2024-10

Phenotypic characterization of indigenous chickens and evaluation of Enteromorpha prolifera polysaccharide supplementation on growth performance and anticoccidial efficacy

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PhD Program

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PhD Dissertation

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Animal Genetics and Breeding

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October, 2024
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We hereby certify that we have supervised, read and evaluated this Dissertation titled "Indigenous Chicken Ecotypes and their Production Systems, and Genetic Resistance for Sustainahle Chicken Production in Northwest Ethiopia" by Bekalu Muluneh Ayalew prepared under our guidance. We recommend the dissertation be submitted fororal defense.

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As members of the board of examiners, we examined this dissertation titled ‘‘**Phenotypic characterization of indigenous chickens and evaluation of *Enteromorpha prolifera* polysaccharide supplementation on growth performance and anticoccidial efficacy**’’ by Bekalu Muluneh Ayalew. We hereby certify that the dissertation is accepted for fulfilling the requirements for the award of ‘‘Doctor of Philosophy in Animal Genetics and Breeding’’.

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DECLARATION

I affirm that this dissertation is entirely my work, and I have appropriately acknowledged all sources of information used in it through proper citations. I have conducted, gathered, analyzed, and concluded the research for this dissertation in accordance with ethical guidelines applicable to scholarly work. The dissertation incorporates numerous scholarly sources, all of which have been duly acknowledged through citations. Throughout the writing process, diligent efforts have been made to ensure the prevention of any form of plagiarism. This dissertation entitled "Indigenous Chicken Ecotypes and their Production Systems, and Genetic Resistance for Sustainable Chicken Production in Northwest Ethiopia" submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D.) in Animal Genetics and Breeding to the Department Graduate Council of animal sciences, Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University by Bekalu Muluneh Ayalew is an authentic work carried out by him under our supervision. To the best of our knowledge and understanding, the content presented in this dissertation has not been previously submitted for the purpose of obtaining a degree or diploma. Short quotations from this dissertation may be utilized without explicit permission, provided that proper and comprehensive source attribution is provided. Requests for permission to extensively quote or reproduce substantial portions of this dissertation, either in whole or in part, may be considered by the responsible academic unit if they deem the proposed use to be in the interest of scholarly pursuits.

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ACKNOWLEDGEMENTS

Above all, I want to thank Almighty God for his abundant blessings, unwavering guidance, and the strength he has bestowed upon me throughout this journey.

I extend my deepest appreciation to my major supervisor, Dr. Mengistie Taye, for his invaluable support and encouragement from the very inception to the successful completion of this dissertation. His mentorship and guidance have been invaluable, and I am immensely grateful for the knowledge and wisdom gained under his tutelage. I am also indebted to my esteemed co-supervisors, Dr. Tadelle Dessie and Dr. Dessie Salilew Wondim, for their constructive criticism, unwavering support, and guidance throughout the research process.

I am thankful to the staff members at the Bahir Dar University School of Animal Sciences and Veterinary Medicine, including Mr. Esubalew Adimasu (Department Head of Animal Science), Dr. Aschalew Shitu, and Mr. Halo Yohans, for their moral and technical support. Special thanks go to Professor Kefyalew Alemayehu, Dr. Solomon Abegaz, Dr. Bizuayehu Kerisew, and Mr. Fentahun Miheret for their professional assistance during my course work. I express my gratitude to Dr. Teketay Wassie and Professor Xin Wu for their technical support and provision of *Enteromorpha prolifera* polysaccharides for the disease challenge and feed experiments.

My heartfelt thanks also go to Wolaita Sodo University for granting me a study leave and providing financial support for my research endeavours. I extend my appreciation to the staff members at the Dawuro Tarcha campus of Wolaita Sodo University for their unreserved support and encouragement during my study.

I would like to thank Bahir Dar University Institute of Biotechnology (IOB-BDU) for providing financial support for my molecular work, the Bahir Dar University Agricultural Development Centre for providing me with a research site at Zenzelima campus, Professor Asaminew Tassew, Dr. Semahegn Yilkal, and all farm workers for their assistance and cooperation.

I am grateful to Bahir Dar Animal Disease Investigation and Diagnostic Laboratory and Bahir Dar University Veterinary Parasitology and Agricultural Biotechnology Laboratory for providing facilities for disease challenge studies, and I extend my thanks to Dr. Fikirtemariam Aregay, Dr. Almaz Habtamu, and other staff members of Bahir Dar Animal Disease Investigation and Diagnostic Laboratory for their technical support.

I express my gratitude to the Amhara Public Health Institute, Regional Artificial Insemination Centre, and the International Livestock Research Institute for their assistance and provision of facilities during various stages of my research. Special thanks go to the International Livestock Research Institute for hosting me during DNA extraction and financing DNA sequencing costs, and to Dr. Mekonnen Girma, Ms. Bersabhe Solomon, Dr. Wondmeneh Esatu, Professor Olivier Hanotte, and other staff members for their moral and technical support and encouragement during DNA extraction and gel-electrophoresis.

I extend my gratitude to my colleagues and PhD study classmates, Dr. Andualem Tenagne, Mr. Siferaw Yifrut, Mr. Endalew Wallelign, Dr. Oumer Sheriff, Dr. Abiy Shenkut, Dr. Sisay Asmare, Mr. Abrham Mulu, and Mr. Endalkachew Birilie for their technical and professional support and encouragement. My sincere appreciation also goes to all regional, zonal, district, and *kebele* office experts and smallholder farmers for their cooperation during survey data and egg collection. My special thanks go to Samuel Wodiferaw (Eng.) for providing an incubator for chick hatching and to Ms. Alemitu Kassahun (Shaki) for her moral and financial support.

I am thankful to the Bahir Dar Polytechnic College for granting me access to their library and internet resources during the dissertation writing process.

Lastly, I extend my heartfelt gratitude to my wife, Abeba Baynes, my child, Hasset Bekalu, my father, Muluneh Ayalew, my mother, Workie Wubie, my sisters, Haymanot Muluneh and Tigist Muluneh, and all other family members and friends who have supported and encouraged me throughout my study. Their unwavering love, encouragement, and support have been the driving force behind my success, and I am immensely grateful for their presence in my life.

DEDICATION

Dedicated to my wife: Abeba Baynes and my kids: Hasset Bekalu and Amen Bekalu.

In **memory** of innocent Ethiopians who lost their lives due to the civil war and conflicts (**2021-2024**).

ABBREVIATIONS

ACI	Anti Coccidial Index
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AFM	Age at First Mating
CAT	Catalase
CRD	Completely Randomized Design
CSA	Central Statistical Authority
CV	Coefficient of variation
FAO	Food and Agricultural Organization of the United Nations
FCR	Feed Conversion Ratio
GLM	General Linear Model
GWAS	Genome Wide Association Study
HCPC	Hierarchical Clustering on Principal Component
HPLC	High Performance Liquid Chromatography
IFN-γ	Interferon Gamma
IL	Interleukin
LSM	Least Square Mean
mRNA	Messenger Ribo Nucleic Acid
OPG	Oocysts Per Gram
PA	Peasant Association
PBS	Phosphate Buffer Saline
SAS	Statistical Analysis System
SCFAs	Short Chain Fatty Acids
SD	Standard deviation
SE	Standard Error
SNP	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Sciences
TLR	Toll Like Receptor

ABSTRACT

Phenotypic characterization of indigenous chickens and evaluation of *Enteromorpha prolifera* polysaccharide supplementation on growth performance and anticoccidial efficacy

By Bekalu Muluneh

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Investigating chicken production systems, along with the ecotype's productive and morphological traits, as well as the challenges and opportunities in chicken production is crucial for developing sustainable breeding programs. The aim of the dissertation was to phenotypically characterize the indigenous chicken ecotypes and evaluate the effect of *Enteromorpha prolifera* polysaccharide on growth performance and anticoccidial efficacy. The dissertation is based on four manuscripts mentioned as chapters (**Chapter 4 - Chapter 7**). The first manuscript (**Chapter 4**) explores the husbandry practices and selection criteria of chicken producers in Northwest Ethiopia. In this chapter, data collected from a total of 360 households from six districts (stratified into lowland, midland, and highland agro-ecologies) was analyzed. In the study area, egg production performance was a priority by chicken owners when selecting female chickens across all agro-ecologies. For male chickens, plumage color, appearance, and growth rate were the main traits considered. Farmers primarily raised chickens for income generation through the sale of eggs and birds. There were significant differences among agro-ecologies in terms of chicken feeding management, housing, chicken flock composition, brood modification methods, and reproductive performance traits. The inbreeding coefficient was higher in the highland agro-ecology compared to the midland and lowland areas. To enhance chicken productivity sustainably, breeding programs should align with farmers' production objectives and trait preferences specific to each agro-ecology. The second manuscript (**Chapter 5**) focuses on characterizing the morpho-biometric variations of indigenous chicken ecotypes in Northwest Ethiopia. Sampling was done using a multi-stage purposive, stratified, and random approach. Morpho-biometric data collected from a total of 1200 adult chickens were analyzed for 12 qualitative and 11 quantitative traits. Red plumage color, white and red earlobe color, and yellow shank color were the most prevalent trait categories. Body measurements were significantly influenced by sex, agro-ecology, location, and the interaction of sex and location. Shank traits (Shank length and circumference)

exhibited high discriminatory power in both sexes. Female and male sample populations were classified with overall rates of 57.47% and 69.97%, respectively. Significant squared Mahalanobis distances were observed between locations/sites. Canonical variates analysis separated sample populations based on location. Cluster analysis classified the indigenous chickens into four ecotypes: ecotype 1 (Banja, Dembecha, and Aneded), ecotype 2 (North Achefer), ecotype 3 (Sinan), and ecotype 4 (Jawi). The third manuscript (**Chapter 6**) was based on an on-station comparison of the growth performance of indigenous chickens and evaluates the effect of *Enteromorpha* polysaccharide (EP) supplementation on their growth. A total of 180 indigenous chicken ecotypes (Sinan, Dembecha, North Achefer, and Jawi) were used in the study. Both ecotype and sex had a significant effect on body weight and average daily gain (ADG). Jawi ecotype exhibited the highest final body weight and ADG, followed by North Achefer. Males had higher body weight and ADG than females. *Enteromorpha* polysaccharide supplementation resulted in significantly higher body weight and ADG compared to non-supplemented chickens. The interaction between ecotype and feed type was not significant, except at week nine. Sinan ecotype showed the highest survivability rate in the first four weeks. Overall, Jawi and North Achefer ecotypes performed better in terms of growth, while Sinan ecotype showed relatively higher survivability. Supplementation of EP showed potential for improving chicken growth. The fourth manuscript (**Chapter 7**) was about the anticoccidial potential of *Enteromorpha prolifera* polysaccharide in indigenous chickens in Northwest Ethiopia. The study included 78 male indigenous chickens divided into two treatment groups: EP non-supplemented and EP supplemented. Each group had five replications. After 14 days of EP supplementation, chickens were inoculated with 1.5×10^4 mixed *Eimeria* oocysts. The EP-supplemented chickens exhibited significantly lower oocyst counts and less severe lesion scores compared to non-supplemented chickens. The EP supplementation provided relatively a higher protection rate against lesions and resulted in higher weight gain. *Enteromorpha* polysaccharide showed promise as a potential strategy for combating coccidiosis in chickens. Further research is needed to understand the mechanisms underlying its protective effects.

Keywords: Agro-ecology, Anticoccidial, Body weight, Breeding objective, Characterization, Coccidiosis, Disease, Ecotype, *Enteromorpha* polysaccharide, Growth, Indigenous chicken, Morpho-biometric, Squared Mahalanobis, Trait

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CHAPTER 1: GENERAL INTRODUCTION

Indigenous chickens (*Gallus domesticus*) possess adaptive characteristics that enable them to thrive in under various environmental conditions, including extensive small-scale village settings, free-range environments, and organic production systems (Padhi, 2016; Manyelo *et al*., 2020; Kpomasse *et al*., 2023). Indigenous chicken production is widely practiced in rural areas of developing countries including Ethiopia. These chickens hold significant socioeconomic importance, serving as crucial contributors to food security, sources of income generation, participants in religious rituals (Gulilat *et al*., 2021), and providers of employment opportunities (Tolasa, 2021). Ethiopia's poultry population is estimated at approximately 57 million, with the majority being laying hens (34.26%) and chicks (32.86%). In terms of breed distribution, 78.85% of the total poultry are indigenous, 9.14% are exotic, and 12.03% are hybrid (CSA, 2021).

Identification of indigenous chicken breeds, along with data on their population sizes, geographic distributions, and genetic diversity, is essential for their improvement, and implementing targeted conservation and breeding programs (Melesse *et al*., 2021; Kpomasse *et al*., 2023). Indigenous chickens exhibit distinctive morphological features and possess genes that confer adaptive advantages in their specific environments, including resistance to local diseases (Aklilu *et al*., 2013) and they have been chosen based on these traits (Mpenda *et al*., 2019). This selective process has resulted in the existence of a diverse array of chicken breeds and ecotypes globally (Di Lorenzo *et al*., 2015). In evolutionary biology, ‘ecotype’ is described as a population that is genetically adapted to specific environmental conditions (Vallejo-Trujillo *et al*., 2022). Indigenous chickens display significant phenotypic variation in plumage, shank coloration, eye color, earlobe size, comb type, skin pigmentation, feather distribution, and body size, which can be attributed to the prevailing climatic conditions (Manyelo *et al*., 2020). The indigenous chickens in Ethiopia are named either after the specific locations where they are predominantly found or based on their plumage color type (Muluneh *et al*., 2023). Ethiopian indigenous chickens possess a rich genetic diversity and demonstrate strong local adaptation capabilities, forming the foundation for selective breeding and genetic improvement approaches (Melesse *et al*., 2021). Nevertheless, despite their abundant resources and remarkable adaptability to local environmental conditions, indigenous

chickens exhibit suboptimal performance within smallholder production systems, with variations observed across different regions of Ethiopia (Matawork, 2018).

In the traditional production system, smallholder farmers maintain flocks consisting of diverse phenotypes to fulfill their multifaceted requirements (Dana *et al*., 2010a). In addition to fulfilling economic requirements, smallholder farmers keep indigenous chickens to meet their cultural and religious needs (Dana *et al*., 2010a; Melesse & Negesse, 2011). For instance, Dana *et al*. (2010b) emphasized the importance of plumage color in indigenous chicken rearing, since this trait is considered by many farmers for its impact on consumer preferences and market prices. To develop a successful animal breeding program for village chicken production systems, it is essential to comprehend the management practices, production environment, breeding goals, and trait preferences (Tilahun *et al*., 2022). Notably, the inclusion of economically significant traits in designing of breeding program is of utmost importance for the conservation of chicken breeds, as well as for enhancing productivity and establishing sustainable breeding strategies (Wong *et al*., 2017). In Ethiopia, efforts have been made to enhance the productivity of the poultry industry by introducing exotic breeds. However, these attempts not resulted in the anticipated outcomes due to the insufficient management practices (Esatu *et al*., 2022). To improve local poultry production, a potential intervention strategy should initially focus on indigenous breeds, taking into consideration the specific needs and preferences of smallholder farmers. As suggested by Wilson (2010), the most effective approach to achieving higher output and productivity is to enhance the genetic characteristics of local chickens and implement changes in management practices.

Indigenous chicken production encounters various challenges, including limited availability and high cost of feedstuffs, climate-related difficulties leading to frequent diseases and increased flock mortality rates, as well as poor feed efficiency (Mahoro *et al*., 2017; Kpomasse *et al*., 2023). Genetics, nutrition, and health collectively influence the production performance of animals (Dana *et al*., 2010a). Breeding and genetic improvement of indigenous chickens is crucial for agricultural sustainability, involving the selection of suitable breeds and a thorough understanding of their specific attributes or phenotypes (Manyelo *et al*., 2020). In addition to breeding and genetic improvement, providing optimal nutrition plays a vital role in promoting the growth, production, and overall health of poultry. Indigenous chickens possess inherent resilience against persistent

pathogen exposure. However, in rural areas, the lack of adequate bio-security measures heightened susceptibility to infectious diseases and limited access to appropriate treatments, such as vaccines and drugs contribute to elevated mortality rates (Mpenda *et al*., 2019). Diseases, such as Newcastle disease, Gumboro disease, and coccidiosis are recognized as major causes of mortality in different regions of Africa (Kpomasse *et al*., 2023). This might be attributed to the high cost and lack of access to vaccines and the emergence of drug-resistant disease-causing strains.

To mitigate the access and affordability of vaccines, overcome drug-resistant strains, and drug residues, various alternative disease control methods such as probiotics, organic acids, essential oils, antioxidants, and the development of disease-resistant chicken breeds are being implemented (Kalkal *et al*., 2021; Gul *et al*., 2022; Raza *et al*., 2022). Studies of Wassie *et al*. (2021b) and Zhao *et al*. (2021) demonstrated that the addition of *Enteromorpha prolifera* (EP), a type of seaweed-green algae, enhanced the performance of chickens in various aspects, including growth and production, breast muscle yield, egg quality, antioxidant capacity, and intestinal morphology. Another promising alternative approach to enhance prophylactic measures is genetic resistance and it can be achieved through genetic breeding and genetic modification techniques (Gul *et al*., 2022).

In the Northwest part of Ethiopia, particularly in the West Gojjam zone, Awi zone, and East Gojjam zone, there is a significant genetic resource of indigenous chickens, estimated at approximately 5.8 million (CSA, 2021). However, there is a lack of scientific information regarding the morphological characteristics of the chickens, selection criteria, and production/breeding objectives of the households keeping these indigenous chickens among farmers. Moreover, the production and reproduction performances of these chickens have not been adequately studied at different environmental conditions. Therefore, this study aims to characterize indigenous chicken ecotypes, explore their production systems, and evaluate the effect of *Enteromorpha prolifera* polysaccharide supplementation on growth and anticoccidial efficacy of indigenous chickens.

The following specific objectives were addressed in order to achieve the overall objective.

1. To identify the selection criteria and husbandry practices of indigenous chicken producers in Northwest Ethiopia.
2. To morpho-biometrically characterize the indigenous chicken ecotypes in Northwest Ethiopia.
3. To evaluate the effect of ecotype and *Enteromorpha prolifera* polysaccharide supplementation on the growth performance of indigenous chicken ecotypes in Northwest Ethiopia.
4. To evaluate anticoccidial efficacy of *Enteromorpha prolifera* polysaccharide in indigenous chickens of Northwest Ethiopia.

CHAPTER 2: LITERATURE REVIEW

2.1. Origin, Domestication and Distribution of Chickens

The origins of domestic chickens (*Gallus g. domesticus*) have been debated ever since Darwin (Sawai *et al*., 2010). The domestication of chickens is widely believed to have occurred in multiple centers, as supported by archaeological and historical evidences (Storey *et al*., 2012). Archaeological and molecular evidence supports the notion that domesticated chicken populations in Asia emerged from a shared ancestor, specifically the red jungle fowl *Gallus gallus* (Storey *et al*., 2012; Al-Jumaili *et al*., 2020). Chickens were likely domesticated in Southeast Asia, particularly in regions like the Indus Valley, with evidence pointing to multiple domestication events across Asia, including India (Kanginakudru *et al*., 2008; Lawal & Hanotte, 2021). The cultural and religious importance of chickens has led to their widespread distribution, with descendants of early domestic fowl being dispersed globally over at least two millennia through multiple means (Belew, 2018). The African local chicken breeds are thought to have originated from South-East Asia, China, and India (Muchadeyi *et al*., 2008; Mtileni *et al*., 2011). There is limited documentation regarding the introduction of domesticated chickens into Africa (Dessie *et al*., 2012). According to Mwacharo *et al*. (2013), the history of introduction and dispersal of village chickens across the African continent is a subject of intense argument and speculation among scholars. Socio-cultural, linguistic, archaeological, and historical evidence collectively indicate that the introduction of chickens to Africa occurred through multiple maritime and/or terrestrial routes over a span of time (Alemayhu, 2003).

2.2. Chicken Production Systems in Ethiopia

Poultry in Ethiopia are raised across diverse management systems, classified based on factors like breed, flock size, housing, feeding, health practices, bio-security levels, and technology utilization. The systems include extensive scavenging, semi-intensive, small-scale intensive, and large-scale commercial poultry production (Esatu *et al*., 2017; FAO, 2019b).

In the extensive or scavenging system, few birds are kept with scarce specific poultry house. Birds scavenge and are occasionally fed home grains and refuse, with no regular health programs, bio-security measures, or formal marketing channels. Native dual-use breeds are common in this system, adapted to scavenging environments. In contrast, hybrid and exotic breeds are prevalent in other systems except for the extensive one (Emebet *et al*., 2016; Esatu *et al*., 2017). This system, prevalent in rural areas, aims for household consumption and supplementary income, covering 95-98% of the country's poultry production and typically lacks profitability, with minimal supplementary feeds, housing, and natural incubation for chicks. High chicken mortality rates, often exceeding 70%, occur due to inadequate healthcare and veterinary services (FAO, 2019; Entity, 2021). Primarily managed by women for supplemental income, poultry in this system contributes significantly to household finances (Ayele & Rich, 2010). The income generated often supports school expenses, underlining the importance of empowering rural women in family poultry development for both women and their children's education (FAO, 2019; Entity, 2021).

The semi-intensive system involves flocks of 50-200 birds, combining scavenging with regular supplementation and improved housing and healthcare, resulting in mortality rates from 20 to over 50% (Entity, 2021). The Ethiopian Livestock Master Plan estimated around 120,000 households in this system in 2014 (Shapiro *et al*., 2015). Small-scale intensive production employs specialized breeds, balanced rations, and high-quality housing, with access to veterinary care and low to medium mortality rates (<20%). This system is popular in urban areas, serves as a vital income source for many families and plays a role in youth employment (Urgesa, 2023). Large-scale commercial poultry production, highly intensive, involves over 10,000 birds under controlled conditions, heavily relying on imported exotic breeds and modern management practices. Market-oriented, it caters to urban poultry demands (Fitsum & Aliy, 2014). The sector is growing, with a significant share in meat and egg production, and relies on its parent stock for sustainable day-old-chick production. It accounts for nearly 2% of the national poultry population (Belew, 2018).

2.3. Breeding Objectives of Indigenous Chicken Producers

The traits to be improved, the cost of production, and the income from the product sales related to a genetic change in each trait are referred to as breeding objectives (Musa *et al*., 2023). The determination of the ideal magnitude and direction of genetic changes in traits within a breeding program can be significantly influenced by breeding objectives, although they are not the sole determining factor (Byrne & Amer, 2018). They enable both breeders and commercial producers to direct breeding emphasis towards specific market outcomes or address key performance aspects of their production system. Under the scavenging production system, smallholder farmers maintain flocks with diverse phenotypes to fulfill their multipurpose needs, reflecting their broad breeding objectives (Chebo *et al*., 2022). In developing countries, indigenous chickens are commonly raised for the purpose of egg and meat production, primarily for home consumption, reproduction, or generating income (Mujyambere *et al*., 2021).

Enhancing the productivity and adaptability of indigenous chickens in diverse environments hinges on the critical selection criteria employed. Indigenous chickens exhibit diverse physical traits such as comb type, shank color, and plumage patterns, serving as key indicators for farmers in selecting for fertility and adaptability in rural settings (Chebo *et al*., 2022). Research suggests that focusing on within-breed selection can notably improve characteristics such as body weight and egg yield, while genomic selection can further amplify genetic advancements and diminish inbreeding rates (Ndung'u *et al*., 2022). Selective breeding has been minimally practiced in indigenous chickens (Desta & Wakeyo, 2024). The diverse breeding goals of small-scale farmers (Bett *et al*., 2011) and the continuous exposure of indigenous chickens to natural selection have mitigated the impact of human-driven selection. Small-scale farmers exhibit a wide range of preferences based on traits that align with their socio-cultural and economic requirements, with a common emphasis on resilience traits (Desta & Wakeyo, 2024). Indigenous chickens, characterized by unique attributes, offer a range of products and services. Both consumers and subsistence farmers express strong demand for meat and eggs with enhanced flavor and diverse morphological traits typical of indigenous chickens (Yakubu *et al*., 2020). The choice of breeds and traits among small-scale farmers is shaped by community needs, production system dynamics, and local interactions. For small-scale farmers raising indigenous chickens, meeting their varied needs and preferences lacks a

standardized approach. Beyond serving as sources of protein, indigenous chickens hold cultural and ritual significance, providing diverse socio-economic benefits to small-scale farmers (Desta, 2021a; Kapella *et al*., 2023). Small-scale farmers intentionally select indigenous chickens to align with their broad breeding goals and adapt to local conditions (Desta & Wakeyo, 2024).

2.4. Morphological Characterization of Indigenous Chickens

The genetic composition and morphology of today's chicken populations have been influenced by the selection process for adaptive traits, preferred by producers and consumers, and the varied production environments (Lawal and Hanotte, 2021). Indigenous chickens, primarily raised by rural communities in Africa and Asia, exhibit significant diversity in body size, plumage color, comb type, and other physical attributes that hold promise for conservation and genetic enhancement (Habimana *et al*., 2021; Maharani *et al*., 2021; Winaya *et al*., 2023). These chickens display a range of morphological features and genetic traits that contribute to their ability to adapt to specific environments and resist diseases (Mekonnen *et al*., 2023). Variations in characteristics such as plumage color and body size can vary significantly across indigenous chicken populations, reflecting adaptations to local climates, predator threats, and other environmental conditions (Mekonnen *et al*., 2023).

The development of strategies for genetic improvement and sustainable conservation of indigenous animal genetic resources should rely on the evaluation of the phenotypic characteristics of the populations being studied (Melesse *et al*., 2021). Evaluation of the genetic attributes of populations is essential before implementing effective strategies for enhancing genetic improvement programs (Azimu *et al*., 2018; Habimana *et al*., 2020). However, in areas where molecular-based genetic analysis is not cost-effective, morphological measurements have been employed to investigate the attributes of indigenous livestock populations (Melesse *et al*., 2021). Morphological characterization involves the assessment of various traits to understand diversity and adaptation, aiding in the identification and documentation of variations within and between distinct breeds based on observable attributes (FAO, 2012). Morphological characterization studies of chickens involve assessing quantitative traits like body weight, body length, chest circumference, shank length, and wingspan, as well as qualitative traits like plumage

color, comb type, feather morphology and distribution, ear-lobe color, shank color, and skin color (FAO, 2012).

2.5. Performances of Indigenous Chickens

2.5.1. Growth performance

Growth refers to the progression in size or weight of farm animals over time until they reach maturity (Kgwatalala & Segokgo, 2013). Body weight serves as a direct indicator of growth and significantly impacts the production and reproductive traits of birds (Mulugeta *et al*., 2020). Body weight and body weight gain are crucial economically important traits when evaluating different genotypes. These traits are influenced by a combination of genetic and environmental factors (Negash *et al*., 2023). Feed efficiency, as measured by the feed conversion ratio, is an important factor that can explain variations in growth performance between genotypes, as it is closely related to body weight and body weight gain (Prakash *et al*., 2020). The feed conversion ratio (FCR) in chickens reflects the efficiency of feed utilization and is determined by the ratio of feed consumption to body weight gain within a specific timeframe. A lower FCR indicates more efficient feed utilization, while a higher FCR suggests potential overfeeding (Yi *et al*., 2018; Bai *et al*., 2022; Kpomasse *et al*., 2023). Indigenous breeds typically exhibit a lower growth rate in comparison to introduced commercial strains (Wondmeneh, 2015; Alemu *et al*., 2021; Tadele *et al*., 2023). The lower growth rate of local chickens results in reduced yields and profitability for farmers (Kpomasse *et al*., 2023). In Ethiopia, scholars (Alewi & Melesse, 2013; Wondmeneh, 2015; Shewangizaw *et al*., 2021; Aleme, 2022; Chebo *et al*., 2022; Tadele *et al*., 2023), reported a range of body weight values of 924 g–1500 g in local chickens aged 18–24 weeks. Indigenous chickens exhibited lower feed intake in comparison to the commercial chicken genotype, which can be attributed to the negative association between weight gain and feed intake, as well as feed efficiency (Bamidele *et al*., 2020; Shewangizaw *et al*., 2021; Tadele *et al*., 2023). Studies conducted by Alam *et al*. (2021) and Zelleke *et al*. (2022) reported lower feed conversion ratio (FCR) values in exotic genotypes compared to indigenous chickens. The selection of a chicken genotype with a favorable feed conversion ratio (FCR) leads to improved feed utilization, decreased feed intake, and enhanced growth rate (Aggrey *et al*., 2010).

2.5.2. Reproductive performance

The success of poultry breeding is influenced by a range of parameters, including age at sexual maturity, fertility, hatchability, clutch size, and clutch length, which collectively define and evaluate the reproductive performance of birds (Mersha & Senbeta, 2020). Among these reproductive traits, sexual maturity holds paramount significance in driving advancements in poultry breeding (Nwogwugwu *et al*., 2018). The delayed sexual maturity of native chickens compared to exotic breeds poses challenges to their reproductive potential (Mekonnen *et al*., 2023), impacting their overall breeding success. In Ethiopia, the reported average age at sexual maturity varies across different regions, ranging from 19.6 to 26.8 weeks for males and 19.7 to 34.05 weeks for females (Mersha & Senbeta, 2020). Fertility refers to the percentage of incubated eggs that are fertile, while hatchability represents the percentage of fertile eggs that successfully hatch (Aleme, 2022). Hatchability plays a crucial role in determining the reproductive output within a specific timeframe, as it influences the quantity of breeding stock (Ajayi & Agaviezor, 2016). To achieve high production numbers, it is essential to maintain a higher hatchability rates for breeder stock and ensure the survival of chicks (Ndofor-Foleng *et al*., 2015). A Hatchability and a clutch size ranging from 59.6% to 93.2% and 2.7 to 4.3 per year, respectively were reported for Ethiopian indigenous chickens in small holder systems (Mersha & Senbeta, 2020). Clutch length, which refers to the duration of a clutch, has been reported differently for indigenous chickens in various regions, such as 22.54 days (Gebremariam *et al*., 2017), 26 days (Yemane *et al*., 2013), and 15 to 28 days (Alem, 2014).

2.5.3. Disease and parasite resistance performance

The susceptibility or resistance of poultry to specific diseases is intricately linked to their genetic makeup (Chen, 2024). Recent research indicates that genetic selection and breeding methods can notably enhance poultry's resistance to particular diseases. Inadequate health management practices resulted in elevated mortality rates and reduced production performance among the birds (Mujyambere *et al*., 2022). Commonly used disease control methods in poultry, like vaccination, antibiotics, and culling, often have limited effectiveness (Gul *et al*., 2022). Genetic resistance, achievable through breeding and genetic modification, offers a promising alternative for enhancing preventive

measures (Gul *et al*., 2022). While indigenous chickens are recognized for their favorable traits, such as disease resistance and adaptability to their environment (Abdelqader *et al*., 2007; Yemane *et al*., 2013), a review by Mersha and Senbeta (2020) revealed higher mortality rates (25.3% to 61.15%) for Ethiopian indigenous chickens kept under backyard management conditions. In rural farming systems, substandard feed quality, insufficient feed quantity, and inadequate farm management practices contributed to increased chicken mortality rates (Francis *et al*., 2016; Goraga *et al*., 2016).

2.6. Improvement of Indigenous Chicken Performances

Assessing the performance of indigenous chickens is crucial before implementing any improvement programs, yet data on the performances of many ecotypes are limited (Kpomasse *et al*., 2023). Enhancing the productivity of local chickens requires careful attention to nutrition, breeding, and health aspects (Manyelo *et al*., 2020). Genetic and breeding programs, along with appropriate nutritional strategies, can contribute to improving the performance of indigenous chickens. When targeting the improvement of indigenous chicken breeds, it is recommended to prioritize within-breed selection rather than crossbreeding with commercial chicken breeds (Padhi, 2016). This approach helps preserve the unique attributes of indigenous chickens that are valued by producers, preventing genetic erosion and dilution and contributing to their conservation (Okeno *et al*., 2012). Although improvement through selection may be a slow process, it results in sustainable changes in production while maintaining the distinctive characteristics of native and indigenous breeds (Padhi, 2016). Genetic selection for feed conversion ratio presents difficulties due to its strong correlation with feed intake and body weight gain (Kpomasse *et al*., 2023). Residual feed intake has been identified as a more suitable criterion for the genetic improvement of energy efficiency in chicken breeding in numerous studies (Yuan *et al*., 2015; Begli *et al*., 2016). Poor-quality feeds and incorrect nutrient levels in diets, such as energy and protein, can lead to nutritional stress and health issues among local chickens (Panda *et al*., 2012). Supplementation with various plant-derived biomolecules, such as *Enteromorpha prolifera* polysaccharide and *Aloe vera*, has been shown to improve growth performance and immune status in chickens (Wassie *et al*., 2021b; Khan *et al*., 2022; Wassie *et al*., 2022a; Wassie *et al*., 2022b; Zhang *et al*., 2022). Implementing proper bio-security measures and vaccination protocols are primary strategies for disease prevention in poultry flocks (Churchil, 2023).

2.7. Chicken Disease Resistance and Genetic Improvement

Disease resistance in chickens refers to their ability to prevent infection or control the life cycle of pathogens when exposed to pathogens (Zanella, 2016). Disease resistance is a complex trait due to the complex interplay of biological networks, host-pathogen interactions, and susceptibility to environmental stressors (Cheng *et al*., 2013). The poultry industry is facing a constant threat of infectious diseases caused by bacterial, viral, and protozoal pathogens, leading to reduced growth yield and profitability. Traditional control strategies, such as vaccination and antibiotics, have limitations of access and cost, necessitating the exploration of alternative approaches (Pinard-van Der Laan *et al*., 2009; Gogoi *et al*., 2022). The misuse of antibiotics has also resulted in the emergence of antimicrobial resistance, posing a global challenge. Consequently, poultry producers are adopting antibiotic-free (ABF) and organic practices, including the use of probiotics and essential oils, to address this issue. However, still, the impact of ABF and organic practices on antimicrobial resistance profiles in the poultry gut microbiome is not well understood (Mak *et al*., 2022; Panyako *et al*., 2022). In addition, recently, selective breeding based on genomic data offers a promising approach to enhance chicken resistance to infectious diseases (Cheng *et al*., 2013).

Disease resistance in chickens is a complex trait influenced by multiple genes and immune components, such as major histocompatibility molecules, immunoglobulins, cytokines, T and B cells (Gul *et al*., 2022; Miyumo *et al*., 2023). These genes encode antibodies, microRNAs, and other factors that contribute to the host's ability to resist pathogen-induced damage (Dar *et al*., 2018). Recent advances in molecular biology have led to the discovery of numerous disease-resistant genes, providing valuable insights for molecular breeders (Dar *et al*., 2018). Genomic selection for disease resistance is a sustainable strategy, although it presents challenges in collecting sufficient genomic and phenotypic data from small populations of indigenous chicken ecotypes (Hearn & Cheng, 2023). It is pointed out that the joint analysis of data from different ecotypes could improve the accuracy of genomic selection for disease resistance (Hearn & Cheng, 2023).

Genetic factors play a significant role in chicken disease resistance, as the breed's genome contributes to its unique characteristics and the transmission of desirable traits across generations (Mak *et al*., 2022). Understanding the genetics of disease resistance,

epigenetic mechanisms, and quantitative trait loci enables the identification of resistance markers and the development of disease-resistant breeds (Banos *et al*., 2020). According to Dar *et al*. (2018), recent advancements in genotyping technology and genomic selection approaches have brought about significant advancements in animal breeding. Moreover, the availability of the chicken genome, transcriptome, and proteome has provided valuable insights into the genetic mechanisms that contribute to susceptibility and resistance to various diseases. Understanding these mechanisms is crucial for genetic enhancement of the immune response, which has the potential to improve vaccine efficacy and reduce drug residues in food. In order to achieve breeding success in chickens with enhanced disease resistance, marker-assisted selection utilizing indicator traits or genetic markers is essential (Dar *et al*., 2018).

2.7.1. Identification of candidate genes

Various methods have been employed to identify disease-resistance genes in chickens, including deep RNA sequencing, whole genome sequencing, amplicon sequencing, signature of selection analysis and genome-wide association studies (Bedier *et al*., 2022; Gul *et al*., 2022; Kim *et al*., 2022; Dar *et al*., 2023). These methods involve analyzing genetic variations, such as single nucleotide polymorphisms (SNPs) and other genetic markers across different chicken breeds to identify genes associated with disease resistance. RNA sequencing enables the detection of SNPs and INDELs in genes linked to disease resistance (Smith *et al*., 2020). Whole genome sequencing provides a comprehensive view of the entire genome, facilitating the identification of genetic variations associated with disease resistance (Gul *et al*., 2022). Amplicon sequencing is utilized to identify genetic polymorphisms in specific genes, such as the shadow of the prion protein gene (SPRN) (Kim *et al*., 2022). Genome-wide association studies (GWAS) involves analyzing genetic variations across multiple chicken breeds to identify genomic regions associated with disease resistance (Dar *et al*., 2023). These methods contribute to a deeper understanding of the genetic foundation of disease resistance in chickens and can assist in the development of disease-resistant breeds.

2.7.2. Genetic resistance to chicken diseases

The poultry industry is highly vulnerable to infectious diseases caused by bacterial, viral, and protozoal pathogens, leading to decreased growth, productivity, and profitability (Gul *et al*., 2022). To control infections in poultry, preventive measures such as vaccination, antibiotic usage, disinfectants, and culling are implemented (Gogoi *et al*., 2022). However, existing vaccines often lack comprehensive protection against multiple strains of different pathogens. Moreover, the mutagenic nature of viruses has resulted in the emergence of highly virulent strains (Mountford *et al*., 2022). To address the challenge posed by emerging pathogens, it is crucial to develop genetically resistant breeds that can prevent outbreaks, ensure sustained economic viability, and maintain consumer confidence in poultry products. By raising flocks with genetic modifications that confer disease resistance, it is possible to obtain a breed capable of withstanding infectious diseases and pathogens (Gul *et al*., 2022). The genetic makeup of a bird has been found to significantly influence its ability to resist diseases (Deist *et al*., 2017). However, the number of studies conducted to date, especially those involving genome-wide scans, remains limited due to the requirement for controlled disease challenges in molecular genetics research (Churchil, 2023). Various researchers have investigated several potential candidate genes associated with the resistance of chickens to protozoal, viral, and bacterial pathogens. Table 2.1 presents some of these candidate genes identified in previous studies.

i) Avian coccidiosis

Avian coccidiosis, an important parasitic disease caused by apicomplexan parasites, has a detrimental effect on the reproductive ability and growth performance of poultry birds (Mumtaz *et al*., 2021). This infectious disease is primarily transmitted by *Eimeria* species, a type of protozoa with various species. It is a major contributor of reduced efficiency in chicken production (Chapman, 2014). Economic losses in chickens are attributed to seven host-specific *Eimeria* species: *E. acervulina, E. maxima, E. tenella, E. brunetti, E. necatrix, E. praecox, and E. mitis* (Blake *et al*., 2020). Globally, this parasitic disease is estimated to cause economic losses ranging from 2.4 to 3 billion dollars annually (Kadykalo *et al*., 2018; Gordillo Jaramillo *et al*., 2021;Rizwan *et al*., 2022; Zhang *et al*., 2023).The main approaches for prevention and control the disease include the use of anticoccidials and vaccinations, although their success rates vary (Khater *et al*.,

2020).These approaches, however, raised concerns regarding the emergence of drug-resistant strains, high vaccine costs, and the presence of drug residues in meat and eggs, leading to a growing interest in alternative control strategies (Awais *et al*., 2011). Gul *et al*. (2022) reviewed the genetic resilience of chickens against various parasites, including avian coccidiosis. Kim *et al*. (2010) found a statistically significant association between myeloid leukemia factor 2 (MLF2) SNP and fecal oocyst shedding, a major indicator of coccidiosis resistance. The MLF2 SNP is associated with body weight which further supports the genetic link between MLF2 and coccidiosis resistance. Another candidate gene, zyxin (ZYX), located on chromosome 1, has been found associated with increased resistance to coccidiosis in birds (Hong *et al*., 2009). Zyxin encodes a protein that plays a role in focal adhesion complexes, regulating actin filament assembly and promoting attachment of epithelial cells to the extracellular matrix (Zaidel-Bar *et al*., 2003). It is speculated that one of the host responses to *Eimeria* infection involves up regulation of genes like zyxin, which mediate focal adhesion, thereby enhancing the formation of the intestinal epithelial barrier against parasite invasion.

ii) Salmonellosis

Salmonella is a significant infectious disease in poultry which leads to substantial economic losses in terms of mortality and morbidity, particularly in countries with inadequate vaccination programs (Dar *et al*., 2023). Salmonella infections pose a threat to both the poultry industry and public health (Wang *et al*., 2023). While spontaneous Salmonella spp. infection may not cause a significant number of chicken deaths, it has a profound negative impact on poultry production capacity and health. Moreover, as a Zoonotic disease, it poses a significant risk to public health and safety (Dar *et al*., 2023).

It is found that the resistance to Salmonellosis varies greatly among different chicken lines (Calenge *et al*., 2010). The IL18 (interleukin-18) gene has been identified to have high-impact SNPs associated with Salmonella disease resistance in chickens (Dar *et al*., 2023). Genetic variations in IL18 have been linked to an increased risk of atopy and asthma (Izakovicova Holla, 2003; Imboden *et al*., 2006). Additionally, IL18 polymorphism has been associated with either an increased or decreased progression of hepatocellular carcinoma (Bakr *et al*., 2018). Through the integration of gene function and RNAseq data, Li *et al*. (2019) identified FBXW7 (F-box and WD repeat domain containing 7) gene as a

candidate gene potentially responsible for resistance to *Salmonella pullorum*. Intriguingly, this gene has been reported to participate in the NF-κB signaling pathway (Fukushima *et al*., 2012), suggesting the involvement of this pathway in the host's resistance to Salmonella infection. NF-κB is a major transcription factor which regulates numerous genes involved in both the innate and adaptive immune response (Zhang *et al*., 2017).

The natural resistance-associated macrophage protein 1 (NRAMP1) genes influences host innate immunity against intracellular bacteria by transporting divalent cations in late endosomes/lysosomes (Liu, 2002). Exploring the association between the NRAMP1 gene and the chicken's innate immune response to *Salmonella enteriditis* might contribute to the understanding and enhancing genetic resistance to *Salmonella enteriditis* in chickens. The Ser379 position SNP of the NRAMP1 gene has been associated with spleen bacterial load following exposure to pathogenic *Salmonella enteriditis*, as well as antibody production in response to Salmonella enteriditis vaccination (Liu, 2002). The transforming growth factor-beta 2 gene (TGF-β2) belongs to the cytokine gene group (Akramullah *et al*., 2020). The same author reported that based on molecular and biological testing, tolaki chickens of all genotypes were found resistant to *Salmonella pullorum*, and the TGF-β2 gene could potentially serve as a genetic marker for resistance to *Salmonella pullorum* bacterial infection in these chickens. Wang *et al*. (2023) identified an extracellular fatty acid binding protein (EXFABP) gene as a potential candidate gene and transcript (co-) factors for resistance to Salmonella infection. Hu *et al*. (2022) found that *Salmonella Enteriditis* infection in chickens leads to increased transcription of EXFABP, which sequesters siderophores produced by enteric bacteria and Gram-positive bacilli like *Escherichia-Shigella* and *Enterococcus*, resulting in a decrease in their abundance.

iii) Newcastle disease

Newcastle disease (NCD) is a highly destructive viral disease that affects chickens, particularly in low- and middle-income countries where backyard production systems are prevalent (Mpenda *et al*., 2019). NCD is of a significant concern due to its endemic nature in tropical regions and the substantial production and economic losses it causes in the industry (Alders *et al*., 2018). Although bio-security measures and vaccinations have proven effective in controlling NCD, their impact can be temporary and heavily influenced by environmental factors (Zanella, 2016). Conversely, the use of antimicrobial drugs for

control purposes is beneficial but often leads to concerns about product safety due to misuse (Lamont, 2010). Marker-assisted selection of chickens resistant to Newcastle disease virus (NDV) holds promise as a strategy worth exploring (Mpenda *et al*., 2019). The NRAMP-1 gene has been associated with defense mechanisms against bacterial and viral infections, and its genotype has shown an association with immunoglobulin Y (IgY) concentration and specific antibodies against Newcastle disease (Ardiyana *et al*., 2020). According to Ardiyana *et al*. (2020), the NRAMP-1|SacI exon 1124 in SenSi-1 Agrinak chickens exhibited polymorphism, with a higher frequency of the C allele compared to the T allele in relation to immunoglobulin Y (IgY) concentration and antibody titers against Newcastle disease. The interferon regulatory factor 1 (IRF1) gene has also been identified as a candidate gene for Newcastle disease resistance, as it inhibits the replication of Newcastle disease virus (Liu *et al*., 2018).

iv) Avian influenza

Chickens serve as natural hosts for various viruses, including the influenza virus, which can lead to severe illness or mortality in these birds (Alam *et al*., 2022). The selective breeding of chickens that are resistant to Avian Influenza Virus would be advantageous for both the chicken industry and public health. The role of the Mx protein in conferring resistance to Avian Influenza virus has been extensively studied (Benfield *et al*., 2008; Sironi *et al*., 2008; Susanti *et al*., 2017; Alam *et al*., 2022). Multiple single-nucleotide polymorphism (SNP) variations in the Myxovirus resistance (Mx) gene have been reported to influence susceptibility to avian influenza, although conflicting findings have also been reported (Sironi *et al*., 2008). Alam *et al*. (2022) identified three genotypes of the Mx gene, with homozygous genotypes AA being resistant and GG being sensitive to avian influenza. Fulton *et al*. (2014) demonstrated that the antiviral activity of the Mx protein is attributed to structural variations in exon 14 of the Mx gene. Sartika *et al*. (2011) observed slightly better age at first egg, egg weight, body weight of hens at first laying, and egg production in the susceptible genotype (Mx-) compared to the resistant genotype (Mx+).

v) Marek's disease

Marek's disease (MD) is a significant global concern in terms of both economics and animal welfare, causing an estimated annual economic loss of 2 billion USD to the poultry

industry (Smith *et al*., 2020). The causative agent, Marek's disease virus (MDV), is an α-herpes virus that initially infects B-cells, undergoes a latency period, and can subsequently develop into an oncogenic disease upon infecting T-cells (Nair, 2005). Vaccinated flocks were found to harbor both vaccine and pathogenic strains of MDV, leading to the emergence of increasingly virulent strains (Read *et al*., 2015). Consequently, the effectiveness of vaccine treatments is diminishing as more virulent strains arose (Boodhoo *et al*., 2016). In addition to vaccination, an alternative approach for controlling Marek's disease is the selection and breeding of chickens with genetic resistance (Churchil, 2023). Recent studies utilizing genome-wide chicken genotyping arrays have identified genes like the SRY-box 1 (SOX1), present in regions of homozygosity, that contribute to immunology and survival against Marek's disease (Xu *et al*., 2018). The transferrin gene, located on chromosome 9, has also been shown to possess antiviral properties against Marek's disease (Giansanti *et al*., 2002; Gul *et al*., 2021). Furthermore, Thy-1 cell surface antigen (THY1) has been implicated in Marek's disease resistance, as reported by Liu *et al*. (2001). Other genes previously associated with Marek's disease resistance include GH1 (growth hormone) and CD79B (B-cell antigen) (Smith *et al*., 2020).

vi) Infectious bursal disease

Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious acute disease that affects chickens worldwide. It is primarily transmitted by two serotypes, Serotype 1 and Serotype 2 (Azli *et al*., 2021). The Infectious Bursal Disease Virus (IBDV) possesses a bi-segmented, double-stranded RNA genome and its lack of a lipid envelope contributes to its high resistance to environmental conditions (Guzmán *et al*., 2022). Currently, vaccination is the primary method for controlling the disease, but researchers are also exploring alternative measures, such as breeding for enhanced genetic resistance (Smith *et al*., 2015). The involvement of the major histocompatibility complex (MHC) in resistance to IBDV has been a subject of debate, but it does seem to play a role (Juul-Madsen *et al*., 2002). In studies conducted on 61 Brown Leghorn chicken lines, the BLB1 gene (MHC class II antigen B-F minor heavy chain) and TLR2B gene (toll-like receptor 2, type 2) were identified as potential candidate genes for infectious bursal disease resistance (Smith *et al*., 2015).

Table 2.1. The candidate genes in chicken disease resistance which are identified by different scholars

Gene ID	Gene symbol	Gene Name	Identified disease	Chromosomal Location	References
418300	ZYX	Zyxin	Coccidiosis Resistance	Chromosome 1	Hong *et al*. (2009)
418284	MLF2	myeloid leukemia factor 2	Coccidiosis Resistance	Chromosome 1	Kim *et al*., (2010); Hong *et al*., (2011)
422481	FBXW7	F-box and WD repeat domain containing 7	Salmonella Resistance	Chromosome 4	Li *et al*. (2019)
396393	EXFABP	extracellular fatty acid binding protein	Salmonella Resistance	Chromosome 17	Wang *et al*. (2023)
395312	IL18	interleukin-18	Salmonella Resistance	Chromosome 24	Dar *et al*. (2023)
395811	NRAMPl	Natural Resistance Associated Macrophage Proteins-1	Salmonella Resistance	Chromosome 7	Liu (2002)
421352	TGF-β2	Transforming growth factor-beta 2	Salmonella Resistance	Chromosome 3	Akramullah *et al*. (2020)
395811	NRAMPl	Natural Resistance Associated Macrophage Proteins-1	Newcastle Disease Resistance	Chromosome 7	Ardiyana *et al*. (2020)
396384	IRF1	interferon regulatory factor 1	Newcastle Disease Resistance	Chromosome 13	Liu *et al*. (2018)
395313	Mx1	myxovirus (influenza virus) resistance 1	Avian Influenza Virus Resistance	Chromosome 1	Benfield et al., (2008); Sironi et al., (2008); Susanti *et al*., (2017); Alam et al.,(2022)
396241	TF	transferrin	Marek's Disease Resistance	Chromosome 9	Giansanti *et al*., (2002); Gul *et al*., (2022)
378897	THY1	Thy-1 cell surface antigen	Marek's Disease Resistance	Chromosome 24	Liu *et al*. (2001)
374240	SOX1	SRY-box 1	Marek's Disease Resistance	Chromosome 1	Xu *et al*. (2018)
378781	GH1	growth hormone-1	Marek's Disease Resistance	Chromosome 27	Smith *et al*. (2020)
41994	CD79B	CD79b molecule	Marek's Disease Resistance	Chromosome 27	Smith *et al*. (2020)
693256	BLB1	MHC class II antigen B-F minor heavy chain	Infectious Bursal Disease Resistance	Chromosome 16	Smith *et al*. (2015)
769014	TLR2B	toll-like receptor 2, type 2	Infectious Bursal Disease Resistance	Chromosome 4	Smith *et al*. (2015)

2.7.3. Roles of candidate genes in chicken breeding strategy

Disease resistance in chickens is attributed to the genetics of the animals, the environmental system where the animals are raised and the interaction between these two. Understanding the genetic bases of disease resistance and integrating this information in breeding programs is essential to significantly impact chicken production reducing reliance on antibiotics and vaccinations (Smith *et al*., 2020). Advanced technologies such as whole genome sequencing, RNA sequencing, and high-density SNP genotyping have revolutionized the identification of disease resistance genes (Smith *et al*., 2020). Furthermore, employing techniques like fine chromosomal mapping, QTL mapping, and genome-wide association studies (GWAS), has enabled researchers to identify candidate genes and causative mutations associated with economically important traits (Banos *et al*., 2020).

Integrating the genetic information of disease resistance into breeding programs facilitate and enhance disease resistance, growth rates, feeding efficiency, egg traits, and overall productivity in chickens. Marker-Assisted Selection, Genomic Selection, crossbreeding, and functional genomics offer promising avenues for developing resilient poultry lines. Maintaining genetic diversity, understanding epigenetic influences, and balancing health with productivity traits are essential components of successful breeding programs. As research in this field progresses, the ability to produce disease-resistant chickens will continue to advance, contributing to more sustainable and efficient poultry production systems.

CHAPTER 3: GENERAL MATERIALS AND METHODS

The dissertation is based on four manuscripts, each of them are mentioned as Chapters (**Chapter 4 - Chapter 7**). The general materials and methods are given in this section and the detailed methodologies are available in the specific manuscripts (Chapters) imbedded herein.

3.1. Description of the Study Area

The chicken production system and morpho-biometric characterization studies (**Chapter 4 and Chapter 5**) were conducted in six selected districts (Banja, Jawi, Sinan, Aneded, Dembecha, and North Achefer) of the Amhara region in Northwest Ethiopia (Figure 3.1).

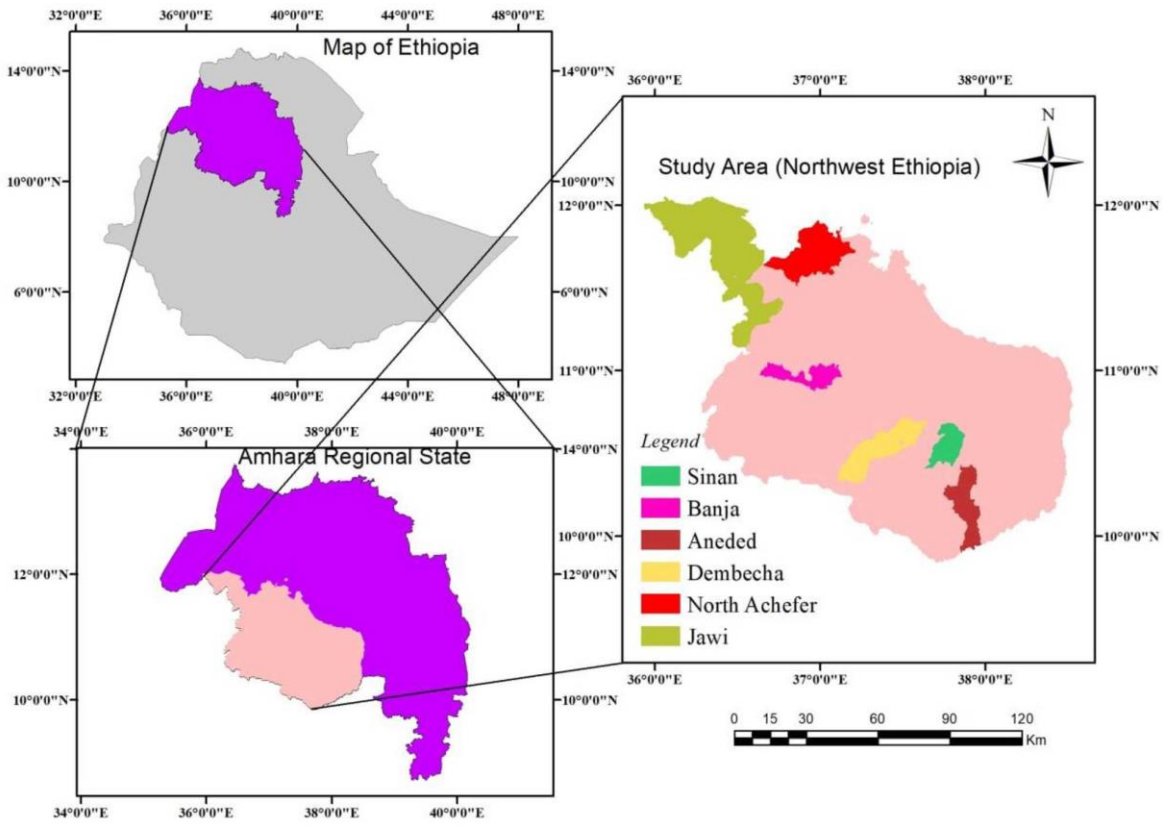

Figure 3.1. Map of the study areas for on-farm characterization study

The on-station studies (**Chapter 6 and Chapter 7**) were conducted at Zenzelima campus of Bahir Dar University, located in Northwest Ethiopia (Figure 3.2).

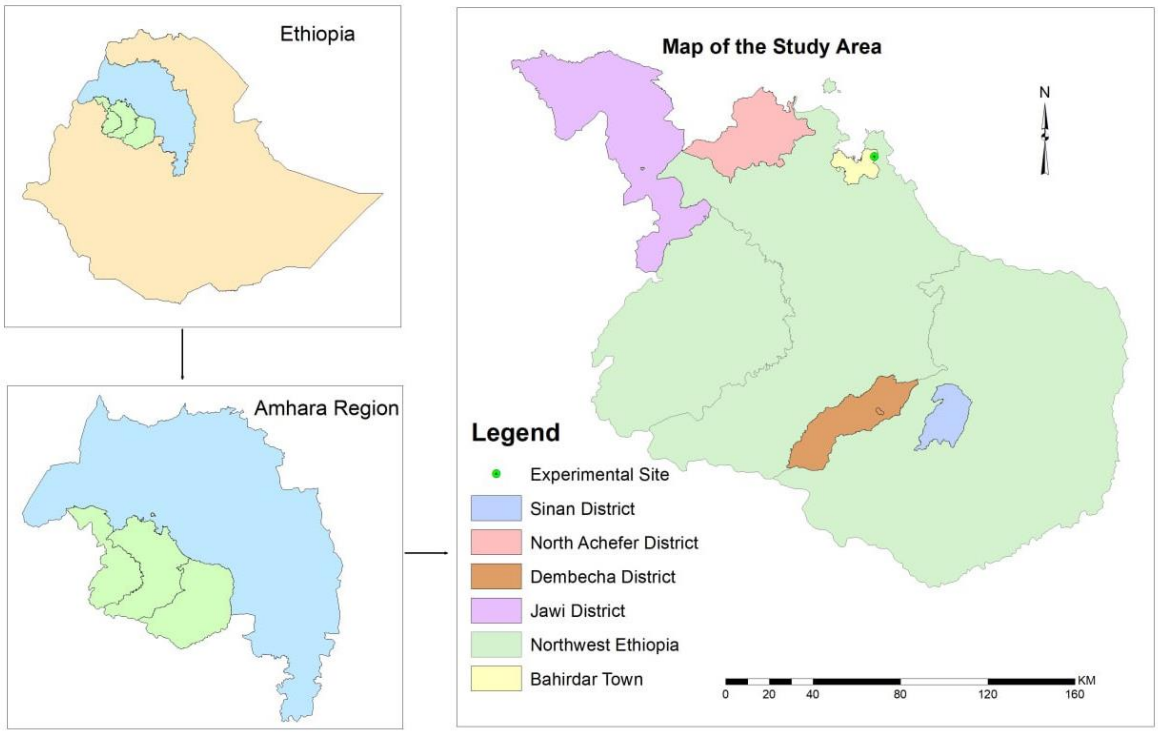

Figure 3.2. Map of the study areas for on-station study

3.2. Sampling Method and Procedures

Study sites and households were selected based on a discussion with the agricultural officers at zonal, district, and *kebele* (the smallest administrative level) levels. A multi-stage purposive, random, and stratified sampling methods were employed for the selection of sites, households, and chickens. In the first stage, three zones (Awi zone, East Gojjam zone, and West Gojjam zone) were selected based on research gaps. In the second stage, districts and *kebeles*/peasant associations were stratified based on agro-ecology, and six districts (two districts from each agro-ecology and three *kebeles* from each district) were selected based on their indigenous chicken potential. Finally, based on the FAO (2012) guidelines for phenotypic characterization and Yamane (1967) sample size determination formula, a total of 360 households and 1200 adult chickens were selected for the household interview (**Chapter 4**), and morpho-biometric characterization studies (**Chapter 5**), respectively.

$n = \frac{N}{1+N\times e^2}$ (Yamane, 1967)

Where

n = the sample size to be determined

N = the size of actual households and animal population

e = margin of error 8% (0.08)

1= Probability of the event occurring

For manuscript 3, a total of 500 chicken eggs (Sinan ecotype=120; Dembecha ecotype=120; North Achefer ecotype=130; Jawi ecotype=130) were collected from smallholder farmers for the performance evaluation study (**Chapter 6**). Of all eggs collected, 180 unsexed chicks were hatched/obtained and divided into four treatment groups (Sinan ecotype, Dembecha ecotype, North Achefer ecotype, and Jawi ecotype), each with ten replications. A completely randomized design (CRD) was used, and each pen was consisted of with a proportional number of chickens from each ecotype. After reaching five weeks of age, the feed experiment began with two treatment groups (EP-supplemented and non-supplemented) and five replications. Half of the chickens from each ecotype received a basal diet supplemented with 400 mg EP/kg diet, while others received only the basal diet.

For the parasite challenge study (**Chapter 7**), a total of 78 adult indigenous male chickens were used. Prior to allocating the animals into each pen, their body weights were measured, and they were randomly assigned into two treatment groups of 39 chickens each, with five replications using a CRD. The body weights in each treatment group were similar. The first group (control group) was fed a commercial ration, and the second group (EP group) received a commercial ration supplemented with 400 mg EP/kg diet for 14 days. Feed amounts were adjusted weekly based on chicken developmental stages. After fourteen days, the EP supplemented, and non-supplemented groups were infected with 1.5×10^4 oocysts of mixed *Eimeria*. Chicken body weights were recorded before inoculation and on the 12^{th} day post-inoculation. Fecal counts were taken on days 5, 7, 9, 11, and 12 post-inoculations. A total of 24 chickens (12 from each treatment groups) were slaughtered for lesion evaluation.

3.3. Data Collection

Survey data

A semi-structured questionnaire was administered using 360 households and six focal group discussions were held to collect data (**Chapter 4** and **Chapter 5**). Body weight, ten linear body measurements, and twelve morphological features recorded on 1200 adult chickens (**Chapter 5**).

On-station research

Effect of EP supplementation on growth performance

For growth performance evaluation test (**Chapter 6**), initial body weight (body weight at hatch) measurements were made on each chicken before placement in the experimental pens. Body weight was taken by two-week intervals and mortality was recorded as it occurred. The average daily gain (ADG), the average daily feed intake (ADFI), feed conversion ratio (FCR) was obtained by using different formulas and recorded.

Parasite-challenge using *Eimeria*

For parasite-challenge study (**Chapter 7**), the initial body weights, final body weights, feed offered, feed leftovers, mortality, fecal egg count, and lesion score were recorded to calculate the average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and the anti-coccidial index (ACI).

3.4. Data Management and Analyses

The survey data (**Chapter 4**) was analyzed using SPSS version 26. Descriptive statistics, chi-square test, Cramer's V test (to know the strength of the association between categorical variables) were utilized. According to Akoglu (2018), a Cramer's V value greater than 0.25 indicates a very strong association, while a value greater than 0.15 suggests a strong association, a value greater than 0.10 indicates a moderate association, a value greater than 0.05 implies a weak association, and a value of 0 or very close to 0

indicates no or very weak association. Indices were calculated using the formula given by Kosgey (2004); Index = Sum of [3 for rank 1 + 2 for rank 2 + 1 for rank 3] given for an individual reason divided by the sum of [3 for rank 1 + 2 for rank 2 + 1 for rank 3] for overall reasons. For the analysis of chicken flock composition, and chicken performance traits, the Generalized Linear Model (GLM) was used fitting agro-ecology as a fixed effect.

$Y_{ij} = \mu + A_i + \varepsilon_{ij}$; Where, Y_{ij}= the response variables;

μ= overall mean;

A_i =effect of the i^{th} agro-ecology (highland, midland, and lowland) on the respective variables;

ε_{ij}= residual error term

The coefficient of inbreeding (ΔF) was calculated with the formula given by Falconer and Mackey (1996); $\Delta F = 1/(2\ Ne)$; $Ne = (4Nm \times Nf)/(Nm + Nf)$; Where, ΔF = the inbreeding coefficient; Ne = the effective population number; Nm = number of breeding males; Nf = number of breeding females. The data collected on morpho-biometric characterization (**Chapter 5**) were analyzed with SAS version 9.4 and R version 4.1.3 (for graphical presentation) software. The chi-square (χ^2) test and multiple correspondence analyses were used for qualitative traits. The general linear model and different multivariate analyses (discriminant, canonical, and cluster) methods were used in quantitative morphological traits. "FactoMineR", "factoextra", and "Cluster" packages were used for analysis and plot visualization in R-software. The following model was utilized;

$Y_{ijk} = \mu + A_i + L_j + S_k + (LS)_{jk} + e_{ijk}$, Where:

Y_{ijk}= response of individual phenotypic observation

μ = the overall mean;

A_i = the fixed effect of agro-ecology (i= Highland, Midland, and Lowland);

L_j = the fixed effect of location (j= Banja, Sinan, Dembecha, Aneded, Jawi and North Achefer);

S_k = the fixed effect of sex (k= Male and Female);

LS_{jk} =the interaction effect of location with sex and

e_{ijk} = the effect of random error.

The growth performance data (**Chapter 6**) was analysed by using SAS version 9.4.

The models used for the statistical analysis were:

Model 1 (before EP experiment started):-$Y_{ijk}= \mu + E_i + S_j + e_{ijk}$,

Model 2 (after EP experiment started):- $Y_{ijkl}= \mu + E_i + S_j + F_k + EF_{ik} + e_{ijkl}$

Where: Y_{ijk} and Y_{ijkl}= the response variable (the measurement of BW, ADG, and FCR);

μ = the overall mean;

E_i = the fixed effect of ecotype (i= Sinan, North Achefer, Jawi, and Dembecha);

S_j = the fixed effect of sex (j= Male and Female);

F_k = the fixed effect of EP supplementation (k= EP^+ and control groups);

EF_{ik} = the interaction effect of ecotype and feed type;

e_{ijk} and e_{ijkl} = the effect of random error.

The mean comparisons were made using Tukey procedures for **Chapter 4, Chapter 5, and Chapter 6.**

The data was analysed by using Mann-Whitney U test in R software version 4.1.3 (**Chapter 7**). The model used for statistical analysis was:

$Y_{ij}= \mu + F_i + e_{ij}$, Where:

Y_{ij}= the response variable

μ = the overall mean;

F_i = the fixed effect of feed type (i= *Enteromorpha* polysaccharide supplemented and non-supplemented);

e_{ij} = the effect of random error.

The origin software was used to construct graphs (**Chapter 6** and **Chapter 7**).

CHAPTER 4: SELECTION CRITERIA AND HUSBANDRY PRACTICES OF INDIGENOUS CHICKEN PRODUCERS IN NORTHWEST ETHIOPIA

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Contents lists available at ScienceDirect

Heliyon

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Research article

Selection criteria and husbandry practices of indigenous chicken producers in Northwest Ethiopia

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Abstract

This study was conducted to identify the selection criteria and husbandry practices of chicken producers in different agro-ecological zones of Northwest Ethiopia as input for designing a breeding program. The study employed a purposive selection of districts and peasant associations with high indigenous chicken potential. The study areas were stratified based on the major agro-ecologies (highland, midland, and lowland). A total of 360 households were included in the study, and data on chicken breeding practices, selection criteria, and reproductive performance were collected and analyzed using SPSS software. In all agro-ecologies, egg production was prioritized by chicken owners when

choosing female chickens. For male chickens, plumage color (index = 0.27), appearance (index = 0.24), and growth rate (index = 0.23) were the main selection factors. Farmers kept chickens primarily to generate cash through the sale of eggs and live animals (male chickens). There was a significant difference ($p<0.01$) among agro-ecologies in nutritional management and housing of chickens. Chicken flock composition showed a highly significant difference ($p<0.001$) among agro-ecologies, except layers. Most of the farmers had their own cock born in the flock. Chicken owners found in all agro-ecologies were practicing culling unwanted chickens. All the reproductive performance traits have shown a highly significant ($p< 0.001$) difference among agro-ecologies. A relatively higher inbreeding coefficient (0.18) was obtained in the highland agro-ecology compared to midland (0.16) and lowland (0.12). The study highlighted the importance of designing breeding programs that align with farmers' production objectives and trait preferences based on specific agro-ecologies for sustainable increases in chicken productivity.

Keywords: Agro-ecology, Breeding objective, Chicken selection, Indigenous chicken, Trait

4.1. Introduction

Poultry production plays a crucial role in reducing poverty, ensuring food security, and generating income in developing countries (Bettridge *et al*., 2018; Zelleke *et al*., 2022). It also provides opportunities for socio-economic improvement and inclusivity, benefiting low- and middle-income households, as well as vulnerable groups such as women, people with disabilities, orphans, and the unemployed (Manyelo *et al*., 2020; Kleyn & Ciacciariello, 2021). Similarly, in Ethiopia, chickens hold significant socioeconomic importance, contributing to food security, income generation, religious rituals, and employment opportunities (Gulilat *et al*., 2021; Tolasa, 2021). Rural Ethiopian families commonly raise both indigenous and exotic chicken breeds, with an estimated total poultry population of approximately 57 million, consisting of 78.85% indigenous breeds, 9.14% exotic breeds, and 12.03% hybrid breeds (CSA, 2021).

Smallholder farmers maintain diverse flocks of indigenous chickens, meeting various economic, cultural, and religious needs (Dana *et al*., 2010a; Melesse & Negesse, 2011). Designing effective animal breeding programs for village chicken production requires a

comprehensive understanding of farmers' management practices, production environment, breeding objectives, and trait preferences (Tilahun *et al*., 2022). It is essential to incorporate economically significant traits into breeding programs to conserve chicken breeds, enhance productivity, and establish sustainable breeding strategies (Wong *et al*., 2017). Although efforts have been made in Ethiopia to improve poultry productivity through the introduction of exotic breeds, these initiatives have faced challenges due to the intensive management requirements of such chickens (Esatu *et al*., 2022). Therefore, prioritizing indigenous breeds and considering the specific needs and preferences of smallholder farmers is a potential intervention strategy to enhance village poultry production.

The breeding objectives, husbandry practices, and trait preferences of chicken producers residing in diverse agro-ecological regions can vary due to differences in attitudes and environmental conditions, including area-specific farming practices. Moreover, variations in attitudes and market factors can lead to differences in these aspects between chicken owners living near urban areas and those in rural or remote locations. Several scholars have conducted studies to identify selection criteria, breeding objectives, and management practices of farmers who raise indigenous chickens in various parts of Ethiopia. However, there is a scanty of information available regarding the selection criteria and breeding objectives of farmers in the northwest region of Ethiopia, particularly in the Awi zone, West Gojjam zone, and East Gojjam zone, where a significant genetic resource of approximately 5.8 million indigenous chickens exists (CSA, 2021). Furthermore, the previous studies have not addressed pocket or remote areas within the districts/zones, leaving a gap in knowledge. Therefore, the current study was set to identify the selection criteria and husbandry practices of farmers keeping indigenous chicken ecotypes found in different agro-ecologies of Northwest Ethiopia. The findings of this study will provide valuable input for the design of a sustainable breeding program tailored to the specific needs of farmers in the region.

4.2. Material and Methods

4.2.1. Description of the study areas

The study was conducted in selected districts (Banja, Jawi, Sinan, Aneded, Dembecha, and North Achefer) from Amhara National Regional State, Ethiopia (Figure 3.1). The agro-ecological description, the number of chickens found in the study area and the major feed resources for the chickens are presented in Table 4.1.

Table 4.1. Agro-ecological description, number of indigenous chickens, and major feed resources for chickens in Northwest Ethiopia

District/site	PA/ *Kebele*	Agro-ecology	Altitude (m.a.s.l.)	Annual rainfall (mm)	Annual temperature (^{O}C)	Indigenous Chicken number	Major feed resources for chickens
Banja	1 2 3	Highland Highland Highland	3028 2685 2723	2200-2560	7-25	36,894	wheat, maize, barley, oat, *injera*
Jawi	1 2 3	Lowland Lowland Lowland	995 1171 1365	650-1250	12-40	256,000	sorghum, finger-millet, maize, rice, *Gobe*, groundnut
Sinan	1 2 3	Highland Highland Highland	3214 3192 3081	900-1500	0-15	19,652	barley, maize, wheat
Aneded	1 2 3	Midland Midland Midland	2203 2071 2142	1200-1660	10-23	48,440	maize, wheat, *injera*, barley, *Engido*
Dembecha	1 2 3	Midland Midland Midland	1857 2287 1979	980-1100	18-27	113,219	wheat, maize, barley, finger-millet, *teff*
North Achefer	1 2 3	Lowland Lowland Lowland	1480 1495 1386	1100-1420	23-33	248,671	finger-millet, maize, sorghum, *injera*

PA= Peasant Association; m.a.s.l. = meter above sea level
Source: districts agricultural bureau, 2021

4.2.2. Sampling method

For this study, districts and peasant associations were selected purposively based on the chicken potential as per the information obtained from zonal agricultural office. The

districts that are known for high indigenous chicken production and not sufficiently studied earlier were considered for the study. The districts were stratified based on major agro-ecologies (highland, midland, and lowland). Secondly, potential peasant associations (six from each agro-ecology) were selected based on information on the dissemination of exotic chickens in the past. Peasant associations, particularly found in pocket areas with no/very low distribution of exotic chickens were considered for the study. Finally, random sampling was held with the aid of agricultural extension workers among indigenous chicken owners to select a total of 360 (120 from each agro-ecology) households for interview based on Yamane (1967) formula for sample size determination.

4.2.3. Data collection

At each sampling site, farmers were briefed about the objective of the study before starting the data collection. A semi-structured questionnaire was designed and translated in to local language (Amharic) to address the major management practices, chicken composition, and the purpose of keeping chickens. The data was collected by trained enumerators with close supervision of investigators. The major chicken breeding practices, culling practices, and trait preferences of farmers, including their criteria to select breeding cocks and hens, were assessed. In addition, information on the number of breeding animals and the reproductive and production performance of chickens was collected from the chicken owners using a semi-structured questionnaire and focal group discussions with extension workers and model farmers.

4.2.4. Data management and analysis

The data was organized and analyzed using MS Excel and Statistical Package for Social Sciences (SPSS) version 26. Preliminary data analysis, such as the normality test was employed before conducting the main data analysis. Descriptive statistics, such as mean, frequency, and percentage were utilized in analyses of the data. The Pearson Chi-square test was employed to compare the qualitative variables between different agro-ecologies and Cramer’s V was used to determine the magnitude of the association between variables. According to Akoglu (2018), a Cramer's V value greater than 0.25 indicates a very strong association, while a value greater than 0.15 suggests a strong association, a

value greater than 0.10 indicates a moderate association, a value greater than 0.05 implies a weak association, and a value of 0 or very close to 0 indicates no or very weak association. Mean comparisons were made using Tukey test. Indices were calculated to provide a ranking of the reasons for keeping chickens, farmers' trait preferences, and selection criteria according to the formula give by Kosgey (2004).

$$\text{Index} = \frac{\sum[(3 \times \text{rank } 1) + (2 \times \text{rank } 2) + (1 \times \text{rank } 3)] \text{ individual trait}}{\sum[(3 \times \text{rank } 1) + (2 \times \text{rank } 2) + (1 \times \text{rank } 3)] \text{ overall traits}}$$

For the analysis of chicken flock composition, and chicken performance traits, the Generalized Linear Model (GLM) was used fitting agro-ecology as a fixed effect.

$Y_{ij} = \mu + A_i + \varepsilon_{ij}$; Where,

Y_{ij}= the response variables;

μ= overall mean;

A_i =effect of the i^{th} agro-ecology (highland, midland, and lowland);

ε_{ij}= residual error term

The coefficient of inbreeding (ΔF) was calculated from the effective number of breeding animals. Estimates of the average change in percentage inbreeding were made with the formula given by Falconer & Mackey (1996).

$$\text{Ne} = \frac{4\text{Nm} \times \text{Nf}}{\text{Nm} + \text{Nf}}; \Delta\text{F} = \frac{1}{2\text{Ne}}$$

Where, ΔF = the inbreeding coefficient; Ne = the effective population number; Nm = number of breeding males; Nf = number of breeding females.

4.3. Results and Discussions

4.3.1. Major indigenous chicken management practices

There was a significant difference ($p<0.01$) observed in the nutritional management of chickens across different agro-ecologies, as shown in Table 4.2. The majority of respondents (98.6%) provided supplementary feed for their chickens. Similarly, more than 94% of farmers in various parts of Ethiopia also offered supplementary feed to their chickens (Bekele *et al*., 2020). The availability of different grains resulting from mixed crop and livestock farming systems, along with farmers' desire to enhance productivity, likely contributes to the trend of supplementing chickens. Regarding housing, a significant difference ($p<0.01$) was found among the agro-ecologies, possibly due to variations in

farmers' perceptions and the availability of local construction materials for chicken housing. Figure 4.1 illustrates distinct chicken housing structures in highland agro-ecology (a and b), midland agro-ecology (c and d), lowland agro-ecology (e), and a chicken shelter located within a family house in lowland agro-ecology (f). In the study area, a significant proportion of farmers (approximately 53.34%) constructed separate shelters for their chickens (Table 4.2). This might be due to their good awareness in the importance of separate house construction for minimizing the risk of transmissible and Zoonotic diseases. A similar finding was reported by Dana *et al*. (2010a), where the majority (80%) of chicken owners in Konso had separate shelters for their chickens. In terms of vaccination, a higher proportion (71.9%) of the respondents in the study area vaccinates their chickens, demonstrating a good understanding among farmers regarding the use of vaccination to prevent diseases. In contrast, a study by Dana *et al*. (2010a) revealed that about 95% of respondents raising village chickens in Farta, Mandura, Horro, Konso, and Sheka did not immunize or vaccinate their chickens.

Table 4.2. The major management practices of indigenous chicken producers in Northwest Ethiopia

Management Practices	**Agro-ecology**							
	Highland N=120		Midland N=120		Lowland N=120		Overall	
	N	%	N	%	N	%	N	%
Nutritional management								
Scavenging	0	0.00	0	0.00	5	4.2	5	1.4
Scavenging with supplement	120	100	120	100	115	95.8	355	98.6
Complete ration	0	0.00	0	0.00	0	0.00	0	0.00
*χ^2=10.14**; Cramer's V=0.168*								
Housing								
In the family house	27	22.5	38	31.7	42	35	107	29.7
Separate shelter	60	50	67	55.8	65	54.2	192	53.3
Separate house with other animals	33	27.5	15	12.5	10	8.3	58	16.1
Others/On the tree	0	0.00	0	0.00	3	2.5	3	0.83
*χ^2=24.93**; Cramer's V=0.186*								
Vaccination								
Yes	92	76.7	79	65.8	88	73.3	259	71.9
No	28	23.3	41	34.2	32	26.7	101	28.1
$\chi^2=3.66^{NS}$; Cramer's V=0.101								

N= Number of households; χ^2 and *Cramer's V* values shows the association of agro-ecology with specific management practice; NS=Non-significant; **P<0.01

Figure 4.1. Separate chicken house in highland (a and b), midland (c and d) and lowland area (e); chicken shelter inside the family house in lowland agro-ecology (f)

4.3.2. Composition of chicken flocks

There was a highly significant difference ($p<0.001$) observed in the composition of chicken flocks among agro-ecologies, except for layers, as shown in Table 4.3. In contrast to the findings of this study, Goraga *et al*. (2018) reported that the average number of chicken flocks per household did not differ between lowland and highland agro-ecologies in different regions of Ethiopia. In highland and midland agro-ecologies, a larger number of chicks and pullets were found in addition to layers. The higher number of layers suggests that farmers primarily keep indigenous chickens for egg production. However, in the lowland agro-ecology, a higher proportion of chicks (34.9%) per household were recorded compared to layers (23.2%) and pullets (19.7%). Similarly, a higher number of young chicks, followed by hens, were reported in the Burie district of Northwest Ethiopia (Moges *et al*., 2010a) and the Indian Himalayan region (Singh *et al*., 2023). The higher proportion of layers and chicks in the study area may be attributed to the farmers' aim of

producing a larger number of eggs for sale and hatching for stock replacement. The lower proportion of cocks, particularly in the highland agro-ecology, could be due to selling cockerels and cocks for income generation and/or utilizing neighboring cocks for breeding purposes. This lower proportion of cocks and cockerels in the area may lead to increased inbreeding and the production of more unfertile eggs. The significant difference in chicken flock composition among different agro-ecologies may be influenced by variations in the breeding objectives of farmers who keep the chicken flocks.

Table 4.3. Average chicken flock holding per household in different agro-ecologies of Northwest Ethiopia

Chicken flock	Agro-ecology						Overall		p-value
	Highland (N=120)		Midland (N=120		Lowland (N=120				
	Mean±SD	%	Mean±SD	%	Mean±SD	%	Mean±SD	%	
Layers	4.38±2.0	43.5	4.8±2.6	41	4.18±2.74	23.2	4.45±2.47	33.5	NS
Cocks	0.82±0.89[b]	8.1	1.24±0.93[a]	10.6	1.44±0.99[a]	7.99	1.17±0.97	8.8	***
Pullets	1.29±2.04[c]	12.8	2.24±2.09[b]	19.2	3.55±2.47[a]	19.7	2.36±2.39	17.8	***
Cockerel	0.64±1.15[b]	6.4	1.12±1.71[b]	9.5	2.58±2.46[a]	14.3	1.44±2.02	10.9	***
Chicks	2.94±4.37[b]	29.2	2.31±3.59[b]	19.7	6.3±6.68[a]	34.9	3.85±5.34	29	***

N= Number of households; SD=Standard deviation; NS=Non-significant; [a,b,c]Means across a row with different superscript letters denote significant differences at P <0.001; Flock: a group of chicken with different age and sex categories

4.3.3. Purpose of keeping chickens

The table presented, Table 4.4, outlines the ranking of chicken production objectives as perceived by smallholder farmers. Across all agro-ecologies, the main reasons for keeping male chickens were income generation (0.36), breeding for replacement stock (0.30), and meat consumption (0.21). These findings align with the results of Moges *et al*. (2010a), who reported that the primary purposes of chicken rearing in the Burie district were income generation (51%), breeding (45%), and home consumption (44%). In the highland and midland agro-ecologies, the primary reason for keeping female chickens was egg production for selling, with index values of 0.41 and 0.39, respectively. Similarly, in various agro-ecologies of Ethiopia, indigenous chickens were mainly kept for income generation through the sale of eggs (Bekele *et al*., 2020). In the lowland agro-ecology, the main purposes of keeping chickens were egg production for home consumption (0.27) and selling (0.26), followed by breeding (0.25). These findings consistent with Fekede and Tadesse (2021), who reported that indigenous chicken rearing in the West Oromia region of Ethiopia primarily focused on egg consumption and egg selling. The variation in

chicken functions among different agro-ecologies can be attributed to socio-economic, socio-cultural, and perception differences among chicken owners. For example, in some areas studied, there was no tradition of consuming eggs, and even during the fasting season, eggs intended for hatching purposes were observed to rot instead of being provided to babies.

Table 4.4. Farmers' purpose of keeping male and female chickens in Northwest Ethiopia

Purpose	Agro-ecology												
	Highland (N=120)				Midland (N=120)				Lowland (N=120)				Overall Index (N=360)
	Rank			Index	Rank			Index	Rank			Index	
	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
Male chickens													
Income (animal sale)	53	56	8	0.39	50	55	5	0.37	35	59	15	0.33	0.36
Breeding	50	26	23	0.31	60	15	14	0.32	39	17	47	0.28	0.30
Meat(home consumption)	14	31	37	0.20	6	26	42	0.16	40	28	23	0.28	0.21
Saving	3	6	38	0.08	3	14	21	0.07	2	6	25	0.06	0.07
Manure	0	0	4	0.01	0	1	19	0.03	0	0	6	0.01	0.02
Ceremony	0	1	10	0.01	1	8	18	0.05	4	10	4	0.05	0.04
Female chickens													
Income (Egg sale)	77	24	17	0.41	72	29	10	0.39	38	28	16	0.26	0.35
Egg (home consumption)	27	52	14	0.28	21	41	21	0.24	26	51	17	0.27	0.26
Income (animal sale)	4	27	55	0.17	8	36	30	0.18	7	18	48	0.14	0.16
Breeding	10	13	26	0.11	14	11	23	0.12	48	5	29	0.25	0.16
Meat (home)	0	0	5	0.01	5	2	12	0.04	1	14	5	0.05	0.03
Saving	2	1	3	0.02	0	1	14	0.02	0	0	5	0.01	0.02
Manure	0	3	1	0.01	0	0	6	0.01	0	0	0	0.00	0.01
Ceremony	0	0	0	0.00	0	0	4	0.01	0	4	0	0.02	0.01

N= Number of households or respondents

4.3.4. Selection criteria for breeding cocks and hens

The selection criteria for breeding cocks varied among different agro-ecologies, with the highland agro-ecology prioritizing growth rate/body weight (0.29), appearance (0.28), and plumage color (0.24), as indicated in Table 4.5. In the midland agro-ecology, comb type (0.26), plumage color (0.23), and growth rate (0.21) were considered the most important traits for selecting breeding cocks. In the lowland agro-ecology, plumage color (0.33), appearance (0.25), and comb type (0.17) were the major criteria for selection. Consistent with the current study, farmers in the Amhara (Farta) and Oromia (Horro) regions also emphasized plumage color as the primary selection criterion (Dana *et al*., 2010a). However, farmers in Kenya (Bett *et al*., 2011) and Rwanda (Mahoro *et al*., 2018) did not

prioritize plumage color in their selection of chickens, which contrasts with the findings of this study. The preference for morphological traits such as plumage color and comb type over growth and adaptive traits (disease resistance, mothering ability, and scavenging ability) in the current study area can be attributed to socio-cultural factors, where farmers place importance on the visual aesthetics of chickens. For instance, red plumage and a double comb are favored over other traits for male chickens in most surveyed areas, and these traits can significantly influence the market price of chickens.

In terms of selecting breeding hens, the most important traits were egg number (0.29), appearance (0.17), and plumage color (0.15), as shown in Table 4.5. This aligns with the findings of Fekede and Tadesse (2021) in the BakoTibe district of the Western Oromia region, where respondents utilized egg production as a key criterion for selecting and retaining hens in the flock. The selection of chickens based on egg production traits indicates that the primary breeding objective of farmers in the study areas is focused on egg production. In the highland agro-ecology, the most important traits for selecting breeding hens were egg number (0.34), appearance (0.20), comb type (0.12), and plumage color (0.12). In the midland agro-ecology, egg number (0.34), egg fertility (0.13), and broodiness (0.12) were the primary considerations. Farmers in the midland agro-ecology preferred hens with low brooding frequency to maximize egg production. In the lowland agro-ecology, plumage color (0.21), appearance (0.21), and egg number (0.20) were the most important traits for selecting breeding hens. In contrast to the current study, Tilahun *et al*. (2022) reported that farmers in the Gurage zone of Ethiopia prioritized adaptive traits (disease and scavenging ability) over production traits in all agro-ecologies. Similarly, a study conducted in Kenya found that egg yield, mothering ability, and body size were the most preferred traits among chicken farmers (Okeno *et al*., 2012). Studies conducted in the Indian Himalayan region (Singh *et al*., 2023) revealed that most households chose to rear indigenous chickens due to their adaptability and survivability. This preference suggests that farmers, especially those in the sub-temperate agro-ecology of the Himalayan region, prioritize these traits when selecting indigenous chickens, considering the challenging climatic conditions. Ethiopia's indigenous chicken breeds are known for their mothering ability, disease resistance, and the taste of their meat and eggs (Terfa *et al*., 2019). The lack of emphasis on adaptive traits in the current study area might be due to the presence of these unique characteristics in the indigenous chickens, which directs farmers' focus towards other traits. Considering farmers' preferences for specific traits is vital in

designing sustainable improvement programs (Mbuthia *et al*., 2015). Failure to incorporate stakeholders' needs in breeding programs can lead to rejection by end users, as highlighted by Yakubu *et al*. (2013).

Table 4.5. Ranking of selection criteria for breeding cocks and hens in Northwest Ethiopia

Class and section criteria	Agro-ecology												Overall index
	Highland (N=120)				Midland (N=120)				Lowland (N=120)				
	Rank			Index	Rank			Index	Rank			Index	
	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
Breeding cocks													
Plumage color	21	34	39	0.24	33	23	18	0.23	53	22	31	0.33	0.27
Appearance	35	31	33	0.28	13	31	26	0.18	16	44	45	0.25	0.24
Growth rate	50	24	13	0.29	27	19	31	0.21	23	24	16	0.18	0.23
Comb type	14	21	30	0.16	36	24	31	0.26	26	26	19	0.21	0.21
Disease resistance	0	8	3	0.03	1	15	7	0.06	0	4	5	0.02	0.04
Scavenging ability	0	1	2	0.01	2	6	6	0.03	0	0	4	0.01	0.02
Longevity	0	2	0	0.01	8	1	3	0.04	2	0	0	0.01	0.02
Breeding hens													
Egg number	56	26	22	0.34	74	5	15	0.34	22	26	30	0.20	0.29
Appearance	30	17	20	0.20	6	18	13	0.09	32	19	18	0.21	0.17
Plumage color	11	20	15	0.12	5	24	16	0.11	32	15	23	0.21	0.15
Broodiness	1	7	10	0.04	9	18	22	0.12	12	28	10	0.14	0.10
Egg fertility	9	17	6	0.09	15	19	11	0.13	8	14	6	0.08	0.10
Comb type	11	17	18	0.12	11	9	13	0.09	1	9	20	0.06	0.09
Mothering ability	0	8	9	0.03	0	15	20	0.07	13	4	4	0.07	0.06
Disease resistance	1	5	13	0.04	0	9	4	0.03	0	5	2	0.02	0.03
Longevity	1	2	5	0.02	0	3	6	0.02	0	0	6	0.01	0.02
Scavenging ability	0	1	3	0.01	0	0	0	0.00	0	0	1	0.001	0.00

N= Number of households or respondents

Figure 4.2 shows the plumage color preferences of chicken owners. Across all agro-ecologies, the most favored color types were red, white, and *Gebisma* (wheaten strips on a black background, grayish with varying mixture, and red brownish with black). However, in the lowland agro-ecology, *Teterima* (black spot on white, black with white tips, and white with black or red spots) were more preferred than white. Likewise, the chicken owners in Benshangul-Gumuz (Mandura), Oromia (Horro), and Southern Regions (Konso and Sheka) preferred red plumage color, while farmers in Amhara (Farta) favored white plumage color, as noted by Dana *et al*. (2010a). On the other study, farmers in Mezhenger, Sheka, and Benchi-Maji zones of southwestern Ethiopia predominantly preferred red

plumage color (Yadessa *et al*., 2017). However, the same study also reported black as the second most preferred color for selecting cocks, which contradicts the findings of the current study. In the present study, black plumage color was not preferred by farmers in any agro-ecology due to its cultural significance. Farmers generally avoid slaughtering chickens with black plumage color, especially during holidays, and these chickens tend to have lower market prices compared to others. In some areas, farmers refrain from choosing chickens with white plumage color because they are more visible and can be easily targeted by predators. Furthermore, some chicken owners hold the belief that white plumage color is attractive and that the "evil eye" of people could affect the growth and production of the chickens. These factors contribute to the dominance of red plumage color across all agro-ecologies.

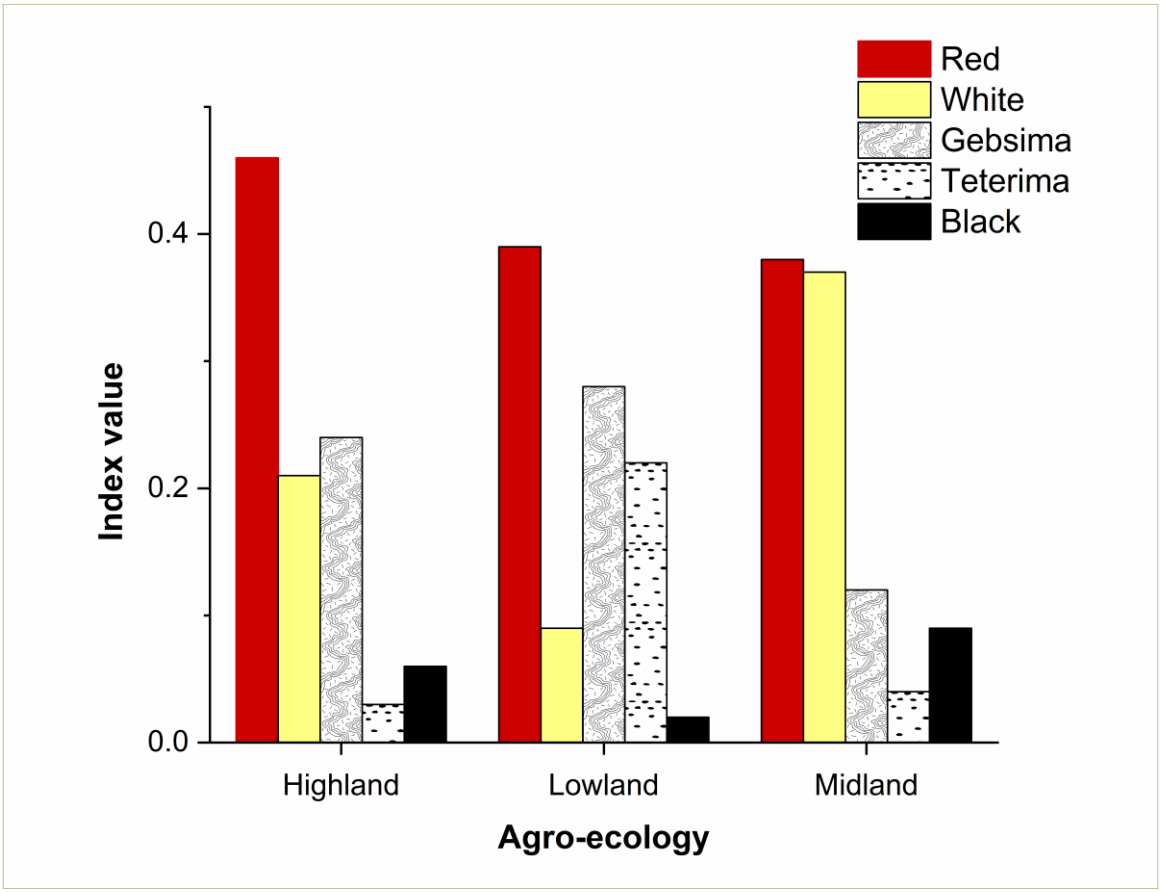

Figure 4.2. Ranking index value for plumage color preference of farmers keeping indigenous chicken in different agro-ecologies of northwest Ethiopia. *Gebsima* = wheaten strips on a black background; *Teterima* = black spot on white.

4.3.5. Breeding and culling practices

A highly significant difference ($p<0.001$) was observed across different agro-ecologies in terms of brood modification methods. In the highland and midland agro-ecologies, the majority of farmers (31.7% and 56.7%, respectively) practiced hanging their broody hen's upside-down to modify brooding (Table 4.6). This aligns with the findings of Yadessa *et al*. (2017) in Mezhenger, Sheka, and Benchi-Maji zones of southwestern Ethiopia, where 38.2% of chicken owners employed the upside-down hanging method for brood modification. In contrast, most lowland chicken owners in the current study (43.3%) opted to change the brooding place as their preferred method. The variation in brood modification methods among agro-ecologies could be attributed to differences in farmers' perceptions. Some farmers in the study area consider hanging chickens for brooding modification as unethical, and there is a decreasing trend in the use of this method over time. The study revealed that almost all farmers practiced an uncontrolled (natural) mating system. Out of the households surveyed, 76.9% owned their own breeding cock, and the majority (70%) of these cocks was obtained from their own flock. While owning a breeding cock can positively impact the fertility of eggs from hens, utilizing cocks from their own flock can increase the risk of inbreeding. Similar findings were reported in the Burie district of Northwest Ethiopia, where 70.7% of chicken owners had their own cocks, primarily sourced from their own flocks (Moges *et al*., 2010a).

All chicken owners in the study area practiced culling unwanted chickens from their flocks, primarily due to old age (36.1%), poor productivity (35.8%), sickness (17.8%), or lack of broodiness (10.3%). A similar study conducted in different agro-ecologies of Ethiopia reported that the majority (91%) of farmers practiced culling their chickens due to old age, sickness, low production, and brooding frequency (Bekele *et al*., 2020). The majority of respondents culled male (49.4%) and female (50.8%) chickens when they reached more than three years of age, as indicated in Table 4.6. This corresponds with the overall mean culling age of 3.37±1.24 years found in southwestern Ethiopia (Yadessa *et al*., 2017). In contrast, larger (4.3 years) and smaller (2.7 years) average culling ages were reported in Southern Ethiopia (Getiso *et al*., 2015) and Northwest Ethiopia (Moges *et al*., 2010a), respectively. The variation in culling age among different areas reflects the differing purposes and functions of chickens for farmers. Some farmers may choose to cull chickens earlier than expected for income generation purposes. The primary purposes for

culling chickens among chicken owners were selling (65.6%) and home consumption (31.9%). This aligns with previous studies conducted in the Haramaya district of Eastern Ethiopia (Abera *et al*., 2014), where selling and home consumption were identified as the major reasons for culling.

Table 4.6. Breeding and culling practices of indigenous chicken producing farmers in Northwest Ethiopia

Breeding Practices	**Agro-ecology**							
	Highland (N=120)		Midland (N=120)		Lowland (N=120)		Overall	
	N	%	N	%	N	%	N	%
Brood modification								
Hanging upside-down	38	31.7	68	56.7	12	10	118	32.78
Change brooding place (In house)	36	30	27	22.5	52	43.3	115	31.94
Moving to neighbor houses	35	29.2	22	18.3	46	38.3	103	28.61
Do nothing	4	3.3	2	1.7	10	8.3	16	4.44
Submerge into water up to the breast	6	5	1	0.8	0	0.00	7	1.94
Other/tie wings separately	1	0.8	0	0.00	0	0.00	1	0.28
*χ^2=74.06***; Cramer's V=0.321*								
Own cock								
Yes	67	55.8	103	85.8	107	89.2	277	76.9
No	53	44.2	17	14.2	13	10.8	83	23.1
*χ^2=45.597***; Cramer's V=0.356*								
Sources of breeding cock								
Own (private flock)	42	35	74	61.7	78	65	194	70
Purchased (Market)	25	20.8	29	24.2	29	24.2	83	30
χ^2=2.302^{NS}; Cramer's V=0.091								
If no cock, how do you breed your hens								
From neighbor	49	40.8	17	14.2	13	10.8	79	95.2
I do not need a cock for my hens	4	3.3	0	0.00	0	0.00	4	4.8
χ^2=2.379^{NS}; Cramer's V=0.169								
Culling criteria								
Old age	44	36.7	41	34.2	45	37.5	44	36.1
Poor productivity	46	38.3	40	33.3	43	35.8	43	35.8
Sickness	20	16.7	25	20.8	19	15.8	21	17.8
Lack of broodiness	10	8.3	14	11.7	13	10.8	12	10.3
χ^2=2.29^{NS}; Cramer's V=0.891								
Culling method								
Home consumption	35	29.2	41	34.2	39	32.5	115	31.9
Sale	83	69.2	74	61.7	79	65.8	236	65.6
Sacrifice/cultural purpose	2	1.7	5	4.2	2	1.7	9	2.5
χ^2=3.004^{NS}; Cramer's V=0.065								
Culling age (male)								
≥2 years	39	35.5	29	24.2	46	38.3	114	31.7
≥3 years	68	56.7	58	48.3	52	43.3	178	49.4
≥4 years	12	10	14	11.7	16	13.3	42	11.7
≥5 years	1	8	19	15.8	6	5	26	7.2
*χ^2=43.584***; Cramer's V=0.246*								
Culling age (female)								
≥2 years	30	25	29	24.2	46	38.3	105	29.2
≥3 years	73	60.8	58	48.3	52	43.3	183	50.8
≥4 years	15	12.5	14	11.7	16	13.3	45	12.5
≥5 years	2	1.7	19	15.8	6	5	27	7.5
*χ^2=26.725***; Cramer's V=0.193*								

N=Number of households; χ^2 and *Cramer's V* values shows the association of agro-ecology with specific breeding practice NS=Non-significant;***P<0.001

4.3.6. Reproductive and production performance of indigenous chickens

All the reproductive and production performance traits showed a highly significant difference (p<0.001) among the agro-ecologies (Table 4.7). The average age at first mating for hens was 5.07±0.74 months in the highland area, 4.93±0.65 months in the midland area, and 5.39±1.22 months in the lowland area. These findings are consistent with Zewdu *et al*. (2013), who reported an average age of 5.2 months for indigenous pullets at first mating in Northwest Ethiopia. Other studies also reported ages at first mating of 6.51 months in Horro district (Aklilu *et al*., 2013) and 26.15 weeks in BakoTibe and Dano districts (Fekede and Tadesse, 2021). The estimated average age for male chickens to reach sexual maturity was 4.81±0.79 months in the highland area, 4.86±0.78 months in the midland area, and 5.31±1.11 months in the lowland area (Table 4.7). In comparison, Yadessa *et al*. (2017) reported an overall mean age of 4.9 months for cocks at first mating in southwestern Ethiopia. The observed differences in the age of sexual maturity for hens and cocks among the agro-ecologies could be attributed to genetic and environmental factors. In this study, the average age for chickens to lay their first egg was 5.61±0.93 months. This is shorter than the findings of Bekele *et al*. (2020), who reported an average of 6.54±0.063 months for pullets to lay their first egg in different agro-ecologies of Ethiopia.

The highland agro-ecology exhibited a higher number of eggs per hen per clutch (16.48±3.13) compared to other agro-ecologies, with an overall value of 14.99±3.59 eggs. This is higher than the reported value of 13.38 eggs per hen per clutch in the Western Oromia region of Ethiopia (Fekede and Tadesse, 2021). According to the respondents, the total number of eggs per hen per year was higher in the highland agro-ecology (83.4±32.29) compared to the midland and lowland areas (82.27±23.37 and 64.36±15.9, respectively), as shown in Table 4.7. In contrast, Bekele *et al*. (2020) reported that the midland agro-ecology had a higher number of eggs compared to other agro-ecologies, with a total annual egg production of 61.89 eggs. A study conducted by Yadessa *et al*. (2017) reported a relatively lower number of eggs per hen per year (54.7) in southwestern Ethiopia. The improved performance of chickens in the current study compared to

previous studies could be attributed to better management and breeding practices by farmers, as well as genetic variations among indigenous chickens.

Table 4.7. The mean reproductive and production performance of indigenous chickens as estimated by farmers among different agro-ecologies in Northwest Ethiopia

Parameters	Agro-ecology				
	Highland N=120 Mean±SD	Midland N=120 Mean±SD	Lowland N=120 Mean±SD	Overall N=360 Mean±SD	p-value
AFM (months, female)	5.07±0.74[b]	4.93±0.65[b]	5.39±1.22[a]	5.13±0.93	***
AFM(months, male)	4.81±0.79[b]	4.86±0.78[b]	5.31±1.11[a]	4.99±0.93	***
Age at first egg (months)	5.49±0.73[b]	5.38±0.69[b]	5.95±1.12[a]	5.61±0.93	***
Eggs per hen per clutch	16.48±3.13[a]	15.38±4.02[b]	14.1±2.61[c]	14.99±3.59	***
Eggs per hen per year	83.39±32.29[a]	82.27±23.37[a]	64.36±15.9[b]	76.67±26.2	***

N=Number of households; AFM = Age at 1st mating; SD=Standard deviation; [a,b,c]Means across a row with different superscript letters denote significant differences at P <0.001

4.3.7. Effective population size and level of inbreeding

The number of breeding male and female chickens, the effective population size, and the rate of inbreeding of indigenous chickens in the study area are presented in Table 4.8. The lowland agro-ecology exhibited a lower inbreeding coefficient (0.12) compared to other agro-ecologies. In contrast, the highland agro-ecology showed a higher coefficient of inbreeding (0.18). The higher inbreeding coefficient in the highland agro-ecology could be attributed to the presence of a smaller number of chickens within households. Increasing the number of chickens would result in a larger effective population size, as it would increase the likelihood of having a greater number of breeding animals. This finding differs from a study conducted in the highland agro-ecology of the Gurage zone, which reported a smaller inbreeding coefficient (0.06) compared to other agro-ecologies (Tilahun *et al*., 2022). However, the present study is consistent with the findings of Bekele *et al*. (2020) for the midland agro-ecology, which reported a 12.8% inbreeding coefficient. Nevertheless, the inbreeding coefficients in the highland (7%) and lowland (11.3%) agro-ecologies were lower than those found in the current study. Another study by Dana *et al*. (2010a) reported inbreeding coefficients of 0.096, 0.10, 0.12, 0.144, and 0.157 for Konso, Horro, Mandura, Farta, and Sheka chickens, respectively. The significant variation in inbreeding coefficient values among agro-ecologies and different studies may be attributed to variations in estimation methods and breeding practices employed by farmers. Factors such as the absence of a breeding male within the flock, uncontrolled mating, limited

awareness about inbreeding, and small flock sizes can contribute to the accumulation of inbreeding and a decrease in genetic diversity (Falconer & Mackey, 1996). The inbreeding coefficient values obtained in the current study exceeded the maximum acceptable level of 0.063 (Armstrong, 2006). Therefore, increasing the effective population size would be essential to mitigate the risk of inbreeding.

Table 4.8. Effective population size and level of inbreeding in indigenous chickens found in different agro-ecologies of Northwest Ethiopia

Agro-ecology	Nm	Nf	Ne	ΔF
Highland	0.82	4.38	2.76	0.18
Midland	1.24	4.8	3.94	0.13
Lowland	1.44	4.18	4.28	0.12

Nm=Number of males; Nf= Number of females; Ne= Effective population size; ΔF= Inbreeding rate

4.3.8. Major constraints of chicken production

According to the chicken owners in the study area, disease and predators were the main problems as compared to the other constraints with an index value of 0.37 and 0.23, respectively (Table 4.9). In the study area, farmers also mentioned the absence of chickens with good genetic makeup as another important constraint in their chicken production with an overall index value of 0.17. In line with the current study, Yadessa *et al*. (2017) reported that disease was the major problem of chicken owners in Mezhenger, Sheka and Benchi-Maji zones of south western Ethiopia. Correspondingly, Moges *et al*. (2010) reported disease, predators and poor productivity of chickens as the major constraints of chicken production in Burie district. Halima *et al*. (2007a) also reported that predators were one of the major challenges for chicken owners in Northwest Ethiopia. Poor extension services and low awareness of farmers could be the major cause of the disease challenge in the study areas. For instance, unless a sufficient number of farmers are obtained to vaccinate chickens, the drugs couldn't be opened and due to this reason, most of the time farmers miss vaccinating their chickens. The commonly reported chicken diseases in the study areas include Newcastle disease (*Fungil*), Coccidiosis, Fowl Pox, Gumbro, and Fowl Cholera. In addition to modern medicines, most farmers were providing traditional medicines, such as *Semiza, Enkoko,* Neem leaf, *Mitmita,* Garlic, *Endod, Gesho, Tenadam, Feto, Areki (*local brewery*),* Lemon water and *Korch* to cure chickens from disease.

Table 4.9. Major constraints to chicken production in Northwest Ethiopia

Constraints	Agro-ecology												
	Highland (N=120)				Midland (N=120)				Lowland (N=120)				
	Rank			Index	Rank			Index	Rank			Index	Overall index
	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
Disease	74	22	9	0.38	32	55	8	0.30	92	13	3	0.42	0.37
Predator	23	48	14	0.25	32	23	19	0.22	12	47	30	0.22	0.23
Genotype	18	17	49	0.19	19	11	38	0.16	4	34	28	0.15	0.17
Feed shortage	5	24	17	0.11	30	11	8	0.17	4	6	14	0.05	0.11
Water shortage	0	2	14	0.03	6	10	7	0.06	7	2	6	0.04	0.04
Market problem	0	6	12	0.03	0	9	33	0.07	1	13	11	0.06	0.05
Labor	0	1	5	0.01	1	1	7	0.02	0	5	28	0.06	0.03

N= Number of households or respondents

4.4. Conclusions and Recommendations

In conclusion, this study emphasizes that farmers prioritize egg production and growth rate when selecting chickens for income generation. Agro-ecology has a slight influence on trait preferences, management practices, and breeding objectives among households raising indigenous chickens. To achieve sustainable improvements in indigenous chicken productivity, it is crucial to consider farmers' production objectives and trait preferences within their specific production environment and agro-ecology.

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CHAPTER 5: MORPHO-BIOMETRIC CHARACTERIZATION OF INDIGENOUS CHICKEN ECOTYPES IN NORTHWEST ETHIOPIA

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PLOS ONE

RESEARCH ARTICLE

Morpho-biometric characterization of indigenous chicken ecotypes in north-western Ethiopia

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Abstract

Morphological characterization of Animal Genetic Resources is the first step to documenting diversity and designing breed specific breeding programs. The current study characterized the morpho-biometric variation of indigenous chicken ecotypes prevailing in northwestern Ethiopia. A multi-stage purposive, stratified, and random sampling method was employed to select the study areas and chickens. A total of 1200 adult chickens were sampled and characterized for 12 qualitative and 11 quantitative traits. Univariate and multivariate data analysis methods were employed to analyze the data using SAS and R statistical software. Red plumage colour (33.2%), white and red earlobe colour (73.8%) and yellow shank colour (57.0%) were the most predominant colour trait categories. Sex,

agro-ecology, location, and the interaction of sex and location had a highly significant ($p<0.001$) effect on all body measurements. Shank traits were found to have the highest discriminating power in both sexes. The overall classification rates for the female and male sample populations were 57.47% and 69.97%, respectively. The squared Mahalanobis distances between sites were significant ($p<0.001$) for both sexes. The longest distance was obtained between North Achefer and Banja (19.25) and between North Achefer and Dembecha (16.80) in female and male chickens, respectively. In female chickens, canonical variates 1 (CAN 1) and 2 (CAN 2) explained 82% of the total variation and distinctly separated the sample populations of North Achefer and Jawi from others. In male chickens, 90% of the total variance is explained by CAN1, CAN2, and CAN3, which distinctly separate the sample populations of the North Achefer, Sinan, and Jawi, among others. Using cluster analysis, the indigenous chickens found in the study area could be classified into four ecotypes: ecotype 1 (Banja, Dembecha, and Aneded), ecotype 2 (North Achefer), ecotype 3 (Sinan), and ecotype 4 (Jawi).

Keywords: Characterization, Chicken, Indigenous, Squared Mahalanobis, Morpho-biometric

5.1. Introduction

Indigenous chicken production is a common practice in rural, resource-poor households in developing countries like Ethiopia. The majority of families at the village level, including the landless and the poorest, are owners of poultry (Wong *et al*., 2017). The total poultry population in Ethiopia is estimated to be about 57 million, most of which are laying hens (34.26 %) followed by chicks (32.86 %). With regard to breed, 78.85%, 9.14%, and 12.03% of the total poultry were reported to be indigenous, exotic, and hybrid, respectively (CSA, 2021). Although indigenous chicken breeds are claimed to be slow growers and poor producers of small eggs, they are characterized by better egg and meat flavour, top brooding and natural incubation capacity, and high dressing percentages (Wong *et al*., 2017). Characterization and identification of the chicken genetic resources require information on the population, adaptation to a specific environment, possession of traits of future value, and their socio-cultural importance (Weigend & Romanov, 2001). Dorji and Sunar (2014) stated that phenotypic approaches are fundamental in chicken breed management because they are fast, easy, and cost-effective. Indigenous chickens

have various unique morphological features and possess genes that have adaptive values for their environment and local diseases (Aklilu *et al*., 2013). Local chicken populations are often described and grouped based on their location or phenotypic features, while their classification into breeds is inadequate (Manyelo *et al*., 2020). In the literature, the terms "ecotype" and "breed" are used interchangeably. "Ecotype" is described in evolutionary ecology as a population that is genetically adapted to specific environmental conditions (Vallejo-Trujillo *et al*., 2022). On the other hand, "ecotype is described as "a non-static adaptive variation over many traits across the natural landscape with no discernible boundaries" (Lowry, 2012). The terms 'indigenous', 'native', 'local' or 'traditional' are also used interchangeably. For instance, Dessie *et al*. (2003) used “local chicken ecotypes” while Halima *et al*. (2007b) called them “native chicken populations”. The indigenous chickens of Ethiopia are named after the location where they are prevalent including Jarso, Chefe, Tilili, Tepi and Horro (Dessie *et al*., 2003); Guangua, Tilili, Debre-Elias, Melo-Hamusit, Gelila, Gassay and Mecha (Halima *et al*., 2007b); Mandura, Horro, Farta, Konso and sheka (Dana *et al*., 2010b); Gasgie, and Gugut (Getu *et al*., 2014). Based on their plumage colour type, scholars named local chickens as *Gebsima*, Red, White, Black, Grey, *Kokima*, *Key Teterima*, *Lebework*, *Tikur Gebsat*, Brown, and Netch *teterima* (Bekele *et al*., 2021). In Ethiopia, several scholars (Melesse & Negesse, 2011; Getu *et al*., 2014; Negassa *et al*., 2014; Getachew *et al*., 2017; Melesse *et al*., 2021; Bekele *et al*., 2021) conducted phenotypic characterization of indigenous chickens. However, there is still a dearth of information in the Northwestern parts of Ethiopia, where the presence of huge genetic resources is anticipated. Therefore, this study was aimed at characterizing the morpho-biometric variation of indigenous chicken ecotypes found in Northwest Ethiopia as an input for designing breeding and conservation programs.

5.2. Material and Methods

5.2.1. Description of the study areas

This study was conducted in three zones (Awi zone, West Gojjam zone, and East Gojjam zone) of the Amhara National Regional State, Ethiopia. Banja and Jawi districts from the Awi administrative zone, Sinan and Aneded from the East Gojjam zone, and Dembecha and North Achefer from the West Gojjam zone were considered for the study (Figure 3.1).

The agro-ecological description, the number of chickens found in the study area and the major feed resources for the chickens are presented in Table 4.1.

5.2.2. Sampling and data collection

A multi-stage purposive, random, and stratified sampling method was employed for the selection of zones, districts, households, and chickens for the study. In the first stage, three zones (Awi zone, East Gojjam zone, and West Gojjam zone) were purposefully selected based on research gaps (not sufficiently addressed) and chicken potential. In the second stage, districts and *kebeles/*peasant associations were stratified based on agro-ecology, and six districts (two districts from each agro-ecology and three *kebeles* from each district) were selected considering their chicken population numbers. Banja and Sinan from highland agro-ecology, Dembecha and Aneded from midland agro-ecology, and Jawi and North Achefer from lowland agro-ecology were considered. At the third stage, households that have only indigenous chickens were purposefully selected to avoid biases during the measurement of chickens. Last, chickens (200 chickens from each district) were randomly sampled based on the FAO guidelines for phenotypic characterization (FAO, 2012). Accordingly, a total of 1200 adult chickens older than seven months (877 females and 323 males) were sampled. While sampling chickens from each household, the distance between households was considered to minimize the error, which might come due to the genetic relatedness between chickens. Each chicken was identified by its sex and location.

Chicken were characterized for ten linear body measurements: wing span (WS), shank length (SL), body length (BL), beak length (BKL), neck length (NL), comb length (CL), shank circumference (SC), chest circumference (CC), thigh circumference (TC), and comb height (CH) using a textile measuring tape (Figure 5.1) and body weight was measured using a suspended spring. Visual observation was made, and 12 morphological features (plumage colour pattern, plumage colour type, shank colour, earlobe colour, skin colour, earlobe presence, wattle presence, shank feather presence, comb type, head shape, feather morphology, and feather distribution) were also recorded based on the FAO breed morphological characteristics descriptor list (FAO, 2012).

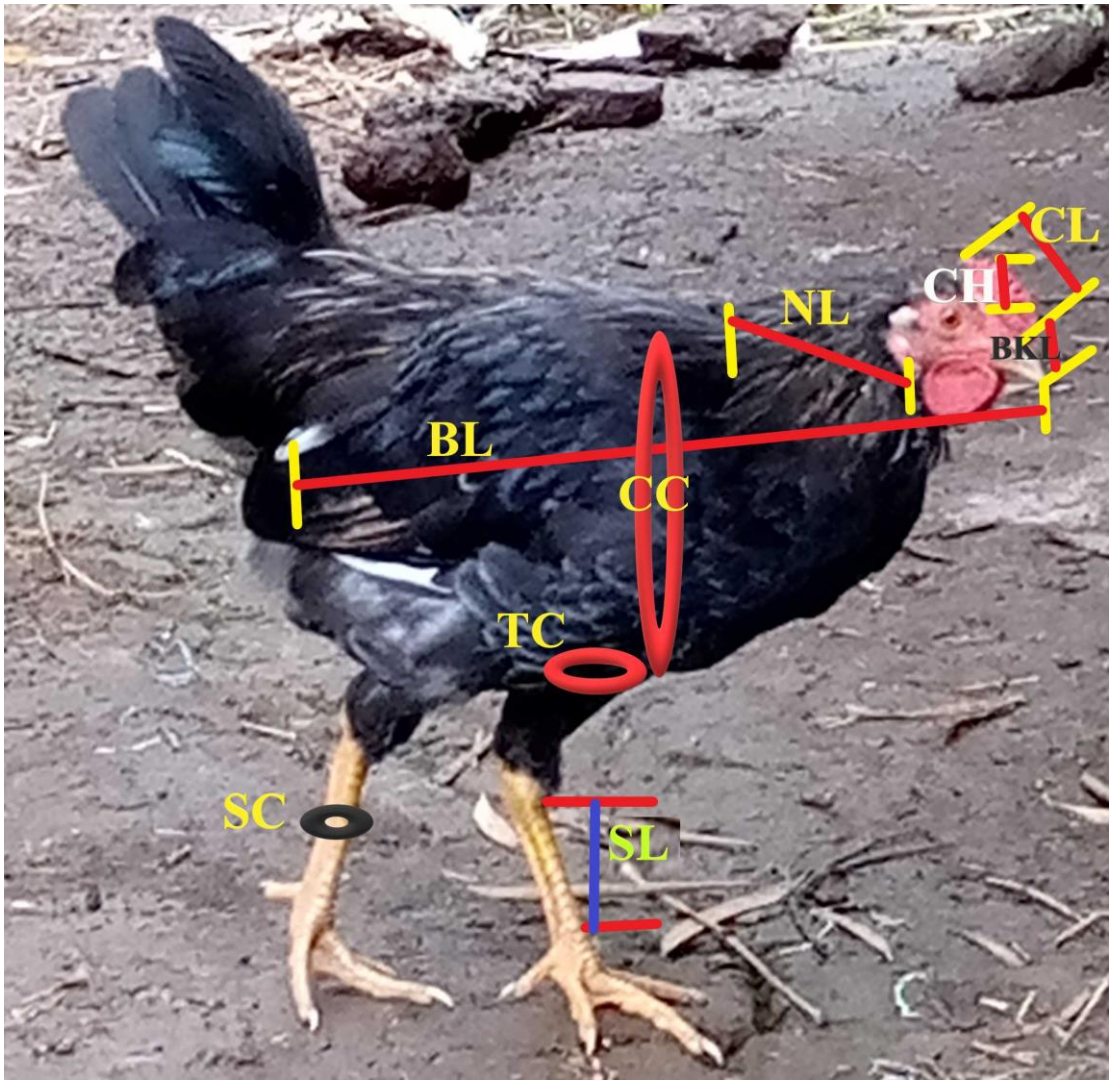

Figure 5.1. Location of linear body measurements (measurement points)

5.2.3. Statistical analysis

All data were coded and recorded in a Microsoft Excel sheet and saved as a "csv" file. Statistical analyses were made via SAS (SAS, 2012). R-software (Team, 2021) was used for graphical presentation/data visualization. Homogeneity tests, normality tests, and screening of outliers were employed before conducting the main data analysis work.

Qualitative morphological traits analysis

Univariate Analysis: Qualitative morphological traits were subjected to the frequency procedure of the chi-square (χ^2) test to assess the statistical significance among qualitative variables.

Multivariate Analysis: The associations among qualitative morphological traits were assessed via a multiple correspondence analysis. "FactoMineR" and "factoextra" packages were used for analysis and plot visualization.

Quantitative morphological traits analysis

Univariate Analysis: quantitative morphological traits were analyzed using the general linear model procedure to determine the effects of agro-ecology, location, sex, and location with sex interaction. Agro-ecology, location, and sex of the indigenous chickens were fitted as fixed independent variables. The effects of class variables and their interaction were expressed as least square means (LSM) ± SE. Mean comparisons were made using Tukey's test method at $P<0.05$. The following model was utilized to show the effect of agro-ecology, location, and sex on different linear body measurements and body weight of indigenous chickens.

$Y_{ijk}= \mu + A_i + L_j + S_k + (LS)_{jk}+e_{ijk}$, Where:

Y_{ijk}= individual phenotypic observation;

μ = the overall mean;

A_i = the fixed effect of agro-ecology (i= Highland, Midland, and Lowland);

L_j = the fixed effect of location/site (j= Banja, Sinan, Dembecha, Aneded, Jawi and North Achefer);

S_k = the fixed effect of sex (k= Male and Female);

LS_{jk} =the interaction effect of location with sex and

e_{ijk} = the effect of random error.

Multivariate analysis: The stepwise discriminant analysis procedure was run to rank the quantitative morphological traits by their discriminating power. Selected significant traits from stepwise discriminant analysis were then subjected to canonical discriminant analysis. The quantitative variables from male and female chickens were separately subjected to discriminate analysis to ascertain the existence of population-level phenotypic differences among them. Non-parametric discriminant analysis was also done for male and female chickens together to classify them based on qualitative traits. The degree of morphological similarity between the chickens was determined using Euclidean and Ward's option of R in hierarchical clustering and hierarchical clustering on principal components analysis (HCPC). "Cluster", "factoextra" and "FactoMineR" packages were utilized in the clustering procedures. The hierarchical clustering on principal components

analysis was done by using the quantitative traits, which have high discriminating power in the classification of chickens. In clustering of female chickens, wing span, shank length, beak length, neck length, shank circumference, and chest circumference were considered. In male chickens, wing span, shank length, shank circumference, comb length, body weight, and chest circumference were included.

5.3. Results

5.3.1. Qualitative morphological traits

The frequency and percent of qualitative traits observed in male and female chickens are presented in Table 5.1. The most frequently observed plumage colour pattern in the study area was plain (55.3%), followed by patchy (34.8%). Red and *Gebisma (*white strips on black background, grayish with varying mixtures, mixtures of white and black with varying shades of multicolours and red brownish with black) were the most frequently observed plumage colour types in almost all study areas, with the overall values of 33.2% and 30.1%, respectively. The dominant shank colours found in the study areas were yellow (57%) and white (32.9%). Some of the indigenous chickens, around 1%, had uncommon shank colour, such as blue and green. About 70.8% of indigenous chickens have earlobes, and white with red earlobe colour (73.8%) was mostly observed in the study area, followed by red earlobe colour (18.4%).

Almost all (99.6%) of the sample chickens had wattles, and only 1.9% of them possessed shank feathers (Figure 5.2). White skin colour was predominant (88.4%) in the sample chicken population. Uncommon skin colour types of black and blue-black (1.8%) were also observed. Among female and male sample chickens, the majority (37.8%) possessed the rose comb type, followed by single and pea comb types with 29.8% and 17.9%, respectively. The plain head shape was most frequently observed, accounting for 51.9% of the sampled population, whereas 47.2% of chickens had a crest head shape. The majority (86.3%) of chickens had normal feather morphology. However, there were still very few chickens (0.5%) with frizzle feather morphology. All chickens had normal feather distribution except in lowland areas (Jawi and North Achefer), which possessed a small number of naked neck chickens (Figure 5.2).

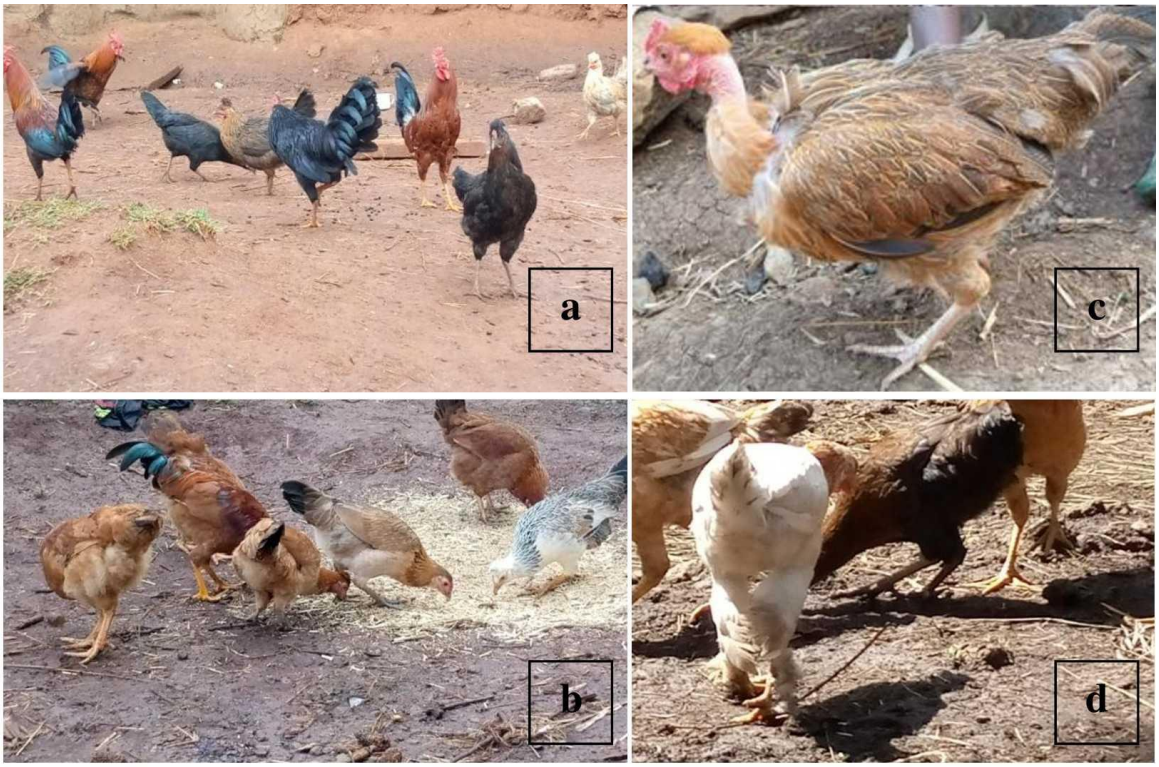

Figure 5.2. Indigenous chickens with different plumage colour in Aneded (a) and Dembecha (b), naked-neck chicken in Jawi (c), and white chicken with a shank feather in Banja (d)

Table 5.1. Qualitative morphological traits characteristics of indigenous chicken ecotypes in Northwest Ethiopia

Morphological character	Locations/sites												
	Banja		Sinan		Dembecha		Aneded		Jawi		North Achefer		Overall
	M N (%)	F N (%)	M N (%)	F N (%)	M N (%)	F N (%)	M N (%)	F N (%)	M N (%)	F N (%)	M N (%)	F N (%)	N (%)
Plumage colour pattern													
Plain	39(78)	99(59.6)	50(79.4)	79(49.7)	25(58.1)	82(62.6)	44(80.0)	72(47.1)	45(75.0)	57(39.0)	40(76.9)	31(25.4)	663(55.3)
Patchy	8(16)	50(30.1)	11(17.5)	68(42.8)	14(32.6)	29(22.1)	5(9.1)	68(44.4)	10(16.7)	57(39.0)	12(23.1)	86(70.5)	418(34.8)
Spotted	3(6)	17(10.2)	2(3.2)	12(7.5)	4(9.3)	20(15.3)	6(10.9)	13(8.5)	5(8.3)	32(21.9)	0(0.0)	5(4.1)	119(9.9)
					*χ^2=72.694*** ; Cramer's V=0.174*								
Plumage colour type													
Plain white	9(18.0)	36(21.7)	0(0.0)	8(5.0)	0(0.0)	24(18.3)	10(18.2)	30(19.6)	0(0.0)	5(3.4)	0(0.0)	5(4.1)	127(10.6)
Plain black	8(16.0)	22(13.3)	4(6.3)	19(11.9)	0(0.0)	0(0.0)	5(9.1)	10(6.5)	0(0.0)	20(13.7)	0(0.0)	0(0.0)	88(7.3)
Plain red	22(44.0	41(24.7)	33(52.4)	43(27.0)	25(58.1)	38(29.0)	25(45.5)	21(13.7)	45(75.0)	27(18.5)	46(88.5)	32(26.2)	398(33.2)
Gebisma	8(16.0)	50(30.1)	11(17.5)	75(47.2)	10(23.3)	29(22.1)	5(9.1)	67(43.8)	5(8.3)	42(28.8)	6(11.5)	53(43.4)	361(30.1)
Multi-colour	0(0.0)	11(6.6)	0(0.0)	0(0.0)	4(9.3)	14(10.7)	5(9.1)	6(3.9)	0(0.0)	15(10.3)	0(0.0)	22(18.0)	77(6.4)
Teterima	3(6.0)	6(3.6)	6(9.5)	7(4.4)	0(0.0)	6(4.6)	0(0.0)	6(3.9)	10(16.7)	37(25.3)	0(0.0)	10(8.2)	91(7.6)
Wosera	0(0.0)	0(0.0)	5(7.9)	7(4.4)	4(9.3)	20(15.3)	5(9.1)	13(8.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	54(4.5)
Sora	0(0.0)	0(0.0)	4(6.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(0.3)
					*χ^2=339.85*** ; Cramer's V=0.238*								
Shank colour													
Yellow	50(100)	98(59.0)	51(81.0)	65(40.9)	29(67.4)	32(24.4)	55(100)	115(75.2)	45(75.0)	43(29.5)	46(88.5)	55(45.1)	684(57.0)
Black	0(0.0)	26(15.7)	5(7.9)	21(13.2)	0(0.0)	0(0.0)	0(0.0)	15(9.8)	0(0.0)	31(21.2)	0(0.0)	11(9.0)	109(9.1)
White	0(0.0)	42(25.3)	7(11.1)	73(45.9)	14(32.6)	99(75.6)	0(0.0)	23(15)	15(25)	72(49.3)	0(0.0)	50(41.0)	395(32.9)
Others	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(11.5)	6(4.9)	12(1.0)
					*χ^2=254.32*** ; Cramer's V=0.266*								
Earlobe colour													
White	0(0.0)	0(0.0)	0(0.0)	6(7.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	16(20.5)	0(0.0)	44(45.8)	66(7.8)
Red	24(59.0)	0(0.0)	24(45.0)	0(0.0)	12(28.0)	0(0.0)	24(43.6)	8(7.0)	10(18.1)	0(0.0)	47(90.4)	7(7.2)	156(18.4)
White and red	17(41.0)	89(100)	29(55.0)	80(93.0)	31(72.0)	86(100)	31(56.4)	107(93.0)	45(81.9)	62(79.5)	5(9.6)	45(47.0)	627(73.8)
					*χ^2=295.24*** ; Cramer's V=0.286*								
Skin colour													
White	36(72.0)	138(83.1)	35(55.6)	154(96.9)	14(32.6)	131(100)	35(63.6)	145(94.8)	60(100)	146(100)	52(100)	115(94.3)	1061(88.4)
Yellow	11(22.0)	17(10.2)	28(44.4)	5(3.1)	29(67.4)	0(0.0)	20(36.4)	8(5.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	118(9.8)
Others	3(6.0)	11(6.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	7(5.7)	21(1.8)
					*χ^2=109.85*** ; Cramer's V=0.214*								
Earlobe													
Present	41(82.0)	89(53.6)	53(84.1)	86(54.1)	43(100)	86(65.6)	55(100)	115(75.2)	55(91.7)	78(53.4)	52(100)	96(78.7)	849(70.8)
Absent	9(18.0)	77(46.4)	10(15.9)	73(45.9)	0(0.0)	45(34.4)	0(0.0)	38(24.8)	5(8.3)	68(46.6)	0(0.0)	26(21.3)	351(29.3)
					*χ^2=52.86*** ; Cramer's V=0.21*								

Morphological character	Locations/sites												
	Banja		Sinan		Dembecha		Aneded		Jawi		North Achefer		Overall
	M	F	M	F	M	F	M	F	M	F	M	F	
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Wattle													
Present	50(100)	166(100)	63(100)	159(100)	43(100)	131(100)	55(100)	153(100)	55(91.7)	146(100)	52(100)	122(100)	1195(99.6)
Absent	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(8.3)	0(0.0)	0(0.0)	0(0.0)	5(0.4)
					*χ^2=24.23*** ; Cramer's V=0.142*								
Shank feather													
Present	0(0.0)	3(1.8)	0(0.0)	0(0.0)	7(16.3)	10(7.6)	2(3.6)	0(0.0)	0(0.0)	1(0.7)	0(0.0)	0(0.0)	23(1.9)
Absent	50(100)	163(98.2)	63(100)	159(100)	36(83.7)	121(92.4)	53(96.4)	153(100)	60(100)	145(99.3)	52(100)	122(100)	1177(98.1)
					*χ^2=68.39*** ; Cramer's V=0.239*								
Comb type													
Rose	38(76.0)	55(33.1)	33(52.4)	61(38.4)	29(67.4)	59(45.0)	25(45.5)	51(33.3)	25(41.7)	49(33.6)	13(25.0)	16(13.1)	454(37.8)
Pea	0(0.0)	45(27.1)	3(4.8)	14(8.8)	0(0.0)	26(19.8)	0(0.0)	39(25.5)	5(8.3)	56(38.4)	0(0.0)	27(22.1)	215(17.9)
Single	12(24.0)	51(30.7)	21(33.3)	47(29.6)	4(9.3)	39(29.8)	25(45.5)	44(28.8)	15(25.0)	25(17.1)	23(44.2)	52(42.6)	358(29.8)
Others	0(0.00)	15(9.0)	6(9.5)	37(23.3)	10(23.3)	7(5.3)	5(9.1)	19(12.4)	15(25.0)	16(11.0)	16(30.8)	27(22.1)	173(14.4)
					*χ^2=111.35*** ; Cramer's V=0.176*								
Head shape													
Plain(Ebab-eras)	34(68.0)	99(59.6)	25(39.7)	80(50.3)	33(76.7)	66(50.4)	50(90.9)	55(35.9)	45(75)	61(41.8)	22(42.3)	53(43.4)	623(51.9)
Crest(Guteya)	16(32.0)	67(40.4)	38(60.3)	79(49.7)	10(23.3)	65(49.6)	5(9.1)	98(64.1)	15(25)	85(58.2)	24(46.2)	64(52.5)	566(47.2)
Others	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(11.5)	5(4.1)	11(0.9)
					*χ^2=79.49*** ; Cramer's V=0.182*								
Feather morphology													
Normal	39(78.0)	166(100)	39(61.9)	148(93.1)	10(23.3)	127(97.0)	11(20.0)	153(100)	55(91.7)	146(100)	38(73.1)	104(85.2)	1036(86.3)
Frizzle	0(0.0)	0(0.0)	0(0.0)	6(3.8)	0(0.0)	0(0.00)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(0.5)
Silky	11(22.0)	0(0.0)	24(38.1)	0(0.0)	33(76.7)	4(3.0)	44(80.0)	0(0.0)	5(8.3)	0(0.0)	14(26.9)	18(14.8)	158(13.2)
					*χ^2=85.46*** ; Cramer's V=0.189*								
Feather distribution													
Normal	50(100)	166(100)	63(100)	159(100)	43(100)	131(100)	55(100)	153(100)	55(91.7)	141(96.6)	47(90.4)	112(91.8)	1175(97.9)
Naked neck	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(8.3)	5(3.4)	5(9.6)	10(8.2)	25(2.1)
					*χ^2=61.65*** ; Cramer's V=0.227*								

N= Number of chickens; M= Male; F= Female; *p<0.05; **p<0.01; ***p<0.001

Multiple correspondence analyses were executed on twelve qualitative traits, and a bi-dimensional plot representing the associations among the various categories of qualitative traits is presented in Figure 5.3. About 14.8% of the total variation was explained by the first two dimensions, 8.1% by the first and 6.7% by the second. The indigenous chickens in North Achefer were clustered together with multicolour plumage colour types and head shapes other than plain and crest. Some of these chickens were also clustered by shank colour, such as blue and green, and by naked neck feather distribution. Indigenous chickens in Jawi were closely associated with pea comb type; some of the chickens had no earlobe and *teterima (*black spot on white, black with white tips, and white with black or red spots) plumage colour type. In the Dembecha site, the chickens were closely associated with white with red plumage colour type, silky feather morphology, and yellow skin colour. Yellow skin colour and silky feather morphology were also closely associated with the chickens found at the Aneded site. Indigenous chickens in Sinan were clustered together with normal feather distribution and an absence of shank feathers. In Banja, the indigenous chickens were closely associated with black plumage.

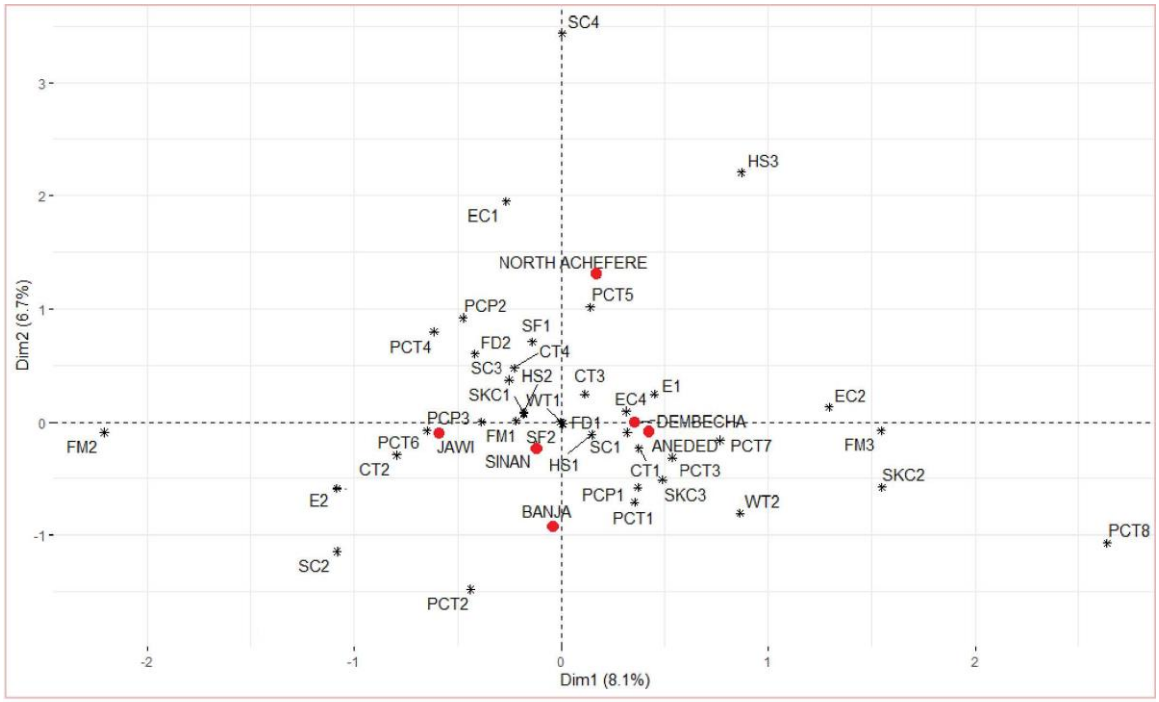

Figure 5.3. Bi-dimensional plot showing the associations among the categories of the different morphological variables. Plumage colour pattern: PCP1=plain, PCP2=patchy, PCP3=spotted; Plumage colour type: PCT1=completely white, PCT2=completely black, PCT3=completely red, PCT4=*Gebisma*, PCT5=multicolour, PCT6=*teterima*, PCT7=white with red, PCT8=*sora;* Shank colour: SC1=yellow, SC2=black, SC3=white, SC4=others (blue, green...); Earlobe colour: EC1=White, EC2=red, EC4=white and red; Skin colour:

SKC1=white, SKC2=yellow, SKC3=others; Comb type: CT1=rose, CT2=pea, CT3=single, CT4=others; Head shape: HS1=plain, HS2=crest, HS3=others; Earlobe: E1=present, E2=absent; Wattle: WT1=present, WT2=absent; Shank feather: SF1=present, SF2=absent; Feather morphology: FM1=normal, FM2=frizzle, FM3=silky; Feather distribution: FD1=normal, FD2=naked neck

5.3.2. Body weight and linear body measurements

The least squares means and standard errors for the effects of agro-ecology, sex, and location on body weight and other body measurements are presented in Table 5.2. In all study areas, sex had a significant ($p<0.001$) effect on body weight and all linear body measurements. Male chickens had consistently higher values than females. All body measurements were significantly affected by agro-ecology and location. However, the significant variation was also observed among chickens found in similar agro-ecologies. Indigenous chickens found in the lowland areas (Jawi and North Achefer) showed higher values than others for the majority of body measurements. The North Achefer chickens had significantly ($p<0.001$) higher average shank length, chest circumference, thigh circumference, comb height, and body weight than others. Chickens from Jawi had a higher value in body length, beak length, and shank circumference than others. The interaction of sex group and location was significant ($p<0.001$) for body weight and all other body measurements (Table 5.2). Indigenous female chickens from Banja had significantly ($p<0.001$) higher measurements in most of the variables. Whereas, the North Achefer male chickens showed significantly ($p<0.001$) higher values in wing span, shank length, neck length, chest circumference, thigh circumference, body weight, and comb height.

Table 5.2. Least squares mean (± SE) body weight (kg) and other linear body measurements (cm) by agro-ecology, sex and location of indigenous chicken ecotypes in Northwest Ethiopia

Effect and levels	WS	SL	BL	BKL	NL	CL	SC	CC	TC	CH	BW
	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Overall	42.9±0.12	7.38±0.03	35.9±0.10	2.44±0.01	10.7±0.05	3.32±0.05	3.2±0.02	24.9±0.06	9.91±0.05	1.27±0.03	1.36±0.01
CV %	6.38	9.49	7.09	11.04	11.41	30.4	17.2	7.33	11.80	19.90	16.50
Sex	***	***	***	***	***	***	***	***	***	***	***
Male	47.47±0.16^{a}	8.47±0.04^{a}	39.3±0.14^{a}	2.6±0.01^{a}	11.8±0.07^{a}	5.71±0.06^{a}	3.66±0.03^{a}	26.17±0.10^{a}	11.4±0.07^{a}	2.36±0.05^{a}	1.59±0.01^{a}
Female	41.23±0.09^{b}	6.99±0.02^{b}	34.6±0.08^{b}	2.38±0.01^{b}	10.2±0.04^{b}	2.4±0.04^{b}	3.0±0.02^{b}	24.4±0.06^{b}	9.36±0.04^{b}	0.85±0.03^{b}	1.27±0.01^{b}
Agro-ecology	***	***	***	***	***	***	**	***	***	***	***
Highland	43.70±0.15^{c}	7.41±0.03^{b}	36.98±0.14^{b}	2.49±0.02^{ab}	11.3±0.07^{a}	3.99±0.05^{b}	3.26±0.03^{b}	24.6±0.10^{c}	9.96±0.06^{b}	1.56±0.04^{b}	1.39±0.01^{b}
Midland	44.36±0.16^{b}	7.34±0.03^{b}	36.18±0.15^{c}	2.46±0.02^{b}	11.1±0.07^{a}	4.06±0.06^{ab}	3.43±0.04^{a}	25.3±0.11^{b}	10.6±0.07^{a}	1.43±0.04^{c}	1.37±0.01^{b}
Lowland	44.82±0.16^{a}	8.40±0.03^{a}	37.56±0.15^{a}	2.51±0.02^{a}	10.6±0.07^{b}	4.08±0.06^{a}	3.31±0.04^{ab}	25.9±0.10^{a}	10.5±0.07^{a}	1.70±0.04^{a}	1.50±0.01^{a}
Location	***	***	***	***	***	**	***	***	**	***	***
Banja	44.8±0.23^{b}	7.59±0.06^{b}	37.4±0.21^{a}	2.52±0.02^{b}	11.5±0.09^{a}	4.15±0.08^{ab}	3.37±0.04^{b}	25±0.15^{bc}	10.3±0.10^{b}	1.6±0.07^{b}	1.41±0.01^{b}
Sinan	42.1±0.21^{d}	7.14±0.05^{c}	35.7±0.20^{cd}	2.39±0.02^{c}	10.8±0.09^{b}	3.70±0.08^{bc}	3.12±0.04^{c}	23.8±0.13^{d}	9.6±0.09^{c}	1.65±0.07^{ab}	1.33±0.01^{c}
Dembecha	45.2±0.25^{ab}	7.38±0.06^{c}	37.2±0.23^{bc}	2.51±0.02^{c}	11.1±0.10^{b}	3.38±0.09^{c}	3.44±0.05^{b}	25.5±0.16^{bc}	10.4±0.10^{ab}	1.1±0.08^{c}	1.48±0.02^{ab}
Aneded	43.2±0.22^{c}	7.25±0.05^{c}	35.5±0.20^{d}	2.45±0.02^{c}	11±0.09^{b}	4.54±0.08^{a}	3.38±0.04^{b}	24.9±0.14^{c}	10.6±0.10^{a}	1.6±0.07^{bc}	1.28±0.01^{c}
Jawi	46.1±0.22^{a}	8.48±0.05^{a}	38.6±0.20^{a}	2.66±0.02^{a}	10.9±0.09^{b}	4.38±0.08^{a}	3.97±0.04^{a}	26±0.14^{ab}	10.6±0.10^{a}	1.7±0.07^{ab}	1.54±0.01^{a}
N/Achefer	44.7±0.23^{c}	8.53±0.06^{a}	37.4±0.21^{b}	2.35±0.02^{d}	10.5±0.10^{c}	4.21±0.09^{ab}	2.72±0.05^{d}	26.5±0.15^{a}	10.8±0.10^{ab}	1.95±0.08^{a}	1.55±0.01^{ab}
Location*sex	***	***	***	**	***	***	***	***	***	***	***
Banja, M	47.19±0.39	8.17±0.10	38.38±0.37	2.54±0.04	11.97±0.17	5.77±0.15	3.65±0.08	24.91±0.26	11.18±0.17	2.28±0.13	1.46±0.03
Banja, F	42.41±0.22	7.0±0.05	36.40±0.21	2.51±0.02	11.08±0.09	2.54±0.08	3.09±0.04	24.9±0.14	9.37±0.09	0.91±0.07	1.36±0.02
Sinan, M	44.68±0.35	7.76±0.09	37.26±0.33	2.48±0.03	11.49±0.15	4.96±0.13	3.34±0.07	24.46±0.23	10.44±0.15	2.42±0.11	1.47±0.03
Sinan, F	39.58±0.22	6.52±0.06	34.08±0.20	2.31±0.02	10.25±0.09	2.45±0.08	2.90±0.04	23.1±0.14	8.77±0.09	0.89±0.07	1.18±0.02
Dembecha, M	47.60±0.43	7.99±0.10	40.23±0.39	2.70±0.04	11.76±0.18	4.23±0.16	3.69±0.08	26.34±0.28	11.17±0.18	1.38±0.14	1.67±0.03
Dembecha, F	42.75±0.25	6.76±0.06	34.14±0.23	2.32±0.02	10.42±0.10	2.53±0.09	3.18±0.05	24.65±0.16	9.7±0.10	0.83±0.08	1.30±0.03
Aneded, M	45.59±0.38	7.90±0.09	37.16±0.35	2.56±0.04	11.67±0.16	6.59±0.14	3.61±0.07	25.32±0.24	11.42±0.16	2.41±0.12	1.36±0.03
Aneded, F	40.88±0.23	6.6±0.05	33.87±0.21	2.34±0.02	10.37±0.09	2.48±0.08	3.15±0.04	24.62±0.15	9.74±0.09	0.78±0.07	1.19±0.01
Jawi, M	49.54±0.36	9.41±0.09	41.57±0.33	2.72±0.03	11.22±0.15	6.38±0.13	4.51±0.07	27.49±0.23	11.71±0.16	2.63±0.12	1.77±0.03
Jawi, F	42.66±0.23	7.55±0.06	35.69±0.21	2.61±0.02	10.58±0.10	2.37±0.08	3.43±0.04	24.56±0.15	9.56±0.10	0.82±0.07	1.32±0.02
N/Achefer, M	50.22±0.39	9.56±0.10	41.27±0.36	2.57±0.04	12.71±0.17	6.36±0.15	3.17±0.08	28.49±0.25	12.66±0.17	3.06±0.12	1.84±0.03
N/Achefer, F	39.13±0.25	7.51±0.06	33.59±0.23	2.14±0.02	8.34±0.11	2.06±0.09	2.26±0.05	24.42±0.16	9.0±0.11	0.86±0.08	1.27±0.02

LSM= Least Square Mean; SE= Standard error; M= Male; F= Female; WS= Wing Span; SL= Shank Length; BL= Body Length; BKL= Beak Length; NL= Neck Length; CL= Comb Length; SC= Shank Circumference; CC= Chest Circumference; TC= Thigh Circumference; CH= Comb Height; BW= Body Weight; *p<0.05; **p<0.01; ***p<0.001

5.3.3. Step-wise discriminant analysis

The stepwise discriminant analysis procedure revealed that, in both sexes, all the variables had a significant discriminating power between the sample populations. For both the female and male sample populations, shank traits (shank circumference and shank length) had the highest discriminating power, with a standard deviation of 0.59 and 1.07 for the female and male populations, respectively (Table 5.3).

Table 5.3. Traits used in discriminating the chicken population from different sites in stepwise discriminant analysis

Sex	Variable	Total standard deviation	Partial R^2	F-value	Pr>F	Wilks' Lambda	Pr < Lambda	ASCC	Pr > ASCC
	SC	0.59	0.34	90.47	<.0001	0.66	<.0001	0.07	<.0001
	SL	0.79	0.29	70.27	<.0001	0.47	<.0001	0.12	<.0001
	NL	1.46	0.28	67.73	<.0001	0.34	<.0001	0.16	<.0001
Female	BKL	0.30	0.13	26.42	<.0001	0.29	<.0001	0.18	<.0001
	CC	1.85	0.12	23.39	<.0001	0.26	<.0001	0.21	<.0001
	WS	3.17	0.11	20.35	<.0001	0.23	<.0001	0.22	<.0001
	BL	2.69	0.09	17.46	<.0001	0.21	<.0001	0.24	<.0001
	TC	1.18	0.10	19.90	<.0001	0.19	<.0001	0.25	<.0001
	BW	0.23	0.08	15.36	<.0001	0.17	<.0001	0.27	<.0001
	CL	0.74	0.05	9.59	<.0001	0.16	<.0001	0.27	<.0001
	SL	1.07	0.48	58.90	<.0001	0.52	<.0001	0.10	<.0001
	SC	0.86	0.27	23.71	<.0001	0.38	<.0001	0.15	<.0001
	CL	1.85	0.18	14.27	<.0001	0.31	<.0001	0.19	<.0001
Male	BW	0.32	0.20	15.72	<.0001	0.25	<.0001	0.22	<.0001
	CC	2.49	0.19	14.31	<.0001	0.20	<.0001	0.25	<.0001
	WS	3.44	0.15	10.95	<.0001	0.17	<.0001	0.27	<.0001
	NL	1.31	0.14	9.89	<.0001	0.15	<.0001	0.30	<.0001
	CH	1.52	0.09	6.41	<.0001	0.13	<.0001	0.31	<.0001
	TC	1.58	0.08	5.29	.0001	0.12	<.0001	0.32	<.0001
	BL	3.42	0.08	5.11	.0002	0.11	<.0001	0.33	<.0001
	BKL	0.33	0.08	5.19	.0001	0.10	<.0001	0.34	<.0001

*p<0.05; **p<0.01; ***p<0.001; ASCC=Average Squared Canonical Correlation

5.3.4. Discriminant analysis

The validity of the discriminant analysis procedure was assessed by means of reclassification statistics (Table 5.4). The overall classification rates (hit rate) of the female and male sample populations were 57.47% and 69.97%, respectively (Table 5.4). The overall hit rate in the female population was not high, as the hit rates observed for Banja (48.80%) and Dembecha (34.35%) were small. In the case of male chickens, the lowest classification rate was obtained for Banja with 42.00%, followed by Dembecha with about 65.00% (Table 5.4).

Table 5.4. Number of observations and percent (bracket) correct classified for female and male sample population using discriminant analysis

Sex	Site	Banja	Sinan	Dembecha	Aneded	Jawi	N/Achefer	Total
Female	**Banja**	81(48.80)	27(16.27)	23(13.86)	13(7.83)	22(13.25)	0(0.00)	166(100)
	Sinan	23(14.47)	99(62.26)	4(2.52)	24(15.09)	3(1.89)	6(3.77)	159(100)
	Dembecha	25(19.08)	27(20.61)	45(34.35)	25(19.08)	9(6.87)	0(0.00)	131(100)
	Aneded	13(8.50)	27(17.65)	18(11.76)	89(58.17)	6(3.92)	0(0.00)	153(100)
	Jawi	19(13.01)	16(10.96)	6(4.11)	11(7.53)	92(63.01)	2(1.37)	146(100)
	N/Achefer	1(0.82)	8(6.56)	5(4.10)	1(0.82)	9(7.38)	98(80.33)	122(100)
Male	**Banja**	21(42.00)	10(20.00)	2(4.00)	10(20.00)	5(10.00)	2(4.00)	50(100)
	Sinan	13(20.63)	44(69.84)	3(4.76)	3(4.76)	0(0.00)	0(0.00)	63(100)
	Dembecha	0(0.00)	9(20.93)	28(65.12)	0(0.00)	4(9.30)	2(4.65)	43(100)
	Aneded	6(10.91)	0(0.00)	0(0.00)	47(85.45)	0(0.00)	2(3.64)	55(100)
	Jawi	0(0.00)	0(0.00)	10(16.67)	0(0.00)	50(83.33)	0(0.00)	60(100)
	N/Achefer	0(0.00)	1(1.92)	0(0.00)	0(0.00)	15(28.85)	36(69.23)	52(100)

5.3.5. Non-parametric discriminant analysis

All the categorical variables that were used for the chi-square test were included in the nonparametric analysis to see how the sample populations differed as group entities. Both male and female populations were merged and analyzed together, and the result is presented in Table 5.5. The overall classification rate estimate was 55.42%.

Table 5.5. Number of observations and percent-classified (in brackets) into the site using a non-parametric discriminant for both male and female sample chicken populations

From site	Banja	Sinan	Dembecha	Aneded	Jawi	North Achefer
Banja	81(37.50)	44(20.37)	35(16.20)	25(11.57)	27(12.50)	4(1.85)
Sinan	21(9.46)	139(62.61)	13(5.86)	39(17.57)	9(4.05)	1(0.45)
Dembecha	25(14.37)	40(22.99)	52(29.89)	36(20.69)	18(10.34)	3(1.72)
Aneded	9(4.33)	28(13.46)	23(11.06)	142(68.27)	4(1.92)	2(0.96)
Jawi	25(12.14)	15(7.28)	12(5.83)	17(8.25)	132(64.08)	5(2.43)
North Achefer	8(4.60)	9(5.17)	5(2.87)	4(2.30)	29(16.67)	119(68.39)

5.3.6. Canonical discriminant analysis

The squared Mahalanobis distances between sites (Table 5.6) for female sample populations were significant ($p<0.001$). The shortest distance (4.73) was measured between Aneded and Dembecha, and the longest distance (19.25) was measured between Banja and North Achefer. In male sample populations, the shortest distance was between Banja and Aneded, with a value of 5.87 standard units, and the longest was between Dembecha and North Achefer, with a value of 16.8 (Table 5.6). The multivariate statistics for differences between the districts were also significant ($p<0.001$) in all four multivariate

tests (Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace, and Roy's Greatest Root). Similarly, the univariate statistic testing the hypothesis that class means are equal showed that the means of each variable are significantly different ($p<0.001$) between sites. The values of Wilks' lambda were 0.16 and 0.10 for the female and male sample populations, respectively.

Table 5.6. The squared Mahalanobis distance between sites for the female (below the diagonal) and male (above the diagonal) sample chickens

From site	Banja	Sinan	Dembecha	Aneded	Jawi	North Achefer
Banja	*	7.35	8.27	5.87	8.79	12.48
Sinan	5.33	*	7.41	9.38	12.09	15.32
Dembecha	5.80	5.96	*	12.55	10.17	16.80
Aneded	6.52	5.97	4.73	*	11.99	15.09
Jawi	5.70	7.55	7.84	8.06	*	12.10
North Achefer	19.25	16.56	18.77	18.00	18.22	*

The procedure of canonical discriminant analysis extracted five canonical variates for the female chicken. For female sample populations, the ratio of between-group variability to within-group variability detected by canonical variate 1 (CAN1) was much larger than the other four canonical variates. The five canonical variates had different discriminatory powers, with a larger proportion (82%) of the total variance explained by the first two canonical variates (Table 5.7). Unlike the female sample population, the Eigen values for the male populations were larger for CAN1, CAN2, and CAN3, indicating their better discriminating capacities (Table 5.7). These three variates explained 90% of the total variance in the population.

Table 5.7. Summary of canonical correlations in female and male chickens

Sex	Functions	Canonical correlation	Eigen values				Likelihood ratio	Approximate F value	Pr>F
			Eigen value	Difference	Proportion	Cumulative			
Female	**CAN1**	0.79	1.65	1.15	0.63	0.63	0.16	38.35	<.0001
	CAN2	0.58	0.50	0.21	0.19	0.82	0.43	22.50	<.0001
	CAN3	0.47	0.28	0.16	0.11	0.93	0.65	16.81	<.0001
	CAN4	0.34	0.13	0.06	0.05	0.98	0.83	11.82	<.0001
	CAN5	0.25	0.07		0.02	1.00	0.94	9.43	<.0001
Male	**CAN1**	0.76	1.34	0.51	0.42	0.42	0.10	16.30	<.0001
	CAN2	0.67	0.82	0.16	0.26	0.68	0.24	13.18	<.0001
	CAN3	0.63	0.67	0.44	0.21	0.90	0.44	10.70	<.0001
	CAN4	0.43	0.23	0.14	0.07	0.97	0.74	6.25	<.0001
	CAN5	0.29	0.10		0.03	1.00	0.91	4.23	0.00

CAN = Canonical

The first two canonical variates (CAN 1 and CAN 2) distinctly separated the female sample populations from North Achefer and Jawi, which were found in lowland areas (Table 5.8). In male chickens, the first three canonical variates (CAN1, CAN2, and CAN3) distinctly separated the sample populations of the North Achefer, Sinan, and Jawi (Table 5.8).

Table 5.8. Class means on canonical variables of female and male chickens

Sex	District/Site	CAN1	CAN2	CAN3	CAN4	CAN5
Female	Banja	0.75	0.48	-0.39	0.57	0.15
	Sinan	0.30	-0.23	-0.88	-0.43	-0.09
	Dembecha	0.59	-0.65	0.41	0.24	-0.48
	Aneded	0.49	-0.88	0.46	-0.14	0.37
	Jawi	0.41	1.21	0.54	-0.34	-0.04
	North Achefer	-3.16	0.01	0.02	0.10	0.01
Male	Banja	-0.41	-0.39	-0.36	-0.14	-0.67
	Sinan	-1.12	-0.01	1.01	0.59	0.06
	Dembecha	-0.77	1.16	0.47	-0.93	0.14
	Aneded	-0.64	-1.28	-0.89	-0.15	0.33
	Jawi	0.74	1.19	-0.96	0.41	0.06
	North Achefer	2.22	-0.59	0.78	-0.13	0.03

CAN = Canonical

5.3.7. Cluster analysis

The hierarchical clustering map for female sample chickens (Figure 5.4) was established using principal components and showed three clusters. About 84% of the variation was explained by dimension 1 (55.4%) and dimension 2 (28.54%). In males, about 82% of the total variation was explained by the first two dimensions (Figure 5.5). While female and male chickens were analyzed together, four distinct clusters were formed (Figure 5.6). Chickens from Banja, Dembecha, and Aneded were clustered together, and chickens from North Achefer, Sinan, and Jawi were clustered independently.

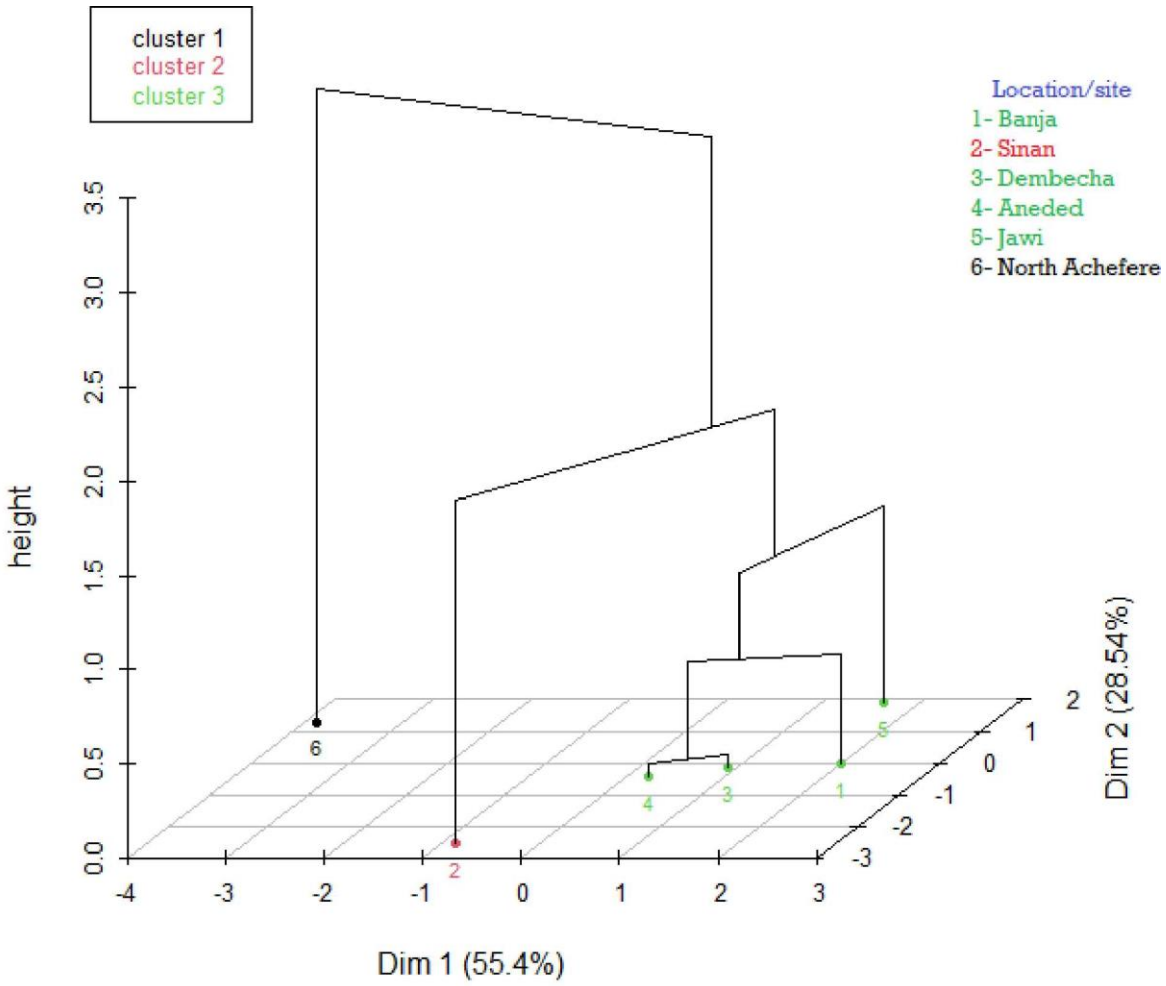

Figure 5.4. Hierarchical clustering on the factor map (3D map) for female chickens by using quantitative traits having a high discriminating power in the classification of chickens

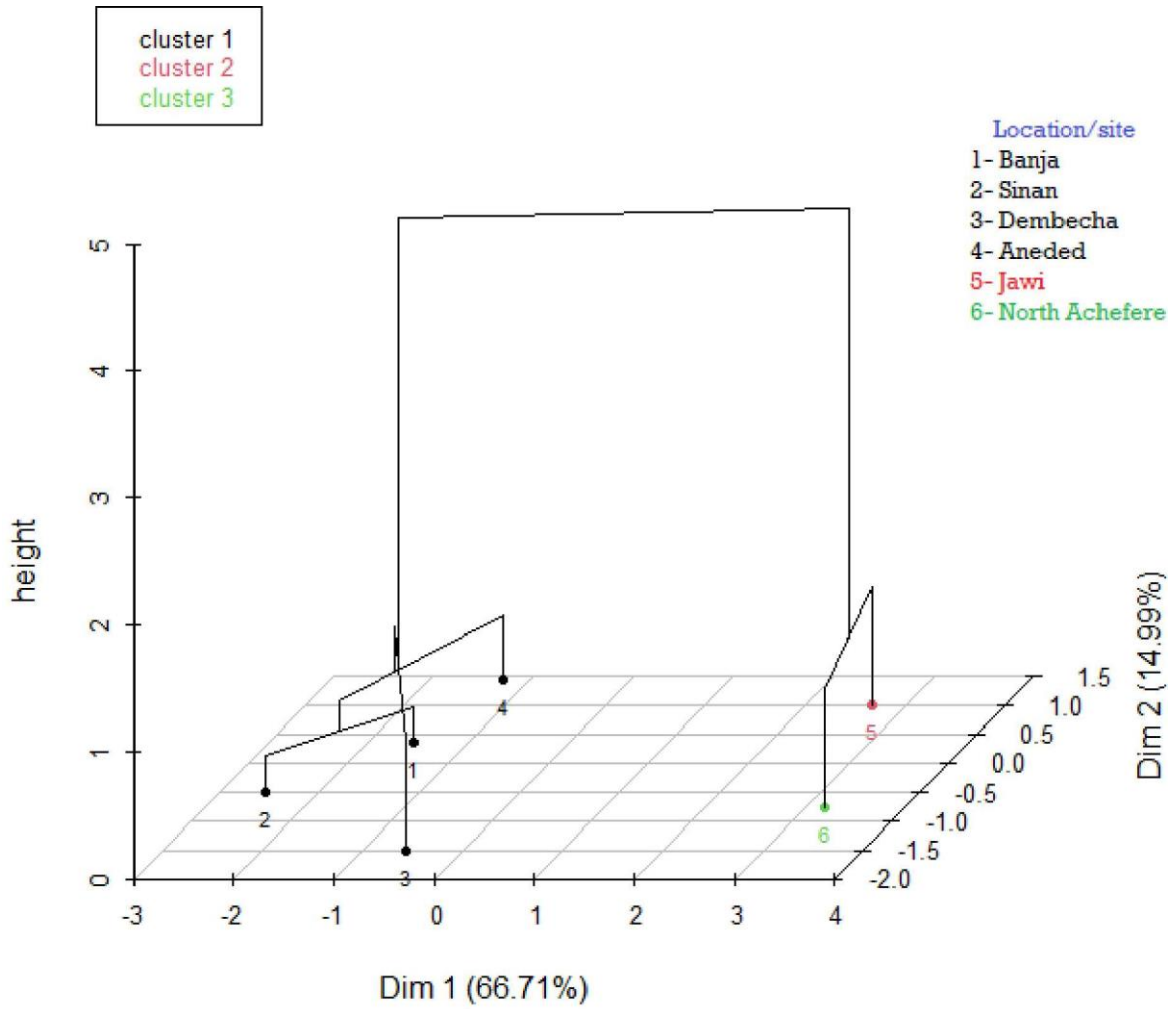

Figure 5.5. Hierarchical clustering on the factor map (3D map) for male chickens by using quantitative traits having a high discriminating power in the classification of chickens

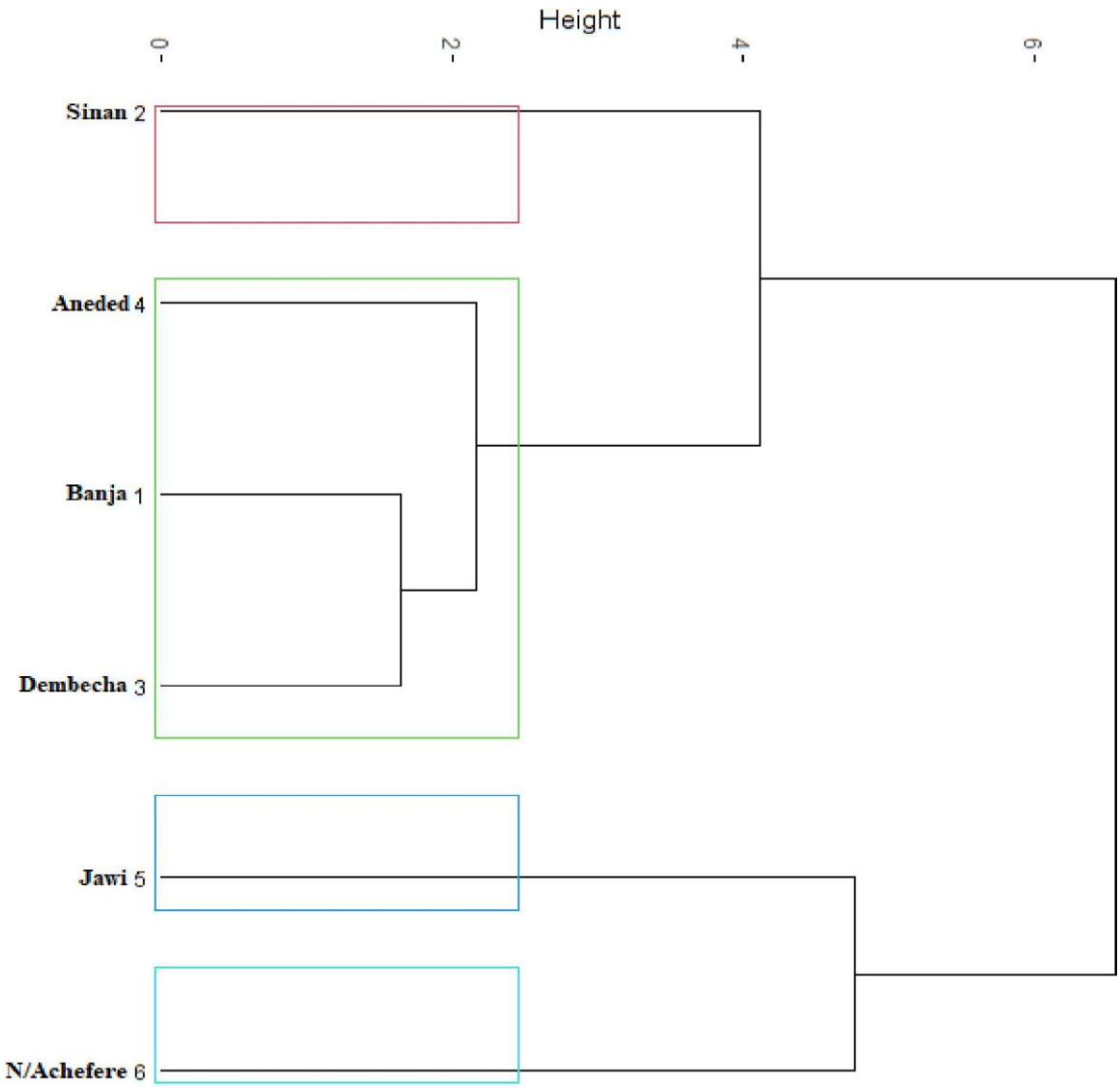

Figure 5.6. Dendrogram constructed based on quantitative traits having a high discriminating power in classification of chickens for two sexes

5.4. Discussions

5.4.1. Qualitative morphological traits

The red plumage colour was the most frequently observed in almost all study areas. Similar results were reported in southern Ethiopia (Melesse & Negesse, 2011), southeastern Oromia, Ethiopia (Negassa *et al*., 2014), and Gambella region (G. Bekele *et al*., 2021). In line with the present study, red was reported as the most dominant plumage colour in those chicken populations from the southern part of Ethiopia, Oromia, and Benshangul-Gumuz regions (Dana *et al*., 2010a). In contrast to the present finding,

25.49% of indigenous chickens in the Northwest parts of Ethiopia had white plumage (Halima *et al*., 2007b). The dominancy of white plumage colour across all chicken populations in Burkina Faso has also been reported (Zare *et al*., 2021). The predominant occurrence of indigenous chickens with red and *Gebisma* plumage colour in the studied area is possibly attributed to farmers' preferences. In some study areas, farmers' do not prefer white colour due to its capability of attracting people, and they believe the eyes of human beings may negatively affect the growth and egg production of the chicken. In addition, some farmers prefer chicken with colour that is not bright to protect it from predators'.

The majority of chickens in this study had yellow shank colour. The result of the current study is in line with the findings in Northwest Ethiopia (Halima *et al*., 2007b), Southern Ethiopia (Melesse & Negesse, 2011), Southeastern Oromia region (Negassa *et al*., 2014) Gambella regions of Ethiopia (G. Bekele *et al*., 2021), and Rwanda (Habimana *et al*., 2021), in which the majority of indigenous chickens had a yellow shank colour. On the contrary, the frequent occurrence of grey and green shank colours in some areas of Southeastern Oromia was reported (Negassa *et al*., 2014). Shank colour indicates the chicken's foraging efficiency, nutritional and immune status, and sexual desirability (Eriksson *et al*., 2008). In the current study, the presence of yellow shank colour was higher in males than females in all the districts. This might indicate the foraging efficiency of males, which enables them to get high carotenes. Similarly, a report of Negassa *et al*. (2014) suggested that cocks might have good access to feed resources than hens as they can compete better. The mixture of white and red earlobe colour was predominant in the indigenous chickens. In contrast to the current study, the majority of indigenous chickens from southern Ethiopia (Melesse & Negesse, 2011) and southeastern Oromia (Negassa *et al*., 2014) had red earlobes. On the other hand, the predominance of yellow earlobes was reported in the Gambella region of Ethiopia (G. Bekele *et al*., 2021). The difference in earlobe colour might be due to their adaptation to the local environment and differences in ancestral lineages (Halima *et al*., 2007b). The nutritional status of birds could also be a possible cause for this variation (Negassa *et al*., 2014). The rare incidence of shank feather in this study is in line with the report in Northwest Ethiopia (Halima *et al*., 2007b), and Gambella region (G. Bekele *et al*., 2021). The predominant white skin colour in this study is different from the reports from different regions of Ethiopia (Dana *et al*., 2010b) and Southeastern Oromia (Negassa *et al*., 2014), which indicated the frequent occurrence of

yellow skin colour. A high proportion of yellow skin colour was found relatively in highland areas (Banja and Sinan). Similar results were obtained in different regions of Ethiopia (Dana *et al*., 2010b), in which the populations in the high altitudes (Sheka, Farta and Horro) were comprised a huge proportions of chickens with yellow skin relative to the others. Besides the agro-ecological region, the proportion of males with yellow skin colour was higher than that of females. This is probably because the scavenging feed resource base is relatively better in the high altitude regions, and cocks have a stronger foraging behavior than the hens.

The present study showed that the majority of the indigenous male chicken possessed rose comb, which is in line with the findings found in Southeastern Oromia (Negassa *et al*., 2014). In contrast to the current study, 55% of chickens were single combed in Southern Ethiopia (Melesse & Negesse, 2011). Similarly, in Gambella region of Ethiopia (G. Bekele *et al*., 2021), the frequent occurrence of single combed indigenous chickens reported. The majority of indigenous chickens found in Northwest Ethiopia (Halima *et al*., 2007b) and different regions of Ethiopia (Sheka, Mandura, Konso, and Farta) (Dana *et al*., 2010b) had pea combs. Single-comb male chickens were culturally not preferred by farmers in all study areas, and this might be the possible driving force behind the low frequency of single-comb chickens. In harmony with the current study, Halima *et al*. (2007b) reported about 51% of indigenous chickens in Northwestern Ethiopia had a plain head shape. Similarly, the frequent occurrence of indigenous chickens with plain heads reported in the Gambella region (G. Bekele *et al*., 2021). On the contrary, most of the chickens in Farta were identified with crest heads (Dana *et al*., 2010b). On the other hand, the majority of indigenous chickens in Southeastern Oromia had snake head shape (Negassa *et al*., 2014). The current result agrees with the findings of Melesse and Negesse (2011), in which the highest proportion of chickens in southern Ethiopia had a normal feather distribution and morphology. Similar results were also reported in the Gambella region of Ethiopia (G. Bekele *et al*., 2021). The naked-neck gene is one of the major genes in indigenous chickens and is important for heat tolerance and adult fitness (Melesse *et al*., 2005). In line with the above statement, small proportions of naked neck chickens were found in lowland areas (Jawi and North Achefer). Unattractive aesthetic nature of these chickens by farmers' might be the possible reason for low distribution of these chickens in the study area.

5.4.2. Quantitative morphological traits

Sex had significant effect on body weight and all linear body measurements. Male chickens were showing higher value for all body measurements than females. In line with the current study, the effect of sex was significant for all traits being higher in males than female indigenous chickens found in Ethiopia (Melesse *et al*., 2021). Similarly, sex had a significant effect ($p<0.05$) on all linear body measurement and body weight of indigenous chickens of Ethiopia with the higher value of male chickens (Bekele *et al*., 2021). The superiority of males over females could be attributed to sexual dimorphism due to differences in the level of male sex hormones, which is responsible for larger muscle development in males than in females (Rotimi *et al*., 2016). The average live weight values of 1.59 and 1.27 kilograms for males and females, respectively, in the current study are comparable with those reported by Melesse *et al*. (2021) in Ethiopian indigenous chickens. But, the values were higher than those reported in Northwest Ethiopia (Halima *et al*., 2007b), Southeaster Oromia (Negassa *et al*., 2014), and Gambella region (G. Bekele *et al*., 2021). The chest circumference reported by Bekele *et al*. (2021) and the wing span reported by Melesse *et al*. (2021) is in line with the current findings. Nevertheless, body length values of indigenous chickens of Ethiopia reported by Bekele *et al*. (2021) were lower, 26.07 cm and 23.52cm for male and female chickens, respectively. On the other study, the higher values of wing span (65.77 cm for males and 56.83 cm for females), shank length (10.19 cm and 8.36 cm), and neck length (18.47 cm and 16.92 cm) reported for the indigenous chickens of Gambella (G. Bekele *et al*., 2021). These variations in traits could be attributed to the genetic background of local chickens and the quality and quantity of the available scavengeble feed resources in different regions.

Agro-ecology and location significantly affected all body measurements. Accordingly, indigenous chickens found in lowland areas (Jawi and North Achefer) had a higher value than others for the majority of their body measurements. However, the Banja indigenous chickens had the longest necks. Similarly, the Aneded chickens had the longest combs compared to the indigenous chickens found at the other sites. These significant differences observed between indigenous chickens from different locations could be due to management practices (Moges, 2010b), and genetic make-up (Apuno *et al*., 2011). The body measurement differences of chickens found in similar agro-ecologies also might be due to the management system used by farmers and the genetic make-up of chickens.

All variables that were subjected to the stepwise discriminant analysis procedure had a significant discriminating power for both sexes. Shank circumference for females and shank length for males showed a high discriminating power. Comb length and beak length had the least discriminating power in female and male chickens, respectively. On the contrary, the body length has shown the highest level of significant discriminating power, while shank length had the least impact in differentiating the chicken populations of indigenous chickens found in the other part of Ethiopia (Melesse *et al*., 2021). In the other study, chest circumference and body weight showed a high discriminating power in indigenous male and female chickens, respectively, which were located in the Metekel zone of Northwestern Ethiopia (Getachew *et al*., 2017). Consistent with the current findings, Getu *et al*. (2014) reported that shank length was the most important variable to discriminate among three chicken ecotypes reared in North Gondar, Ethiopia. The variation of variables in discriminating power for the classification of chickens in different studies might be due to the analysis method utilized and the unique importance of these variables in the classification of chickens in the specific study areas. The overall classification rates for the female and male sample populations were 57.47% and 69.97%, respectively. Comparable to the current study, the correct classification ranged from 41-84% in the case of female chickens found in West Hararghe zone (wolde Kawole *et al*., 2019). In the correct classification percent table, the diagonal values represent the proportion of observations that were correctly classified as belonging to a particular group. Whereas the above and below diagonal values enable us to know the misclassified percentage of groups. The difference between the above and below diagonal values might be due to the size and distribution of the groups being classified or the difference in the sample size between groups.

The female sample populations from Banja, Dembecha, and Aneded and the male sample populations from Banja and Dembecha were more heterogeneous on the quantitative variables, as can be witnessed from their respective low hit ratios. It indicates the populations from these sites were morphologically more closely related to each other. For instance, the discriminant analysis correctly classified 48.8% of chickens in Banja into their respective origin populations, with 16.27%, 13.86%, 7.83%, 13.25%, and 0.00% being misclassified as Sinan, Dembecha, Aneded, Jawi, and North Achefer, respectively. Whereas, in male chickens, the analysis correctly classified 42% of chickens in Banja into their respective populations, with 20%, 4%, 20%, 10%, and 4% being misclassified as

Sinan, Dembecha, Aneded, Jawi, and North Achefer, respectively. The higher classification rates were recorded on North Achefer and Jawi for female chickens. This indicates the homogeneous and distinctive nature of the chicken populations found in these areas.

In the current study, all the squared Mahalanobis distances were highly significant ($P < 0.001$). This revealed that the population from each site is distinct and has its own measurable differences from others. These observations are in good agreement with those obtained in different parts of Ethiopia (Melesse *et al*., 2021) and Metekel zone (Getachew *et al*., 2017). The multivariate statistics for differences between the sites was also significant ($p<0.001$) in all of the four multivariate tests (Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root. The value of Wilks' lambda for the female sample populations was 0.16. This shows that most (84%) of the variability in the discriminator variables was due to differences between populations rather than variation within populations. In females, the shortest distance was measured between Aneded and Dembecha, and the longest distance was measured between Banja and North Achefer. Whereas, in male sample populations, the shortest distance was between Banja and Aneded and the longest was between Dembecha and North Achefer. The shortest distance observed between chickens might be attributed to the sharing of similar genetic identities as a result of non-selection, inbreeding, and migration among these chickens over many generations (Daikwo *et al*., 2015). The squared Mahalanobis distances reported among the Ethiopian indigenous chickens (Melesse *et al*., 2021) ranged from 4.39 to 23.9, which is comparable with the current findings (ranging from 4.73 to 19.25). On the other hand, lower squared Mahalanobis distances ranging from 1.19 to 4.8 and 2.19 to 7.95 for female and male chickens, respectively, were reported for indigenous chickens in West Hararghe zone (wolde Kawole *et al*., 2019). Such variations might arise in the methods applied for computing the Mahalanobis distances and the number of samples used in the discriminant analysis, being higher in a smaller sample size than in a larger (Melesse *et al*., 2021).

The canonical discriminant analysis extracted five canonical variables for female chickens, of which CAN1 and CAN2 accounted for 63% and 19% of the total variations, respectively. In males, three canonical variates, CAN1, CAN2, and CAN3, accounted for 42%, 26%, and 21%, respectively. These canonical variates in female and male chickens were distinctly separated among indigenous chickens found in North Achefer, Sinan, and

Jawi. This observation is somehow comparable with that of indigenous chicken populations in Northwestern Ethiopia, which reported that CAN1 and CAN2 accounted for 66.75% and 33.3% of the total variation, respectively (Getu *et al*., 2014). The higher CAN1 values of 73.2% (Melesse *et al*., 2021), 91.46% (wolde Kawole *et al*., 2019), and 99.36% (Getachew *et al*., 2017) were reported for indigenous chickens in Ethiopia. Three clusters were found for each male and female chicken by utilizing quantitative traits that have high discriminating power in the classification of chickens. Some grouping variation was found in male and female chickens, and it might be due to sample size differences. On the other hand, four distinct clusters were found while the quantitative traits had high discriminating power in classifying male and female chickens together. By combining the above two clustering results, the chickens in the study area could be clustered into four; cluster one (North Achefer), cluster two (Sinan), cluster three (Jawi), and cluster four (Banja, Dembecha, and Aneded).

5.5. Conclusions and Recommendation

The univariate and multivariate analysis of qualitative and quantitative traits revealed the existence of morphological differences between indigenous chickens in different districts. Generally, the results of the analyses enabled us to classify the indigenous chickens found in the study area into four ecotypes: ecotype 1 (Banja, Dembecha, and Aneded), ecotype 2 (North Achefer), ecotype 3 (Sinan), and ecotype 4 (Jawi). Molecular-based characterization studies should be conducted to strengthen the current findings.

CHAPTER 6: EFFECT OF ECOTYPE AND *ENTEROMORPHA* POLYSACCHARIDE SUPPLEMENTATION ON THE GROWTH PERFORMANCE OF INDIGENOUS CHICKENS IN NORTHWEST ETHIOPIA

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Tropical Animal Health and Production (2024) 56:293
https://doi.org/10.1007/s11250-024-04178-6

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Effect of ecotype and *Enteromorpha* polysaccharide supplementation on the growth performance of indigenous chickens in Northwest Ethiopia

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Received: 19 January 2024 / Accepted: 17 September 2024

Abstract

The study was conducted to compare the growth performance of indigenous chickens and evaluate the effect of *Enteromorpha* polysaccharide (EP) supplementation on the growth of chickens. A total of 180 indigenous chicken ecotypes (Sinan, Dembecha, North Achefer, and Jawi) were used in the study. Chicken ecotype and sex had a highly

significant ($p<0.001$) effect on body weight and average daily gain (ADG). The highest final body weight (1811.5±16.6 g) and ADG at twenty weeks of age (12.76±0.12 g) were recorded for the Jawi ecotype, followed by the North Achefer. A significantly higher body weight and ADG in male chickens than female chickens were observed. The EP supplemented (EP+) chickens showed a significantly ($P < 0.05$) higher body weight and ADG than the non-supplemented (EP-) chickens. The interaction effect of ecotype and feed type was not statistically significant on body weight in the starter and grower phases, except at week 9. In the first four weeks, the highest (100%) and lowest (91.7%) survivability rates were recorded for the Sinan ecotype and the Jawi ecotype, respectively. In general, the Jawi and North Achefer ecotypes had better growth performance, and the Sinan ecotype relatively showed better survivability. EP supplementation could improve the growth performance of chickens.

Keywords: Body weight, Ecotype, Enteromorpha polysaccharide, Growth, Indigenous chicken

6.1. Introduction

Poultry production plays a significant role in poverty reduction, food security, and income generation in developing countries including, Africa (Kingori *et al*, 2010; Bettridge *et al*., 2018; Zelleke *et al*., 2022). However, the production of indigenous chickens in these regions encounters several challenges. These challenges include limited access to and high costs of feed resources, climate-related difficulties leading to frequent diseases and increased flock mortality rates, predation, as well as subpar feed efficiency (Mahoro *et al*., 2017; Ngongolo & Chota, 2021; Mujyambere *et al*., 2022; Kpomasse *et al*., 2023). The poultry industry helps the low- and middle-income households to improve their socio-economic conditions and promotes the inclusion of vulnerable groups such as women, people living with disabilities, orphans, and the unemployed (Manyelo *et al*., 2020; Kleyn & Ciacciariello, 2021). Similarly, in Ethiopia, chickens are of significant socioeconomic importance for food security, income generation, as rituals for religious purposes (Gulilat *et al*., 2021), and provide employment opportunities (Tolasa, 2021). Most rural Ethiopian families raise a small to a big number of indigenous and exotic chickens. The total poultry population in Ethiopia is estimated to be about 57 million heads. The percentages of

indigenous, exotic, and hybrid breeds in the total poultry were reported to be 78.85%, 9.14%, and 12.03%, respectively (CSA, 2021).

Despite the enormous population and significant contribution, the performance of local chickens in the smallholder production systems is poor and varies across Ethiopia (Matawork, 2018). The productivity of indigenous chickens can be improved by providing better housing, disease prevention and control, enhanced genetics, and nutrition. According to recent research findings, seaweed polysaccharides improved the performance of chickens in terms of growth and production, breast muscle yield, egg quality, antioxidant capacity, and intestinal morphology (Guo *et al*., 2020; Liu *et al*., 2020; Liu *et al*., 2021; Wassie *et al*., 2021b; Zhao *et al*., 2021). *Enteromorpha prolifera* (EP) is a type of green algae or seaweed that has been utilized for centuries in traditional medicine and food (Wassie *et al*., 2021b). In addition to its nutritional value, the polysaccharides found in *E. prolifera* have been discovered to possess diverse bioactive properties. These include the ability to modulate the immune system, assist in managing diabetes, act as antioxidants, lower lipid levels, exhibit antimicrobial activity, and influence the composition of the gut microbiota (Xu *et al*., 2015; Wassie *et al*., 2021a).

Efforts have been made in Ethiopia to enhance the productivity of the poultry industry by introducing exotic breeds. However, these attempts did not yield the anticipated outcomes primarily due to the demanding management requirements of the chickens (Esatu *et al*., 2022). Long-term breeding programs for chickens shouldn't solely rely on the importation of non-adapted exotic chickens (Belew, 2018). Improvement of indigenous chicken through selective breeding can better achieve the goal for low input and tropical systems (Belew, 2018). Yonas (2020) also suggested that improving the indigenous chicken through selective breeding is better than crossbreeding with non-adapted exotic breeds. The Ethiopian livestock master plan made clear the importance of enhancing indigenous chicken genotypes through selective breeding to boost their genetic potential and ensure sustainable use (Shapiro *et al*., 2015). Increasing the productivity of the indigenous chickens by keeping their better adaptive traits, such as disease resistance and tolerance to harsh environments is crucial. To obtain a better outcome from the selective breeding of indigenous chickens, understanding the real production potential through performance evaluation at the station and farmer level is essential.

In Ethiopia, different scholars, such as Halima *et al*. (2006); Shewangizaw *et al*. (2021), Shewangizaw *et al*. (2022), and Tadele *et al*. (2023) have conducted performance evaluations of indigenous and exotic chicken ecotypes in different management conditions. However, there is a dearth of information on the growth performances of indigenous chickens under intensive management conditions, and up to date, EP's effect on the growth performance of indigenous chickens has not been studied. Therefore, this study aimed to compare the growth performance of indigenous chicken ecotypes and evaluate the effect of EP supplementation on their growth, which could be used as input for designing a sustainable breeding program.

6.2. Material and Methods

6.2.1. Study locations

The experiment was conducted at the Zenzelima campus of Bahir Dar University, located in Northwest Ethiopia at 11° 37′ north latitude and 37° 29′ east longitude and an elevation of 1912 meters above sea level (Figure 3.2).

6.2.2. Source of *Enteromorpha* Polysaccharide (EP)

The polysaccharide was extracted from marine algae *E. prolifera* and provided by Qingdao Seawin Biotechnology Group Co., Ltd. (Qingdao, China). Briefly, the water-soluble sulfated polysaccharides of EP were extracted from E. *prolifera* using an enzymatic method (Liu *et al*., 2021). The algae were washed in distilled water, dried at 60°C, and then minced to create homogenate powder. After the algae powder was soaked in water, it underwent a step-by-step enzymatic treatment using pectinase, cellulase, and papain at 50°C for 1.5 hours. To obtain the polysaccharide products, the enzyme reaction was first inactivated by heating the reaction at 90 to 100 °C for 10 minutes, followed by an instantaneous cooling on an ice bath, centrifugal concentration, ethanol precipitation, and spray drying (Lv *et al*., 2013). The EP contained the monosaccharides rhamnose, glucuronic acid, xylose, glucose, and galactose (Table 6.1).

Table 6.1. The composition of Enteromorpha prolifera polysaccharide supplemented for chickens

Monosaccharides	% composition
Rhamnose (Rha)	40.6
Glucuronic acid (GlcA)	38.2
Xylose (Xyl)	9.3
Glucose (Glc)	5.6
Galactose (Gal)	6.3

Source: Wassie *et al*., 2021b

6.2.3. Experimental birds and management

A total of 500 chicken eggs were collected from smallholder farmers in Sinan (Sinan ecotype = 120), Dembecha (Dembecha ecotype = 120), North Achefer (North Achefer ecotype = 130), and Jawi (Jawi ecotype = 130) from four ecotypes that are identified in the Northwestern part of Ethiopia (Muluneh *et al*., 2023). A total of 180 unsexed chicks were obtained from the hatching, and they were randomly divided into four treatment groups (Sinan ecotype, Dembecha ecotype, North Achefer ecotype, and Jawi ecotype), each of which had ten replications. Each pen or replication consisted of 17-19 chickens, a proportional number of chickens (identified by leg band) from each ecotype. A completely randomized design (CRD) was utilized in the experiment, in which chickens were allocated to each pen randomly. Each pen was consisted from all ecotypes which identified by leg band. The sex of chicks was identified after three weeks of age; of 180 chicks, 88 were females and 92 were males. After the chicks reached five weeks of age, the feed experiment was started, and the experimental design was rearranged with two additional treatment groups (EP-supplemented and non-supplemented). The two treatment groups had five replications. Half of the chickens from each ecotype received a basal diet supplemented with 400 mg EP/kg diet, according to the dose recommended (Guo *et al*., 2020), while others received a basal diet only. The pens, drinkers, feeders, and other equipment were cleaned, formalin-disinfected, and dried before the chicks were moved to the experimental pens. The floor of the deep litter system, in which the chicks were raised, was covered with teff straw at a depth of about 6 cm. Adequate floor space, light, temperature, ventilation, and relative humidity were provided for each treatment. The birds were given vaccinations for the major diseases (Marek's, Newcastle Disease, Gumboro, Fowl Typhoid, and Fowl Pox) as per the recommended vaccination schedule, and they also received treatment as required. All chicken ecotypes were fed commercial

starter ration (Table 6.2) up to week 8 and grower ration from week 9 to week 20 throughout the trial, and they were all given clean and fresh water *ad libitum*. The amount of feed was adjusted weekly by considering the development stages of the chickens (NRC, 1994).

Table 6.2. Nutrient composition of the diet fed to experimental chickens

Nutrients	Starter	Grower
Crude protein (%DM)	20.5	18.5
Crude fat (%DM)	6.5	5.0
Crude fiber (%DM)	5.5	5.8
Calcium (%DM)	0.9	0.9
Metabolizable energy (ME-Kcal/kg)	3000.0	2950.0
Moisture (%)	10.0	10.0

DM= Dry matter; ME-Kcal/kg = Metabolizable energy in Kilo calorie per kg of feed

6.2.4. Data collection

Initial body weight (body weight at hatch) measurements were made on each chicken before placement in the experimental pens, and then chickens were individually weighed at two-week intervals using a sensitive balance for the entire experimental period. The body weight recorded was used to calculate the average daily gain (ADG). There was no data available for the body weight of chicken at week one (BW1) due to technical issues. Feed offered and feed leftovers were measured daily using a sensitive balance to calculate the average daily feed intake (ADFI) and feed conversion ratio (FCR). FCR was calculated as ADFI/ADG. Mortality was recorded as it occurred.

6.2.5. Statistical analysis

The collected data were analyzed using the General Linear Model (GLM) in the SAS 9.4 package to determine the effect of ecotype, sex, and feed type on the growth parameters. The mean comparison was done using the Tukey procedures. The growth performance data are presented as the LSM ± standard error, and statistical significance was considered when $P < 0.05$. Origin software was used to construct graphs.

The models used for the statistical analysis were:

Model 1 (before EP experiment started):-$Y_{ijk} = \mu + E_i + S_j + e_{ijk}$,

Model 2 (after EP experiment started):- $Y_{ijkl} = \mu + E_i + S_j + F_k + EF_{ik} + e_{ijkl}$

Where: Y_{ijk} and Y_{ijkl}= the response variable (the measurement of BW, ADG, and FCR);

μ = the overall mean;

E_i = the fixed effect of ecotype (i= Sinan, North Achefer, Jawi, and Dembecha);

S_j = the fixed effect of sex (j= Male and Female);

F_k = the fixed effect of EP supplementation (k= EP supplemented and non-supplemented);

EF_{ik} = the interaction effect of ecotype and feed type;

e_{ijk} and e_{ijkl} = the effect of random error.

6.3. Results

6.3.1. Ecotype and sex effect on body weights

Ecotype and sex exerted a highly significant ($p<0.001$) effect on body weight (Table 6.3). The overall body weight of indigenous chickens at twenty weeks of age obtained in this experiment was 1640.1g (Table 6.3). The higher (28.3±0.29 g) and lower (25.8±0.38 g) body weights at day old age were recorded for the North Achefer and Jawi ecotypes, respectively (Table 3). Jawi ecotypes recorded the highest final body weight (1811.5±16.6 g), followed by North Achefer (1725.8±12.6 g). A significantly higher ($P<0.001$) body weight was recorded in male chickens than female chickens (Table 6.3).

6.3.2. Feed effect on body weight

There were notable distinctions ($P<0.05$) in body weight during week seven between EP+ and EP- groups, with EP+ exhibiting the highest recorded body weight. The EP-supplemented (EP+) chickens showed a highly significant ($P<0.001$) and higher body weight than the non-supplemented (EP-) chickens in the entire grower phase (Table 6.3).

Table 6.3. The body weight (LSM±SE) of indigenous chicken ecotypes in Northwest Ethiopia at different weeks of ages

Effect and levels	N	BW0	BW3	BW5	BW7	BW9	BW13	BW17	BW20
Overall		26.9±2.13	127.1±21.1	196.2±36.1	368.8±44.9	608.5±64.1	1016.7±82.9	1401.9±84.9	1640.1±92.1
CV %		7.93	16.65	18.4	12.19	10.5	8.15	6.05	5.61
Ecotype		***	***	***	***	***	***	***	***
Sinan	51	26.4±0.30^{b}	122±2.96^{ab}	181.1±5.0^{b}	333.4±6.31^{b}	580.1±8.98^{b}	931.7±11.6^{c}	1297.6±11.9^{c}	1508.5±12.9^{c}
North Achfer	59	28.3±0.29^{a}	135.9±2.89^{a}	203.6±4.93^{ab}	397.5±6.14^{a}	627.5±8.75^{a}	1071±11.3^{b}	1472.7±11.6^{b}	1725.8±12.6^{b}
Jawi	39	25.8±0.38^{b}	116.5±3.82^{b}	189.8±6.52^{b}	406.6±8.11^{a}	660.7±11.6^{a}	1135±14.9^{a}	1550.8±15.3^{a}	1811.5±16.6^{a}
Dembecha	31	26.1±0.44^{b}	129.0±4.45^{ab}	219.7±7.60^{a}	326.7±9.46^{b}	547.1±13.5^{b}	897.3±17.4^{c}	1243.0±17.9^{d}	1477.5±19.4^{c}
Sex		NA	***	***	***	***	***	***	***
Male	92	NA	138.9±2.45^{a}	213.9±4.18^{a}	391.2±5.20^{a}	645.1±7.41^{a}	1071.2±9.59^{a}	1471.6±9.8^{a}	1724.6±10.6^{a}
Female	88	NA	112.9±2.47^{b}	183.2±4.23^{b}	340.9±5.26^{b}	562.6±7.50^{b}	946.5±9.69^{b}	1310.5±9.9^{b}	1537.1±10.8^{b}
Feed type		NA	NA	NS	*	***	***	***	***
EP+		NA	NA	200.6±4.2^{a}	375.8±5.31^{a}	652.6±7.56^{a}	1058.7±9.78^{a}	1439.6±10.0^{a}	1670.7±10.9^{a}
EP-		NA	NA	196.6±4.4^{a}	356.3±5.47^{b}	555.0±7.80^{b}	959.0±10.1^{b}	1342.4±10.3^{b}	1591.0±11.2^{b}
Ecotype*Feed type		NA	NA	NS	NS	*	NS	NS	NS
Sinan, EP+		NA	NA	176.5±7.24	331.8±9.0	650.3±12.8	982.8±16.6	1333.4±17.0	1545.1±18.4
Sinan, EP-		NA	NA	185.7±7.09	335.0±8.82	509.8±12.6	880.6±16.3	1261.8±16.7	1472.0±18.1
North Achfer, EP+		NA	NA	205.1±6.71	403.0±8.35	660.9±11.9	1119.3±15.4	1533.2±15.8	1776.9±17.1
North Achfer, EP-		NA	NA	202.1±7.24	391.9±9.0	594.1±12.8	1023.6±16.6	1412.2±17.0	1674.7±18.4
Jawi, EP+		NA	NA	199.8±9.66	429.9±12.0	702.2±17.1	1163.2±22.2	1586.6±22.7	1838.3±24.6
Jawi, EP-		NA	NA	179.9±8.77	383.3±10.9	619.2±15.5	1106.5±20.1	1515.1±20.6	1784.7±22.3
Dembecha, EP+		NA	NA	220.9±10.0	338.5±12.5	597.2±17.8	969.4±23.0	1305.2±23.6	1522.7±25.6
Dembecha, EP-		NA	NA	218.5±11.4	315.0±14.2	497.0±20.3	825.3±26.2	1180.7±26.9	1432.4±29.1

BW= Body weight; LSM= Least square mean; CV= Coefficient of variation; NA= Not available; EP+ = Enteromorpha polysaccharide supplemented; EP- = Non-supplemented; N= Number of chickens; ^{a,b,c}Means across a row with different superscript letters denote significant differences at P <0.05; NS = Non-significant.

The body weight difference between the ecotypes for both male and female chickens was not clearly identified in most weeks of the starter phase, with some overlapping of the trend observed (Figure 6.1). But, starting from around week seven, it became clearly identified, and the Jawi and North Achefer male and female ecotypes showed consistently higher body weight than the Sinan and Dembecha ecotypes (Figure 6.1).

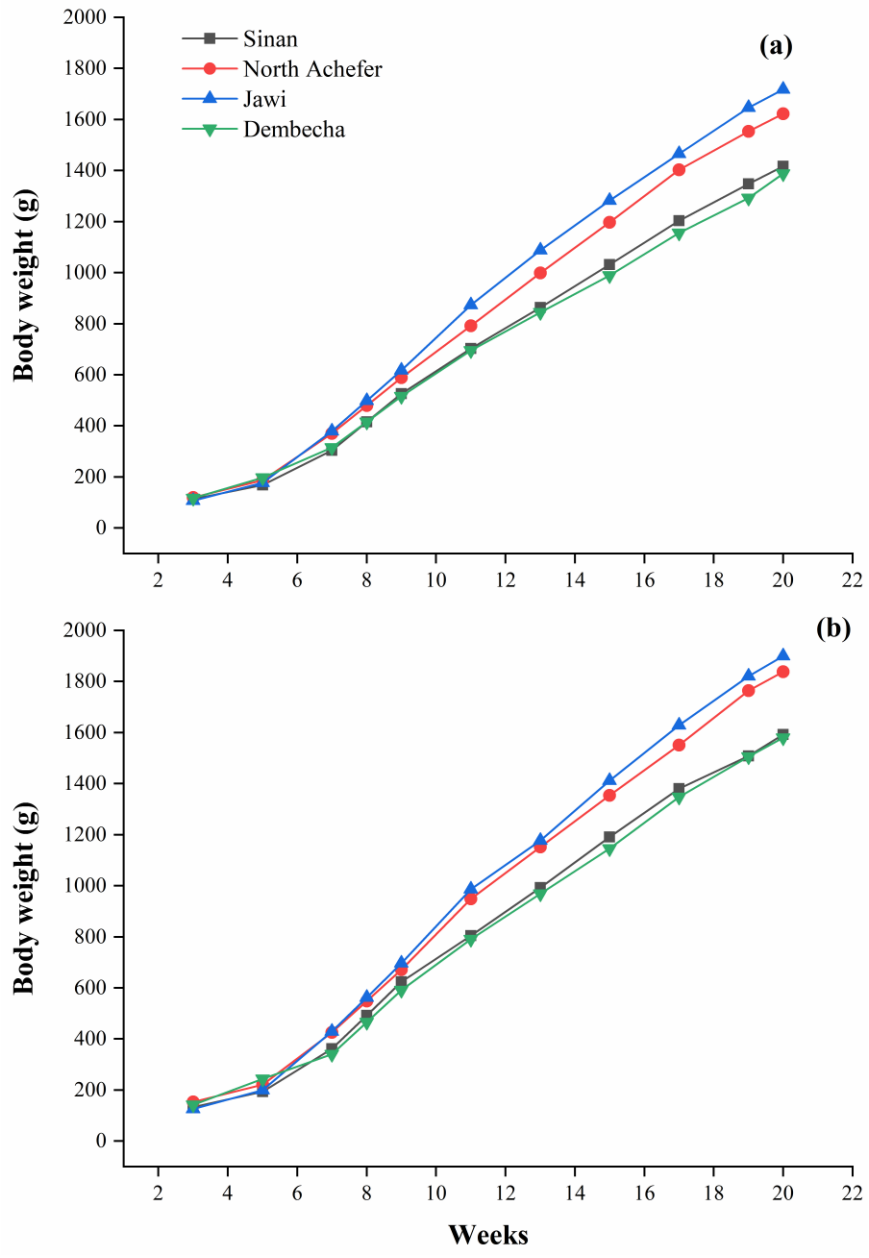

Figure 6.1. The growth of chicks (body weight, g) at different ages for the four ecotypes; (a) female; and (b) male chickens

6.3.3. Ecotype and sex effect on average daily gain

The effect of ecotype and sex on average daily gain (ADG) is demonstrated in Figure 6.2. The current study results indicate that the Jawi chicken ecotype showed a significantly higher average daily gain at twenty weeks in both male (12.60±0.16 g) and female (11.37±0.17 g) sexes compared to the other ecotypes (Figure 6.2). In all ecotypes, male chickens exhibited significantly higher ($p < 0.001$) ADG at twenty weeks than their female counterparts.

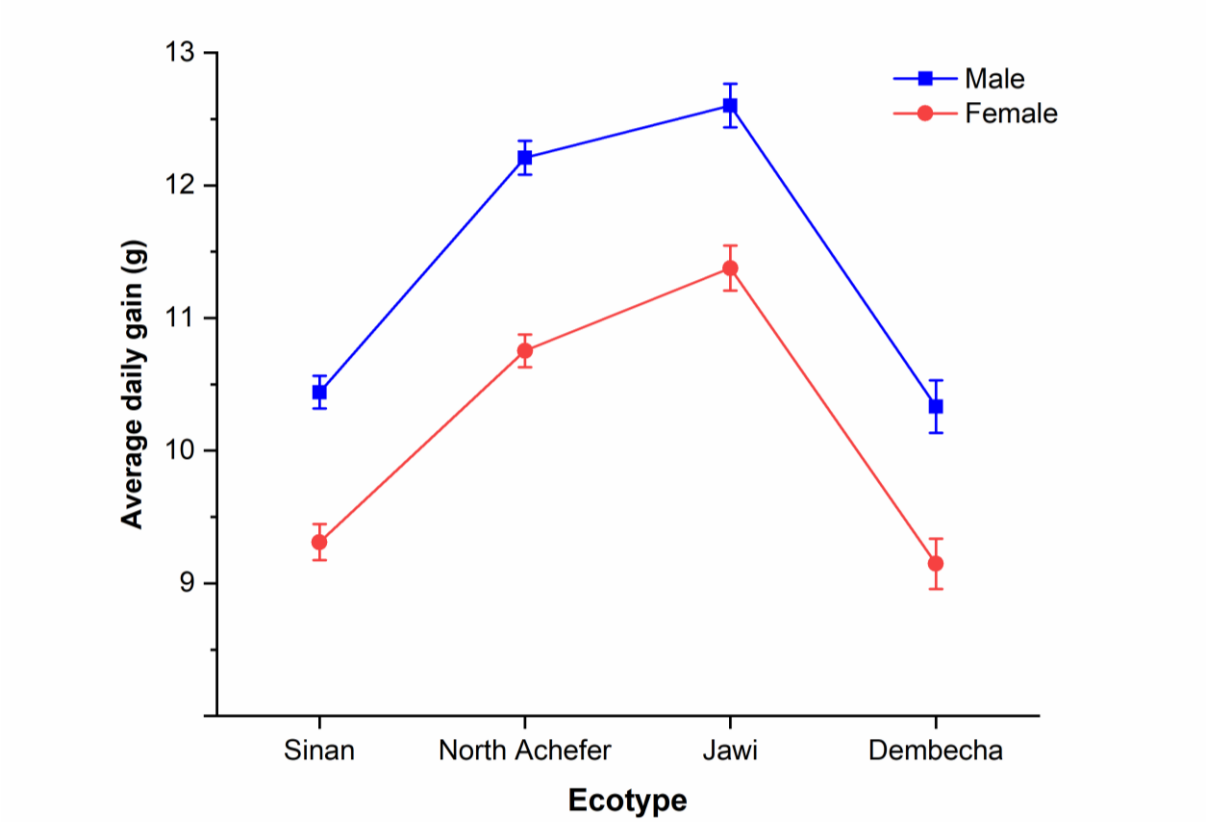

Figure 6.2. The average daily gain (ADG) at 20 weeks for male and female indigenous chicken ecotypes in Northwest Ethiopia

The ADG of chicken ecotypes in starter and grower phases is presented in Figure 6.3. In the current study, Jawi and North Achefer ecotypes showed a significantly higher ($p < 0.001$) average daily gains than Sinan and Dembecha ecotypes in both phases. The ADG ranged from 7.40±0.25 g (Dembecha chicken) to 9.01±0.21 g (Jawi chicken) obtained during the starter phase. In the grower phase, an ADG of 12.07±0.17 g (Sinan chicken) to 14.96±0.22 g (Jawi chicken) was recorded. In the present study, relatively a higher ADG was obtained in the grower phase than in the starter phase for all ecotypes (Figure 6.3).

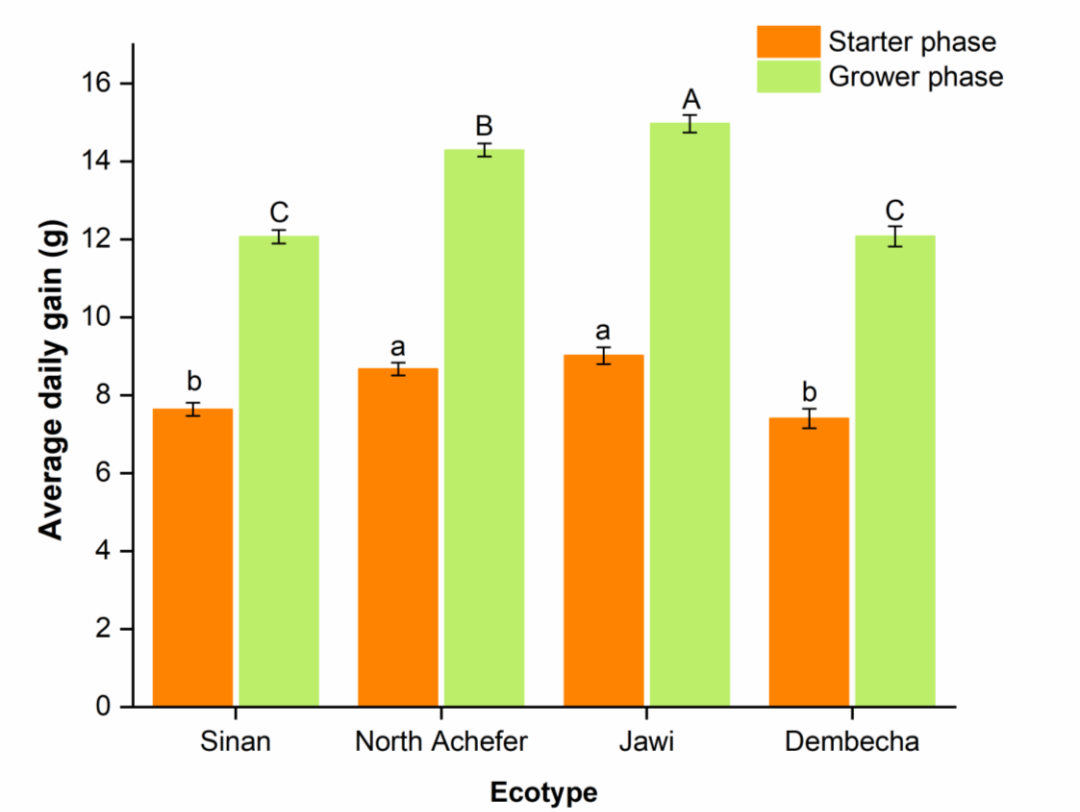

Figure 6.3. The average daily gain of chickens in the starter phase (0-8 weeks) and grower phase (9-20 weeks)

6.3.4. Feed effect on average daily gain and feed conversion ratio

The effects of EP supplementation on average daily gain and feed conversion ratio are shown in Table 6.4. Chickens fed EP had significantly higher ($p < 0.05$) average daily gains than chickens fed basal diet only. However, there were no significant differences ($p>0.05$) in the average daily feed intake and feed conversion ratio between the treatment groups (Table 6.4).

Table 6.4. The effect of EP on the growth performance of indigenous chicken ecotypes in Northwest Ethiopia from five to twenty weeks of age fed on a commercial diet

Parameters	Overall LSM±SE	Treatment group		p-value
		EP+ LSM±SE	EP- LSM±SE	
Initial body weight (g)	195±21.7	198±9.7	193±9.71	0.692
Final body weight (g)	1638±25.9	1678±11.6[a]	1599±11.6[b]	0.001
Average daily gain(g/day)	13.7±0.27	14.1±0.12[a]	13.4±0.12[b]	0.003
ADFI (g/day)	69.7±1.67	69.9±0.74	69.5±0.74	0.690
Feed conversion ratio	5.08±0.18	4.96±0.08	5.19±0.08	0.083

ADFI, Average Daily Feed Intake; EP+, EP supplemented; EP-, non-supplemented; [a,b]Means across a row with different superscript letters denote significant differences at P <0.05

6.3.5. Survivability

The survivability ranged from 91.7% (Jawi ecotype) in week four up to 100% (Sinan ecotype) from week one to week four (Figure 6.4). All Sinan chicken ecotypes survived.

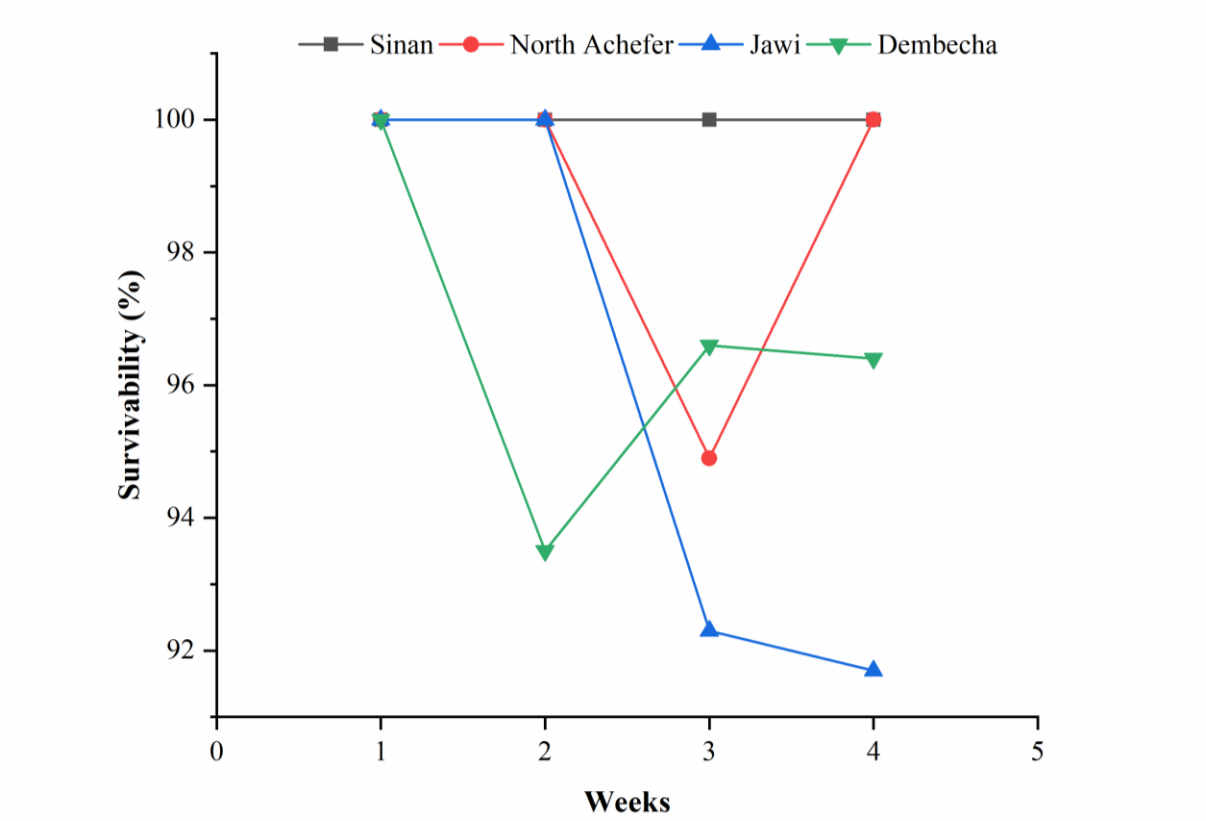

Figure 6.4. The percent survivability (from week one to week four) of indigenous chicken ecotypes in Northwest Ethiopia

6.4. Discussions

The significant effects of ecotype on body weight at different ages might imply the genetic difference of the ecotypes. The significant differences in body weight among the chickens demonstrated that genetic influences are a major contributor to this trait (Mulugeta *et al*., 2020). Similar results were reported by various scholars (Momoh *et al*., 2010; Siwendu *et al*., 2013; Adedeji *et al*., 2015; Musa *et al*., 2015; Amin *et al*., 2017; Alemu *et al*., 2021; Balcha *et al*., 2021; Negash *et al*., 2023), that body weight is significantly affected by genotype. It is worth noting that the final body weights (20 weeks) obtained in this study were higher than those reported by Halima *et al*. (2006) at 22 weeks and Tadele *et al*. (2023) at 18 weeks in different parts of Ethiopia, but lower than those reported by Adomako *et al*. (2014) for naked-neck chickens in Ghana at 20 weeks of age (1895.8 g).

The variations in final body weight between studies could be attributed to several factors, including genetic makeup, environmental conditions, age, and feed quality. In line with the current study, Muluneh *et al*. (2023) reported the highest body weight at about seven months of age for North Achefer (1550±0.01 g) and Jawi (1540±0.01 g) indigenous chickens kept by farmers. The higher body weights obtained in the current study than in the on-farm study could be due to the difference in management of these chickens. Although Jawi ecotypes had the lowest body weight at hatch (day old), they showed the highest body weight at the age of 20 weeks than other ecotypes. The possible reason for the lowest day-old weight might be due to maternal influence due to the size/weight of the egg (Momoh *et al*., 2010). The genetic potential of Jawi chicken could not be expressed at a farmer level, but, in intensive management conditions due to the effect of genetic×environment, the better genetic performance of the Jawi ecotype could be expressed. Hence, the highest final body weight observed in this chicken ecotype.

The clearly identified body weight at the last weeks of the starter phase (weeks seven and eight) and the grower phase but not in the majority of weeks of the starter phase implies that there were differences in the growth rates of these ecotypes, with some exhibiting faster growth rates than others. The observed body weight difference between male and female chickens could be attributed to sexual dimorphism due to differences in the level of male sex hormones and differences in nutritional requirements (Adeleke *et al*., 2011; Benyi *et al*., 2015; Rotimi *et al*., 2016). In harmony with the current findings, Rajkumar *et al*. (2017) and Negash *et al*. (2023) reported significant differences between male and female birds, in which males showed a higher body weight than their female counterparts.

At week five (BW5), the difference between EP+ and EP- was not statistically significant, and it indicated the effect of the polysaccharide in the physiology of the chicken not having started at this stage. In line with the current study, Wassie *et al*. (2022b) reported that EP-fed chickens had a higher ($P < 0.05$) final body weight compared with non-supplemented chickens. The increase in body weight of EP-supplemented chickens observed in this study might be due to an improvement in nutrient absorption through improved gut morphology and function (Wassie *et al*., 2022b). Similarly, other studies (Liu *et al*., 2020; Wassie *et al*., 2021b) have been showed the growth-promoting effects of algae-derived polysaccharides. The absence of a statistically significant interaction effect between ecotype and feed type on body weight during both the starter and grower phases,

except at week 9 (the initiation of the grower phase), may be attributed to a distinct growth trajectory difference. It is plausible that a particular ecotype or a specific combination of ecotypes demonstrates a varied response to the feed type at this specific time point, resulting in the observed statistical significance. The physiological developmental variations due to genetic difference among ecotypes might lead to differences in feed absorption, consequently yielding a significant interaction effect of ecotype and feed type on body weight.

The significant effect of ecotype and sex on ADG suggests the presence of genetic differences and sex dimorphism in this important trait, in line with previous studies by Wondmeneh (2015), Bamidele *et al*. (2020), Shewangizaw *et al*. (2022), Negash *et al*. (2023), and Tadele *et al*. (2023). However, the study by Benyi *et al*. (2015) reported contradictory results, with no observed influence of genotype on body weight gain during the starter period. Although the Dembecha and Sinan ecotypes had a higher day-old body weight than the Jawi ecotype, their ADG was lower than that of the Jawi ecotype. This suggests that high early weight does not necessarily translate to the highest weight gains later in a chicken's life. Consistent with our findings, Benyi *et al*. (2015) reported a significant effect of sex on body weight gain. Notably, Negash *et al*. (2023) also reported a significant effect of sex on body weight gain, with male chickens exhibiting greater gains than females at eight weeks and beyond. Similar to the current study, Mohammed and Dei (2017) recorded an ADG of 7.8 g for the first eight weeks of local Guinea fowl brooded under an intensive management system. On the other hand, Sanka and Mbaga (2014) reported an ADG of 15.2 g in Tanzania for local male chickens under intensive management at 27 months of age. The variation in average daily gain (ADG) between the current chicken ecotypes, among others, can be attributed to a combination of genetic factors, environmental factors (such as feeding and stress), and compensatory growth. These differences in ADG are influenced by variations in the genetic regulation of growth hormones such as growth hormone (GH) and insulin-like growth factors (IGFs) pathways, as well as genetic variations related to skeletal and muscular development, energy metabolism, and nutrient absorption and utilization pathways. Chicken genotypes with the highest body weight had the highest body weight gain, and the lightest genotypes gained the least body weight (Khawaja *et al*., 2013). Even though the study was conducted in a different environment, researchers such as Osei-Amponsah *et al*. (2012), Tolasa (2021),

and Negash *et al*. (2023) found that the heaviest chickens gained more weight than the lightest ones.

The increase in feed consumption, improved digestive capacity, and hormonal changes might be the reasons for a higher ADG in the grower phase than the starter phase. In accordance with this, Momoh *et al*. (2010) reported a relatively higher ADG during the grower phase. In contradiction to this, Zelleke *et al*. (2022) reported a higher weight gain during the starter phase, and the author stated that the ADG in the grower phase decreased due to the reduction of crude protein to 18.5%. The difference in the value of ADG between the starter phase and the grower phase in different studies might be due to the difference in the genetic makeup and composition of feed provided for chickens at these phases. The proportion of each nutrient composition could affect the ADG, and if the chicken is provided with the required amount of nutrients, such as crude protein, any further increase in the nutrient will not increase the ADG.

The significantly higher average daily gains of EP supplemented chickens than chickens fed basal diet only indicate that adding EP to the diet might help to improve growth performance of chickens. Similar findings were reached that polysaccharides produced from algae, including EP could enhance growth performance in chicken (Ribeiro *et al*., 2013; Evans & Critchley, 2014; Liu *et al*., 2020; Wassie *et al*., 2021b). The growth promoting effect of EP may be associated with its enhancement of nutrient absorption by improving intestinal function and morphology, such as by preserving the integrity of the intestinal barrier, promoting the growth of beneficial bacteria in the intestine, by suppressing inflammation, and promoting the growth of intestinal villi and crypts (Wassie *et al*., 2021b). Stimulation of digestive enzyme activities (Long *et al*., 2020), promotion of nutrient digestibility (Liu *et al*., 2019), and the absorption of amino acids (Kong *et al*., 2009) might be the reasons for the growth-promoting effects of polysaccharides.

The mortality rate of 3.4% (Dembecha ecotype at week three) to 8.3% (Jawi ecotype at week four) was in good agreement with the rates reported by Adomako *et al*. (2014) and Zelleke *et al*. (2022), which were 7.89–9.1% and 5.3–9.3%, respectively. Contrary to the present study, Halima *et al*. (2006) reported a higher mortality rate ranging from 52.8-82.4% in indigenous chicken lines in Northwest Ethiopia. The overall lower mortality rate in the current study could disprove the suggestion reported by Halima *et al*. (2006), which

says the confinement of the indigenous chickens results in high stress levels and high morbidity and mortality. The variation in survival rate between the current ecotypes and other genotypes reported by earlier researchers (Lemlem & Tesfay, 2010; Shewangizaw *et al*., 2021; Zelleke *et al*., 2022) could be due to genetic differences, maternal immunity, stress response, or adaptation.

6.5. Conclusion and Recommendations

In conclusion, the study findings indicate that the Jawi and North Achefer ecotypes exhibited better growth performance, while the Sinan ecotype demonstrated relatively higher survivability compared to the other ecotypes. Additionally, the supplementation of EP was found to improve the growth performance of chickens. Before going to the extent of importing exotic chicken breeds, the maximum productivity of the indigenous chickens should be known by improving their environment, such as feed, and using optimum selective breeding.

CHAPTER 7: ANTICOCCIDIAL EFFICACY OF *ENTEROMORPHA PROLIFERA* POLYSACCHARIDE IN INDIGENOUS CHICKENS OF NORTHWEST ETHIOPIA

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DOI: 10.1002/vms3.70037

ORIGINAL ARTICLE

POULTRY

WILEY

Anti-coccidial efficacy of *Enteromorpha prolifera* polysaccharide in indigenous chickens of Northwest Ethiopia

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Abstract

A variety of bioactive compounds isolated from various botanical sources have been found to have therapeutic and immunotherapeutic effects on chicken coccidiosis. This study aimed to evaluate the anticoccidial potential of *Enteromorpha* polysaccharide (EP) in indigenous chickens in Northwest Ethiopia. A total of 78 male indigenous chickens were used for this study. The study had two treatment groups: 1) the EP non-supplemented group (those fed on diets without EP and *Eimeria* oocyst inoculated), and 2) the EP group (those receiving diets supplemented with 400 mg EP/kg diet and *Eimeria* oocyst inoculated). Each treatment group had five replications. Following fourteen days of EP supplementation, 1.5×10^4oocysts of mixed *Eimeria* species were inoculated into individual birds. Results indicated that EP supplemented chicken showed significantly

lower ($P<0.05$) oocyst counts compared to non-supplemented ones on 9 and 11 days post-challenge. In addition, chickens in the EP supplemented group showed less severe lesion scores, with an average score of 1.33. Chickens that received EP showed a maximum of 27.27% protection against lesions. In contrast, the non-supplemented chickens had a lower percentage of protection (19.83%). A relatively higher anti-coccidial index value (147) was obtained from EP-supplemented chickens. Chickens in the EP supplemented group exhibited a significantly higher ($P<0.05$) weight gain. Overall, the inclusion of *Enteromorpha* polysaccharide in chickens' diets shows promise as a potential anticoccidial strategy. However, additional research is required to explore the mechanisms by which *Enteromorpha* polysaccharide in chickens' diet could involve in increasing the protection ability of chickens against coccidiosis.

Keywords: Anticoccidial, Chicken, Coccidiosis, Efficacy, Enteromorpha polysaccharide

7.1. Introduction

Avian coccidiosis is an important apicomplexan parasitic disease of poultry birds, having a negative correlation with the bird's ability to reproduce and perform well in terms of growth (Mumtaz *et al*., 2021). The immune suppression may be brought on in a subclinical form, followed by secondary diseases (Shahid *et al*., 2020). It is one of the main infectious diseases that affect chicken production efficiency and is spread by *Eimeria* (a type of protozoa), having different species (Chapman, 2002). It has been established that seven host-specific *Eimeria* species; *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. necatrix*, *E. praecox*, and *E. mitis* are responsible for economic losses in chickens (Blake *et al*., 2020). In every region of the world, coccidiosis results in a significant financial loss for the production of poultry. This parasitic disease could cause an estimated global economic loss of 2.4-3 billion dollars per year (Kadykalo *et al*., 2018; Gordillo Jaramillo *et al*., 2021; Rizwan *et al*., 2022; Zhang *et al*., 2023). Inadequate ventilation, the presence of sporulated oocysts in the environment for long period, the absence of an all-in-all-out system, and additional stressors such as dietary changes and immunosuppressive effects are all potential risk factors for this disease (Adjei-Mensah & Atuahene, 2023).

Anticoccidial drugs and vaccinations are the main contributors to the prevention and control of this issue but with varying degrees of success (Khater *et al*., 2020). Due to the

associated drawbacks of existing control tactics, such as the growth of drug-resistant strains, the high cost of vaccines, and drug residues in meat and eggs, there is also a growing concern about the use of alternative control approaches (Awais *et al*., 2011). The most popular and widely used technique for the prevention and control of avian coccidiosis is the use of preventive and therapeutic anticoccidial medications (Snyder *et al*., 2021). Numerous alternative methods of control, such as the utilization of probiotics, organic acids, essential oils, and antioxidants, are presently the subject of investigation (Kalkal *et al*., 2021; Raza *et al*., 2022). Nevertheless, polysaccharides derived from plants, algae, and cereals, such as the polysaccharide extract from *Enteromorpha prolifera*, are gaining increasing attention in comparison to these alternative extracts and substances. This heightened interest is attributed to their potential for wide-ranging effectiveness and non-specific immunity. Recent research has underscored the importance of polysaccharides obtained from various plant origins, showcasing their varied biological functionalities. These encompass hypolipidemic, hypoglycemic, antioxidant, anti-tumor, anti-viral, anticoagulant, anti-inflammatory, and immunomodulatory properties (Ahmad, 2021; Long *et al*., 2021; Luo *et al*., 2021; Zhu *et al*., 2021). In addition, they represent prevalent and significant biological macromolecules present in plants, and they are generally acknowledged as safe with minimal toxicity (Ahmad, 2021).

Enteromorpha prolifera (*E. prolifera*) is a seaweed-green algae with a long history of use as a traditional medicine and food (Wassie *et al*., 2021b). One of the primary biologically active components of *E. prolifera* is a sulfated polysaccharide, which is responsible for the immune-modulating, hypolipidemic, antitumor, anti-aging, antibacterial, anticoagulant, antiviral, and anticancer properties (Wassie *et al*., 2021b; Zhang *et al*., 2022). Previous studies indicated that chickens fed seaweed polysaccharides performed better in terms of growth and production, breast muscle yield, egg quality, antioxidant capacity, and intestinal morphology (Guo *et al*., 2020; Liu *et al*., 2020; Liu *et al*., 2021; Wassie *et al*., 2021b; Zhao *et al*., 2021). Even though several bioactive molecules, such as polysaccharides extracted from various botanical sources, have been discovered for their therapeutic and immunotherapeutic effects on chicken coccidiosis, the anticoccidial effects of *Enteromorpha prolifera* polysaccharide (EP) have not yet been well investigated. Therefore, this study aimed to evaluate the anticoccidial potential of EP in chickens.

7.2. Materials and Methods

7.2.1. Study location

The experiment was conducted at Zenzelima campus of Bahir Dar University, located in Northwest Ethiopia at 11° 37′ north latitude and 37° 29′ east longitude and an elevation of 1912 meters above sea level (Figure 3.2).

7.2.2. Source of *Enteromorpha* polysaccharide (EP)

The EP, extracted from the marine algae *E. prolifera*, provided by Qingdao Seawin Biotechnology Group Co., Ltd. (Qingdao, China) was used for the experiment. The EP was extracted following a procedure previously reported by Liu *et al*. (2021). Accordingly, the water-soluble sulfated polysaccharides of EP were extracted from E. *prolifera* using an enzymatic method. Briefly, the algae were washed in distilled water, dried at 60°C, and then minced to create homogenate powder. After the algae powder was soaked in water, it underwent a step-by-step enzymatic treatment using pectinase, cellulase, and papain at 50°C for 1.5 hours. To obtain the polysaccharide products, the enzyme reaction was first inactivated by heating the reaction at 90 to 100 °C for 10 minutes, followed by an instantaneous cooling on an ice bath, centrifugal concentration, ethanol precipitation, and spray drying (Lv *et al*., 2013). High-performance liquid chromatography (HPLC), in accordance with the previously reported protocols (Yu *et al*., 2017), was used to determine the monosaccharide composition. According to the HPLC analytical findings, the EP employed in this study contained the monosaccharides rhamnose (Rha), glucuronic acid (GlcA), xylose (Xyl), glucose (Glc), and galactose (Gal), with the molar percentages being 40.6%, 38.2%, 9.3%, 5.6%, and 6.3%, respectively.

7.2.3. Preparation of parasite for infection

Fecal samples were collected from the local chicken suspected of being naturally infected with coccidiosis around Bahir Dar, Ethiopia. The samples were checked for the presence of coccidial eggs (oocysts) using the floatation technique, following the procedure used by Wondimu *et al*. (2019). The absence of other protozoal oocysts and helminth eggs were checked during various stages of examination, such as direct fecal smear and flotation

techniques, sporulation assessment, and oocyst dose estimation using the McMaster egg counting slide. To ensure the uniform concentration of *Eimeria* oocysts in all chicken droppings, we thoroughly mixed all positive samples using a pestle and mortar, adding 2.5% potassium dichromate ($K_2Cr_2O_7$) for sporulation using the standard guidelines provided by Ryley *et al*. (1976). The sporulation of oocysts was confirmed with direct microscopic examination. The number of oocysts per gram (OPG) of droppings was estimated using the McMaster slide. Sporulated oocysts were washed with phosphate-buffered saline and used in the challenge experiment by adjusting the dose to 1.5×10^4 with phosphate-buffered saline (PBS) following previous studies by Reid (1970) and Chapman (2002). Each challenge dose was taken with shaking to prevent variations in the number of oocysts in the suspension.

7.2.4. Experimental design and chicken management

A total of 78 adult indigenous male chickens were used in the study. Until they reached this age, they were kept in an intensive management system and received vaccinations for the major diseases (Marek's, Newcastle Disease, Gumboro, Fowl Typhoid, and Fowl Pox) as per the recommended vaccination schedule, and they also received treatment as required. The chickens were fed a commercial starter ration (up to week 8) and a grower ration (from week 9 to week 20), which were obtained from Alema Koudjis Feed PLC., and they were given clean and fresh water *ad libitum*. Prior to allocation to each pen, the body weights of all chickens were measured and then chickens were randomly assigned into two treatment groups consisting of 39 chickens each, with five replications using a Completely Randomized Design. The body weight of the chickens in each treatment group was kept similar. Eight chickens were allocated to each pen, except for two pens that contained seven chickens each. The first group (Control group) was fed a commercial ration, and the second group received a commercial ration supplemented with 400 mg EP/kg diet (EP group), according to the recommended dose (Guo *et al*., 2020), for 14 consecutive days. The amount of feed was adjusted weekly by considering the developmental stages of the chickens (NRC, 1994). Chickens were checked for coccidiosis before the start of the experiment and after fourteen days of the start of the experiment, the EP supplemented and non-supplemented groups were infected with 1.5×10^4oocysts of mixed *Eimeria,* including *E. acervulina, E. necatrix, E. maxima*, and *E. tenella* per bird

with PBS. The body weight of chickens was recorded before inoculation, and on the 12th day post-inoculation (the final body weight). Since egg shedding was expected after four days (Mesa-Pineda *et al*., 2021), a fecal (dropping oocyst) count was taken on days 5, 7, 9, 11, and 12 post-inoculation. A total of 24 chickens (12 from the EP supplemented group and 12 from the non supplemented group) were slaughtered for small intestine and ceca lesion evaluation.

7.2.5. Data collection

The anticoccidial efficacy of EP was assessed based on body weight gain, feed conversion ratio (FCR), survival rate, oocyst value, lesion score, percent protection against lesions, and, the anti-coccidial index (ACI). The initial body weights (IBW) and final body weight (FBW) were used to calculate the average daily gain (ADG).

$ADG = (FBW - IBW)/(\text{No. of experimental days})$.

Feed offered and feed leftovers were measured daily using a sensitive balance to calculate the average daily feed intake (ADFI) and feed conversion ratio (FCR). FCR was calculated as ADFI/ADG. The survival rate was estimated by dividing the number of survived chickens by the initial number of chickens. The relative weight gain rate was determined by dividing the weight gains by initial body weights. The oocyst value was calculated using the formula described previously (Shah *et al*., 2010). The percent protection against lesions was calculated using the formula;

$\text{Protection against lesions\%} = (\text{IUG} - \text{ITG/IUG}) \times 100$; Where IUG = Infected Untreated/EP Non-supplemented Group; ITG = Infected Treated/ EP supplemented Group (Khaliq *et al*., 2017). Lesion scores of the chickens from both groups were calculated by the method of Reid (1970); 0 for healthy or showing no signs of infection; 1 for mild lesions; 2 for moderate lesions; 3 for severe lesions; and 4 for extremely severe lesions.

The Anti-coccidial index (ACI) was calculated based on the formula described by Zhi-Qiang *et al*. (2022):

$ACI = (\text{relative weight gain rate} + \text{survival rate}) - (\text{lesion rate} + \text{oocyst rate})$.

7.2.6. Statistical analysis

The collected data were analyzed by using R 4.1.3 software through the Mann-Whitney U test. Origin software was used to construct graphs. Data presented as a percentage, index

value, and mean value was considered statistically significant when $P < 0.05$. The model used for statistical analysis was:

$Y_{ij} = \mu + F_i + e_{ij}$, Where:

Y_{ij}= the response variable

μ = the overall mean;

F_i = the fixed effect of feed type (i= EP supplemented and non-supplemented);

e_{ij} = the effect of random error.

7.3. Results

7.3.1. Oocyst count

The EP administered chickens showed significantly lower ($P<0.05$) oocyst counts as compared to non-supplemented chickens on days 9 and 11 post-challenge (Figure 7.1).

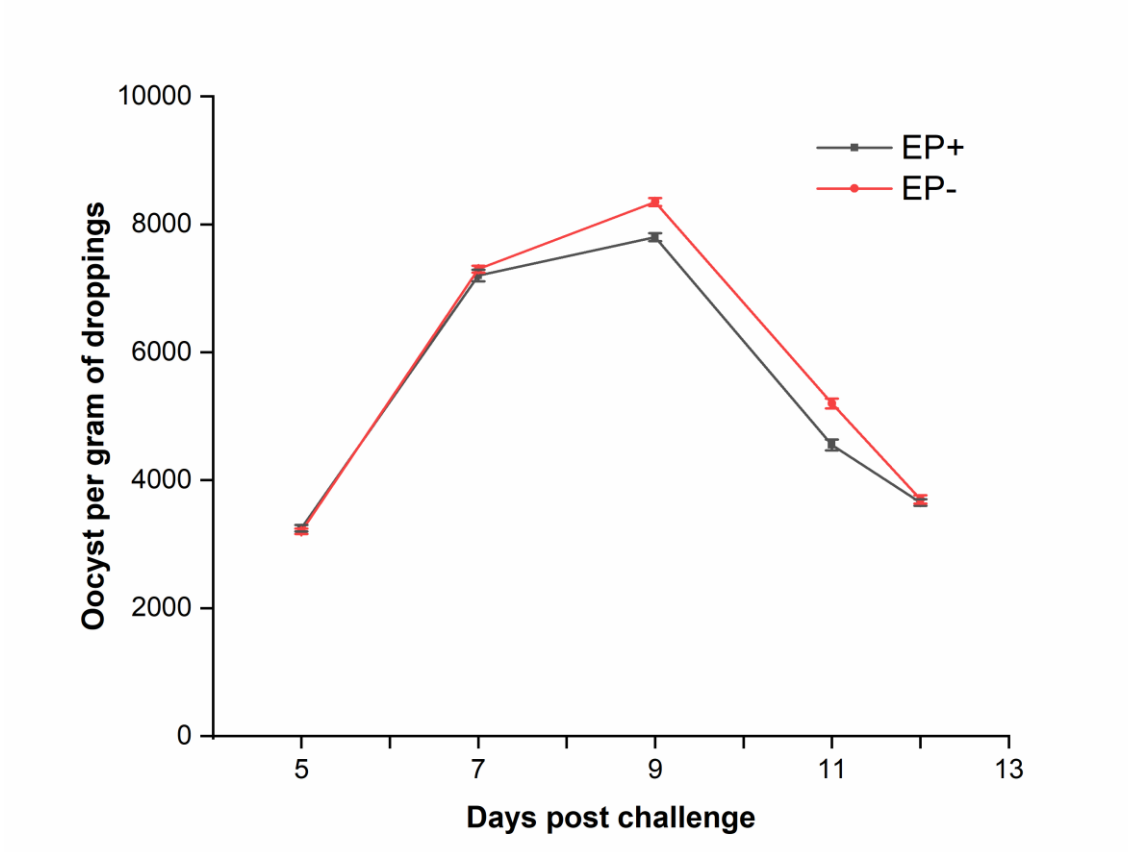

Figure 7.1. Oocyst per gram of droppings in *Enteromorpha* polysaccharide (EP) supplemented (EP^+) and non-supplemented (EP^-) chicken groups challenged with *Eimeria* species

7.3.2. Lesion scores and protection against lesions

Chickens from the EP supplemented group had significantly lower ($P < 0.05$) severe lesion score (1.33) than non-supplemented ones, which showed a relatively higher lesion score (1.83). A significant ($P < 0.05$) higher percent protection against lesions (27.27%) was observed in chickens supplemented with EP than non-supplemented chickens (Table 7.1).

Table 7.1. The lesion scoring and percent protection against lesions for Enteromorpha polysaccharide (EP) supplemented (EP+) and non-supplemented (EP-) chicken groups

	Total lesion scoring of chickens					Protection against
Treatment groups	**0**	**1**	**2**	**3**	**4**	lesions (%)
EP^+	0	8	4	0	0	27.27^a
EP^-	0	2	10	0	0	19.83^b

[a,b]Means across a row with different superscript letters denote significant differences at $P < 0.05$; A lesion score from 0 (No lesion) to 4 (extremely sever lesion) was given based on Reid (1970)

7.3.3. Anti-coccidial indices

Relative weight gain rate, survival rate, lesion value, and oocyst value were determined to calculate the anti-coccidial index (Table 7.2). A relatively higher anticoccidial index value (147) was obtained from EP-supplemented chickens than from EP-non-supplemented chickens.

Table 7.2. Anticoccidial evaluation parameters used for calculation of ACI in *Enteromorpha* polysaccharide (EP)-supplemented and non-supplemented indigenous chickens of Northwest Ethiopia

Parameters	Treatment groups	
	EP^+	EP^-
Relative weight gain rate (%)	53.0^a	25.0^b
Survival rate (%)	100	100
Lesion value	1.33^b	1.83^a
Oocyst value	4.68	4.91
Anti-coccidial index (ACI)	147	118

[a,b]Means across a row with different superscript letters denote significant differences at $P < 0.05$

7.3.4. Post-challenge average daily gain and feed conversion ratio

The average daily weight gains and feed conversion ratio of EP supplemented and non-supplemented chickens during the coccidiosis challenge were calculated (Figure 7.2). The weight gain difference between chickens in the EP^+ group and EP^- group was statistically significant ($P < 0.05$) but EP supplementation had no significant effect on the FCR of chickens.

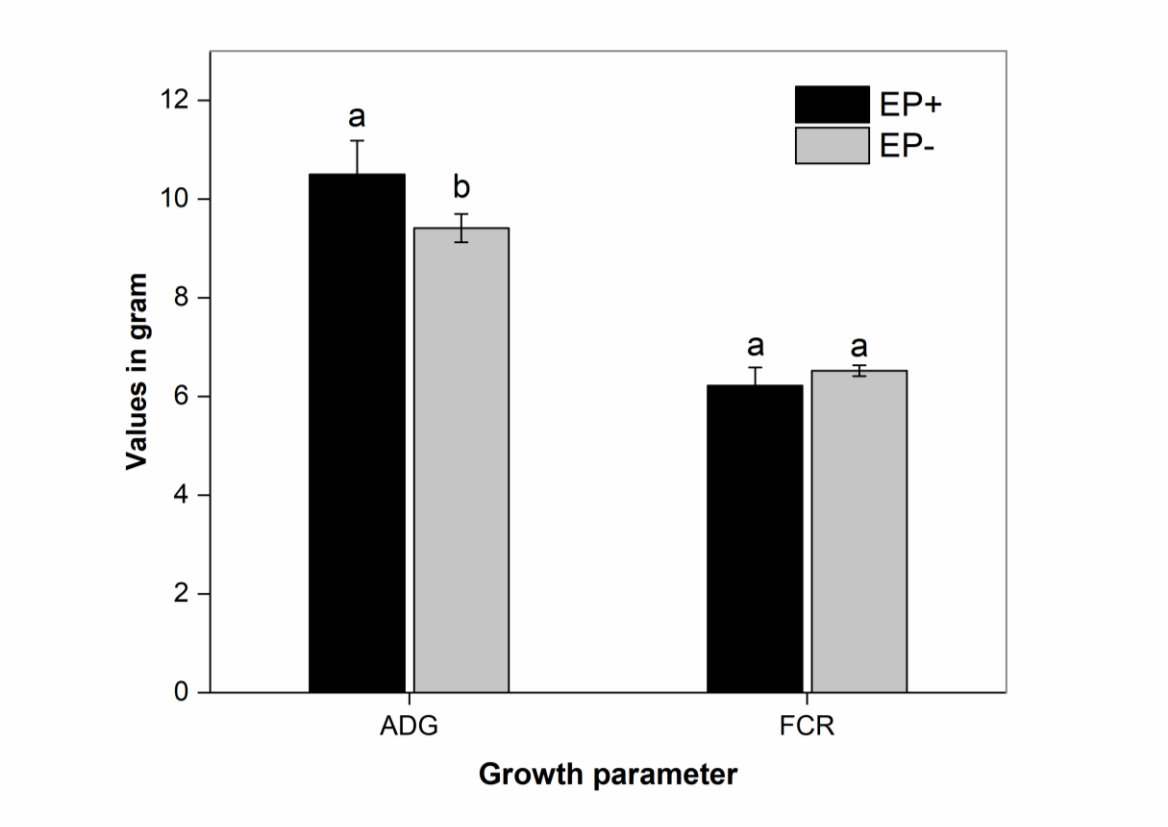

Figure 7.2. The average daily gain (ADG) and feed conversion ratio (FCR) of EP supplemented (EP^+) and non-supplemented (EP^-) chicken groups during the coccidiosis challenge

7.4. Discussions

Previous findings demonstrated that sulfated polysaccharides from marine algae elicit a variety of biological actions, including immune modulation, antioxidant, anti-diabetic, and hypolipidemic effects, in addition to its usage as food and traditional medicine (Wassie *et al.*, 2021a).

In the current study, the chickens fed EP had relatively reduced oocyst counts. Similar results were obtained by another study that focused on various plant-derived polysaccharides and herbs (Ullah *et al*., 2014; Ali *et al*., 2019; Zhang *et al*., 2020). The activation of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) by EP may have an anti-sporulation impact by interfering with the parasite life cycle, such as blocking oxygen access and inhibiting certain sporulation-related enzymes. The TLR4, and NF-B signaling pathways are activated by EP supplementation, which prompts an immunological response (Zhang *et al*., 2022). The reduced oocyst count in the chickens receiving EP supplements might be related to the improvement of cytokines (IL-2, IL-4, and IFN-γ) mRNA expression and Toll-like receptor (TLR-4) genes, which increase mucosal immunity. In another study, Zhi-Qiang *et al*. (2022) reported that IL-2, IFN-*γ*, and IL-17 cytokines play important roles in resistance to coccidiosis infection.

The chickens fed EP had fewer intestinal lesions compared to the EP non-supplemented chickens. Similar conclusions were made by Ullah *et al*. (2014) and Amerah and Ravindran (2015), who reported that chickens fed different plant-derived polysaccharides and compounds, exhibit lower lesion scores than control ones. According to Khaliq *et al*. (2017), the effects of polysaccharides on the microbes of the intestinal tract, reduced bowel putrefaction that subsided or decreased inflammation, or the lining of the intestine layer with aloe biomolecules could all be contributing factors to these less severe intestinal lesions. In a previous investigation, mucin-2 and occluding-1 mRNA expressions were shown to be increased in the ileum and jejunum of chickens receiving EP supplements (Wassie *et al*., 2021b). This phenomenon could decrease the intestinal lesion in EP-consuming chickens as the mucus secreted by mucin-2 protects the epithelial cells from pathogenic microbes. Dietary supplementation of plant-derived polysaccharides increases the serum concentration of IgA (Zhang *et al*., 2022). This IgA can lessen the number of bacteria by binding them to the designated antigens, limit their access to the mucosal membrane of the intestine, and also lessen the severity of lesions (Kulkarni *et al*., 2010).

A higher percent protection against lesions was observed on chickens supplemented with EP than non-supplemented chickens. Previous studies also reported that *A.vera* polysaccharides increased intestinal macrophage and T-lymphocyte activity to prevent the penetration of *Eimeria* species, leading to higher protection rates and lower lesion scores in infected birds (Khaliq *et al*., 2017). EP supplementation could increase caecal Short

Chain Fatty Acids (SCFAs) content, especially acetate, butyrate, and propionate, indicating that EP improved caecal metabolites (Wassie *et al*., 2021b). The SCFAs, particularly butyrate, and propionate, provide energy for the immune cell by triggering intestinal gluconeogenesis and enhancing the synthesis of inflammatory and effector cytokines and antigen presentation (Shi *et al*., 2011). The relatively higher percent protection against lesions in chickens supplemented with EP might be due to the enhancement of SCFAs in the gut that contributes to intestinal immune response and gut barrier function (Yu *et al*., 2004). The anti-coccidial index (ACI) reflects the comprehensive ability of any compound against coccidial infection. According to Zhi-Qiang *et al*. (2022), values lower than 120 depict that the compound or drug has no anticoccidial activity, whereas a medium effect ACI is between 120 and 160; a good effect ACI is between 160 and 180; and an excellent effect ACI is more than 180. In the present study, EP administered chickens exhibited an ACI value of 146.98, so EP can be considered a partially effective therapeutic/immunotherapeutic regime against coccidiosis. An ACI value of 118.26 was also recorded for EP^- chicken group, which could be due to the self-limiting nature of the *Eimeria* infection in chicken due to genetic and environmental factors, such as host age and infective dose.

In the current study, the EP group exhibited relatively a higher average daily weight gain post- challenge. Similarly, Awais *et al*. (2018) reported higher daily weight gains in chickens administered with polysaccharides from sugarcane bagasse. Improvement of intestinal health in EP administered groups might be the reason for their relatively higher weight gain. The non-significant difference in feed conversion ratio among treatment groups might be due to the effect of other environmental factors, such as feed intake and nutrient utilization.

7.5. Conclusions and Recommendation

The results of this study suggest that incorporating *Enteromorpha* polysaccharides into the diet of chickens may serve as an effective anticoccidial strategy. Therefore, the current findings provide new insights for using EP as an immune stimulant in chickens. However, further investigations focusing on microbiota profiles are necessary to elucidate the underlying mechanism of action.

CHAPTER 8: GENERAL DISCUSSION

The study encompassed a multifaceted exploration of indigenous chicken production in Northwest Ethiopia, shedding light on crucial aspects ranging from selection criteria and husbandry practices to morpho-biometric characteristics of indigenous chickens, growth performance evaluation, and anticoccidial efficacy of *Enteromorpha prolifera* polysaccharide. The interconnected objectives of the study form a cohesive framework aimed at enhancing sustainable indigenous chicken production. By delving into the selection criteria and husbandry practices of indigenous chicken producers, the study provides insights into locally adapted breeding and management strategies crucial for maintaining genetic diversity and resilience. Morpho-biometric characterization further aids in understanding the phenotypic diversity within indigenous chicken ecotypes, facilitating informed breeding decisions for improved productivity and adaptability. Evaluating the effects of ecotype and *Enteromorpha prolifera* polysaccharide supplementation on growth performance not only enhances efficiency but also highlights sustainable practices that optimize resource utilization and health outcomes. Lastly, the assessment of anticoccidial efficacy addresses a critical aspect of disease management, promoting healthier flocks and reducing reliance on conventional medications, thereby contributing to the long-term sustainability of indigenous chicken production. Together, these objectives underscore the importance of a holistic approach that integrates traditional knowledge with scientific advancements to foster resilience, productivity, and welfare in indigenous chicken farming systems, ensuring a sustainable future for the chicken production sector.

Indigenous chicken producers in Northwest Ethiopia prioritize egg production potential and better growth rate for income generation when selecting their breeding stock. These priorities were influenced by agro-ecology which shapes their trait preferences and breeding goals to align with local conditions and farmer needs, ultimately enhancing flock productivity and sustainability (Desta & Wakeyo, 2024). Nutritional regimens, housing setups, and vaccination strategies are crucial for sustainable chicken farming. The diversity in chicken flock compositions reflects varied breeding objectives impacting egg production, genetic diversity, and fertility. Socio-cultural influences and farming imperatives shape farmers' preferences regarding plumage color, breeding practices, and

culling criteria in Ethiopia, with a focus on traits like plumage color and body weight, particularly favoring red plumage (Tilahun *et al*., 2022; Muluneh *et al*., 2023). Farmers' choices regarding plumage color, breeding practices, and culling criteria are influenced by economic motives, cultural values, and the perceived resilience of local chickens to diseases (Kapella *et al*., 2023). Challenges such as inbreeding, disease susceptibility, and predation risks underscore the importance of extension services, awareness campaigns, and vaccination drives to ensure chicken welfare. The integration of traditional and modern medicinal practices showcases farmers' adaptability in managing their flocks' health demands.

The morpho-biometric study reveals prevalent traits among indigenous chicken ecotypes in Northwest Ethiopia. Variations in body measurements among indigenous chickens from different locations stem from factors like agro-ecology, management practices, and genetic makeup. It is worth noting that while agro-ecology plays a crucial role, the management practices employed by farmers and the genetic diversity of the chicken populations are also influential factors. Consequently, in the current study, the emphasis on analyzing differences based on location rather than agro-ecology is justified by the distinct variations observed within apparently similar agro-ecological settings. Environmental conditions significantly influence the distinct morphological traits of indigenous chickens, demonstrating variations in body size and shape influenced by factors like altitude (Melesse *et al*., 2021; Kebede *et al*., 2024). Management strategies such as breeding and feeding practices significantly impact the growth and development of indigenous chickens, leading to observable differences in body measurements across regions (Muluneh *et al*., 2023; Xie *et al*., 2024). The interplay of management practices with local ecological conditions complicates phenotypic outcomes, emphasizing the genetic factors' role in indigenous chickens' adaptability, with genetic diversity crucial for survival in different environments. Genetic makeup, management practices, and genetic diversity collectively shape the phenotypic characteristics of indigenous chicken populations. Farmers' preferences for plumage color influenced by socio-cultural factors and market demands, may confer survival advantages through signaling and potential links to foraging efficiency. Specific genes influence color traits, suggesting a genetic basis for coloration variations. The presence of carotenoid pigments in plumage reflects foraging success and stress management abilities, further associating color with foraging efficiency. Varied morphological traits, such as earlobe colors, comb types, and head shapes underline the

genetic diversity and environmental influence on indigenous chicken populations, with location-specific variations and sex disparities highlighted through discriminant analyses and clustering methods.

The performance evaluation study emphasized the significant impact of ecotype on body weight and growth patterns, reflecting the underlying genetic diversity. Genetic factors were identified as crucial determinants of variations in body weight, aligning with previous research findings. A study on Chinese indigenous chickens highlighted key genes like NELL1, XYLT1, and NCAPG/LCORL, which are strongly associated with body weight traits (Wang *et al*., 2023). In Ghanaian local chickens, high heritability estimates (0.51-0.69) for body weight traits were discovered, with specific genomic regions explaining a substantial portion of genetic variance (Kanlisi *et al*., 2022; Kanlisi *et al*., 2024). Research on Mazandaran indigenous chickens revealed sexual dimorphism in body weight, with distinct heritability estimates for males and females, suggesting independent selection potential (Esmaeili *et al*., 2024). A genome-wide study across four Chinese breeds indicated a polygenic basis for body weight, with different genetic loci contributing to phenotypic variability, underscoring the necessity for tailored breeding strategies (Yuan *et al*., 2018). Variations in growth rates and average daily gain (ADG) underscored the complex interplay of genetic factors, environmental influences, and hormonal dynamics. Supplementation with *Enteromorpha* polysaccharides showcased a significant impact on growth performance by enhancing nutrient absorption and growth trajectories. Studies demonstrated that *Enteromorpha* polysaccharide supplementation led to improved ADG and feed conversion ratios in broilers, particularly under heat stress conditions (Liu *et al*., 2020; Liu *et al*., 2023). Dietary inclusion of EP-Zn was shown to notably increase body weight and muscle antioxidant activity while enhancing amino acid digestibility (Wassie *et al*., 2022). The positive effects of EP supplementation extended to the expression of genes related to amino acid transport and metabolism, ultimately improving nutrient absorption (Zhao *et al*., 2021; Wassie *et al*., 2022). Additionally, EP supplementation was associated with enhanced intestinal barrier function, crucial for effective nutrient absorption, and contributed to reduced oxidative stress and improved gut health, ultimately supporting growth performance (Liu *et al*., 2020; Akter *et al*., 2024).

Research on the anticoccidial efficacy of *Enteromorpha prolifera* polysaccharide highlighted its potential in mitigating coccidiosis infections in indigenous chicken

ecotypes. The study revealed reduced oocyst counts, diminished intestinal lesions, and enhanced weight gain, indicating the moderate anticoccidial effects of *Enteromorpha* polysaccharide supplementation. The immunomodulatory properties of *Enteromorpha* polysaccharides were suggested to enhance immunity, activate antioxidants, and promote gut health. Dietary supplementation of EP was found to increase the immune organ index and levels of key immune markers such as secretory immunoglobulin A (SIgA), interferon-gamma (IFN-γ), and interleukin-2 (IL-2) in broilers, boosting their response to infections and vaccinations (Sun *et al*., 2017). Under heat-stressed conditions, EP improved the expression of tight junction proteins, reducing intestinal inflammation and apoptosis, crucial for maintaining gut health (Liu *et al*., 2023). The inclusion of EP in the diet was associated with increased activities of antioxidant enzymes like superoxide dismutase and catalase while decreasing malondialdehyde levels, indicating reduced oxidative stress (Liu *et al*., 2020; Liu *et al*., 2023). EP also positively influenced the composition of gut microbiota, promoting beneficial bacteria like Lactobacillus, known to enhance growth performance and gut integrity (Jiang *et al*., 2023; Liu *et al*., 2023).

CHAPTER 9: GENERAL CONCLUSIONS AND RECOMMENDATIONS

Farmers' attention is given to morphological traits when choosing chickens for breeding, in addition to egg production and growth rate. Cash generation through the sale of eggs and male chickens is the primary purpose for keeping chickens. Agro-ecologies significantly affect nutritional management, housing, flock composition, and brood modification methods. It has also a slight influence on trait preferences, management practices, and breeding objectives of indigenous chicken owners. To design a sustainable breeding program for indigenous chickens, it is essential to consider farmers' production objectives and trait preferences specific to the production environment and agro-ecology, and emphasize economically important traits alongside morphological traits **(Chapter 4)**. The univariate and multivariate analysis of phenotypic traits identified morphological differences among indigenous chickens from different districts, allowing the classification of the study area's indigenous chickens into four ecotypes: ecotype 1 (Banja, Dembecha, and Aneded), ecotype 2 (North Achefer), ecotype 3 (Sinan), and ecotype 4 (Jawi). To strengthen the current findings, molecular-based characterization studies should be conducted **(Chapter 5)**.

The study findings indicate that the Jawi and North Achefer ecotypes exhibited superior growth performance, while the Sinan ecotype demonstrated relatively higher survivability; furthermore, the supplementation of EP was found to enhance the growth performance of chickens. Prior to considering the importation of exotic chicken breeds, it is advisable to optimize the productivity of indigenous chickens by improving their environment, including feed quality and implementing optimal selective breeding practices **(Chapter 6)**. The study results suggest that the incorporation of EP into chicken diets can be an effective strategy against coccidiosis and highlight the potential of EP as an immune stimulant in chickens. However, further research on microbiota profiles is needed to understand the underlying mechanism of action and optimize the application of EP in poultry production **(Chapter 7)**.

REFERENCES

Abdelqader, A., Wollny, C., & Gauly, M. (2007). Characterization of local chicken production systems and their potential under different levels of management practice in Jordan. *Tropical Animal Health and Production, 39*, 155-164. https://doi.org/10.1007/s11250-007-9000-x.

Abera, B., & Geta, T. (2014). Study on challenges and opportunities of village chicken production in Haramaya District, Eastern Ethiopia. *International Journal of Science Research Publication, 4*(12), 2250-3153.

Adedeji, T. A., Amusan, S. A., & Adebambo, O. A. (2015). Effect of chicken genotype on growth performance of pure and crossbred progenies in the development of a broiler line. *International Journal of Agriculture Innovations and Research, 4*(1), 134-138.

Adeleke, M., Peters, S., Ozoje, M., Ikeobi, C., Bamgbose, A., & Adebambo, O. A. (2011). Growth performance of Nigerian local chickens in crosses involving an exotic broiler breeder. *Tropical Animal Health and Production, 43*, 643-650. https://doi.org/10.1007/s11250-010-9747-3.

Adjei-Mensah, B., & Atuahene, C. (2023). Avian coccidiosis and anticoccidial potential of garlic (Allium sativum L.) in broiler production: a review. *Journal of Applied Poultry Research, 32*(1). https://doi.org/10.1016/j.japr.2022.100314.

Adomako, K., Olympio, O., Hagan, J., & Hamidu, J. (2014). Growth performance of crossbred naked neck and normal feathered laying hens kept in tropical villages. *British Poultry Science, 55*(6), 701-708. https://doi.org/10.1080/00071668.2014.960805.

Aggrey, S. E., Karnuah, A. B., Sebastian, B., & Anthony, N. B. (2010). Genetic properties of feed efficiency parameters in meat-type chickens. *Genetics selection evolution, 42*, 1-5. https://doi.org/10.1186/1297-9686-42-25.

Ahmad, M. M. (2021). Recent trends in chemical modification and antioxidant activities of plants-based polysaccharides: A review. *Carbohydrate Polymer Technologies and Applications, 2*. https://doi.org/10.1016/j.carpta.2021.100045.

Ajayi, F., & Agaviezor, B. (2016). Fertility and hatchability performance of pure and crossbred indigenous chicken strains in the high rainforest zone of Nigeria. *International Journal of Livestock Production, 7*(12), 141-144. https://doi.org/10.5897/IJLP2016.0308.

Aklilu, E., Kebede, K., Dessie, T., & Banerjee, A. (2013). Phenotypic characterization of indigenous chicken population in Ethiopia. *International Journal of Interdisciplinary and Multidisciplinary Studies, 1*(1), 24-32.

Akoglu, H. (2018). User's guide to correlation coefficients. *Turkish journal of emergency medicine, 18*(3), 91-93. https://doi.org/10.1016/j.tjem.2018.08.001.

Akramullah, M., Sumantri, C., Ulupi, N., & Pagala, M. A. (2020). Association of TGF-β2 Gene Polymorphism with Salmonella pullorum Bacterial Infection Resistance in Tolaki Chickens. *International Journal of Scientific Research in Science, Engineering and Technology, 7*(1), 46-54. https://doi.org/10.32628/IJSRSET20716.

Akter, L., Kalam, M. A., Ayman, U., Islam, R., Nasrin, M., Bhakta, S., ... & Haque, Z. (2024). Marine macroalgae (Enteromorpha intestinalis) for improving the growth performance, meat quality traits, and serum biochemical parameters in broilers. *Journal of Advanced Veterinary and Animal Research, 11*(2), 524. https://doi.org/10.5455%2Fjavar.2024.k802.

Al-Jumaili, A. S., Boudali, S. F., Kebede, A., Al-Bayatti, S. A., Essa, A. A., Ahbara, A., . . . Bjørnstad, G. (2020). The maternal origin of indigenous domestic chicken from the Middle East, the north and the horn of Africa. *BMC genetics, 21*, 1-16. https://doi.org/10.1186/s12863-020-0830-0.

Alam, J., Rahman, M. M., Halder, J., Islam, M. R., Sarkar, N., Jabeen, I., . . . Bhuyan, A. A. (2022). Myxovirus resistance (Mx) gene diversity in avian influenza virus infections. *Biomedicines, 10*(11), 2717. https://doi.org/10.3390/biomedicines10112717.

Alam, M., Chand, N., Khan, S., & Suhail, S. (2021). Growth performance, proximate composition and immune competence of naked neck, Rhode Island Red and their F1 crossbred chickens in a tropical climate. *J. Anim. Health Prod, 9*(3), 303-311. http://dx.doi.org/10.17582/journal.jahp/2021/9.3.303.311.

Alders, R. G., Dumas, S. E., Rukambile, E., Magoke, G., Maulaga, W., Jong, J., & Costa, R. 2018. Family poultry: M ultiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. *Maternal & child nutrition 14*.

Alem, T. (2014). Production and reproduction performance of rural poultry in lowland and midland agro-ecological zones of central Tigray, Northern Ethiopia. *African Journal of Agricultural Research, 9*(49), 3531-3539. https://doi.org/10.5897/AJAR2013.7351.

Alemayhu, T. (2003). Phenotypic and genetic characterization of local chicken ecotypes in Ethiopia.

Aleme, M. (2022). Review on production and reproduction performance of indigenous chicken in Ethiopia. *East African Journal of Agriculture and Biotechnology, 5*(1), 90-107. https://doi.org/10.37284/eajab.5.1.638.

Alemu, S. W., Hanotte, O., Kebede, F. G., Esatu, W., Abegaz, S., Bruno, J. E., . . . Dessie, T. (2021). Evaluation of live-body weight and the number of eggs produced for introduced and local chickens in Ethiopia. *Acta Agriculturae Scandinavica, Section A—Animal Science, 70*(2), 71-77. https://doi.org/10.1080/09064702.2021.1891278.

Alewi, M., & Melesse, A. (2013). Evaluating the growth performance of local kei chickens and their f1-crosses with Rhode Island Red and Fayoumi breeds in watershed areas of guraghe administrative zone, Southern Ethiopia. *Tropical and Subtropical Agroecosystems, 16*(1).

Amerah, A., & Ravindran, V. (2015). Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. *Poultry Science, 94*(4), 673-680. https://doi.org/10.3382/ps/pev022.

Amin, E., El-Dlebshany, A. E., & Debes, A. (2017). Using general and specific combining abilities to expected breeding values, genetic values and hybrid performance in chickens. *Egypt. Poult. Sci. J, 37*, 1289-1302. https://dx.doi.org/10.21608/epsj.2017.5654.

Apuno, A., Mbap, S., & Ibrahim, T. (2011). Characterization of local chickens (Gallus gallus domesticus) in shelleng and song local government areas of Adamawa State, Nigeria. *Agriculture and Biology Journal of North America, 2*(1), 6-14. https://dx.doi:10.5251/abjna.2011.2.1.6.14.

Ardiyana, M., Gunawan, A., Murtini, S., Sartika, T., & Sumantri, C. (2020). Polymorphisms and associations of the Nramp-1 and inos genes on Newcastle disease and Salmonella enteritidis resistances in sensi-1 Agrinak Chickens. *Tropical Animal Science Journal, 43*(2), 95-102. https://doi.org/10.5398/tasj.2020.43.2.95.

Armstrong, J. (2006). Inbreeding: Why we will not do it. *Accessed on September, 15*, 2008.

Awais, M. M., Akhtar, M., Anwar, M. I., & Khaliq, K. (2018). Evaluation of Saccharum officinarum L. bagasse-derived polysaccharides as native immunomodulatory and anticoccidial agents in broilers. *Veterinary parasitology, 249*, 74-81. https://doi.org/10.1016/j.vetpar.2017.11.012.

Awais, M. M., Akhtar, M., Muhammad, F., ul Haq, A., & Anwar, M. I. (2011). Immunotherapeutic effects of some sugar cane (Saccharum officinarum L.) extracts against coccidiosis in industrial broiler chickens. *Experimental Parasitology, 128*(2), 104-110. https://doi.org/10.1016/j.exppara.2011.02.024.

Ayele, G., & Rich, K. M. (2010). Poultry value chains and HPAI in Ethiopia. *HPAI Africa/Indonesia Team Working Paper*.

Azimu, W., Manatbay, B., Li, Y., Kaimaerdan, D., Wang, H., Reheman, A., & Muhatai, G. (2018). Genetic diversity and population structure analysis of eight local chicken breeds of Southern Xinjiang. *British Poultry Science, 59*(6), 629-635. https://doi.org/10.1080/00071668.2018.1523537.

Azli, B., Ravi, S., Hair-Bejo, M., Omar, A. R., Ideris, A., & Mat Isa, N. 2021. Functional prediction of de novo uni-genes from chicken transcriptomic data following infectious bursal disease virus at 3-days post-infection. *BMC genomics 22*(1): 461.

Bai, S. C., Hardy, R. W., & Hamidoghli, A. (2022). Diet analysis and evaluation *Fish nutrition*. 709-743. https://doi.org/10.1016/B978-0-12-819587-1.00010-0.

Bakr, N. M., Awad, A., & A. Moustafa, E. 2018. Association of genetic variants in the interleukin-18 gene promoter with risk of hepatocellular carcinoma and metastasis in patients with hepatitis C virus infection. *IUBMB life 70*(2): 165-174.

Balcha, K. A., Mengesha, Y. T., Senbeta, E. K., & Zeleke, N. A. (2021). Evaluation of different traits from day-old to age at first eggs of Fayoumi and White leghorn chickens and their reciprocal crossbreeds. *Journal of Advanced Veterinary and Animal Research, 8*(1), 1. https://doi.org/10.5455%2Fjavar.2021.h478.

Bamidele, O., Sonaiya, E., Adebambo, O., & Dessie, T. (2020). On-station performance evaluation of improved tropically adapted chicken breeds for smallholder poultry production systems in Nigeria. *Tropical Animal Health and Production, 52*, 1541-1548. https://doi.org/10.1007/s11250-019-02158-9.

Banos, G., Lindsay, V., Desta, T. T., Bettridge, J., Sanchez-Molano, E., Vallejo-Trujillo, A., . . . Christley, R. M. (2020). Integrating genetic and genomic analyses of combined health data across ecotypes to improve disease resistance in indigenous African chickens. *Frontiers in genetics, 11*. https://doi.org/10.3389/fgene.2020.543890.

Bedier, E., Labib, S., & Ahmed, A. (2022). Characterization of Antimicrobial Resistance Genes of Pasteurella multocida Isolated from Diseased Chickens in Egypt. *Journal of the Hellenic Veterinary Medical Society, 73*(2), 4165-4172. https://orcid.org/0000-0002-0034-5071.

Begli, H. E., Torshizi, R. V., Masoudi, A. A., Ehsani, A., & Jensen, J. (2016). Longitudinal analysis of body weight, feed intake and residual feed intake in F2 chickens. *Livestock Science, 184*, 28-34. https://doi.org/10.1016/j.livsci.2015.11.018.

Bekele, B., Melesse, A., Banerjee, S., Esatu, W., & Dessie, T. (2020). Farmers preferences towards breeding objective for indigenous chickens in different agro-ecologies of Ethiopia. *African Journal of Agricultural Research, 16*(12), 1681-1690. https://doi.org/10.5897/AJAR2020.14784.

Bekele, B., Melesse, A., Esatu, W., & Dessie, T. (2021). Statistical Modeling of Live Body Weight and Linear Body Measurements of Local Chicken at Different Agro-Ecologies of Ethiopia. *International Journal of Poultry Science, 20*(4), 146-151. https://doi.org/10.3923/ijps.2021.146.151.

Bekele, G., Goshu, G., Melesse, A., Esatu, W., & Dessie, T. (2021). On-Farm Phenotypic and Morphological Characterization of Indigenous Chicken Populations in Gambella Region, Ethiopia. *International Journal of Poultry Science, 20*(1), 27-38. https://doi.org/10.3923/ijps.2021.27.38.

Belew, A. K. (2018). *Whole genome based characterization of indigenous chicken populations in Ethiopia.* Addis Ababa University.

Benfield, C. T., Lyall, J. W., Kochs, G., & Tiley, L. S. 2008. Asparagine 631 variants of the chicken Mx protein do not inhibit influenza virus replication in primary chicken embryo fibroblasts or in vitro surrogate assays. *Journal of virology 82*(15): 7533-7539.

Benyi, K., Tshilate, T. S., Netshipale, A. J., & Mahlako, K. T. (2015). Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. *Tropical Animal Health and Production, 47*, 1225-1231. https://doi.org/10.1007/s11250-015-0850-3.

Bett, H. K., Bett, R. C., Peters, K. J., Kahi, A. K., & Bokelmann, W. (2011). Estimating farmers' preferences in selection of indigenous chicken genetic resources using non-market attributes. *Animal Genetic Resources/Resources Génétiques Animales/Recursos Genéticos Animales, 49*, 51–63.

Bett, H., Peters, K., & Bokelmann, W. (2011). Hedonic price analysis to guide in breeding and production of Indigenous chicken in Kenya. *Livestock Research for Rural Development, 23*(6), 2011.

Bettridge, J. M., Psifidi, A., Terfa, Z. G., Desta, T. T., Lozano-Jaramillo, M., Dessie, T., . . . Christley, R. M. (2018). The role of local adaptation in sustainable production of village chickens. *Nature Sustainability*, 1(10), 574-582. https://doi.org/10.1038/s41893-018-0150-9.

Blake, D. P., Knox, J., Dehaeck, B., Huntington, B., Rathinam, T., Ravipati, V., . . . Jatau, I. D. (2020). Re-calculating the cost of coccidiosis in chickens. *Veterinary Research, 51*, 1-14. https://doi.org/10.1186/s13567-020-00837-2.

Boodhoo, N., Gurung, A., Sharif, S., & Behboudi, S. 2016. Marek's disease in chickens: a review with focus on immunology. *Veterinary Research 47*: 1-19.

Byrne, T., & Amer, P. (2018). *Breeding objectives in an era of transformational breeding technologies.* Paper presented at the Proc. World Congress on Genetics Applied to Livestock Production, Auckland, New Zealand.

Calenge, F., Kaiser, P., Vignal, A., & Beaumont, C. 2010. Genetic control of resistance to salmonellosis and to Salmonella carrier-state in fowl: a review. *Genetics selection evolution 42*: 1-11.

Chapman, H. 2014. Milestones in avian coccidiosis research: a review. *Poultry Science 93*(3): 501-511.

Chapman, H. D., Barta, J. R., Hafeez, M. A., Matsler, P. L., and Rathinam, T. (2002). Suppression of Oocyst Production in Chickens Vaccinated with Eimeria necatrix, Eimeria tenella and Eimeria acervulina. *Avian Diseases, 46*(4), 865-869.

Chebo, C., Betsha, S., & Melesse, A. (2022). Chicken genetic diversity, improvement strategies and impacts on egg productivity in Ethiopia: a review. *World's Poultry Science Journal, 78*(3), 803-821. https://doi.org/10.1080/00439339.2022.2067020.

Chen, M. (2024). Genetic Resolution of Disease Resistance in Poultry: A Genome-Wide Association Study Perspective. *Animal Molecular Breeding, 14*.

Cheng, H. H., Kaiser, P., & Lamont, S. J. (2013). Integrated genomic approaches to enhance genetic resistance in chickens. *Annu. Rev. Anim. Biosci., 1*(1), 239-260. https://doi.org/10.1146/annurev-animal-031412-103701.

Churchil, R. R. (2023). Genetics, genomics and breeding for disease resistance in poultry. *Ind. J. Vet. & Anim. Sci. Res, 52*(2), 1-7.

Central Statistical Agency (CSA). 2021. Agricultural Sample Survey 2020/21 (2013 E.C). Report on Livestock and Livestock Characteristics. II, 589.

Daikwo, S., Dike, U., & Dim, N. (2015). Discriminant analysis of morphometric differences in the normal feathered and frizzle feathered chickens of north central Nigeria. *Agro-Science, 14*(3), 12-15. https://doi.org/10.4314/as.v14i3.3.

Dana, N., Dessie, T., van der Waaij, L. H., & van Arendonk, J. A. M. (2010b). Morphological features of indigenous chicken populations of Ethiopia. *Animal Genetic Resources/Ressources génétiques animales/Recursos genéticos animales, 46*, 11-23. https://doi.org/10.1017/s2078633610000652.

Dana, N., Van der Waaij, L. H., Dessie, T., & Van Arendonk, J. A. (2010a). Production objectives and trait preferences of village poultry producers of Ethiopia: implications for designing breeding schemes utilizing indigenous chicken genetic resources. *Tropical Animal Health and Production, 42*(7), 1519-1529. https://doi.org/10.1007/s11250-010-9602-6.

Dar, M. A., Bhat, B., Nazir, J., Saleem, A., Manzoor, T., Khan, M., . . . Ahmad, S. M. (2023). Identification of SNPs Related to Salmonella Resistance in Chickens Using RNA-Seq and Integrated Bioinformatics Approach. *Genes, 14*(6), 1283. https://doi.org/10.3390/genes14061283.

Dar, M. A., Mumtaz, P. T., Bhat, S. A., Nabi, M., Taban, Q., Shah, R. A., . . . Ahmad, S. M. (2018). Genetics of disease resistance in chicken. *Application of Genetics and Genomics in Poultry Science*, 163-174. https://dx.doi.org/10.5772/intechopen.77088.

Deist, M. S., Gallardo, R. A., Bunn, D. A., Dekkers, J. C., Zhou, H., & Lamont, S. J. 2017. Resistant and susceptible chicken lines show distinctive responses to Newcastle disease virus infection in the lung transcriptome. *BMC genomics 18*: 1-15.

Dessie, T., Dana, N., Ayalew, W., & Hanotte, O. (2012). Current state of knowledge on indigenous chicken genetic resources of the tropics: domestication, distribution and documentation of information on the genetic resources. *World's Poultry Science Journal, 68*(1), 11-20. https://doi.org/10.1017/S0043933912000025.

Desta, T. T. (2021a). Indigenous village chicken production: A tool for poverty alleviation, the empowerment of women, and rural development. *Tropical Animal Health and Production*, *53*(1), 1–16. https://doi.org/10.1007/s11250-020-02433-0.

Desta, T. T., & Wakeyo, O. (2024). Breeding practice of indigenous village chickens, and traits and breed preferences of smallholder farmers. *Veterinary Medicine and Science*, *10*(4). https://doi.org/10.1002/vms3.1517.

DI Lorenzo, P., Ceccobelli, S., Panella, F., Attard, G., & Lasagna, E. (2015). The role of mitochondrial DNA to determine the origin of domestic chicken. *World's Poultry Science Journal, 71*(2), 311-318. https://doi.org/10.1017/S0043933915000318.

Dorji, N., & Sunar, S. (2014). Short communication Morphometric variations among five Bhutanese indigenous chickens (Gallus domesticus). *Journal of Animal and Poultry Sciences, 3*(3), 76-85.

Entity, M. (2021). Livestock Systems: Overview and Areas of Inquiry. *Feed the Future Innovation Lab for Livestock Systems, Gainesville, FL, USA*.

Eriksson, J., Larson, G., Gunnarsson, U., Bed'Hom, B., Tixier-Boichard, M., Strömstedt, L., . . . Randi, E. (2008). Identification of the yellow skin gene reveals a hybrid

origin of the domestic chicken. *PLoS genetics, 4*(2). https://doi.org/10.1371/journal.pgen.1000010.

Esatu, W., Kassa, A., Lulie, B., Kebede, A., Genetu, G., Yismaw, K., . . . Dessie, T. (2022). A guide to setting up a selective indigenous chicken improvement program: The Tilili breed in Ethiopia. *ILRI Manual*.

Esmaeili, M., Gholizadeh, M., Hafezian, H., & Farhadi, A. (2024). Sex-specific genetic parameter estimates of body weight in Mazandaran indigenous chickens. *Journal of Animal Breeding and Genetics*. https://doi.org/10.1111/jbg.12855.

Evans, F., & Critchley, A. (2014). Seaweeds for animal production use. *Journal of applied phycology, 26*, 891-899. https://doi.org/10.1007/s10811-013-0162-9.

Falconer, D., & Mackey, T. (1996). Introduction to quantitative genetics. 228-246: Longman. Essex.

FAO (2012). Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines.

FAO (2019b). Poultry Sector Ethiopia. FAO Animal Production and Health Livestock Country Reviews Rome 11.

Faustin, V., Adégbidi, A. A., Garnett, S. T., Koudandé, D. O., Agbo, V., & Zander, K. K. (2010). Peace, health or fortune?: Preferences for chicken traits in rural Benin. *Ecological Economics, 69*(9), 1848-1857. https://doi.org/10.1016/j.ecolecon.2010.04.027.

Fekede, G., & Tadesse, Y. (2021). Breeding practices and preferred traits of indigenous chicken in Western Oromia region, Ethiopia. *Journal of Livestock Science (ISSN online 2277-6214), 12*, 85-94. https://doi.org/10.33259/JLivestSci.2021.

Fitsum, M., & Aliy, M. (2014). Poultry production system and role of poultry production in Tigray region, Northern Ethiopia: a review. *J Biol Agric Healthc, 4*(27), 154.

Francis, M., Richard, H., Thomas, S., & Denis, M. (2016). Characterization of layer poultry production in Rwanda. *International Journal of Agricultural Sciences, 6*(10), 1148-1156.

Fukushima, H., Matsumoto, A., Inuzuka, H., Zhai, B., Lau, A. W., Wan, L., . . . Gygi, S. P. 2012. SCFFbw7 modulates the NFκB signaling pathway by targeting NFκB2 for ubiquitination and destruction. *Cell reports 1*(5): 434-443.

Fulton, J. E., Arango, J., Ali, R. A., Bohorquez, E. B., Lund, A. R., Ashwell, C. M., . . . Koci, M. D. 2014. Genetic variation within the Mx gene of commercially selected chicken lines reveals multiple haplotypes, recombination and a protein under selection pressure. *PLoS One 9*(9).

Gebremariam, B., Mazengia, H., & Gebremariam, T. (2017). On-farm productive and reproductive performance of local, exotic and crossbred chickens in Southern Tigray, North Ethiopia. *Journal of Biology, Agriculture and Healthcare, 7*(13), 42-50.

Gebremedhin, B., Tesema, E., Tegegne, A., Hoekstra, D., & Nicola, S. (2016). Value chain opportunities for women and young people in livestock production in Ethiopia: Lessons learned. *LIVES Working Paper*.

Getachew, F., Abegaz, S., Assefa, A., Misganaw, M., Emshaw, Y., Hailu, A., . . . Okore, C. (2017). Multivariate analyses of morphological traits in indigenous chicken populations of Metekel zone, Northwestern Ethiopia. *Animal Genetic Resources/Ressources génétiques animales/Recursos genéticos animales, 59*, 15-25. https://doi.org/10.1017/s2078633616000084.

Getiso, A., Tessema, F., Mekonnen, M. M., Jimma, A., & Zeleke, B. (2015). Assessment of Village Chicken Production Systems in Kambata Tambaro and Wolaita Zones, SNNPR, Ethiopia.

Getu, A., Alemayehu, K., & Wuletaw, Z. (2014). Canonical Analysis for Assessment of Genetic Diversity of Three Indigenous Chicken Ecotypesin North Gondar Zone, Ethiopia. *Iranian Journal of Applied Animal Science, 4*(4), 871-876.

Giansanti, F., Rossi, P., Massucci, M. T., Botti, D., Antonini, G., Valenti, P., & Seganti, L. (2002). Antiviral activity of ovotransferrin discloses an evolutionary strategy for the defensive activities of lactoferrin. *Biochemistry and cell biology, 80*(1), 125-130. https://doi.org/10.1139/o01-208.

Gogoi, A., Das, B., Chabukdhara, P., Phookan, A., & Phangchopi, D. (2022). Livestock breeding for disease resistance: a perspective review. *Agricultural Reviews, 43*(1), 116-121. http://dx.doi.org/10.18805/ag.R-2169.

Goraga, Z., Caron, L., Wilbert, C., & Brockmann, G. A. (2016). Characterization of village chicken production systems and challenges across agro-climatic zones in Ethiopia. *International Journal of Livestock Production, 7*(11), 94-105. https://doi.org/10.5897/IJLP2016.0320.

Goraga, Z., Wilbert, C., & Caron, L. (2018). Ethiopian native chicken productivity, aims of production and breeding practices across agro-climatic zones. *International Journal of Livestock Production, 9*(8), 198-205. https://doi.org/10.5897/IJLP2017.0388.

Gordillo Jaramillo, F. X., Kim, D.-H., Lee, S. H., Kwon, S.-K., Jha, R., & Lee, K.-W. (2021). Role of oregano and Citrus species-based essential oil preparation for the control of coccidiosis in broiler chickens. *Journal of Animal Science and Biotechnology, 12*, 1-9. https://doi.org/10.1186/s40104-021-00569-z.

Gul, H., Chen, X., & Geng, Z. (2021). Comparative yolk proteomic analysis of fertilized low and high cholesterol eggs during embryonic development. *Animals, 11*(3), 744. https://doi.org/10.3390/ani11030744.

Gul, H., Habib, G., Khan, I. M., Rahman, S. U., Khan, N. M., Wang, H., . . . Liu, Y. (2022). Genetic resilience in chickens against bacterial, viral and protozoal pathogens. *Frontiers in Veterinary Science, 9*. https://doi.org/10.3389/fvets.2022.1032983.

Gulilat, L., Tegegne, F., & Demeke, S. (2021). Hatchery and broody technologies and least cost ration practice for poultry production improvement in ethiopia. *Cogent Food & Agriculture, 7*(1). https://doi.org/10.1080/23311932.2021.1913793.

Guo, Y., Zhao, Z. H., Pan, Z. Y., An, L. L., Balasubramanian, B., & Liu, W. C. (2020). New insights into the role of dietary marine-derived polysaccharides on productive performance, egg quality, antioxidant capacity, and jejunal morphology in late-phase laying hens. *Poult Sci, 99*(4), 2100-2107. https://doi.org/10.1016/j.psj.2019.12.032.

Guzmán, M., Cádiz, L., Guerrero-Moncayo, A., Cáceres, F., Vidal, S., Lapierre, L., . . . Hidalgo, H. 2022. Molecular characterization of Infectious Bursal Disease Virus isolated in Chile reveals several mutations in VP2 coding region and a reassortment in its genome. *Veterinary Research Communications 46*(4): 1281-1289.

Habimana, R., Ngeno, K., Mahoro, J., Ntawubizi, M., Shumbusho, F., Manzi, M., . . . Okeno, T. (2021). Morphobiometrical characteristics of indigenous chicken ecotype populations in Rwanda. *Tropical Animal Health and Production, 53*(1), 1-11. https://doi.org/10.1007/s11250-020-02475-4.

Habimana, R., Okeno, T. O., Ngeno, K., Mboumba, S., Assami, P., Gbotto, A. A., . . . Yao, N. (2020). Genetic diversity and population structure of indigenous chicken in Rwanda using microsatellite markers. *PLoS One, 15*(4). https://doi.org/10.1371/journal.pone.0225084

Halima, H., Neser, F., De Kock, A., & Van Marle-Köster, E. (2006). Growth performance of indigenous chickens under intensive management conditions in Northwest Ethiopia. *South African Journal of Animal Science, 36* (5), 71-73.

Halima, H., Neser, F., Van Marle-Koster, E., & De Kock, A. (2007a). Village-based indigenous chicken production system in north-west Ethiopia. *Tropical Animal Health and Production, 39*, 189-197. https://doi.org/10.1007/s11250-007-9004-6.

Halima, H., Neser, F. W., van Marle-Koster, E., & de Kock, A. (2007b). Phenotypic variation of native chicken populations in northwest Ethiopia. *Trop Anim Health Prod, 39*(7), 507-513. https://doi.org/10.1007/s11250-007-9032-2.

Hearn, C. J., & Cheng, H. H. (2023). Contribution of the TCRβ Repertoire to Marek's Disease Genetic Resistance in the Chicken. *Viruses, 15*(3), 607. https://doi.org/10.3390/v15030607.

Hong, Y., Kim, E.-S., Lillehoj, H., Lillehoj, E., & Song, K.-D. (2009). Association of resistance to avian coccidiosis with single nucleotide polymorphisms in the zyxin gene. *Poultry Science, 88*(3), 511-518. https://doi.org/10.3382/ps.2008-00344.

Hong, Y. H., Kim, E.-S., & Lillehoj, H. S. (2011). *Identification of parental line specific effects of MLF2 on resistance to coccidiosis in chickens.* Paper presented at the BMC proceedings. https://doi.org/10.1186/1753-6561-5-S4-S21.

Hu, G., Liu, L., Miao, X., Zhao, Y., Peng, Y., Liu, L., & Li, X. 2022. The response of cecal microbiota to inflammatory state induced by Salmonella enterica serovar Enteritidis. *Frontiers in Microbiology 13*.

Imboden, M., Nicod, L., Nieters, A., Glaus, E., Matyas, G., Bircher, A., . . . Team, S. 2006. The common G-allele of interleukin-18 single-nucleotide polymorphism is a genetic risk factor for atopic asthma. The Sapaldia Cohort Study. *Clinical & Experimental Allergy 36*(2): 211-218.

Izakovicova Holla, L. 2003. Interleukin-18 in asthma and other allergies (Vol. 33, pp. 1023-1025): Wiley Online Library.

Jiang, S., Yang, C., Xiao, Y., Zheng, S., Jiang, Q., & Chen, J. (2023). Effects of polysaccharides-rich extract from Gracilaria lemaneiformis on growth performance, antioxidant capacity, immune function, and meat quality in broiler chickens. *The Journal of Poultry Science*, *60*(2). https://doi.org/10.2141/jpsa.2023018.

Juul-Madsen, H. R., Nielsen, O., Krogh-Maibom, T., Rontved, C., Dalgaard, T., Bumstead, N., & Jorgensen, P. 2002. Major histocompatibility complex-linked immune response of young chickens vaccinated with an attenuated live infectious bursal disease virus vaccine followed by an infection. *Poultry Science 81*(5): 649-656.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M., & Espeisse, O. (2018). The value of anticoccidials for sustainable global poultry production. *International Journal of Antimicrobial Agents, 51*(3), 304-310. https://doi.org/10.1016/j.ijantimicag.2017.09.004.

Kalkal, H., Kumar, P., & Vohra, S. (2021). Advances in control strategies and vaccine development of coccidiosis in poultry. *Concern (Peek Landman 2011, Mund al, 2017), 13*, 17.

Kanginakudru, S., Metta, M., Jakati, R., & Nagaraju, J. (2008). Genetic evidence from Indian red jungle fowl corroborates multiple domestication of modern day chicken. *BMC evolutionary biology, 8*, 1-14. https://doi.org/10.1186/1471-2148-8-174.

Kanlisi, R. A., Amuzu-Aweh, E. N., Walugembe, M., Naazie, A., Otsyina, H. R., Chouicha, N., ... & Kayang, B. B. (2022, December). Genetic architecture of body weight and carcass traits in Ghanaian local chickens. In *Proceedings of 12th World*

Congress on Genetics Applied to Livestock Production (WCGALP) Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges (pp. 2433-2436). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-940-4.

Kanlisi, R. A., Amuzu-Aweh, E. N., Naazie, A., Otsyina, H. R., Kelly, T. R., Gallardo, R. A., ... & Kayang, B. B. (2024). Genetic architecture of body weight, carcass, and internal organs traits of Ghanaian local chickens. *Frontiers in Genetics*, *15*. https://doi.org/10.3389/fgene.2024.1297034.

Kapella, L. E., Nyanda, S. S., & Mahonge, C. P. (2023). The influence of trait preferences and breeding practices on local chicken genetic resource conservation in igunga district, Tanzania. *European Journal of Agriculture and Food Sciences*, *5*(1), 51–60. https://doi.org/10.24018/ejfood.2023.5.1.628.

Kebede, F. G., Derks, M. F., Dessie, T., Hanotte, O., Barros, C. P., Crooijmans, R. P., ... & Bastiaansen, J. W. (2024). Landscape genomics reveals regions associated with adaptive phenotypic and genetic variation in Ethiopian indigenous chickens. *BMC genomics*, *25*(1), 284. https://doi.org/10.1186/s12864-024-10193-6

Kgwatalala, P. M., & Segokgo, P. (2013). Growth performance of Australorp x Tswana crossbred chickens under an intensive management system.

Khaliq, K., Akhtar, M., Awais, M. M., & Anwar, M. I. (2017). Evaluation of immunotherapeutic effects of Aloe vera polysaccharides against coccidiosis in chicken. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *23*(6). https://doi.org/10.9775/kvfd.2017.17957.

Khan, R. U., Naz, S., De Marzo, D., Dimuccio, M. M., Bozzo, G., Tufarelli, V., . . . Ragni, M. (2022). Aloe vera: a sustainable green alternative to exclude antibiotics in modern poultry production. *Antibiotics*, *12*(1), 44. https://doi.org/10.3390/antibiotics12010044

Khater, H. F., Ziam, H., Abbas, A., Abbas, R. Z., Raza, M. A., Hussain, K., . . . Selim, A. (2020). Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. *Agrobiol Rec*, *1*, 11-25. https://doi.org/10.47278/journal.abr/2020.003.

Khawaja, T., Khan, S. H., Mukhtar, N., Parveen, A., & Ahmed, T. (2013). Comparative study of growth performance, meat quality and haematological parameters of three-way crossbred chickens with reciprocal F1 crossbred chickens in a subtropical environment. *Journal of Applied Animal Research*, *41*(3), 300-308. https://doi.org/10.1080/09712119.2013.782869.

Kim, E.-S., Hong, Y., & Lillehoj, H. (2010). Genetic effects analysis of myeloid leukemia factor 2 and T cell receptor-β on resistance to coccidiosis in chickens. *Poultry Science*, *89*(1), 20-27. https://doi.org/10.3382/ps.2009-00351.

Kim, Y.-C., Kim, H.-H., & Jeong, B.-H. (2022). The First Report of Polymorphisms and Genetic Characteristics of the Shadow of Prion Protein (SPRN) in Prion Disease-Resistant Animal, Chickens. *Frontiers in Veterinary Science, 9*. https://doi.org/10.3389/fvets.2022.904305.

Kingori, A., Wachira, A., & Tuitoek, J. (2010). Indigenous chicken production in Kenya: a review. *International Journal of Poultry Science*, *9*(4), 309-316.

Kleyn, F., & Ciacciariello, M. (2021). Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? *World's Poultry Science Journal*, 77, 267-278. https://doi.org/10.1080/00439339.2021.1904314.

Kong, X., Yin, F., He, Q., Liu, H., Li, T., Huang, R., . . . Li, P. (2009). Acanthopanax senticosus extract as a dietary additive enhances the apparent ileal digestibility of

amino acids in weaned piglets. *Livestock Science, 123*(2-3), 261-267. https://doi.org/10.1016/j.livsci.2008.11.015.
Kosgey, I. S. (2004). *Breeding objectives and breeding strategies for small ruminants in the tropics*: Wageningen University and Research.
Kpomasse, C. C., Kouame, Y. A. E., N'nanle, O., Houndonougbo, F. M., Tona, K., & Oke, O. E. (2023). The productivity and resilience of the indigenous chickens in the tropical environments: improvement and future perspectives. *Journal of Applied Animal Research, 51*(1), 456-469. https://doi.org/10.1080/09712119.2023.2228374.
Kulkarni, R., Parreira, V., Jiang, Y.-F., & Prescott, J. (2010). A live oral recombinant Salmonella enterica serovar Typhimurium vaccine expressing Clostridium perfringens antigens confers protection against necrotic enteritis in broiler chickens. *Clinical and Vaccine Immunology, 17*(2), 205-214. https://doi.org/10.1128/CVI.00406-09.
Lamont, S. J. 2010. Genetics of disease resistance.
Lawal, R. A., & Hanotte, O. (2021). Domestic chicken diversity: Origin, distribution, and adaptation. *Animal genetics, 52*(4), 385-394. https://doi.org/10.1111/age.13091.
Lemlem, A., & Tesfay, Y. (2010). Performance of exotic and indigenous poultry breeds managed by smallholder farmers in northern Ethiopia. *Livestock Research for Rural Development, 22*(7), 133.
Li, X., Nie, C., Liu, Y., Chen, Y., Lv, X., Wang, L., . . . Ban, L. (2019). A genome-wide association study explores the genetic determinism of host resistance to S almonella pullorum infection in chickens. *Genetics selection evolution, 51*, 1-12. https://doi.org/10.1186/s12711-019-0492-4.
Liu, H.-C., Kung, H.-J., Fulton, J. E., Morgan, R. W., & Cheng, H. H. (2001). Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. *Proceedings of the National Academy of Sciences, 98*(16), 9203-9208. https://doi.org/10.1073/pnas.161466898.
Liu, H., Yang, G., Yu, C., & Wang, S. (2019). Effects of Enteromorpha prolifera polysaccharide on intestinal digestive enzyme activity, microbial number and nutrient apparent utilization of broilers. *Chinese Journal of Animal Nutrition, 31*(2), 956-961. https://doi.org/10.1186/s40104-023-00932-2.
Liu, W. 2002. *A candidate gene analysis for response to Salmonella enteritidis challenge or vaccination in young chicks*: Iowa State University.
Liu, W.-C., Guo, Y., Zhao, Z.-H., Jha, R., & Balasubramanian, B. (2020). Algae-derived polysaccharides promote growth performance by improving antioxidant capacity and intestinal barrier function in broiler chickens. *Frontiers in Veterinary Science, 7*. https://doi.org/10.3389/fvets.2020.601336.
Liu, W.-C., Zhu, Y.-R., Zhao, Z.-H., Jiang, P., & Yin, F.-Q. (2021). Effects of dietary supplementation of algae-derived polysaccharides on morphology, tight junctions, antioxidant capacity and immune response of duodenum in broilers under heat stress. *Animals, 11*(8). https://doi.org/10.3390%2Fani11082279.
Liu, W. (2002). A candidate gene analysis for response to Salmonella enteritidis challenge or vaccination in young chicks: Iowa State University.
Liu, Y., Cheng, Y., Shan, W., Ma, J., Wang, H., Sun, J., & Yan, Y. (2018). Chicken interferon regulatory factor 1 (IRF1) involved in antiviral innate immunity via regulating IFN-β production. *Developmental & Comparative Immunology, 88*, 77-82. https://doi.org/10.1016/j.dci.2018.07.003.
Liu, W., Liu, H., Wang, Y., Zhao, Z., Balasubramanian, B., & Jha, R. (2023). Effects of Enteromorpha prolifera polysaccharides on growth performance, intestinal barrier

function and cecal microbiota in yellow-feathered broilers under heat stress. *Journal of Animal Science and Biotechnology*, *14*(1), 132. https://doi.org/10.1186/s40104-023-00932-2.

Long, L., Kang, B., Jiang, Q., & Chen, J. (2020). Effects of dietary Lycium barbarum polysaccharides on growth performance, digestive enzyme activities, antioxidant status, and immunity of broiler chickens. *Poultry Science*, *99*(2), 744-751. https://doi.org/10.1016%2Fj.psj.2019.10.043.

Long, L., Zhang, H., Wang, F., Yin, Y., Yang, L., & Chen, J. (2021). Research Note: Effects of polysaccharide-enriched Acanthopanax senticosus extract on growth performance, immune function, antioxidation, and ileal microbial populations in broiler chickens. *Poultry Science*, *100*(4). https://doi.org/10.1016/j.psj.2021.101028.

Lowry, D. B. (2012). Ecotypes and the controversy over stages in the formation of new species. *Biological Journal of the Linnean Society*, *106*(2), 241-257. https://doi.org/10.1111/j.1095-8312.2012.01867.x.

Luo, D., Yang, N., Liu, Z., Li, T., Wang, H., Ge, M., & Zhang, R. (2021). Effects of astragalus polysaccharide on intestinal inflammatory damage in goslings infected with gosling plague. *British Poultry Science*, *62*(3), 353-360. https://doi.org/10.1080/00071668.2020.1859094.

Lv, H., Xiao, B., & Gao, Y. (2013). Study on the extraction, purification and structural characterization of polysaccharide from Enteromorpha. *Food Res Dev*, *34*(8), 33-36.

Maharani, D., Mustofa, F., Sari, A. P. Z., Fathoni, A., Sasongko, H., & Hariyono, D. N. H. (2021). Phenotypic characterization and principal component analyses of indigenous chicken breeds in Indonesia. *Veterinary World*, *14*(6), 1665. https://doi.org/10.14202%2Fvetworld.2021.1665-1676.

Mahoro, J., Muasya, T., Mbuza, F., Habimana, R., & Kahi, A. (2017). Characterization of indigenous chicken production systems in Rwanda. *Poultry Science*, *96*(12), 4245-4252. https://doi.org/10.3382/ps/pex240.

Mahoro, J., Muasya, T., Mbuza, F., Mbuthia, J., & Kahi, A. (2018). Farmers' breeding practices and traits of economic importance for indigenous chicken in RWANDA. *Tropical Animal Health and Production*, *50*(1), 121-128. https://doi.org/10.1007/s11250-017-1411-8.

Mak, P. H., Rehman, M. A., Kiarie, E. G., Topp, E., & Diarra, M. S. (2022). Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review. *Journal of Animal Science and Biotechnology*, *13*(1), 148. https://doi.org/10.1186/s40104-022-00786-0.

Manyelo, T. G., Selaledi, L., Hassan, Z. M., & Mabelebele, M. (2020). Local chicken breeds of Africa: their description, uses and conservation methods. *Animals*, *10*(12), 2257. https://doi.org/10.3390/ani10122257.

Matawork, M. (2018). Productive and reproductive performance of indigenous chickens in Ethiopia. *International Journal of Livestock Production*, *9*(10), 253-259. https://doi.org/10.5897/IJLP2018.0451.

Mbuthia, J. M., Rewe, T. O., & Kahi, A. K. (2015). Analysis of pig breeding management and trait preferences in smallholder production systems in Kenya. *Animal Genetic Resources/Resources génétiques animales/Recursos genéticos animales*, *56*, 111-117. https://doi.org/10.1017/S207863361400054X.

Mekonnen, K. T., Lee, D.-H., Cho, Y.-G., & Seo, K.-S. (2023). A Review on Production, Reproduction, Morphometric, and Morphological Characteristics of Ethiopian

Native Chickens. *Journal of World's Poultry Research, 13*(3), 280-291. https://dx.doi.org/10.36380/jwpr.2023.31.

Melesse, A. (2014). Significance of scavenging chicken production in the rural community of Africa for enhanced food security. *World's Poultry Science Journal, 70*(3), 593-606. https://doi.org/10.1017/S0043933914000646.

Melesse, A., Maak, S., & Von Lengerken, G. (2005). The performance of naked neck and their F1 crosses with Lohmann White and New Hampshire chicken breeds under long-term heat stress conditions. *Ethiopian J. Anim. Product, 5*, 91-107.

Melesse, A., & Negesse, T. (2011). Phenotypic and morphological characterization of indigenous chicken populations in southern region of Ethiopia. *Animal Genetic Resources/Ressources génétiques animales/Recursos genéticos animales, 49*, 19-31. https://doi.org/10.1017/s2078633611000099.

Melesse, A., Tadele, A., Assefa, H., Taye, K., Kebede, T., Taye, M., & Betsha, S. (2021). Assessing the Morphological Diversity of Ethiopian Indigenous Chickens Using Multivariate Discriminant Analysis of Morphometric Traits for Sustainable Utilization and Conservation. *Poultry Science Journal, 9*(1), 61-72. https://doi.org/10.22069/psj.2021.18469.1644.

Mersha, S. F., & Senbeta, E. K. (2020). Chicken Reproductive Performance in Ethiopia. *Turkish Journal of Agriculture-Food Science and Technology, 8*(8), 1755-1762. http://orcid.org/0000-0002-4119-9082.

Mesa-Pineda, C., Navarro-Ruíz, J. L., López-Osorio, S., Chaparro-Gutiérrez, J. J., & Gómez-Osorio, L. M. (2021). Chicken coccidiosis: from the parasite lifecycle to control of the disease. *Frontiers in Veterinary Science, 8*. https://doi.org/10.3389/fvets.2021.787653.

Miyumo, S., Wasike, C. B., Ilatsia, E. D., Bennewitz, J., & Chagunda, M. G. (2023). Genetic and non-genetic factors influencing KLH binding natural antibodies and specific antibody response to Newcastle disease in Kenyan chicken populations. *Journal of Animal Breeding and Genetics, 140*(1), 106-120. https://doi.org/10.1111/jbg.12738.

Moges, F. (2010b). Indigenous chicken production and marketing systems in Ethiopia: Characteristics and opportunities for market-oriented development (Vol. 24): ILRI (aka ILCA and ILRAD).

Moges, F., Mellesse, A., & Dessie, T. (2010a). Assessment of village chicken production system and evaluation of the productive and reproductive performance of local chicken ecotype in Bure district, North West Ethiopia. *African Journal of Agricultural Research*, 1739-1748.

Mohammed, A., & Dei, H. (2017). Comparative performance of Guinea keets managed under 2 brooding systems in the Tolon district of Northern region of Ghana. *UDS International Journal of Development, 4*(1), 42-45. https://doi.org/10.47740/156.UDSIJD6i.

Momoh, O., Nwosu, C., & Adeyinka, I. (2010). Comparative evaluation of two Nigerian local chicken ecotypes and their crosses for growth traits. *International Journal of Poultry Science, 9*(8), 738-743. https://doi.org/10.3923/ijps.2010.738.743.

Moreda, E., & Mesekel, K. G. (2016). Importance of Traditional, Small Scale and Commercial Poultry Production in Ethiopia: A Review. *British Journal of Poultry Sciences, 5*(1), 01-08.

Mountford, J., Gheyas, A., Vervelde, L., & Smith, J. 2022. Genetic variation in chicken interferon signalling pathway genes in research lines showing differential viral resistance. *Animal genetics 53*(5): 640-656.

Mpenda, F., Schilling, M., Campbell, Z., Mngumi, E., & Buza, J. (2019). The genetic diversity of local african chickens: A potential for selection of chickens resistant to viral infections. *Journal of Applied Poultry Research, 28*(1), 1-12. https://doi.org/10.3382/japr/pfy063.

Mtileni, B., Muchadeyi, F. C., Maiwashe, A., Chimonyo, M., Groeneveld, E., Weigend, S., & Dzama, K. (2011). Diversity and origin of South African chickens. *Poultry Science, 90*(10), 2189-2194. https://doi.org/10.3382/ps.2011-01505.

Muchadeyi, F., Eding, H., Simianer, H., Wollny, C., Groeneveld, E., & Weigend, S. (2008). Mitochondrial DNA D-loop sequences suggest a Southeast Asian and Indian origin of Zimbabwean village chickens. *Animal genetics, 39*(6), 615-622. https://doi.org/10.1111/j.1365-2052.2008.01785.x.

Mujyambere, V., Adomako, K., Olympio, S., Ntawubizi, M., Nyinawamwiza, L., Mahoro, J., & Conroy, A. (2021). Local chickens in East African region: their production and potential. Polutry Science 2021. *press. https://doi. org/10.1016/j. psj*.

Mujyambere, V., Adomako, K., Olympio, S. O., Ntawubizi, M., Nyinawamwiza, L., Mahoro, J., & Conroy, A. (2022). Local chickens in East African region: Their production and potential. *Poultry Science, 101*(1). https://doi.org/10.1016/j.psj.2021.101547.

Mulugeta, S., Goshu, G., & Esatu, W. (2020). Growth performance of DZ-white and Improved Horro chicken breeds under different agro-ecological zones of Ethiopia. *Journal of Livestock Science*. http://dx.doi.org/10.33259/JLivestSci.2020.45-53.

Muluneh, B., Taye, M., Dessie, T., Wondim, D. S., Kebede, D., & Tenagne, A. (2023). Morpho-biometric characterization of indigenous chicken ecotypes in north-western Ethiopia. *PLoS One, 18*(6). https://doi.org/10.1371/journal.pone.0286299.

Mumtaz, S., Akhtar, M., Awais, M. M., & Anwar, M. I. (2021). Evaluation of immunomodulatory, growth promoting and protective effects of ficus religiosa against coccidiosis in broilers. *Pak. J. Agric. Sci, 58*, 219-228. https://doi.org/10.21162/PAKJAS/21.1331.

Musa, S. A., Kebede, K., & Tadesse, D. (2023). Review on phenotypic characterization and breeding objective traits of indigenous chicken in Ethiopia. *International Journal of Veterinary Science and Research, 9*(3), 041-046. https://doi.org/10.17352/ijvsr.000135.

Musa, A. A., Orunmuyi, M., Akpa, G., Olutunmogun, A., Muhammad, H., & Adedibu, I. (2015). Diallel analysis for bodyweight involving three genotypes of Nigerian indigenous chickens. *South African Journal of Animal Science, 45*(2), 188-197. https://doi.org/10.4314/sajas.v45i2.10.

Mwacharo, J. M., Nomura, K., Hanada, H., Han, J., Amano, T., & Hanotte, O. (2013). Reconstructing the origin and dispersal patterns of village chickens across E ast A frica: insights from autosomal markers. *Molecular Ecology, 22*(10), 2683-2697. https://doi.org/10.1111/mec.12294.

Nair, V. 2005. Evolution of Marek's disease–a paradigm for incessant race between the pathogen and the host. *The Veterinary Journal 170*(2): 175-183.

National Research Council (NRC). (1994). Nutrient requirements of poultry. 9th rev. ed. Washington, DC: National Academy Press.

Ndofor-Foleng, H., Oleforuh-Okoleh, V., Musongong, G., Ohageni, J., & Duru, U. (2015). Evaluation of growth and reproductive traits of Nigerian local chicken and exotic chicken. *Indian Journal of Animal Research, 49*(2), 155-160. http://dx.doi.org/10.5958/0976-0555.2015.00046.1.

Ndung'u, C., Muasya, T., & Okeno, T. (2021). Optimization of response to selection using genomic selection in indigenous chicken breeding programmes. *South African Journal of Animal Science, 51*(6), 723-734. https://doi.org/10.4314/sajas.v51i6.5.

Nebiyu Yemane, N. Y., Berhan Tamir, B. T., & Kelay Belihu, K. B. (2013). Characterization of village chicken production performance under scavenging system in Halaba district of southern Ethiopia. https://doi.org/10.4314/evj.v17i1.6.

Negash, F., Abegaz, S., Tadesse, Y., Jembere, T., Esatu, W., & Dessie, T. (2023). Evaluation of growth performance and feed efficiency in reciprocal crosses of Fayoumi with three exotic chicken breeds. *Acta Agriculturae Scandinavica, Section A—Animal Science, 72*(1-2), 11-24. http://dx.doi.org/10.1080/09064702.2023.2168743.

Negassa, D., Melesse, A., & Banerjee, S. (2014). Phenotypic characterization of indigenous chicken populations in Southeastern Oromia Regional State of Ethiopia. *Animal Genetic Resources/Ressources génétiques animales/Recursos genéticos animales, 55*, 101-113. http://dx.doi.org/10.1017/s2078633614000319.

Ngongolo, K., & Chota, A. (2021). Chicken production, flock size, management systems, and challenges in the Dodoma region in Tanzania. *Poultry Science, 100*(6). https://doi.org/10.1016/j.psj.2021.101136.

Nwogwugwu, C. P., Lee, J., Freedom, E. C., & Lee, S. (2018). Review on the genetic potential of Nigerian local chickens. *J. Anim. Breed. Genomics, 2*(2). https://doi.org/10.12972/jabng.201800XX.

Okeno, T., Kahi, A., & Peters, J. (2010). *Characterization of indigenous chicken production systems in Kenya: Household flock structure, dynamics and breeding practices.* Paper presented at the Biennial conference paper.

Okeno, T. O., Magothe, T. M., Kahi, A. K., & Peters, K. J. (2012). Breeding objectives for indigenous chicken: Model development and application to different production systems. *Tropical Animal Health and Production, 45*, 193-203. https://doi.org/10.1007/s11250-012-0191-4.

Osei-Amponsah, R., Kayang, B. B., & Naazie, A. (2012). Age, genotype and sex effects on growth performance of local chickens kept under improved management in Ghana. *Tropical Animal Health and Production, 44*, 29-34. http://dx.doi.org/10.1007/s11250-011-0010-3.

Padhi, M. K. (2016). Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. *Scientifica, 2016*. https://doi.org/10.1155/2016/2604685.

Panda, A. K., Rama Rao, S. V., Raju, M. V. L. N., Niranjan, M., & Reddy, M. R. (2012). Effect of nutrient density on production performance, egg quality and humoral immune response of brown laying (Dahlem Red) hens in the tropics. *Tropical Animal Health and Production, 44*(2), 293-299. https://doi.org/10.1007/s11250-011-0017-9.

Panyako, P. M., Lichoti, J. K., & Ommeh, S. C. (2022). Antimicrobial drug resistance in poultry pathogens: Challenges and opportunities. *Journal of Agriculture, Science and Technology, 21*(1), 62-82. https://doi.org/10.4314/jagst.v21i1.7.

Pinard-van Der Laan, M.-H., Bed'Hom, B., Coville, J.-L., Pitel, F., Feve, K., Leroux, S., . . . Repérant, J.-M. (2009). Microsatellite mapping of QTLs affecting resistance to coccidiosis (Eimeria tenella) in a Fayoumi× White Leghorn cross. *BMC genomics, 10*, 1-13. https://doi.org/10.1186/1471-2164-10-31.

Prakash, A., Saxena, V. K., & Singh, M. K. (2020). Genetic analysis of residual feed intake, feed conversion ratio and related growth parameters in broiler chicken: a

review. *World's Poultry Science Journal, 76*(2), 304-317. https://doi.org/10.1080/00439339.2020.1735978.

Rajkumar, U., Haunshi, S., Paswan, C., Raju, M., Rao, S. R., & Chatterjee, R. (2017). Characterization of indigenous Aseel chicken breed for morphological, growth, production, and meat composition traits from India. *Poultry Science, 96*(7), 2120-2126. https://doi.org/10.3382/ps/pew492.

Raza, Q. S., Saleemi, M. K., Gul, S., Irshad, H., Fayyaz, A., Zaheer, I., . . . Imran, M. (2022). Role of essential oils/volatile oils in poultry production—A review on present, past and future contemplations. *Agrobiol. Rec, 7*, 40-56. https://doi.org/10.47278/journal.abr/2021.013.

Read, A. F., Baigent, S. J., Powers, C., Kgosana, L. B., Blackwell, L., Smith, L. P., . . . Nair, V. K. 2015. Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS biology 13*(7).

Reid, J. J. a. W. M. (1970). Anticoccidial Drugs: Lesion Scoring Techniques in Battery and Floor-Pen Experiments with Chickens. *Experimental Parasitology, 28*, 30-36. https://doi.org/10.1016/0014-4894(70)90063-9.

Ribeiro, T., Lordelo, M., Alves, S., Bessa, R., Costa, P., Lemos, J., . . . Prates, J. (2013). Direct supplementation of diet is the most efficient way of enriching broiler meat with n-3 long-chain polyunsaturated fatty acids. *British Poultry Science, 54*(6), 753-765. https://doi.org/10.1080/00071668.2013.841861.

Rizwan, H. M., Khan, M. K., Mughal, M. A. S., Abbas, Z., Abbas, R. Z., Sindhu, Z. u. D., . . . Zafar, A. (2022). A new insight in immunomodulatory impact of botanicals in treating avian coccidiosis. *Journal of Parasitic Diseases, 46*(4), 1164-1175. https://doi.org/10.1007/s12639-022-01519-w.

Rotimi, E., Egahi, J., & Adeoye, A. (2016). Phenotypic characterization of indigenous chicken population in Gwer-West, Benue State, Nigeria. *World Scientific News, 53*(3), 343-353.

Ryley, J., Meade, R., Hazelhurst, J., & Robinson, T. E. (1976). Methods in coccidiosis research: separation of oocysts from faeces. *Parasitology, 73*(3), 311-326. https://doi.org/10.1017/S0031182000046990.

Sanka, Y., & Mbaga, S. (2014). Evaluation of Tanzanian local chicken reared under intensive and semi-intensive systems: I. Growth performance and carcass characteristics. *Livestock Research for Rural Development, 26*(7), 127.

Sartika, T., Sulandari, S., & Zein, M. S. A. (2011). *Selection of Mx gene genotype as genetic marker for Avian Influenza resistance in Indonesian native chicken.* Paper presented at the BMC proceedings. 5. (Suppl 4), S37. https://doi.org/10.1186/1753-6561-5-S4-S37.

SAS. (2012). SAS for Windows, ver. 9.4.

Sawai, H., Kim, H. L., Kuno, K., Suzuki, S., Gotoh, H., Takada, M., . . . Akishinonomiya, F. (2010). The origin and genetic variation of domestic chickens with special reference to junglefowls Gallus g. gallus and G. varius. *PLoS One, 5*(5). https://doi.org/10.1371/journal.pone.0010639.

Shah, M. A., Xu, L., Yan, R., Song, X., & Li, X. (2010). Cross immunity of DNA vaccine pVAX1-cSZ2-IL-2 to Eimeria tenella, E. necatrix and E. maxima. *Exp Parasitol, 124*(3), 330-333. https://doi.org/10.1016/j.exppara.2009.11.010.

Shahid, S. R. A., Shah, M. A., Riaz, A., Malik, A. M., Hasan, M., Xiangrui, L., . . . Shahid, S. K. A. (2020). Identification and molecular characterization of Eimeria tenella based on EtMic5 gene in Pakistan. *Pak Vet J, 40*(4), 443-448. https://doi.org/10.29261/pakvetj/2020.063.

Shapiro BI, Gebru G, Desta S, Negassa A, Negussie K, Aboset G, Mechal H. (2015). Ethiopia livestock master plan: roadmaps for growth and transformation. ILRI

Shi, L. Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D. R., & Chi, H. (2011). HIF1α–dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *Journal of Experimental Medicine, 208*(7), 1367-1376. https://doi.org/10.1084/jem.20110278.

Singh M, R Patton, R Mollier, N Pongener, R Yadav, V Singh, R Katiyar, G Singh, S Deori, and S Doley. (2023). Indigenous chicken production system in different agro-ecology of Indian Himalayan Region: implication on food and economic security. *Frontiers in Nutrition*, 10. https://doi.org/10.3389/fnut.2023.1244413.

Sironi, L., Williams, J. L., Moreno-Martin, A. M., Ramelli, P., Stella, A., Jianlin, H., . . . Mariani, P. 2008. Susceptibility of different chicken lines to H7N1 highly pathogenic avian influenza virus and the role of Mx gene polymorphism coding amino acid position 631. *Virology 380*(1): 152-156.

Siwendu, N. A., Norris, D., Ngambi, J. W., Shimelis, H. A., & Benyi, K. (2013). Heterosis and combining ability for body weight in a diallel cross of three chicken genotypes. *Tropical Animal Health and Production, 45*, 965-970. http://dx.doi.org/10.1007/s11250-012-0317-8.

Smith, J., Lipkin, E., Soller, M., Fulton, J. E., & Burt, D. W. (2020). Mapping QTL associated with resistance to avian oncogenic Marek's disease virus (MDV) reveals major candidate genes and variants. *Genes, 11*(9), 1019. https://doi.org/10.3390/genes11091019.

Smith, J., Sadeyen, J.-R., Butter, C., Kaiser, P., & Burt, D. W. (2015). Analysis of the early immune response to infection by infectious bursal disease virus in chickens differing in their resistance to the disease. *Journal of virology, 89*(5), 2469-2482. https://doi.org/10.1128/jvi.02828-14.

Snyder, R., Guerin, M., Hargis, B., Page, G., & Barta, J. (2021). Monitoring coccidia in commercial broiler chicken flocks in Ontario: comparing oocyst cycling patterns in flocks using anticoccidial medications or live vaccination. *Poultry Science, 100*(1), 110-118. https://doi.org/10.1016/j.psj.2020.09.072.

SPSS for windows. (2019). Statistical Package for Social Science (SPSS). Release 26.0. The Apache software foundation.

Storey, A. A., Athens, J. S., Bryant, D., Carson, M., Emery, K., DeFrance, S., . . . Jones, S. (2012). Investigating the global dispersal of chickens in prehistory using ancient mitochondrial DNA signatures. *PLoS One, 7*(7). https://doi.org/10.1371/journal.pone.0039171.

Sun, Q., Shen, M., Li, F., Liu, J., Lu, L., Zhu, M., & Yuan, D. (2017). Immune regulatory effects of enteromorphaclathrata polysaccharides on Nd attenuated vaccine in a chicken model infected with reticuloendotheliosis virus. *Brazilian Journal of Poultry Science, 19*, 601-608. https://doi.org/10.1590/1806-9061-2016-0460.

Susanti, R., Utami, N. R., Utami, N. R., Rahayuningsih, M., Fibriana, F., Rahayuningsih, M., & Fibriana, F. (2017). Identification of avian influenza genetic resistance gene marker in chickens. *Makara Journal of Science*, 169-174. https://doi.org/10.7454/mss.v21i4.7428.

Tadele, A., Berhane, G., Esatu, W., & Wassie, T. (2023). Effect of genotype on hatchability, growth, morphometric and carcass traits of Chicken. *Journal of Agriculture and Food Research, 11*. https://doi.org/10.1016/j.jafr.2023.100531.

Tadelle, D., Kijora, C., & Peters, K. (2003). Indigenous chicken ecotypes in Ethiopia: growth and feed utilization potentials. *International Journal of Poultry Science, 2*(2), 144-152.

Team, R. C. (2021). R: A language and environment for statistical computing [R Foundation for Statistical Computing]. Retrieved from https://www.R-project.org/.

Terfa, Z. G., Garikipati, S., Kassie, G. T., Dessie, T., & Christley, R. (2019). Understanding farmers' preference for traits of chickens in rural Ethiopia. *Agricultural Economics, 50*(4), 451-463. https://doi.org/10.1111/agec.12502.

Tilahun, M., Mitiku, M., & Ayalew, W. (2022). Agroecology Is Affecting Village Chicken Producers' Breeding Objective in Ethiopia. *Scientifica, 2022*, 1-12. https://doi.org/10.1155/2022/9492912.

Tolasa, B. (2021). Current status of indigenous and highly productive chicken breeds in Ethiopia. *Advances in Agriculture, 2021*. https://doi.org/10.1155/2021/8848388.

Ullah, M. I., Akhtar, M., & Iqbal, Z. (2014). Immunotherapeutic activities of mushroom derived polysaccharides in chicken. *International Journal of Agriculture and Biology, 16*(2).

Urgesa, L. (2023). Review on Poultry Production System, Trends and Development Strategies in Ethiopia. *Journal of Aquaculture & Livestock Production. SRC/JALP-138.* https://doi.org/10.*47363/JALP/2023 (4), 125*, 2-5.

Vallejo-Trujillo, A., Kebede, A., Lozano-Jaramillo, M., Dessie, T., Smith, J., Hanotte, O., & Gheyas, A. A. (2022). Ecological niche modelling for delineating livestock ecotypes and exploring environmental genomic adaptation: The example of Ethiopian village chicken. *Frontiers in Ecology and Evolution, 10*. https://doi.org/10.3389/fevo.2022.866587.

Wang, Q., Thiam, M., Barreto Sánchez, A. L., Wang, Z., Zhang, J., Li, Q., . . . Zhao, G. (2023). Gene co-expression network analysis reveals the hub genes and key pathways associated with resistance to Salmonella Enteritidis colonization in chicken. *International Journal of Molecular Sciences, 24*(5), 4824. https://doi.org/10.3390/ijms24054824.

Wang, H., Zhao, X., Wen, J., Wang, C., Zhang, X., Ren, X., ... & Qu, L. (2023). Comparative population genomics analysis uncovers genomic footprints and genes influencing body weight trait in Chinese indigenous chicken. *Poultry Science*, *102*(11). https://doi.org/10.1016/j.psj.2023.103031.

Wassie, T., Cheng, B., Zhou, T., Gao, L., Lu, Z., Wang, J., . . . Wu, X. (2022b). Enteromorpha polysaccharide and yeast glycoprotein mixture improves growth, antioxidant activity, serum lipid profile and regulates lipid metabolism in broiler chickens. *Poultry Science*, *101*(10). https://doi.org/10.1016/j.psj.2022.102064.

Wassie, T., Duan, X., Xie, C., Wang, R., & Wu, X. (2022a). Dietary Enteromorpha polysaccharide-Zn supplementation regulates amino acid and fatty acid metabolism by improving the antioxidant activity in chicken. *Journal of Animal Science and Biotechnology, 13*(1), 18. https://doi.org/10.1186/s40104-021-00648-1.

Wassie, T., Lu, Z., Duan, X., Xie, C., Gebeyew, K., Yumei, Z., . . . Wu, X. (2021b). Dietary Enteromorpha polysaccharide enhances intestinal immune response, integrity, and caecal microbial activity of broiler chickens. *Frontiers in Nutrition*, 938. https://doi.org/10.3389%2Ffnut.2021.783819.

Wassie, T., Niu, K., Xie, C., Wang, H., & Xin, W. (2021a). Extraction techniques, biological activities and health benefits of marine algae Enteromorpha prolifera polysaccharide. *Frontiers in Nutrition, 8*. https://doi.org/10.3389/fnut.2021.747928.

Weigend, S., & Romanov, M. N. (2001). Current strategies for the assessment and evaluation of genetic diversity in chicken resources. *World's Poultry Science Journal, 57*(3), 275-288. https://doi.org/10.1079/WPS20010020.

Wilson, R. (2010). Poultry production and performance in the Federal Democratic Republic of Ethiopia. *World's Poultry Science Journal, 66*(3), 441-454. https://doi.org/10.1017/S0043933910000528.

Winaya, A., Fahmiady, D. I., Suyatno, S., Malik, A., Mahmud, A., & Jaganathan, R. (2023). Morphometric Diversity and Genetic Relationship of" Bangkok" Chicken (Thai Game Fowl) in East Java, Indonesia. *Jordan Journal of Biological Sciences*, *16*(2). https://doi.org/10.54319/jjbs/160203.

Wolde Kawole, B., Mengesha, Y. T., & Zeleke, N. A. (2019). On farm phenotypic characterization of indigenous chicken ecotypes in west Hararghe zone, Oromia region, Ethiopia.

Wolde, S., Mirkena, T., Melesse, A., Dessie, T., & Abegaz, S. (2021). Hatchability and growth performances of normal feathered local, Sasso-RIR and their F1-cross chickens managed under on-station condition in southern Ethiopia. *Tropical Animal Health and Production, 53*, 1-8. https://doi.org/10.1007/s11250-021-02957-z.

Wolde, S., Mirkena, T., Melesse, A., Dessie, T., & Abegaz, S. (2022). Effects of genotype and age on growth performance and carcass traits of chickens in southern ethiopia. *J. Anim. Health Prod, 10*(1), 107-115. http://dx.doi.org/10.17582/journal.jahp/2022/10.1.107.115.

Wondimu, A., Mesfin, E., & Bayu, Y. (2019). Prevalence of poultry coccidiosis and associated risk factors in intensive farming system of Gondar Town, Ethiopia. *Veterinary medicine international, 2019*. https://doi.org/10.1155/2019/5748690.

Wondmeneh, E.W. (2015). *Genetic improvement in indigenous chicken of Ethiopia*. PhD thesis, Wageningen University, The Netherlands.

Wondmeneh E., Alemayehu, A., Bewket S., Tsigereda F. (2017). Status of commercial poultry production in Ethiopia.

Wong, J., de Bruyn, J., Bagnol, B., Grieve, H., Li, M., Pym, R., & Alders, R. (2017). Small-scale poultry and food security in resource-poor settings: A review. *Global Food Security, 15*, 43-52. https://doi.org/10.1016/j.gfs.2017.04.003.

Xie, X. F., Wang, Z. Y., Zhong, Z. Q., Pan, D. Y., Hou, G. Y., & Xiao, Q. (2024). Genome-wide scans for selection signatures in indigenous chickens reveal candidate genes associated with local adaptation. *animal*, *18*(5). https://doi.org/10.1016/j.animal.2024.101151

Xu, J., Xu, L.-L., Zhou, Q.-W., Hao, S.-X., Zhou, T., & Xie, H.-J. (2015). Isolation, purification, and antioxidant activities of degraded polysaccharides from Enteromorpha prolifera. *International journal of biological macromolecules, 81*, 1026-1030. https://doi.org/10.1016/j.ijbiomac.2015.09.055.

Xu, L., He, Y., Ding, Y., Liu, G. E., Zhang, H., Cheng, H. H., . . . Song, J. (2018). Genetic assessment of inbred chicken lines indicates genomic signatures of resistance to Marek's disease. *Journal of Animal Science and Biotechnology, 9*, 1-10. https://doi.org/10.1186/s40104-018-0281-x.

Yadessa, E., Tulu, D., Bogale, A., Mengistu, G., Aleme, M., Shiferawu, S., . . . Amare, A. (2017). Characterization of smallholder poultry production systems in Mezhenger, Sheka and Benchi-Maji zones of south western Ethiopia. *Research Journal of Agricultural Science Research, 5*(1), 2360-7874. https://doi.org/10.14662/ARJASR2016.041.

Yakubu, A., Abimiku, K., Musa-Azara, I., Idahor, K., & Akinsola, O. (2013). Assessment of flock structure, preference in selection and traits of economic importance of domestic turkey (Meleagris gallopavo) genetic resources in Nasarawa state, Nigeria. *Livestock Research for Rural Development, 25*(1), 18.

Yakubu, A., Bamidele, O., Hassan, W. A., Ajayi, F. O., Ogundu, U. E., Alabi, O., Sonaiya, E. B., & Adebambo, O. A. (2020). Farmers' choice of genotypes and trait preferences in tropically adapted chickens in five agroecological zones in Nigeria. *Tropical Animal Health and Production, 52*(1), 95–107. https://doi.org/10.1007/s11250-019-01993-0.

Yamane, Y. (1967). Mathematical formulae for sample size determination. *J. Mathemetics, 1*, 1-29.

Yi, Z., Li, X., Luo, W., Xu, Z., Ji, C., Zhang, Y., . . . Zhang, X. (2018). Feed conversion ratio, residual feed intake and cholecystokinin type A receptor gene polymorphisms are associated with feed intake and average daily gain in a Chinese local chicken population. *Journal of Animal Science and Biotechnology, 9*, 1-9. https://doi.org/10.1186/s40104-018-0261-1.

Yonas, K. (2020). Introduction of the Exotic Breeds and Cross Breeding of Local Chicken in Ethiopia and Solution to Genetic Erosion. *African Journal of Biotechnology, 19*(2), 92-98. https://doi.org/10.5897/AJB2019.16748.

Yu, Y., Li, Y., Du, C., Mou, H., & Wang, P. (2017). Compositional and structural characteristics of sulfated polysaccharide from Enteromorpha prolifera. *Carbohydrate polymers, 165*, 221-228. https://doi.org/10.1016/j.carbpol.2017.02.011.

Yu, Y., Sitaraman, S., & Gewirtz, A. T. (2004). Intestinal epithelial cell regulation of mucosal inflammation. *Immunologic research, 29*, 55-67. https://doi.org/10.1385/IR:29:1-3:055.

Yuan, J., Dou, T., Ma, M., Yi, G., Chen, S., Qu, L., . . . Yang, N. (2015). Genetic parameters of feed efficiency traits in laying period of chickens. *Poultry Science, 94*(7), 1470-1475. https://doi.org/10.3382/ps/pev122.

Yuan, Y., Peng, D., Gu, X., Gong, Y., Sheng, Z., & Hu, X. (2018). Polygenic basis and variable genetic architectures contribute to the complex nature of body weight—a genome-wide study in four Chinese indigenous chicken breeds. *Frontiers in genetics, 9*, 229. https://doi.org/10.3389/fgene.2018.00229.

Zaidel-Bar, R., Ballestrem, C., Kam, Z., & Geiger, B. 2003. Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. *Journal of cell science 116*(22): 4605-4613

Zanella, R. (2016). Genomic tools and animal health. *Veterinary Sciences, 3*(3), 21. https://doi.org/10.3390/vetsci3030021.

Zare, Y., Gnanda, I., Houaga, I., Kere, M., Traore, B., Zongo, M., . . . Rekaya, R. (2021). Morpho-Biometric Evaluation of the Genetic Diversity of Local Chicken Ecotypes in Four Regions (Centre-East, Sahel, Centre-North and South-West) of Burkina Faso. *International Journal of Poultry Science, 20*(6), 231-242. https://doi.org/10.3923/ijps.2021.231.242.

Zelleke, G., Urge, M., Animut, G., Esatu, W., & Dessie, T. (2022). Comparative growth performance and carcass characteristics of guinea fowl (*Numida meleagris*) and three chicken genotypes. *Ethiopian Journal of Science and Technology, 15*(1), 81-99. https://doi.org/10.1007/s11250-010-9747-3.

Zewdu, S., Kassa, B., Agza, B., & Alemu, F. (2013). Village chicken production systems in Metekel zone, Northwest Ethiopia. *Wudpecker Journal of Agricultural Research, 2*(9), 256-262.

Zhang, K., Li, X., Na, C., Abbas, A., Abbas, R.Z., Zaman, M.A. (2020). Anticoccidial effects of *Camellia sinensis* (green tea) extract and its effect on Blood and Serum chemistry of broiler chickens. *Pak Vet ,* 40, 77-80.

Zhang, Q., Lenardo, M. J., & Baltimore, D. 2017. 30 years of NF-κB: a blossoming of relevance to human pathobiology. *Cell 168*(1): 37-57.

Zhang, Y., Duan, X., Wassie, T., Wang, H.-h., Li, T., Xie, C., & Wu, X. (2022). Enteromorpha prolifera polysaccharide–zinc complex modulates the immune response and alleviates LPS-induced intestinal inflammation via inhibiting the TLR4/NF-κB signaling pathway. *Food & Function, 13*(1), 52-63. https://doi.org/10.1039/D1FO02171K.

Zhang, Z., Li, M., Tan, Q., Chen, J., Sun, J., Li, J., . . . Zhao, X. (2023). A moderate anticoccidial effect of cedrol on Eimeria tenella in broiler chickens. *Parasitology International, 97*. https://doi.org/10.1016/j.parint.2023.102779.

Zhao, Y., Balasubramanian, B., Guo, Y., Qiu, S.-J., Jha, R., & Liu, W.-C. (2021). Dietary Enteromorpha polysaccharides supplementation improves breast muscle yield and is associated with modification of mRNA transcriptome in broiler chickens. *Frontiers in Veterinary Science, 8*. https://doi.org/10.3389/fvets.2021.663988.

Zhi-Qiang, Y., Qian-Lin, C., Li-Zhi, F., Wen-Gui, F., Hua, Z., Hong-Mei, T., . . . Chun-Lin, C. (2022). Effect of Qing Chang oral liquid on the treatment of artificially infected chicken coccidiosis and the cellular immunity. *Vet Med Sci, 8*(6), 2504-2510. https://doi.org/10.1002/vms3.922.

Zhu, Y., Wang, X., Zhang, C., Ni, J., Luo, Q., Teng, L., . . . Chen, Y. (2021). Characterizations of glucose-rich polysaccharides from Amomum longiligulare TL Wu fruits and their effects on immunogenicities of infectious bursal disease virus VP2 protein. *International journal of biological macromolecules, 183*, 1574-1584. https://doi.org/10.1016/j.ijbiomac.2021.05.138.

APPENDIX

Appendix Tables

Appendix Table 1. Household characteristics of indigenous chicken producers in Northwest Ethiopia

Variable	Agro-ecology						Overall	
	Highland, N=120		Midland, N=120		Lowland, N=120			
	N	%	N	%	N	%	N	%
Sex structure								
Male	105	87.5	102	85	103	85.8	310	86.1
Female	15	12.5	18	15	17	14.2	50	13.9
	χ^2=0.325^{NS}; Cramer's V=0.03							
Age structure								
<31 years	21	17.5	24	20	11	9.2	56	15.6
31-40 years	41	34.2	38	31.7	47	39.2	126	35
41-50 years	32	26.7	36	30	47	39.2	115	31.9
51-60 years	12	10	17	14.2	9	7.5	38	10.6
>60 years	14	11.7	5	4.2	6	5	25	6.9
	χ^2=119.27^{NS}; Cramer's V=0.407							
Education status								
Illiterate	56	46.7	39	32.5	68	56.7	163	45.3
Write and read	58	48.3	76	63.3	48	40	182	50.6
Grade 10 and above	6	5.0	5	4.2	4	3.3	15	4.2
	*χ^2=14.853**; Cramer's V=0.144*							
Farming activity								
Livestock production	6	5.0	11	9.2	3	2.5	20	5.6
Crop production	5	4.2	9	7.5	1	0.8	15	4.2
Both	109	90.8	100	83.3	116	96.7	325	90.3
	χ^2=12.448; Cramer's V=0.132*							

N= Number of households; NS=Non-significant;*P<0.05;**P<0.01

Appendix Table 2. Coefficient of correlation between body weight and linear body measurements (above diagonal for female chickens and below diagonal for male chickens)

	BW	WS	SL	BL	BKL	NL	CL	SC	CC	TC	CH
BW		0.51***	0.51***	0.49***	0.14***	0.28***	0.35***	0.22***	0.69***	0.53***	0.21***
WS	0.78***		0.46***	0.49***	0.23***	0.48***	0.09**	0.43***	0.47***	0.39***	0.12***
SL	0.64***	0.64***		0.43***	0.19***	0.13***	0.07*	0.09**	0.39***	0.35***	0.06^{NS}
BL	0.69***	0.70***	0.62***		0.24***	0.42***	0.09**	0.15***	0.41***	0.40***	0.49***
BKL	0.22***	0.19***	0.38***	0.16**		0.34***	0.11***	0.31***	0.23***	0.17***	0.14***
NL	0.37***	0.31***	0.17**	0.22***	-0.01^{NS}		0.15***	0.38***	0.28***	0.24***	0.02^{NS}
CL	0.36***	0.38***	0.43***	0.35***	0.07^{NS}	0.27***		0.06^{NS}	0.24***	0.28***	0.69***
SC	0.30***	0.19***	0.35***	0.24***	0.24***	0.02^{NS}	0.29***		0.25***	0.21***	-0.06^{NS}
CC	0.84***	0.72***	0.61***	0.60***	0.16***	0.29***	0.36***	0.27***		0.49***	0.12***
TC	0.75***	0.66***	0.45***	0.58***	0.07^{NS}	0.45***	0.61***	0.20***	0.72***		0.13***
CH	0.27***	0.26***	0.27***	0.20***	-0.04^{NS}	0.13*	0.63***	0.20***	0.23***	0.43***	

BW= Body weight; WS = Wing span; SL = Shank length; BL = Body length; BKL = Beak length; NL = Neck length; CL = Comb length; SC = Shank circumference; CC = Chest circumference; TC = Thigh circumference; CH = Comb height; NS= Non-significant; *p<0.05; **p<0.01; ***p<0.001.

Appendix Table 3. Multivariate statistics and F approximation for female and male chickens

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Female chickens					
Wilks' Lambda	0.16358683	38.35	50	3934.7	<.0001
Pillai's Trace	1.34992872	32.03	50	4330	<.0001
Hotelling-Lawley Trace	2.62114673	45.12	50	2785.2	<.0001
Roy's Greatest Root	1.64648716	142.59	10	866	<.0001
Male chickens					
Wilks' Lambda	0.10441015	16.30	55	1424.6	<.0001
Pillai's Trace	1.69858167	14.55	55	1555	<.0001
Hotelling-Lawley Trace	3.15320810	17.52	55	1012.2	<.0001
Roy's Greatest Root	1.33592326	37.77	11	311	<.0001
Wilks' Lambda	0.10441015	16.30	55	1424.6	<.0001

Appendix Figures

Appendix Figure 1. Photos taken during interviews and focal group discussion with chicken producers in Northwest Ethiopia

Appendix Figure 2. Field photos on morphological measurement of indigenous chickens in Northwest Ethiopia

Appendix Figure 3. Photos shown different activities for growth performance evaluation of indigenous chickens in Northwest Ethiopia

Appendix Figure 4. Photos shown different activities for evaluation of EP on chicken coccidiosis in Northwest Ethiopia