37WOL +15.85EW	38.3	157.7	7
	4.39W4 -121.77SL4 + 0.61WOL +16.73EN	38.2	157.3	8
	3.76W4+11.20SL12 +0.55WOL +12.04EN	37.8	155.6	9
	0.70W12+0.42WOL + 15.13EW – 7.01EN	37.2	153.0	10
5 (TOP10)	4.30W4 – 110.58SL4 +0.65W12 +0.38WOL +15.93EW	44.9	184.9	1
	4.42W4 +0.43W12+ 68.54SL12 + 0.31WOL + 15.43EW	44.7	183.8	2
	5.25W4 -200.70SL4 +0.51W12 +0.60WOL + 12.53EN	44.4	182.7	3

	4.22W4 +0.53W12 +0.37WOL +13.36EW +1.80EN	44.2	182.1	4
	4.46W4 – 101.98SL4 +0.33W12 + 59.50SL12 + 0.51WOL	44.0	181.3	5
	4.67W4 + 0.24W12 + 63.03SL12 +0.48WOL +5.30EN	43.6	179.5	6
	4.29W4 – 92.59SL4 + 0.49WOL + 9.83EW +13.58EN	39.0	160.4	7
	3.95W4 +57.7SL12 + 0.37WOL + 14.511EW + 9.54EN	38.9	160.1	8
	3.37W4 – 0.91SL4 + 58.1SL12 + 0.32WOL +18.47EW	38.5	158.3	9
	4.72W4 – 155.90SL4 +58.67SL12 + 0.59WOL +18.77EN	38.4	158.0	10
6 (ALL 7)	3.15W4 – 168.86SL4 + 0.87W12 + 0.39WOL + 21.92EW + 8.82EN	78.3	442.5	1
	2.62W4 +0.99W12 – 50.67SL12 + 0.35WOL + 21.94EW + 7.49EN	77.8	439.9	2
	3.35W4 – 178.64SL4 +0.55W12 + 26.89SL12 +0.59 WOL + 12.41EN	77.2	436.6	3
	2.24W4 – 139.02SL4 +1.19W12 – 66.62SL12 + 0.37WOL + 24.82EW	76.5	432.6	4
	1.16W12 – 103.4SL4 – 92.70SL12 +0.44WOL +21.05EW + 2.51EN	74.7	422.4	5
	0.83W4 – 159.62SL4 +0.89W12 – 15.90SL12 +8.74EW + 1.48EN	48.5	274.6	6
	2.67W4 – 19.03SL4 +30.10SL12 + 0.41WOL + 14.66EW + 7.69EN	41.1	609	7
7(ONLY 1)	3.25W4 – 176.59SL4 + 0.84W12 + 22.12SL12 + 0.38WOL + 21.89EW + 2.51EN	78.3	442.7	1

Appendix 2: Alternative selection indices (I), expected genetic gain (GA) and percent relative efficiency (PRE) based on broad sense heritability

Trait Combn	Selection Indices (I)	GA	PRE	Ran k
1	0.36WOL	18.9	77.8	1
	0.34W12	7.8	32.2	2
	0.45W4	2.40	10.0	3
	0.22EW	0.17	0.69	4
	0.02EN	0.03	0.11	5
	0.02SL12	0.00 1	0.00 5	6
	0.0005SL4	0.00	0.00	7
2 (Top 10)	1.78W4 +0.32WOL	22.3	91.8	1
	0.33W12 + 0.36WOL	21.6	88.9	2
	0.30WOL + 6.54EW	19.4	80.0	3
	0.37WOL + 2.43EN	19.0	78.2	4
	32.4SL4 + 0.35WOL	19.0	78.1	5
	0.37WOL- 22.58SL12	18.9	78.0	6
	0.92W4 + 0.33W12	10.3	42.4	7
	0.30W12 – 4.56EN	8.8	36.0	8
	0.39W12 - 54.88SL4	8.4	34.8	9
	0.37W12 – 16.5SL12	7.9	32.4	10
3 (Top 10)	2.45W4 + 0.22W12 +0.31WOL	26.2	108. 0	1
	1.97W4 +0.23WOL + 9.50EW	23.1	95.3	2

	$2.31W4 + 0.33WOL + 7.14EN$	23.0	94.5	3
	$1.77W4 - 3.91SL12 + 0.33WOL$	22.3	91.9	4
	$1.76W4 + 5.39SL4 + 0.32WOL$	22.3	91.9	5
	$0.41W12 + 0.29WOL + 6.83EW$	22.0	90.5	6
	$0.41W12 - 49.52SL12 + 0.38WOL$	21.7	89.5	7
	$0.31W12 + 0.35WOL - 2.41EN$	21.7	89.2	8
	$0.35W12 - 25.42SL4 + 0.37WOL$	21.6	89.1	9
	$37.86SL4 + 0.28WOL + 6.80EW$	19.5	80.5	10
4 (Top 10)	$2.50W4 + 0.33W12 + 0.21WOL + 8.91EW$	26.8	110. 2	1
	$2.53W4 - 55.31SL4 + 0.26W12 + 0.33WOL$	26.4	108. 7	2
	$2.66W4 + 0.22W12 + 0.31WOL + 2.88EM$	26.3	108. 2	3
	$2.57W4 + 0.17W12 + 27.86SL12 + 0.29WOL$	26.3	108. 2	4
	$2.34W4 + 0.26WOL + 7.15EW + 5.63EN$	23.5	96.7	5
	$2.06W4 + 35.89SL12 + 0.19WOL + 11.16EW$	23.2	95.7	6
	$2.70W4 - 72.73SL4 + 0.36WOL + 10.01EN$	23.2	95.6	7
	$1.95W4 + 9.45SL4 + 0.22WOL + 9.53EW$	23.1	95.3	8
	$2.33W4 + 8.29SL12 + 0.33WOL + 7.23EN$	23.0	94.6	9
	$1.75W4 + 7.09SL4 - 5.54SL12 + 0.32WOL$	22.3	91.9	
5 (TOP 10)	$2.59W4 - 64.82SL4 + 0.38W12 + 0.23WOL + 9.72EW$	27.0	111. 1	1
	$2.67W4 + 0.25W12 + 41.33SL12 + 0.19WOL + 9.45EW$	26.8	110. 5	2
	$3.19W4 - 119.65SL4 + 0.30W12 + 0.36WOL + 7.622EN$	26.8	110. 4	3

	$2.57W4 + 0.32W12 + 0.22WOL + 8.26EW + 1.13EN$	26.7	110.0	4
	$2.69W4 - 59.07SL4 + 0.19W12 + 35.45SL12 + 0.31WOL$	26.5	109.0	5
	$2.85W4 + 0.14W12 + 38.00SL12 + 0.29WOL + 3.32EN$	26.3	108.5	6
	$2.64W4 - 54.99SL4 + 0.29WOL + 5.92EW + 8.08EN$	23.6	97.2	7
	$2.44W4 + 36.86SL12 + 0.22WOL + 8.83EW + 5.68EN$	23.6	97.1	8
	$2.90W4 - 94.22SL4 + 37.03SL12 + 0.35WOL + 11.30EN$	23.3	96.0	9
	$2.07W4 - 1.09SL4 + 36.18SL12 + 0.19WOL + 11.17EW$	23.2	95.7	10
6 (ALL 7)	$3.08W4 - 110.76SL4 + 0.38W12 + 0.28WOL + 7.09EW + 7.09EN$	27.1	111.8	1
	$2.82W4 - 71.12SL4 + 0.29W12 + 51.90SL12 + 0.19WOL + 10.47EN$	27.1	111.6	2
	$3.67W4 - 142.51SL4 + 0.17W12 + 74.7SL12 + 0.33WOL + 9.39EN$	27.0	111.3	3
	$2.79W4 + 0.23W12 + 45.02SL12 + 0.19WOL + 8.60EW + 1.57EN$	26.8	110.4	4
	$2.94W4 - 82.99SL4 + 59.43SL12 + 0.26WOL + 8.00EW + 9.41EN$	23.8	98.1	5
	$0.48W12 - 1.42SL4 - 39.76SL12 + 0.27WOL + 8.60EW - 3.95EN$	22.3	91.8	6
	$0.86W4 - 58.46SL4 + 0.37W12 + 3.52SL12 + 1.68EW - 2.31EN$	11.1	45.7	7
7(ONLY 1)	$3.58W4 - 134.43SL4 + 0.25W12 + 78.79SL12 + 0.25WOL + 7.44EW + 7.56EN$	27.4	112.8	

Appendix 3: Gulu University Research Ethics Committee (GUREC) Approval



12/09/2021

To: Geoffrey Beyihayo

Gulu University
+256782627576

Type: Initial Review

Re: GUREC-2020-18: Native Chicken Project, 2.0, 2021-09-07

I am pleased to inform you that at the 71st convened meeting on 03/12/2020, the Gulu University REC, committee meeting, etc voted to approve the above referenced application.

Approval of the research is for the period of 12/09/2021 to 12/09/2022.

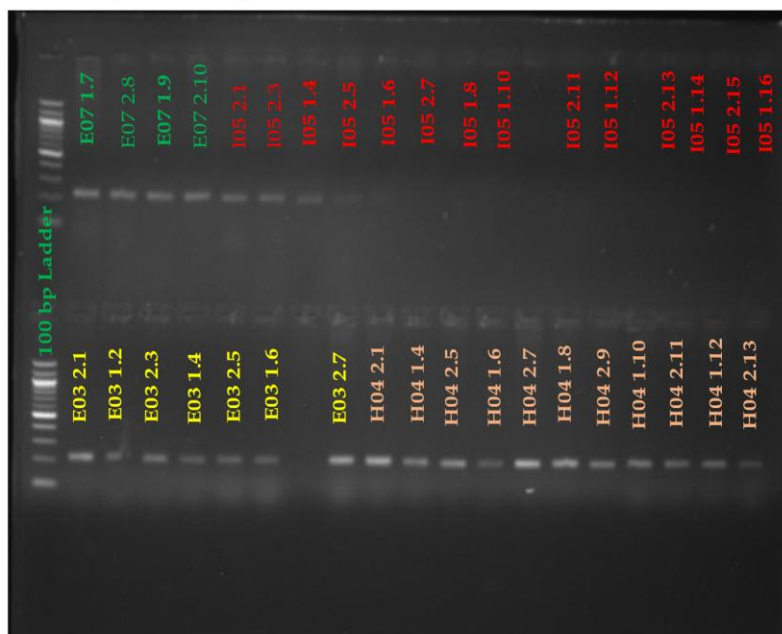
As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval prior to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **12/09/2022** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:

Appendix 4: Photo of allele band size scoring based on MCW0222

MCW0222 (220 Allele size)



E07=Nyoro(Hoima); E03=Madi (Yumbe); I05=Lango (Lira); H04=Acholi (Omoro)

Appendix 5: Informed consent form

VERSION 2.0 DATED 17.05. 2021

INFORMED CONSENT DOCUMENT

Title of the study:

CHARACTERIZATION AND DEVELOPMENT OF SELECTION INDICES FOR THE
GENETIC IMPROVEMENT OF UGANDAN INDIGENOUS CHICKENS

Investigator(s): Beyihayo Geoffrey Akiiki

Institution(s): Gulu University

Introduction

The investigator, **Beyihayo Geoffrey Akiiki**, is a PhD student of Agricultural and Applied Biosciences, Faculty of Agriculture and Environment, Gulu University. Mr Beyihayo Geoffrey Akiiki holds a Master of Science in Animal Science and a Bachelor of Science in Agriculture (Animal Option) all from Makerere University Kampala. The proposed study titled “Marker Assisted Selection for Growth and Egg Production of Indigenous Chicken Eco-Types in Uganda” is under thematic area of “Sustainable Animal Production Systems”.

Under this study, indigenous chicken across the four regions of Uganda will first be characterized, evaluated and then markers for growth and egg production identified. The chicken will be characterized phenotypically; i) quantitatively- live weight and body

measurements ii) qualitative – plumage pattern, skin color beak type etc. Blood samples (for DNA) extraction will be collected from the wing vein. DNA will be analyzed to assess any diversity across the chicken population. Following a growth trial, chicken will be grouped into **Inferior** or ii) **Superior** performers; with respect to growth and egg production. A genome-wide association study (GWAS) utilizing specific SNPs will be used to identify marker genes.

A brief description of the sponsors of the research project

The proposed research is co-funded by AU (Native Chicken Project) and the investigator.

Purpose:

The study seeks to identify markers (phenotypic and genetic markers) for growth and egg production among indigenous chicken ecotypes in Uganda. The findings of this study will help to increase accuracy of selecting highly productive indigenous multipurpose chickens

Procedures:

Your participation in this study will involve selecting a cock and hen from your flock as guided by the survey team.

Who will participate in the study?

You have been chosen to participate in this study because you are one of the stakeholders in the indigenous chicken sector. The interview/survey/sample collection will last for approximately three months and a total of 480 Households will participate in the study country-wide.

Risks/discomforts:

There is no foreseeable risk of harm or discomfort that will arise from your participation in this study. The only risk or discomfort will be the inconvenience in terms of time spent during the survey. The stress imposed on cocks and hens when collecting about two drops of blood from the wing vein is short lived and will not affect their growth or laying potential.

Benefits:

At the end of the study, participants and other chicken farmers will be able to access highly performing breeding indigenous chickens and will be empowered/trained to select replacement elite chickens from their flocks.

Participants will get feedback on the progress and findings of the study. Any new information affecting the study participants (including incidental findings) will be made available to them.

Confidentiality:

Identity of participants will not be revealed to any one as we shall only use codes to identify participants. Information obtained will only be accessible by the research team. Soft copies of the data will be protected by password and hard copy files will be kept under lock and key. Confidential information will only be accessed by the principal investigator.

Alternatives:

Participating in this study is optional. You will not lose any benefit in case of no participation.

Cost:

There will not be any additional cost incurred as a result of participating in this study. Fresh fertile eggs will be bought from consenting households at 1000/= each.

Questions:

If you have any questions related to the study as a research participant, you can contact the principal investigator, Mr. Beyihayo Geoffrey Akiiki on telephone number +256 782 627576 or via email beyigeo@gmail.com

Statement of voluntariness:

Participation in the research study is voluntary and you may join on your own free will. You have a right to withdraw from the study at any time without penalty.

If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson, Gulu University Research Ethics Committee, Dr. Gerald Obai Tel: No., 0772305621; email: lekobai@yahoo.com/lekobai@gmail.com; or the Uganda National Council for Science and Technology, on plot 6 Kimera road, Ntinda, Kampala on Tel 0414705500.

Statement of consent

Beyihayo Geoffrey Akiiki has described to me what is going to be done, the risks, the benefits involved and my rights as a participant in this study. I understand that my decision to participate in this study will not affect me in any way. In the use of this information, my identity will be concealed. I am aware that I may withdraw at any time. I understand that by signing this form,

I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name Signature of participant.....Date
.....

Name.....Signature of
interviewer.....Date.....

Name.....Signature of
witness.....Date:.....

Appendix 6: Photo of the 15 microsattellites packed in vials



Appendix 7: Photos of Indigenous chicken (cocks and hens)



Photo of Indigenous cocks (*Photo by G.A Beyihayo*)



Photo of indigenous hens (*Photo by G.A Beyihayo*)