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Availability and Utilization Practices of Phytogenic Feed Additives and Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf and Garlic Powder

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BAHIR DAR UNIVERSITY

College of Agriculture and Environmental Sciences

Department of Animal Sciences

**Availability and Utilization Practices of Phytogenic Feed Additives and
Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf
and Garlic Powder**

PhD Dissertation

By

Minichle Yegrem Gedamu

December 2025

Bahir Dar, Ethiopia

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College of Agriculture and Environmental Sciences
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Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf
and Garlic Powder**

PhD Dissertation

By

Minichle Yegrem Gedamu

**A Dissertation Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Animal Nutrition**

Advisory Board members:

Principal advisor: Dr. Getachew Animut

Co-advisors: Prof. Yeshambel Mekuriaw

**December, 2025
Bahir Dar, Ethiopia**

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DECLARATION

This is to certify that the thesis entitled “**Availability and Utilization Practices of Phytogenic Feed Additives and Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf and Garlic Powder**”, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Nutrition in Department of Animal science, Bahir Dar University, is a record of original work carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

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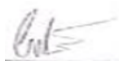
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Approval of Dissertation for Defense

I hereby certify that I have supervised, read, and evaluated this dissertation titled “**Availability and Utilization Practices of Phytogetic Feed Additives and Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf and Garlic Powder**” by Minichle Yegrem Gedamu prepared under my guidance. I recommend the dissertation be submitted for oral defense.

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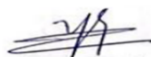
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Approval of Dissertation for Defense Result

As members of the board of examiners, we examined this dissertation titled “**Availability and Utilization Practices of Phytogenic Feed Additives and Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf and Garlic Powder**” by Minichle Yegrem Gedamu. We hereby certify that the dissertation is accepted for fulfilling the requirements for the award of the degree of “Doctor of Philosophy in Animal Nutrition”.

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DEDICATION

To my father, Yegrem Gedamu, whose unwavering belief in the power of education has inspired me; my mother, Mantegibosh Eshetie; and the strongest woman I know, my beloved wife, Maritu Fentahun.

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ABBREVIATIONS AND ACRONYMS

ALT	Alanine Aminotransferase
ALP	Alkaline Phosphate
AGPS	Antibiotic Growth Promoters
AID	Apparent Ileal Digestibility
AST	Aspartate Aminotransferase
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
BDZD	Bahir Dar Zuria District
BLM	Bamboo Leaf Meal
BC	Black Cumin
BW	Body Weight
BWG	Body Weight Gain
CFU	Colony Forming Unit
CRD	Completely Randomized Design
CF	Crude Fiber
CP	Crude Protein
DBWG	Daily Body Weight Gain
EOS	Essential Oils
EE	Ether Extract
EDTA	Ethylene Diamine Tetra Acetic Acid
EFSA	European Food Safety Authority
FCR	Feed Conversion Ratio
FBW	Final Body Weight
GP	Garlic Powder
GIT	Gastrointestinal Tract
HB	Haemoglobin
HU	Haugh Unit

ABBREVIATIONS AND ACRONYMS (*continued*)

HDL	High Density Lipo Protein
HH	Household
IBW	Initial Body Weight
LDL	Low Density Lipo Protein
MCHC	Mean Corpuscular Hemoglobin Concentration
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MP	Mortality Percentage
NLM	Neem Leaf Meal
Ns	Non-Significant
PCV	Packed Cell Volume
PLF	Paw-Paw Leaf Meal
PEAS	Phytogenic Feed Additives
RBC	Red Blood Cell
RP	Red Pepper
RH	Relative Humidity
THI	Temperature-Humidity Index
TLU	Tropical Livestock Unit
XLD	Xylose Lysine Deoxycholate
YCF	Yolk Color Fun

Availability and Utilization Practices of Phytogenic Feed Additives and Performance Evaluation of Chickens Fed Leaf Meals of Neem and Bitter Leaf and Garlic Powder

ABSTRACT

This study aimed to assess the availability and utilization of phytogenic feed additives among chicken producers and to evaluate the effects of neem, bitter-leaf, and garlic on egg production, egg quality, blood parameters, and gut and cecal bacteria in Bovans Brown layer hens, as well as on growth performance, carcass traits, blood parameters, and cecal bacterial load in Koekoek chickens. Data collection methods included farm observations, individual interviews, key informants and focus group discussions, supplemented by secondary information from agricultural office records and research publications. For the second and third research objectives, 128 Bovans Brown layer hens with 1.73 ± 0.05 kg/chicken initial body weight (IBW) were randomly assigned to four dietary treatments, each with four replications in a completely randomized design (CRD). The treatments included a control diet (T1) with no additives and three experimental diets supplemented with 2.5% neem leaf meal (T2), bitter-leaf leaf meal (T3), and garlic powder (T4). Each treatment consisted of 32 hens, and the study lasted for 12 weeks. Data collected included production performance, egg quality, blood parameters and bacterial load. Blood samples were taken for haematological and serological analyses. At the end of the study, two chickens per replicate were slaughtered for bacterial load analysis. For the fourth objective, 192 Koekoek chicks were randomly allocated to four dietary treatments in a completely randomized design (CRD): a basal control diet (T1) and three experimental diets with the inclusions of neem leaf meal (T2), bitter-leaf meal (T3) and garlic powder (T4) at a 2.5% level of inclusion for each. Growth performance, carcass characteristics, blood parameters and cecal bacterial load were assessed. At the end of the study, 32 chickens (8 per treatment) were slaughtered for carcass analysis, and cecal content was collected for microbial assessment. Blood samples were also taken prior to slaughter for haematological and serum chemistry evaluation. The survey result revealed that phytogenic feed additives were used by farmers in urban (58.3%), peri-urban (56.3%), and rural (52.5%) areas, with neem, bitter-leaf, and garlic being the most commonly available and utilized. Awareness of these additives was highest among urban (70.9%) and peri-urban (73.8%) producers. The highest feed intake occurred in T2 (117.77

g/hen) and T4 (117.12 g/hen), with the lowest in T3 (108.20 g/hen) in layer hens. Hen-day egg production was the highest in (T2) (92.45%), followed by (T4) (89.78%), and lowest in (T3) (83.86%). T2 also had the highest egg mass. No significant differences ($P > 0.05$) were observed in other internal and external egg quality parameters. Haematological analysis showed significant differences in packed cell volume (PCV) and haemoglobin concentration. The red blood cell (RBC) count was also highest in T2 ($2.61 \times 10^6/\text{mm}^3$) compared to the control ($1.3 \times 10^6/\text{mm}^3$). However, lymphocytes, MCH, MCHC, and MCV were not significantly affected ($P > 0.05$). Serum biochemical parameters were also unaffected ($P > 0.05$). Gut bacteria results indicated reduced *E. coli* and *Salmonella* counts and increased *Lactobacillus* in treated groups. Phytogenic diets (T2–T4) significantly improved ADBWG, final body weight, and several carcass traits, with T3 showing the best FCR, although dressing percentage remained unchanged. Supplemented groups also showed improved health indicators and elevated total protein in T2. Cecal analysis confirmed enhanced beneficial bacteria. In conclusion, phytogenic additives such as neem, bitter-leaf, and garlic commonly used by chicken producers show strong potential, with neem leaf meal (T2) notably improving egg production and yolk color in layers. The use of 2.5% inclusion rates for neem, bitter-leaf, and garlic showed no adverse effects on blood profiles and in fact, improved haematological parameters and gut microbiota. In Koekoek chickens, these additives improved growth, feed efficiency, and gut health, with no negative effects on performance. Further nationwide studies are needed to standardize protocols and quantify the potential of phytogenic feed additives and bitter-leaf supplementation in chicken production, with a focus on optimizing dosage, assessing long-term effects on health and carcass traits, and evaluating levels below 2.5% in layer hens.

Keywords: Bacterial load, Bitter-leaf plant, Blood profiles, Carcass traits, Chicken nutrition, Egg quality, Garlic, Koekoek chicken performances, Layer hens, Layer performance, Neem, Phytogenic feed additives

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Background and Justification

Chicken is the primary livestock species, contributing more to animal protein consumption than any other species (FAO, 2011; Mseleku *et al.*, 2023). Its production has become a popular option for small- and medium-scale farmers due to its high turnover rate and quick return on investment (Phiri *et al.*, 2023; Urgesa, 2023). The global chicken industry is crucial for food security, providing eggs and meat to a growing population (Mottet and Tempio, 2017). As the world's population grows and urbanizes, the demand for animal-based food continues to rise, underscoring the importance of chicken products in meeting this need. In Ethiopia, where achieving food self-sufficiency and combating malnutrition are critical, chicken production has emerged as a key strategy (Yitbarek, 2013). Chicken meat and eggs are vital sources of edible animal protein (FAO, 2010) and play a significant role in meeting the nutritional needs of the poorest sections of society (Reta, 2009).

Despite indigenous chicken being the dominant breed in Ethiopia, their productivity remains low. However, they possess advantages in disease resistance and adaptability to low-input systems. As an intervention, improved breeds of layer and broiler chickens have been introduced to boost egg and meat production. Various exotic chicken breeds were introduced to Ethiopia and extensively studied in the 1950s (Mazengia *et al.*, 2012; Weyuma *et al.*, 2015). Evidence indicates that imported breeds perform well under intensive management systems. Nevertheless, the contribution of exotic chickens to the Ethiopian economy is significantly lower than in other African countries (Kebede, 2016; Milkias, 2016). The livestock master plan aims to transform existing village production systems to enhance family chicken production, recommending an expansion of commercial systems to meet the increasing demand for chicken meat and eggs (Shapiro *et al.*, 2015). The per capita consumption of chicken meat and eggs in Ethiopia is 0.55 kg and 0.34 kg, respectively (FAOSTAT, 2021). Other estimates indicate that the average annual consumption of chicken meat is approximately 2.85 kg per person, while egg consumption is about 57 eggs per person per year (Sime, 2022).

Chicken production is an integral part of most Ethiopian households in all rural, pre-urban, and urban areas, contributing to poverty alleviation, nutrition, and food security (Habte *et*

al., 2017). Improvements in key chicken management practices, such as enhanced detection and control of avian diseases, have positively affected productivity (Habte *et al.*, 2017; Astill *et al.*, 2020). The productivity of layer and broiler chickens is closely linked to their health and growth, with the digestive system playing a crucial role in nutrient absorption. This underscores the need to develop diets that support gut health and functionality (Adedokun and Olojede, 2019). While antibiotics were previously used as feed additives to promote gut health, concerns about side effects and public health have led to their ban in many countries (Castanon, 2007; El-Deek *et al.*, 2011). As consumer demand for antibiotic-free poultry products increases, there is growing interest in phytogetic feed additives as safer alternatives for enhancing gut health and productivity (Mahlake *et al.*, 2022).

Phytogetic feed additives such as garlic, neem, and bitter-leaf are increasingly used in intensive chicken production as natural growth promoters because of their antimicrobial, antioxidant, immunomodulatory, and performance-enhancing properties (Salisu *et al.*, 2024). Garlic enhances gut health and regulates lipid metabolism but requires careful dosing due to its strong odor and variable bioactive composition (Kharde and Soujanya, 2014). Neem improves growth, feed efficiency, carcass traits, and overall welfare through its broad antimicrobial and immune-stimulating compounds, although its bitterness and inconsistent phytochemical content can limit its application (Sobayo *et al.*, 2016; Salisu *et al.*, 2024). Bitter-leaf supports growth, metabolic health, and carcass leanness but may reduce palatability at high inclusion levels and contains anti-nutritional factors that require management (Adelowo *et al.*, 2020; Yeigba and Tule, 2021). Collectively, these botanicals represent promising natural alternatives to antibiotics, although challenges related to dose optimization, product standardization, and safety validation must be addressed before widespread adoption (Sobayo *et al.*, 2016).

1.2. Statement of the Problem

Although phytogetic feed additives are known for their antimicrobial and growth-promoting properties, research on their practical availability, utilization practices, and the effects of neem, bitter-leaf, and garlic in chicken diets remains limited. Most studies have focused on controlled experimental feeding trials, with little attention given to assessing the availability and utilization practices of phytogetic feed additives, as well as their comparative effects on chicken

performance. This underscores the need to evaluate both the accessibility of phytogetic feed additives and efficacy of neem, bitter-leaf, and garlic under real chicken production conditions.

The restriction of antibiotic use, due to the risk of bacterial resistance and drug residues in chicken products that threaten human health, has intensified the search for alternatives. In response to challenges such as reduced chicken performance, increased disease susceptibility, and the high cost and limited availability of antibiotics, researchers have explored phytogetic feed additives as potential solutions. However, the effectiveness of these alternatives requires thorough evaluation under practical production conditions.

Studies have shown that phytogetic feedstuffs can increase feed efficiency; consequently, farmers are increasingly adopting them to address these issues (Mnisi *et al.*, 2022). The restriction on antibiotic usage in chicken diets has driven the surge in phytogetic feed additive use (Wati *et al.*, 2015). Thus, there is a need to enhance chicken productivity and health through phytogetic feed additives (El-Saadany *et al.*, 2022; El-Sabrouit *et al.*, 2022). Safe compounds derived from medicinal plants that can boost the health status, improve stress resistance, and normalize physiological function should be prioritized when selecting phytogetic feed additives. The inclusion of polyphenols, vitamins, minerals, and other herbal ingredients in feed has been shown to significantly enhance chicken health by mitigating the effects of environmental stress (Abd El-Hack *et al.*, 2020). Phytogetic feed additives with proven pharmacological value, without compromising the nutritional content of feed, have been demonstrated to improve chicken production performance (Olgun *et al.*, 2021).

Phytogetic feed additives represent a new class of growth promoters, attracting significant interest in the chicken feed industry (Gao *et al.*, 2023). Various herbs and medicinal plants such as neem, garlic, and bitter-leaf have been identified as potential feed additives for enhancing the performance of both layer and broiler chickens (Dhana, 2015; Oni *et al.*, 2024). Previous studies support the selected inclusion rates. For neem, studies report improved broiler body weight at 1–3 g/kg (Alam *et al.*, 2015), with 2 g/kg also enhancing feed intake and blood parameters (Shihab *et al.*, 2017). It also positively affected layer feed intake (Singh *et al.*, 2014), suggesting 2 g/kg as the optimal inclusion rate. For bitter-leaf, Tokofai *et al.* (2020) found that 1–3% inclusion affected growth performance, with 2% producing the best results. Garlic supplementation also shows consistent benefits at inclusion level of 1–3%: Asrat *et al.* (2018)

reported improved yolk traits and reduced mortality at 2%, while Zeryehun *et al.* (2017) documented enhanced immunity in White Leghorn chickens at the same level.

Compared to synthetic antibiotics or inorganic chemicals, these plant-derived products offer a safer, residue-free alternative, making them ideal for chicken production (Dalal and Kosti, 2018). Identifying and utilizing major phytogenic feed additives from Ethiopia holds significant potential for expanding chicken production and enhancing both quantity and quality. To achieve higher production and improved quality of chicken products, the production methods play a critical role. In this context, organic chicken production is a promising opportunity for the country, particularly regarding feed costs, human health issues, and the availability of alternative herbal and spice feed additives. Damaziak *et al.* (2017), who found that the inclusion of garlic and onion in the hen diet was beneficial, as it increased egg weight, yolk weight, and the yolk-to-egg weight ratio, and improved albumen quality as expressed by Haugh units. Another study by Girma *et al.* (2021) revealed that a combination of neem and pumpkin seed has the potential to improve health as a feed additive in broiler chicken rations without compromising meat sensory quality.

However, there is a lack of knowledge regarding the effects and utilization practices of phytogenic feed additives. Therefore, it is essential to identify interventions for efficient utilization mechanisms and assess the availability of phytogenic feed additive resources in the selected study areas. Currently, there is no scientific information on accessible phytogenic feed additives and their effects as growth promoters and other enhancements for chicken products in the Northwest Amhara region. Furthermore, the cost of antibiotics used on chicken farms could be reduced by incorporating phytogenic feed additives into chickens' diets, enhancing growth and overall productivity. Therefore, this study aims to provide a comprehensive appreciation of the potential benefits and effect of phytogenic feed additives on chicken performances with the following objectives.

1.3. Research Objectives

- To assess availability, and utilization practices of phytogenic feed additives in selected areas of Northwest Amhara.

- To evaluate the effects of inclusion neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*) leaf meal, and garlic (*Allium sativum*) powder on layer chicken production performance and egg quality.
- To determine the effects of neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*) leaf meal and garlic (*Allium sativum*) powder on the hematological, serum chemistry, and gastrointestinal bacterial counts of layer hens.
- To investigate the effects of neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*) leaf meal and garlic (*Allium sativum*) powder on growth performances, carcass traits, haemato-biochemical indices and cecal bacterial load of Koekoek Chickens.

1.4. Research Questions and Hypotheses

The following research questions were developed to address the objectives of this study:

- i. What is the level of farmers' knowledge about phytogenic feed additives, and what are their availability, preference, and utilization in chicken production practices in Northwest Amhara?
- ii. What are the effects of neem, bitter-leaf lmeal, and garlic powder on the production performance and egg quality parameters of layer chickens?
- iii. How does the inclusion of neem, bitter-leaf, and garlic affect the hematological and serum biochemical parameters, as well as the gastrointestinal bacterial populations of layer hens?
- iv. What are the effects of neem, bitter-leaf, and garlic inclusion on the growth performance, carcass traits, haematobiochemical parameters, and cecal microbial load of Koekoek chickens?

More specifically, this study sought to test the following hypotheses:

- i. Farmers in Northwest Amhara have substantial knowledge and actively utilize phytogenic feed additives.
- ii. The inclusion of neem, bitter-leaf, and garlic powder significantly improves the production performance and egg quality of layer chickens.
- iii. Neem, bitter-leaf, and garlic powder significantly affect the hematological indices, serum chemistry, and reduce harmful gastrointestinal bacterial counts in layer hens.

- iv. Supplementation with neem, bitter-leaf, and garlic improves growth performance, enhances carcass traits, positively affects haematobiochemical indices, and modulates cecal bacterial load in Koekoek chickens.

1.5. Significance of the Study

This study carries both scientific and practical significance in promoting sustainable chicken production through the use of locally available phytogetic feed additives. Assessing farmers' knowledge of preference, availability, and utilization of these additives provides essential insights into current practices, awareness levels, and the potential for their integration into broader chicken management systems. Understanding this local context supports the development of targeted extension services and informed policy-making.

Evaluating the effects of neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*) leaf meal, and garlic (*Allium sativum*) powder on layer and Koekoek chickens will generate valuable data on their influence on productivity, egg quality, health status, and microbial balance. These findings can guide the evidence-based adoption of natural feed additives as alternatives to synthetic growth promoters, helping address issues related to antimicrobial resistance, food safety, and chemical residues in chicken products.

Moreover, the study provides new insights into the hematological and biochemical responses of chickens to these supplements, supporting their safe and effective use in chicken diets. The analysis of growth performance, carcass traits, and gut microbial composition in Koekoek chickens will further inform breed-specific nutritional strategies. Finally, the research aims to benefit chicken farmers, feed manufacturers, veterinarians, and policymakers by promoting cost-effective, health enhancing, and environmentally sustainable chicken production practices.

1.6. Organization of the Dissertation

This dissertation is organized into eight chapters (Figure 1.1). **Chapter 1** introduces the study by outlining the background, problem statement, research objectives, research questions, hypotheses, significance of the study, and an overview of the dissertation structure. **Chapter 2** presents the literature review, highlighting research gaps, theoretical frameworks, and methodological guidance that shape the study. **Chapter 3** describes the materials and methods, justifying the research design, addressing ethical considerations, guiding data analysis, and ensuring alignment with the research questions. **Chapters 4 to 7** consist of individual research papers, each including its own abstract, introduction, materials and methods, results and discussion, and conclusion. Finally, **Chapter 8** summarizes the key results, draws conclusions, and offers recommendations along with directions for future research.

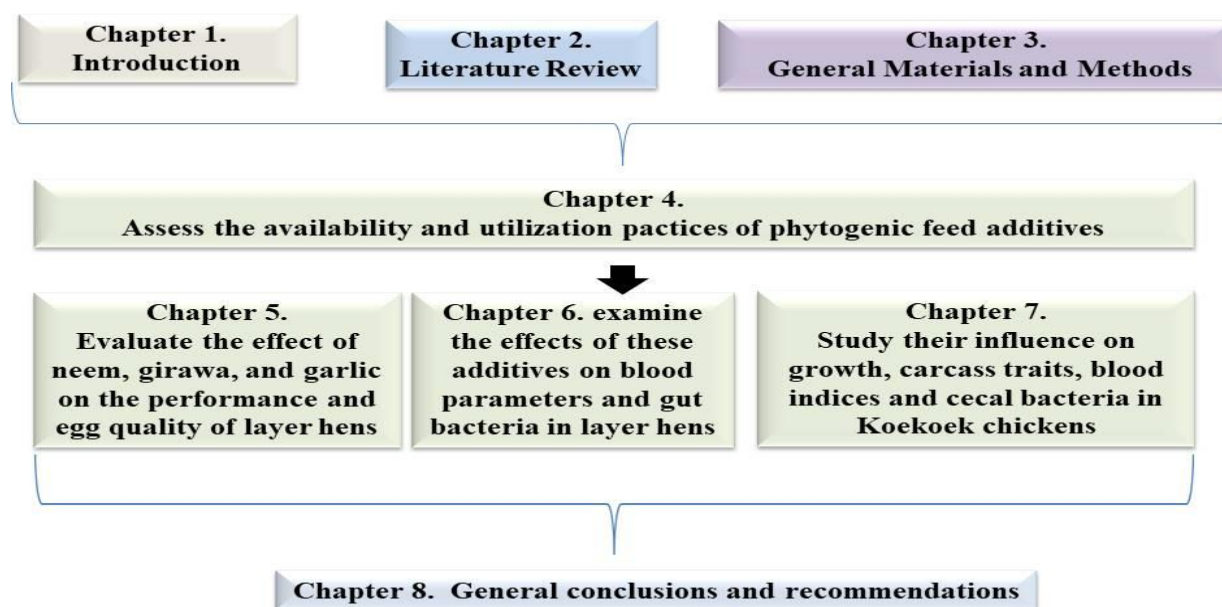


Figure 1.1. Structure of the Dissertation

CHAPTER 2: LITERATURE REVIEW

2.1. Phytogetic Feed Additives in Chicken Nutrition

Phytogetic feed additives, derived from herbs, spices, or aromatic plants, have gained considerable attention in the feed industry (Windisch *et al.*, 2008; Alagawany *et al.*, 2016). These plant-based products are incorporated into diets to improve chicken productivity by enhancing feed properties and overall production performance (Oni *et al.*, 2024). The increasing relevance of phytogetic additives, particularly for swine and poultry, is evident from the significant rise in scientific publications since 2000 (Frankic *et al.*, 2009). This surge in research interest appears to be driven largely by the European Union's ban on most antibiotic feed additives in 1999, which was fully enforced in 2006 due to concerns about human and animal health (Windisch *et al.*, 2008).

More recently, plant-based feed additives known as phytogetics and derived from herbs, spices, and essential oils have attracted attention as effective, natural alternatives to antibiotics (Abdelli *et al.*, 2021). They are considered safe due to their natural origin and the absence of harmful residues (Wang *et al.*, 2024). According to Yang *et al.* (2015), the active compounds in phytogetics are efficiently absorbed in the intestines and rapidly metabolized. Studies by Cardoso *et al.* (2012) and Ndelekwute *et al.* (2015) report that phytogetic additives can significantly improve feed intake, nutrient digestibility, feed conversion ratios, and overall weight gain in broilers. Thus, phytogetics represent a promising and sustainable strategy for enhancing poultry production without relying on synthetic antibiotics. Consequently, there is growing interest in developing new feed additives that do not contribute to bacterial resistance or adverse effects (Wenk, 2003; Abd El-Hack *et al.*, 2022). Phytogetic plants, known for their antimicrobial and antioxidant properties, are increasingly used to improve the health and performance of chickens (Minisi *et al.*, 2022).

The primary role of phytogetic feed additives is to positively influence the gastrointestinal microbiota by controlling potential pathogens (Shehata *et al.*, 2022). This mode of action enhances digestive capacity in the small intestine by stabilizing microbial eubiosis (Trevisi *et al.*, 2021). Maintaining intestinal health is essential for optimal chicken performance, and phytogetics play a crucial role in preventing intestinal disorders and supporting nutrient

absorption (Shehata *et al.*, 2022). As a result, phytochemicals can reduce immune stress in chickens during critical periods, increase the availability of essential nutrients, and support growth within their genetic potential (Abdelli *et al.*, 2021; Shehata *et al.*, 2022). Herbs and spices rich in flavonoids, vitamin C, and carotenoids also possess immune-stimulating properties that benefit chicken health (Rafeeq *et al.*, 2023). For example, neem has been shown to enhance feed utilization and improve immune responses to specific diseases (Kumar *et al.*, 2016), while extracts of bitter-leaf have demonstrated potential in improving blood parameters and combating helminths (Adegboye *et al.*, 2021).

2.2. Availability and Utilization Practices of Phytochemical Feed Additives

Low-cost and residue-free feed additives are essential for enhancing animal productivity (Sonaiya, 1990). To address this need, it is important to explore affordable, abundant, and locally available source of phytochemical feed additives (Odunsi *et al.*, 2002). This is especially relevant to poultry production, which heavily depends on commercial feed additives.

Rising costs and growing resistance to conventional feed additives pose significant challenges to the future of livestock production. Research efforts have not kept pace with the demands of sustainable animal feeding (Ayele *et al.*, 2021). A steady supply of cost-effective additives is therefore vital. Emphasis should be placed on identifying natural, locally sourced alternatives, particularly phytochemical feed additives that can reduce reliance on synthetic additives.

Phytochemical compounds are classified based on their sources herbs, spices, essential oils, or oleoresins. Processing methods such as chopping, grinding, crushing, and solvent extraction can influence the activity and composition of these compounds (Frankic *et al.*, 2009). Additives can be administered to chickens in various forms, including powders, liquid suspensions, and sprays (Azemraw and Sewalem, 2017). The frequency and dosage of administration vary depending on the purpose, some are given once, others daily or weekly (Kumar *et al.*, 2018).

In Ethiopia, many farmers cultivate aromatic herbs and spices for household use and local markets (Halima *et al.*, 2007). However, there is limited awareness among farmers about the potential use of the phytochemical resources in poultry nutrition. Despite this, phytochemical feed

additives are promising due to their natural origin, low toxicity, and absence of harmful residues (Ibrahim *et al.*, 2005).

Traditional practices are widely used in rural poultry health management. Farmers often treat chickens with medicinal plants such as ‘garden cress’ (*Lepidium sativum*) seed powder and ‘African soapberry’ (*Phytolacca dodecandra*) leaf juice (Gashu and Bezabih, 2014). In some areas, garlic and alcohol-soaked injera are fed to sick birds (Mengesha *et al.*, 2011). In Oromia’s Rift Valley, around 44% of farmers use medicine plants for chicken health (Dinka *et al.*, 2010), and similar practices are found in West Amhara (Mogoes, 2010). Other studies (Dagnaw, 2008) confirm the widespread reliance on indigenous knowledge, including the use of herbs like bitter-leaf (*Vernonia amygdalina*) and neem leaves (*Azadirachta indica*). Below is a brief description of three plant species with phytogenic feed additive potential.

I. Neem (*Azadirachta indica*)

Neem (*Azadirachta indica*) is a fast-growing evergreen tree known for its medicinal and nutritional value in poultry production. Neem leaves possess a wide range of therapeutic properties, including antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective, and antiparasitic effects, without causing adverse side effects (Kale *et al.*, 2003; Jawad *et al.*, 2014). These antibacterial properties have been associated with improved growth and feed efficiency in poultry (Prasannabalaji *et al.*, 2012; Oleforuh-Okoleh *et al.*, 2015a).

Neem leaves also exhibit immune-stimulant activity that enhances cell-mediated immunity and improves the bird’s resistance to disease. Obikaonu *et al.* (2011) reported that neem leaf meal could be included in starter broiler diets at levels up to 10% without harmful effects, while inclusion levels below 5% often resulted in improved feed intake and performance. However, neem products are generally recommended for use at low inclusion rates preferably below 5% and only after proper processing (Uko *et al.*, 2008). The inclusion of neem leaves in both drinking water and feed has been shown to enhance feed conversion efficiency and increase body weight in broilers (Shihab *et al.*, 2017).

In addition, neem leaves have been found to improve growth performance, carcass characteristics, and lymphoid organ development, as well as increase total protein, albumin, globulin, and triglyceride levels, while reducing cholesterol and fat deposition in broilers (Tollba

and Mahmoud, 2009). Alam *et al.* (2015) also reported significant improvements in body weight among broilers fed neem leaf powder at inclusion rates of 1, 2, and 3 g/kg. These findings are consistent with those of Shihab *et al.* (2017), who observed enhanced body weight and feed intake when 2 g/kg of neem leaf powder was added to broiler diets. Similarly, Singh *et al.* (2014) documented significant differences in feed intake among layers supplemented with 1, 2, and 3 g/kg of neem leaf powder. Furthermore, Shihab *et al.* (2017) found that supplementing broiler diets with 2 g/kg of neem leaf powder improved total protein, cholesterol, triglycerides, albumin, globulin, and calcium concentrations.

Overall, the literature indicates that neem leaf meal at an inclusion level of 2 g/kg is optimal for enhancing growth performance, blood biochemical parameters, and overall physiological responses in poultry.

II. Bitter-leaf (*Vernonia amygdalina*)

Bitter-leaf (*Vernonia amygdalina*) is a widely distributed medicinal plant in East and West Africa with notable antimicrobial and antioxidant properties (Burkill, 1985; Erasto *et al.*, 2007). Its leaves contain a range of bioactive compounds, including alkaloids, tannins, saponins, flavonoids, and glycosides, which contribute to its pharmacological effects such as antibacterial, antiparasitic, and hepatoprotective activities (Tadesse *et al.*, 1993; Arhoghro *et al.*, 2009).

The aqueous extract of bitter-leaf (*V. amygdalina*) has been shown to stimulate cell-mediated immunity, enhancing resistance to infections, particularly in immunosuppressed birds (Oleforuh-Okoleh *et al.*, 2015a). It also exhibits antioxidant activity, which can reduce oxidative stress and improve overall health, making it a potential alternative to synthetic antibiotics, many of which have been banned due to their residual effects on meat and eggs (Erasto *et al.*, 2006; Windisch *et al.*, 2008; Mehdi *et al.*, 2018).

Nutritionally, bitter-leaf (*V. amygdalina*) leaves provide essential macronutrients and trace elements and can improve feed conversion efficiency in poultry without negative impact (Olobatoke and Oloniruha, 2009; Banjoko *et al.*, 2019). Studies have also reported its efficacy in treating coccidiosis, bacillary white diarrhea, and bronchitis in poultry (Gbolade, 2009). At inclusion rates of 200-400 g per 150 kg of feed, the leaf meal has shown no adverse effects on broiler. Tokofai *et al.* (2020) reported that varying inclusion levels (1%, 2%, and 3%) of bitter-leaf (*Vernonia amygdalina*) leaf meal influenced weight gain, feed intake, and feed conversion

ratio in broiler chickens, and their findings indicated that a 2% inclusion level provided the most favorable improvement in growth performance and physiological responses. The authors suggested that bitter-leaf at 2% could be recommended for poultry production, while noting that additional studies are needed to validate these results.

However, bitter-leaf contains significant levels of anti-nutritional factors such as alkaloids, tannins, saponins, and glycosides, which can reduce feed intake and nutrient utilization if not properly processed and beyond optimal level of inclusions (Mohammed and Zakariya, 2012).

III. Garlic (*Allium sativum*)

Garlic (*Allium sativum*) is widely recognized for its broad spectrum of medicinal properties, including antibacterial, antifungal, antiparasitic, hypocholesterolemic, antioxidant, and immune-stimulatory activities (Amagase *et al.*, 2001). These effects are largely attributed to its rich content of sulfur-containing compounds such as alliin, allicin, ajoene, and diallyl sulfide. The enzyme alliinase, which becomes active when garlic is crushed or processed, converts alliin into allicin one of the most potent antimicrobial agents effective against bacteria, fungi, viruses, and parasites. Garlic has been used since antiquity as both a food additive and a natural remedy. In poultry, its bioactive components; especially allicin have been shown to enhance growth, support immunity, and promote a healthier gut environment (Santhosha *et al.*, 2013).

Garlic powder (GP), which contains bioactive compounds such as allicin, ajoene, and S-allylcysteine, is increasingly recognized as a valuable natural growth promoter in broiler nutrition (Ziarlarimi *et al.*, 2011). These compounds inhibit pathogenic microorganisms in the digestive tract, thereby enhancing nutrient absorption and overall growth performance (Kumar *et al.*, 2010). In poultry, garlic supplementation has demonstrated antimicrobial, antioxidative, antithrombotic, antiplatelet, and antihypertensive effects, which collectively contribute to improved feed efficiency, weight gain, immunity, and general health. Additional bioactive substances, including flavonoids and saponins, further strengthen garlic's antibacterial activity, with flavonoids in particular disrupting bacterial membranes and inhibiting microbial action. Garlic has also been used in layer diets to enhance egg production and improve egg quality.

Beyond its nutritional benefits, garlic plays a role in environmental and management aspects of poultry production. However, despite its economic importance and increasing demand, garlic cultivation faces challenges from various biotic and abiotic stresses, including poor mineral nutrition and adverse weather conditions (Jaleel *et al.*, 2007).

According to Asrat *et al.* (2018), adding 2% garlic powder to layer diets improved yolk weight, yolk diameter, yolk color, chick length, and reduced hen mortality, suggesting that 1-3% garlic powder can enhance egg yolk and eggshell traits without compromising overall performance or egg quality. Similarly, Zeryehun *et al.* (2017) reported that 2% garlic powder significantly increased total immunoglobulin levels and reduced eosinophil counts, indicating that incorporating 1-3% garlic powder into layer diets can improve hematological parameters and strengthen immunity in White Leghorn chickens.

2.3. Nutritional Value of Some Phytogetic Feed Additives

Common herbs and spices used as phytogetic feed additives in chicken production include oregano, thyme, garlic, black cumin, chili, cayenne, peppermint, cinnamon, anise, rosemary, and sage. These compounds provide beneficial properties due to their bioactive molecules (Yitbarek, 2015). Neem leaf meal contains approximately 15% protein and 8.5% ether extract (Bonsu *et al.*, 2012). Lamage and Hido (2021) reported that bitter-leaf (*Vernonia amygdalina*) contains 13.04% ash and 19.25% crude protein (CP) on a dry matter basis. Meza-Rios *et al.* (2024) reported that garlic's chemical composition consists of 1.2% amino acids, 2.3% sulfur compounds, and about 1.5% combined fiber, phenolic compounds, fatty acids, and trace elements.

Spirulina algae are notable for their high protein content, ranging from 50-70% dry weight, and provide essential amino acids along with vitamins, minerals, and carotenoids (Doreau *et al.*, 2010; Guroy *et al.*, 2012). Beyond herbs and spices, certain phytogetic feed additives like alfalfa, clover, and specific herbs are rich sources of vitamins A, E, and K, which are essential for immune function, growth, and overall health. Additionally, many phytogetic feed additives, including rosemary, sage, and oregano, provide essential minerals like iron, zinc, and magnesium (Djuragic *et al.*, 2023).

Many secondary compounds possess antioxidant and anti-inflammatory properties and are prevalent in various plants. Notable examples include flavonoids, tannins, and lignans, found

in rosemary, grape seed, and flaxseed (Sharma *et al.*, 2025). Additionally, nitrogen-containing compounds such as piperine from black pepper and berberine from barberry are recognized for their diverse biological activities, including antimicrobial, antiparasitic, and anti-inflammatory effects. Volatile compounds, often extracted from aromatic plants, are responsible for their characteristic scents and flavors. Examples include carvacrol, thymol, eugenol, and cinnamaldehyde, found in oregano, thyme, clove, and cinnamon, respectively (Dhifi *et al.*, 2016; Abdi-Moghadam *et al.*, 2023).

Secondary compounds can also be characterized by a sugar molecule attached to a non-sugar component, exhibiting a range of biological activities, including anti-inflammatory and antimicrobial effects. Saponins from alfalfa and glucosinolates from mustard are notable examples. Many secondary compounds have demonstrated antimicrobial activity against bacteria, fungi, and parasites, contributing to improved gut health, reduced disease incidence, and enhanced feed efficiency (Amerikova *et al.*, 2019). Furthermore, secondary compounds with antioxidant properties can protect against oxidative stress, which can damage cells and contribute to inflammation and disease, thereby promoting poultry health and immunity (Jomova *et al.*, 2024).

Certain secondary compounds can stimulate digestive enzyme activity, improve gut motility, and modulate gut microbiota, leading to better nutrient absorption and utilization (Wang *et al.*, 2022). Some of these compounds possess anti-inflammatory properties that can reduce inflammation in the gut and other tissues, promoting overall poultry health (Nunes *et al.*, 2020). Moreover, specific secondary compounds can stimulate the immune system, enhancing poultry's ability to fight off infections and diseases (Amerikova *et al.*, 2019). Overall, phytogenic feed additives can bolster the immune system, reduce disease incidence, and improve poultry health (Nunes *et al.*, 2020).

2.4. Effect of Phytogenic Feed Additives on Chicken Production Performance and Egg Quality

Medicinal herbs are widely used as feed additives for chickens due to favorable climatic conditions and their abundance of bioactive compounds (El-Sabrou *et al.*, 2023). The shift toward intensive poultry production has increased the demand for meat and eggs, driving the

development of cost-effective and environmentally friendly feed additives (Dong *et al.*, 2024; Wang *et al.*, 2024). Botanicals are particularly attractive because of their low toxicity and multifunctional properties, as they can enhance feed intake, stimulate digestion and enzyme secretion, improve liver function, and support carbohydrate and protein metabolism, ultimately promoting growth (Abd El-Hack *et al.*, 2022; El-Sabrou *et al.*, 2023). In addition, these herbs exhibit a wide range of biological activities, including immune stimulation, antibacterial, antiviral, anti-inflammatory, antioxidant, coccidiostatic, and antihelminthic effects (Shawon *et al.*, 2024), with active secondary metabolites such as isoprene derivatives, flavonoids, and glucosinolates functioning as natural antibiotics or antioxidants (Frankic *et al.*, 2009).

The timing and route of administration significantly influence the efficacy of phytogetic feed additives (PFAs), with supplementation through feed generally yielding better results than through drinking water, particularly for average daily gain (Abdelli *et al.*, 2021). Early-life supplementation is especially critical, as harmful bacteria can alter gene expression in intestinal epithelial cells, affecting gut development (Timmerman *et al.*, 2006). Multiple studies, summarized (Table 2.1), consistently show that PFAs improve growth, feed efficiency, and overall health. For instance, seaweed and fenugreek seed supplements enhance feed conversion and reduce production costs (Mohammadigheisar *et al.*, 2020; Paneru *et al.*, 2022), while seaweed also improves meat quality by boosting immune function and reducing gut pathogens (Nhlane *et al.*, 2020; Matshogo, 2023). Other botanicals, including anise, garlic, thyme, and black pepper, have demonstrated benefits such as increased weight gain, improved feed efficiency, and enhanced overall production performance (Kaczmarek *et al.*, 2016; Rafeeq *et al.*, 2023).

Specific studies highlight the effectiveness of particular PFAs. For example, thyme supplementation at 0.1–0.5% improves feed conversion and egg production while reducing *E. coli* counts (Gholami-Ahangaran *et al.*, 2022). *Spirulina platensis* at 0.1–0.3% in laying hen diets has been shown to increase body weight, feed intake, and egg production (Selim *et al.*, 2018). Additionally, garlic and thyme improve laying hen performance, including egg quality and weight gain (Gerzilov *et al.*, 2015). Moreover, turmeric at 0.5–1.0% increases egg weight, mass, and yolk pigmentation (Dalal and Kosti, 2018; Kinati *et al.*, 2021). Essential oil-based PFAs, including mint, oregano, thyme, and clove, are particularly effective in stimulating digestion, enhancing nutrient absorption, and boosting broiler growth rates (Mohammadigheisar and Kim,

2018; Oladeji *et al.*, 2019), effects attributed to increased enzyme activity and improved gut microflora (Abdelli *et al.*, 2021). Overall, PFAs improve feed conversion ratios, nutrient digestibility, and weight gain (Flees *et al.*, 2020; Wang *et al.*, 2024), while improved feed palatability reduces feed waste and promotes intake. Their antioxidant and antimicrobial properties further strengthen the immune system and reduce disease incidence, contributing to improved overall poultry health (Abdelli *et al.*, 2021; Wang *et al.*, 2024). In broiler chickens, bitter-leaf (*Vernonia amygdalina*) leaf extract has been shown to improve growth performance. Specifically, inclusions significantly increased body weight gain and improved feed conversion ratio (FCR) (Tokofai *et al.*, 2021).

Several studies in Ethiopia have shown that phytogenic feed additives, particularly garlic (*Allium sativum*) and neem (*Azadirachta indica*), can improve the performance and health of layer chickens. Among these, garlic supplementation at 1-3%, particularly 2%, has been shown to enhance yolk weight and yolk color while reducing hen mortality in White Leghorn layers (Asrat *et al.*, 2018). Similarly, neem supplementation has demonstrated positive effects in both layers and broilers; for instance, Mathewos *et al.* (2019) reported improved gut health and growth performance in Ethiopian broilers fed neem. These findings indicate that garlic and neem are effective natural feed additives that can enhance productivity, egg quality, and overall health in Ethiopian chicken, highlighting their potential for sustainable layer production.

Table 2.1. Effects of dietary supplementation of PFAs on performance, product qualities and immunity of broiler and laying hens

Feed additives	Major compounds	Level of inclusion	Line and Age	Main funding	References
Turmeric (<i>Curcuma longa</i>)	Curcumin	0.5g/kg	Lohmann broiler chicken (22-35 weeks)	↑ FBW, feed digestibility and ↓FCR	(Sugiharto, 2020)
Green tea	Polyphenols	200mg/kg	Hy-line Brown (65–74 weeks)	↑ egg production and ↓FCR	(Wang <i>et al.</i> , 2018)
Neem leaf meal (NLM), bamboo leaf meal (BLM) and paw-paw leaf meal (PLM)	NLM: triterpenoid (Azadirachtin) BLM: silica PLM: Carpaine	5 g/kg for each and 5 g/kg at (1:1:1) for their mixture	Arbor Acres broiler chicken (1-42 weeks)	↑ FBW, ↓serum glucose, trigly cerides and cholesterol, Promo ted haematopoietic activities, boosting immunity and antioxidative status	(Oloruntola <i>et al.</i> , 2019)
Thyme (<i>Thymus vu lgaris</i> <i>L.</i>) and rosemary (<i>Rosmarinus officinalis L.</i>)	Thymol and camphor	1000 mg/kg for each	Bovans laying hens (48-56 weeks)	↑egg production and egg nutritive value and ↓ yolk bad cholesterol and triglyceride levels	(Cimrin, 2019)
Peppermint and Thyme EO	Menthol and menthone Thymol, Terpinen	1000mg/kg	Lohmann LSL-lite (40–48 weeks)	↑egg production and egg mass, ↓FCR	(Akbari <i>et al.</i> , 2016)
Dried grape Pomace	Polyphenols	40,000 and 60,000 mg/kg	Bovans laying hens (80–92 weeks)	=Live weight, feed intake, egg production and feed efficiency	(Kara <i>et al.</i> , 2016)
Fennel seeds (F), black cumin (BC) seeds and hot red pepper (RP)	F: trans-anethole BC: thymoquinone RP: Capsaicin	5000 mg/kg for each	Lohmann Brown laying hens (32–40 weeks)	↑egg weight, egg production, egg mass and ↓FCR by F and RP	(Abou- elkhair <i>et al.</i> , 2018)
Garlic and onion powder	Garlic: Allicin Onion: Quercetin	5 and 10g/kg for garlic, 10g/kg for onion	Bovan Brown layers (30-42 weeks)	↑egg weight, and feed conversion of hens and ↓in blood cholesterol	(Omer <i>et al.</i> , 2019)
peppermint, lemongrass, and thyme	Peppermint: Menthol Lemongrass: Citral Thyme: Thymol	1g/kg for each	Cobb 500 brioler	↑ FCR	Tesfaye <i>et al.</i> , 2023

Table 2.2. Effects of dietary supplementation of PFAs on performance, product qualities and immunity of broiler and laying hens (*continued*)

Papaya seed		Benzyl isothiocyanate	5, 10 and 15 g/kg		↑DMI, egg production, egg mass and FCR	Dissa <i>et al.</i> , 2023
Garlic	and	Garlic: Allicin	2 g/kg	White Novogen laying hens	↑shell and albumen weight, ↓egg yolk cholesterol	Ayed <i>et al.</i> , 2018
Turmeric		Turmeric:Curcumin				
Bitter-leaf		Vernodalin	5-10g/kg	Arbo acre broiler chicks	↑FBW, ↑BWG, ↑ HDL, ↓ LDL, and ↓ total cholesterol	Adelowo <i>et al.</i> , 2020

ADG: Average Daily Gain; ADFI: Average Daily Feed Intake; EO: essential oil; BWG: body weight gain; FBW: final body weight;

FI: Feed Intake; FCR: Feed Conversion Ratio; ↑: increased; ↓: decreased; =: equal.

2.5. Effect of Phytogenic Feed Additives on Chicken Blood Profile

Blood profiles are crucial indicators of a chicken's physiological condition (Khan and Zafar, 2005). Hematological and serum biochemical parameters serve as valuable tools for assessing health status, offering insights into stress, infections, and toxicity (Etim *et al.*, 2014). These blood constituents also provide essential information for clinical evaluations and nutritional assessments (Aderemi, 2004).

The influence of dietary components on hematological indices is critical for determining the suitability of feed ingredients (Etim *et al.*, 2014). Parameters such as packed cell volume (PCV), red blood cell (RBC) count, and hemoglobin (Hb) concentration correlate with production traits and reflect overall health and nutritional status (Mitruka and Rawnsley, 1997; Onasanya *et al.*, 2015). For example, higher PCV and Hb levels are associated with better feed conversion ratios, while increases in white blood cell counts particularly lymphocytes indicate enhanced resilience to stress.

Neem, due to its rich bioactive compounds, can influence hematological and serum biochemical parameters in animals and birds (Jawad *et al.*, 2014). Studies have shown that neem supplementation increases heterophils and lymphocytes while reducing monocytes in the white blood cell profile (Nayaka *et al.*, 2013). Neem has also been reported to elevate hemoglobin levels, with the highest concentrations found in chickens receiving a combination of neem, turmeric, and vitamin E (10.90 g/dl), followed by neem alone (10.50 g/dl) and neem with vitamin E (10.50 g/dl) (Nayaka *et al.*, 2013). However, findings from Biu *et al.* (2009) and Akbari *et al.* (2008) contradict these results, reporting significant reductions in hemoglobin levels when high amounts of neem and vitamin E were included in broiler diets.

Similarly, garlic supplementation has shown positive hematological effects. According to Zeryehun *et al.* (2017), including 1-3% garlic powder in layer diets improves hematological and immunoglobulin values, potentially enhancing blood circulation and immune function in White Leghorn chickens. Numerous studies also demonstrate that phytogenic feed additives (PFAs) can increase RBC count and hemoglobin levels in chickens (Ismail *et al.*, 2021). This improvement is likely due to the antioxidant activity of plant-derived compounds, which protect red blood cells from oxidative damage. Higher hemoglobin levels reflect enhanced oxygen-carrying capacity, leading to better tissue oxygenation and metabolic efficiency (Ben-Gigi *et al.*, 2021).

Bitter leaf (*Vernonia amygdalina*) extract likewise contributes to improved physiological responses in poultry. The extract shows notable hepatoprotective effects; in liver-damaged broilers, it significantly reduced serum ALT, AST, and ALP levels, thereby supporting improved liver function, metabolism, and overall productivity (Tokofai *et al.*, 2021). Furthermore, sesquiterpene lactones and other bitter compounds in *V. amygdalina* demonstrate strong anthelmintic activity, effectively reducing *Ascaridia galli* infestation in chickens (Siamba *et al.*, 2007). Additionally, administering bitter leaf extract through drinking water has been shown to improve hematological parameters such as PCV, Hb, and RBC count when provided at appropriate doses (Ikugbiyi *et al.*, 2024).

Beyond these effects, PFAs can also modulate white blood cell counts, with several studies reporting increases in specific WBC types, including heterophils and lymphocytes (Ismail *et al.*, 2021). This suggests that PFAs may enhance immune function, improving the bird's ability to resist infections and disease. PFAs also influence liver health, as reflected in changes in biochemical markers such as AST, ALT, and ALP (Tokofai *et al.*, 2021; Ashayerizadeh *et al.*, 2023). Reductions in these enzymes indicate improved liver function, likely due to the antioxidant and hepatoprotective properties of certain phytochemicals (Tokofai *et al.*, 2021).

Furthermore, PFAs can affect kidney function, as shown by changes in creatinine and urea levels. Some studies indicate that PFAs help maintain normal renal function or even enhance kidney health (Oladeji *et al.*, 2019; Abdelli *et al.*, 2021; Wang *et al.*, 2024), possibly due to their diuretic effects or their capacity to mitigate oxidative stress. Research also suggests that PFAs can reduce triglyceride levels and increase HDL cholesterol, indicating potential cardiovascular benefits (Griela *et al.*, 2021). Additionally, these additives can influence other biochemical parameters, including blood glucose and protein concentrations (Abdelli *et al.*, 2021).

2.6. Impact of Phytogetic Feed Additives on Chicken Gut Microbial Load

Studies have shown that phytogetic feed additives can significantly affect the composition and diversity of the chicken gut microbiome. Some research has reported an increase in microbial diversity, suggesting a more balanced and resilient gut ecosystem. PFAs enhance nutrient digestibility and absorption by stimulating enzyme production and modifying gut microbial metabolism (Karaskova *et al.*, 2015). Many phytochemicals possess antimicrobial

properties that inhibit the growth of pathogenic bacteria while promoting beneficial ones. Moreover, phytogetic compounds stimulate the production of digestive enzymes, further enhancing nutrient absorption and utilization (Lexing *et al.*, 2021). They act as antioxidants, protecting the gut from oxidative stress and inflammation. Certain phytogetic compounds can also stimulate the immune system, strengthening the gut barrier and enhancing resistance to pathogens (Adele *et al.*, 2024). These additives can alter the relative abundance of specific bacterial groups, increasing the presence of beneficial bacteria like *Lactobacilli* and *Bifidobacteria* while reducing potentially pathogenic bacteria such as *salmonella* and *E. coli* (Wang *et al.*, 2024).

Moreover, phytogetic additives enhance immune function, leading to reduced susceptibility to infections and improved disease resistance (Akosile *et al.*, 2023; Wang *et al.*, 2024). The positive impact on the gut microbiome can translate to improved growth performance in chickens, including increased weight gain and feed efficiency (Yu *et al.*, 2022). Essential oils from plants such as oregano, thyme, and cinnamon have shown promising results in modulating the chicken gut microbiome. Similarly, herbs and spices like ginger, garlic, and turmeric contain bioactive compounds that positively influence gut health. Extracts from plants like rosemary, licorice, and grape seed have also been investigated for their potential to improve gut microbial balance (Grandhay *et al.*, 2020; Anwar *et al.*, 2025).

Tipu *et al.* (2006) noted that medicinal plants and herbs have been used for many years to treat various diseases in both animals and humans. The utilization of these medicinal plants is increasing, especially as they are incorporated into animal feed as growth promoters. With the prohibition of most antimicrobial growth promoters due to their residual effects, plant extracts are becoming more popular (Ivanova *et al.*, 2024). A study using Bitter leaf (*Vernonia amygdalina*) in broiler chickens found improvements in lipid profiles and reductions in pathogenic bacteria, concluding that it has ‘potential uses as a natural feed additive to improve broiler health (Ilesanmi and Fajilade, 2025).

The leaf of Neem (*Azadirachta indica*) are used to feed animals and reduce parasitic loads. Tollba and Mahmoud (2009) conducted an experiment to reduce intestinal pathogenic bacteria populations in broiler chicks under normal or heat stress conditions using neem leaf. Neem leaf extract effectively demonstrated antibacterial and immune stimulant activities in controlling intestinal pathogenic bacteria, thereby improving the performance and physiological and

bacteriological status of broilers (Ali *et al.*, 2021). Therefore, it is advisable to focus more on medicinal plants in broiler diets, under both normal and stressed environmental conditions.

2.7. Role of Phytogetic Feed Additives on Carcass Characteristics of Broiler Chickens

Studies have shown that phytogetic feed additives (PFAs) can positively influence carcass yield in broiler chickens. For instance, Hassan *et al.* (2024) reported that incorporating thyme, ginger, and their mixture into broiler diets significantly increased carcass weight and breast muscle yield compared to control groups. Similarly, El Hamouly *et al.* (2014) found that broiler chicks fed diets containing 0.0%, 2.0%, 3.0%, or 4.0% garlic powder achieved the highest body weight gain, feed intake, feed conversion ratio (FCR), and dressing and breast percentages at the 3.0% inclusion level. Salisu *et al.* (2024) reported that 45-day-old broilers fed 1.0 g/kg neem leaf achieved the highest body weight gain, best FCR, and improved dressed weight, while Sufi *et al.* (2020) found that adding neem and bitter leaf extracts to drinking water at 6 ml/L significantly increased dressed weight and carcass cut weights compared to the control. These improvements have been attributed to enhanced feed digestibility and nutrient absorption promoted by the bioactive compounds in PFAs.

In addition, Kairalla *et al.* (2022) demonstrated that supplementing broiler feed with garlic powder increased carcass weight, breast muscle weight, and thigh weight, likely due to the anti-inflammatory properties of garlic extract, which reduce stress and enhance growth potential. Studies have also shown that incorporating cinnamon and ginger into broiler diets can significantly improve meat tenderness and juiciness, likely through the antioxidant properties of these spices, which protect muscle fibers from oxidative stress during processing (Gaikwad and Fulpagare, 2024). Furthermore, Lokaewmanee and Sirival (2022) observed that supplementing broiler diets with residues of *Zingiber cassumunar*, *Kaempferia galangal*, or *Curcuma aromatica* improved the overall acceptability of raw and cooked chicken breast meat, suggesting that curcumin in turmeric may enhance muscle pigmentation and contribute to a more desirable flavor profile. PFAs have also been reported to improve meat quality parameters such as tenderness, color, and water-holding capacity (Mir *et al.*, 2017).

The positive effects of PFAs on carcass characteristics translate into substantial productivity improvements for broiler producers (Ayalew *et al.*, 2022). Improved carcass yield

and enhanced meat quality increase product value, while better feed utilization reduces feed costs, enhancing overall profitability.

2.8. Bovans Brown Layer Hens and Performance

The Bovans Brown laying hen is well adapted to all production systems, including intensive, semi-extensive, and free-range systems (Solomon *et al.*, 2018). It maintains excellent health, resulting in high livability, and is easy to manage. Bovans Brown hens also demonstrate strong productivity, laying attractive dark brown eggs with good shell quality. This breed is the most dominant in Bahir Dar (Aderaw *et al.*, 2021) and in the urban and peri-urban areas of Dessie town (Solomon *et al.*, 2018), as it is both accessible and highly preferred for egg production.

Bovans Brown chickens are among the most widely used commercial layer strains due to their consistently high egg production, superior feed efficiency, adaptability to diverse production environments, and strong livability under both intensive and semi-intensive systems. As high-performing layers, their productivity depends heavily on diet quality and gut health, prompting producers to explore natural alternatives to antibiotic growth promoters (Tomas *et al.*, 2017). Phytogetic feed additives such as garlic (*Allium sativum*), neem (*Azadirachta indica*), and bitter leaf (*Vernonia amygdalina*) have gained considerable attention because they contain bioactive compounds including allicin, flavonoids, limonoids, saponins, tannins, and various sulfur derivatives that exhibit antimicrobial, antioxidant, immune-modulating, and digestive-enhancing properties.

2.9. Koekoek Chickens and Performance

Koekoek chickens originally developed as a dual-purpose breed for both meat and egg production are widely utilized in smallholder and semi-intensive poultry systems across Africa. Their popularity stems from their proven adaptability to diverse environmental conditions, good survival rates, and relative resistance to common poultry diseases (Gobena, 2020). Although Koekoeks do not grow as rapidly as commercial broiler strains, their robustness and efficient feed utilization make them suitable for low-input production systems. Improving their growth

rate and overall productivity therefore requires nutritional interventions, including the use of natural growth promoters.

While direct studies involving phytogetic feed additives in Koekoek chickens are limited, plant-derived additives such as garlic (*Allium sativum*), neem (*Azadirachta indica*), and bitter leaf (*Vernonia amygdalina*) have been extensively evaluated in commercial broilers and other indigenous breeds. These plants are rich in bioactive compounds including organosulfur compounds, flavonoids, tannins, and saponins which contribute to improved digestion, antimicrobial activity, immune modulation, and enhanced nutrient utilization. For example, garlic powder has been shown to enhance growth performance and improve hematological parameters in broilers (Kairalla *et al.*, 2022). Similarly, neem leaf supplementation has been associated with improved body weight gain, feed conversion efficiency, and immune response (Ogunsipe *et al.*, 2020). Bitter leaf extracts also exhibit beneficial effects on growth performance, gut health, and carcass characteristics (Yeigba and Tule, 2021).

Given the moderate growth rate yet strong resilience of Koekoek chickens, integrating these natural phytogetic additives into their diets may provide a promising, and sustainable strategy to enhance performance while reducing dependence on synthetic growth promoters.

CHAPTER 3: GENERAL MATERIALS AND METHODS

This chapter outlines the general materials and methods used in the research, providing an overview of the survey study, experimental design, and procedures. The study comprises four activities, a survey study and three experimental studies each specifically designed to address key research questions (**Chapters 4 to 7**).

3.1. Study Areas

The survey study was conducted in urban, peri-urban, and rural areas of Bahir Dar city and West Gojjam Zone in Amhara region, Ethiopia. These sites were selected based on chicken production potential, as confirmed by the Amara regional Bureau of Agricultural Offices and the Central Statistical Agency (CSA, 2021).

Bahir Dar is the capital city of Amhara National Regional State, Ethiopia. It's located about 565 km north of Addis Ababa at 11° 38' N latitude and 37° 10' E longitude. The elevation of the city is about 1801 meters above sea level, and it receives an average annual rainfall of 850 mm to 1250 mm, a minimum average daily temperature of 10°C, and a maximum of 32°C.

Bahir Dar Zuria District (BDZD) is found in the West Gojjam Zone. The area is located about 564 km northwest of Addis Ababa. It is situated at an altitude ranging from 1700 to 2300 metres above sea level; and between 11°25'N and 11°55'N latitude and 37°04'E-37°39'E longitude. The mean annual temperature is about 20°C, with a maximum temperature 28.3°C and a minimum of about 10.2°C. The annual rainfall ranges from 800-1250 mm.

Semen Achefer district is located 101 km from Bahir Dar and 591 km from Addis Ababa. Its geographical coordinates are 11° 41' 0" north latitude and 36° 57' 0" east longitude. The elevation of the district is about 1,500-2,500 meters above sea level.

3.2. Sampling Method and Sample Size Determination

A multi-stage sampling procedure was used to collect data. Stage 1: Bahir Dar and West Gojjam Zone were purposively selected based on their chicken production potential. Stage 2: Three districts, Bahir Dar City, Semen Achefer, and Bahir Dar Zuria, were purposively selected. Stage 3: Within these districts, kebeles representing urban, peri-urban, and rural areas were also randomly selected. Finally, 40 households were purposively selected from each selected kebele, resulting in a total of 320 households participating in the study.

3.3. Method of Data Collection

3.3.1. Survey data collection

Households were interviewed using semi-structured questionnaires on the use of phytogenic feed additives. A focus group discussion with community members in the study areas was also conducted to gather information on utilization techniques and the plant parts used. Additionally, key informants including elders, long-time chicken farmers, development agents, an animal production expert, and a veterinarian with experience in poultry production were consulted to assess the availability and utilization practices of phytogenic feed additives. Both primary and secondary data were collected from various sources.

3.3.2. Selection of phytogenic feed additives for analysis

Top five phytogenic feed additives (PFAs) were selected based on farmer rankings in availability and utilization practices. These samples (neem, bitter-leaf, garlic, cabbage, ginger) were collected, dried, ground, and analyzed for contents of dry matter (DM), ash, crude protein (CP), crude fiber (CF), phosphorus (P), and metabolizable energy (ME).

3.4. Experimental Studies

3.4.1. Study site for experiments

The experiments were conducted at Bahir Dar University Chicken Farm in Zenzelma campus (**chapter 5, 6 and 7**), which is located 565 km west of Addis Ababa at an altitude of 1911 meter above sea level, 11° 37' 18"N latitude, and 37°27'47" E longitude. The mean annual rainfall is 1445 mm. The mean minimum and maximum temperatures during the experimental period were 18.5°C and 30.3°C, respectively.

3.4.2. Preparation of phytogenic feed additives

Garlic was purchased from the local market in Bahir Dar city. Fresh bitter-leaf (*Vernonia amygdalina*) and neem (*Azadirachta indica*) leaves were harvested in Woreb Kebele and Maraki Kebele, respectively, both located in Bahir Dar city. To prepare the garlic, the whole part of fresh garlic was chopped into small pieces and spread on a plate, using plastic shade netting to dry under the sun. The dried garlic was then ground into a fine powder using a 2 mm sieve and stored in an airtight polyethylene bag until needed. For the leaves, fresh bitter-leaf and neem leaves were sparsely spread on a plastic mat at room temperature for 8 days, allowing them to dry until crispy and then dirt was handpicked. The leaves were turned regularly to ensure even drying and prevent decay, maintaining their greenish color. Once dry, the crispy leaves were hammer-milled, to pass through a 2 mm sieve, and then stored in airtight plastic sack to prevent moisture absorption. The processed leaves and garlic powder were manually mixed with the commercial diet. A small amount (0.625 kg) of each additive was added per 25 kg of feed, and then blended properly to prepare homogenous mixtures (**chapter 5 and 6**).

3.4.3. Experimental designs

All the two experimental and one laboratory studies were conducted in a completely randomized design, with 4 treatments each with 4 replications. The arrangement of the experimental treatments was as follows:

T1: Basal diet without additive (control).

T2: Basal diet + 2.5% neem

T3: Basal diet + 2.5% bitter-leaf

T4: Basal diet + 2.5% garlic

3.4.4. Laboratory analysis

3.4.4.1. Proximate and some secondary metabolite analysis

Prior to the experiment, samples of neem, bitter-leaf, and garlic powder were collected and analyzed for their chemical composition. All feedstuffs, including these additives and the experimental diets, were ground to pass through a 1 mm screen. Proximate analysis was conducted according to AOAC (1996) methods. DM, Ether Extract (EE), CF, CP and ash were analyzed. The CP content was determined using the Kjeldahl method, with nitrogen content multiplied by 6.25. Calcium was measured using atomic absorption spectrophotometry, and phosphorus was determined colorimetrically. Phytochemical constituents were analyzed following standard procedures. Total phenolic were determined according to Woldegiorgis *et al.* (2014) and Kuyu *et al.* (2016), while total flavonoids were analyzed using the method of Goyeneche *et al.* (2015). Condensed tannins were measured using the method of Maxson and Rooney (1972), and alkaloids were quantified using the procedure described by Harborne (1973). Analyses of DM, CP, and ash were carried out at the Animal Nutrition Laboratory of Bahir Dar University, while the remaining parameters, including secondary metabolites, were analyzed at Jimma University. The metabolizable energy (ME) of the experimental diets was estimated using the equation proposed by Wiseman (2013):

ME (kcal/kg DM) = 3951 + 54.4 EE – 88.7 CF – 40.8 Ash.

3.4.4.2. Blood sample collection and analysis

At the end of the feeding trial, blood samples were collected from both layer hens and Koekoek chickens for hematological and biochemical analysis. Eight birds were randomly selected from each treatment group. Blood was drawn from overnight-fasted hens via the wing

vein by a qualified veterinarian using a 5 mL syringe fitted with a sterile 22-gauge hypodermic needle.

From each bird, the blood was divided into two portions: For hematological analysis, approximately 2 mL of blood was transferred into tubes containing EDTA (ethylene diamine tetraacetic acid) as an anticoagulant. For layer hens, red blood cell (RBC) count, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined using an automatic hematology analyzer (Hemax330) at ANAMEL private clinic. PCV was also measured using the microhematocrit method described by Kelly (1979). For Koekoek chickens, Hb, PCV, RBC count, MCV, MCH, MCHC and total white blood cell (WBC) count were analyzed using the ADVIA 560 automatic hematology analyzer.

For biochemical analysis, approximately 3 mL of blood was collected into plain tubes without anticoagulant. Samples were centrifuged at 5,000 rpm for 5 minutes, and the serum was decanted and transported in an ice box. For both chicken types, serum biochemical parameters were analyzed using an automated chemistry analyzer. These included total protein, albumin, triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). In Koekoek chickens, additional parameters such as uric acid, creatinine, alkaline phosphatase (ALP), and glucose were also measured. Globulin levels were calculated by subtracting albumin from total serum protein (Doumas *et al.*, 1985). All hematological and biochemical analyses of koekoek chickens were performed at Bahir Dar Tibebe Ghion Specialized Referral Hospital, Bahir Dar, Ethiopia.

3.4.4.3. Bacterial counts

At the end of the experiment, bacterial count was conducted on gastrointestinal samples from both layer hens and Koekoek broiler chickens to determine total bacterial counts and the abundance of selected gut bacteria. For layer hens, two birds per replicate (a total of eight hens per treatment group) were slaughtered. Digesta samples were aseptically collected from the crop, gizzard, small intestine, ceca, and feces at the cloaca. Similarly, for Koekoek chickens, two chickens per replicate (eight chickens per treatment group) were slaughtered and cecal digesta

samples were collected for bacterial evaluation from slaughtered chickens for carcass trait analysis.

The ceca were ligated, and their contents were carefully hand-stripped into sterile plastic bags. All samples were aseptically transferred to sterile sampling tubes and immediately transported to the laboratory for bacterial analysis. For both chickens' types, bacterial dilution cultures were prepared by suspending 1 g of each sample in 9 mL of buffered peptone water. Serial dilutions ranging from 10^{-1} to 10^{-10} were prepared. From each dilution, 0.1 mL (or 250 μ L in the case of cecal bacterial analysis) was spread on selective agar for bacterial enumeration. For total bacterial counts were determined using plate count agars. *Escherichia coli* colonies were enumerated on Eosin Methylene Blue (EMB) Agar, incubated aerobically at 37°C for 24 hours. *Lactobacillus* was counted on de-Man, Rogosa, and Sharpe (MRS) Agar, incubated anaerobically at 37°C for 72 hours. *Salmonella* was identified using a colorimetric reaction on Xylose Lysine Deoxycholate (XLD) Agar, incubated at 37°C for 48 hours.

3.5. Statistical Analysis

Data collected from urban, peri-urban, and rural areas were entered and analyzed using the Statistical Package for Social Sciences (SPSS), version 27. The normality of the data was assessed using the Kolmogorov Smirnov test. A Chi-square test was employed to examine associations between categorical variables across the three study areas (urban, peri-urban, and rural). Descriptive statistics (means, and percentages) were used to summarize both qualitative and quantitative data. To compare the means of quantitative variables among the study areas, one-way analysis of variance (ANOVA) was conducted with a model consisting of treatments. The mean comparisons were made using tukey HSD procedures. Indices were calculated using the formula given by Kosgey (2004);

index

$$= \frac{\Sigma (n \times \text{No. of HHs ranked 1st}) + (n - 1) \times \text{No. of HHs ranked 2nd} + \dots + 1 \times \text{No. of HHs ranked last}) \text{ for one factor}}{\Sigma (n \times \text{No. of HHs ranked 1st} + (n - 1) \times \text{No. of HHs ranked 2nd} + \dots + 1 \times \text{No. of HHs ranked last}) \text{ for all factors}}$$

Where; n: value given for the least ranked level (example if the least rank is 5th rank n-1=4, n-

2=3 and.....n=1)

CHAPTER 4: AVAILABILITY, UTILIZATION PRACTICES AND FARMERS' PERCEPTION OF PHYTOGENIC FEED ADDITIVES FOR CHICKEN PRODUCTION IN NORTHWESTERN AMHARA, ETHIOPIA

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AVAILABILITY, UTILIZATION PRACTICES AND FARMERS' PERCEPTION OF PHYTOGENIC FEED ADDITIVES FOR CHICKEN PRODUCTION IN NORTHWESTERN AMHARA, ETHIOPIA

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ABSTRACT

This study aimed to assess the availability, utilization practices, and farmers' perceptions of phytogetic feed additives (PFAs) for chicken production in Bahir Dar City and the West Gojjam Zone of the Amhara Region. The study included three areas (Bahir Dar city, Semen Achefer, and Bahir Dar Zuria districts), from which 320 respondents were selected from eight Kebeles. Data were collected from farm observations, individual interviews, and focus group discussions, supplemented by secondary information from agricultural offices records, and research publications. The study revealed that phytogetic feed additives were used by 58.3%, 56.3%, and 52.5% of farmers in urban, peri-urban, and rural areas, respectively. PFAs such as neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*), and garlic (*Allium sativum*) ranked 1st, 2nd, and 3rd in their availability and 1st, 3rd, and 2nd in their utilization practices, respectively. The large majority of urban and peri urban chicken producers (70.9 and 73.8%, respectively) were aware of the use of phytogetic feed additives in chicken production. The findings of this study show that the uses of PFAs contribute to sustainable chicken production by enhancing food security, public health, environmental sustainability, and economic resilience. Their use supports with national development goals and key sustainable development goals (SDGs), including zero hunger, good health and well-being and climate action. This finding suggests that phytogetic feed additives are readily available and utilized in the study area for chicken production at the farmer's level, although standard dosages were not established among users. Further research is needed to verify the effects of these phytogetic feed additives on

chicken performance, and a nationwide assessment should be conducted to quantify their potential.

Keywords: Farmers' awareness, Bitter-leaf plant, Neem, Phytogenic feed additives, chicken nutrition

4.1. Introduction

Feed accounts for the majority of chicken production costs. The steady increase in the cost of chicken feed ingredients and compounded formulated feed is reducing profits for chicken farmers (Thirumalaisamy *et al.*, 2016). Numerous feed additives have been widely utilized to enhance chicken production and reduce the cost of feed. Due to their therapeutic properties, antibiotics have traditionally been used as feed additives in poultry diets (Mehdi *et al.*, 2018). The improper use of antibiotics can lead to drug-resistant microorganisms and antibiotic residues in chicken products (Mesfin *et al.*, 2024; Ali *et al.*, 2025). Phytogenic feed additives have been shown to support digestive health, enhance immune response, and improve overall performance in poultry (Mandey *et al.*, 2022). Therefore, phytogenic feed additives present a promising alternative for supporting improved and sustainable chicken production (Yitbarek, 2015).

Phytogenic feed additives are gaining interest as alternatives to conventional antibiotics, probiotics, and prebiotics, due to low costs and high productive efficacy (Jachimowicz *et al.*, 2022; Shehata *et al.*, 2022), as consumers may accept their inclusion in chicken diets due to their natural origin (Abou-elkhair *et al.*, 2018). Moreover, certain phytogenic compounds may increase the intestinal surface area, thereby improving Apparent Ileal Digestibility (AID) and nutrient absorption. This improved digestion promotes the development of broilers (Ravindran and Abdollahi, 2021). Overall, phytogenic feed additives could improve feed efficiency in chicken production (Aroche *et al.*, 2018). In the Ethiopian context, traditional medicinal plants continue to play a vital role in solving livestock health challenges, including those affecting chicken (Belayneh *et al.*, 2012). Biological activity has been documented in extracts obtained from a variety of Ethiopian local plants, including antibacterial and anti-inflammatory properties (Ayalew *et al.*, 2022). This evidence suggests that phytogenic feed additives offer a promising pathway toward sustainable chicken production in Ethiopia. Their integration into chicken production systems can contribute to multiple dimensions of development enhancing food

security, protecting public health, mitigating environmental impacts, and strengthening economic resilience. Accordingly, their use aligns with Ethiopia's national development priorities and global commitments under the Sustainable Development Goals (SDGs), particularly Zero Hunger, Good Health and Well-being, and Climate Action.

While studies have explored the effects of PFAs on chicken performance (Shawle *et al.*, 2016; Asrat *et al.*, 2018; Dolle, 2020), little is known about chicken producers' knowledge, perceptions, and utilization practices. This study provides an opportunity to understand chicken producers' knowledge and preferences regarding phytogenic feed additives, which is crucial for developing research and strategies to improve their utilization practices and dissemination among farmers. The study hypothesizes that farmers with greater knowledge of phytogenic feed additives are more likely to use them effectively, perceive them as more available, and show stronger preferences for their use in chicken production. This study aims to fill that gap by assessing chicken producers' knowledge, preferences, and utilization practices of phytogenic feed additives in chicken production, as well as the perceived availability of these additives.

4.2. Materials and Methods

4.2.1. Description of study areas

The study was conducted in urban, peri-urban, and rural areas of Bahir Dar City, Bahir Dar Zuria, and Semen Achefer districts in Amhara Region. These sites were purposively selected based on their potential for chicken production, as identified by the Bureau of Agriculture and CSA reports.

Bahir Dar is the capital city of Amhara National Regional State, Ethiopia. It's located about 565 km north of Addis Ababa at 11° 38' N latitude and 37° 10' E longitude. The elevation of the city is about 1801 meters above sea level, and it receives an average annual rainfall of 850 mm to 1250 mm, a minimum average daily temperature of 10°C, and a maximum of 32°C.

Bahir Dar Zuria District (BDZD) is found in the West Gojjam Zone. The area is located about 564 km Northwest of Addis Ababa. It is situated at an altitude ranging from 1700 to 2300 metres above sea level. Its extension is between 11°25'N and 11°55'N latitude and 37°04'E-37°39'E longitude. The mean annual temperature is about 20°C, with a maximum temperature

slightly above 28.3°C and a minimum of about 10.2°C. The annual rainfall ranges from 800-1250 mm.

Semen Achefer is found about a distance of 101 km and 591 km far from Bahir Dar and Addis Ababa, respectively. Its geographical coordinates are 11° 41' 0" north latitude and 36° 57' 0" east longitude. The altitude of the district ranges from 1,500 to 2,500 m above mean sea level. It is bordered on the south by the South Achefer, on the west by the Central Gondar Zone, on the north by Lake Tana, on the east by Bahir Dar Zuria, and on the southeast by the Mecha district, and one part of the Abay River defines the district's eastern boundary.

4.2.2. Sampling method and sample size determination

A multi-stage sampling procedure was used to collect data. Stage 1: Bahir Dar and West Gojjam zone were purposively selected based on their chicken production potential; Stage 2: Within these areas, three districts were included: Bahir Dar city, Semen Achefer, and Bahir Dar Zuria. From these districts, three urban kebeles (two from Bahir Dar and one from North Achefer), two peri-urban kebeles from Bahir Dar, and three rural kebeles (two from Bahir Dar Zuria and one from Semen Achefer) were randomly selected. In the urban study areas, a list of chicken-producing households was obtained from the agricultural office. In the peri-urban and rural areas, development agents assisted in identifying households with 25 or more chickens. Finally, 40 households from each selected kebele were interviewed, resulting in a total of 320 households participating in the study. All household participants agreed to provide information and images for the study, and the individuals in the pictures consented to their inclusion in the published paper, with the understanding that these materials could be used by the researcher as needed.

4.2.3. Methods of data collection

Respondents ranked the major phyto-genic feed additive sources for chickens based on their preferences and perceived effectiveness. Focus group discussions with community members in the study areas provided information on utilization techniques and the specific plant parts used. One group discussion, consisting of nine participants, was conducted in each kebele, totaling

eight discussions. Semi-structured interviews were also conducted with key informants, including elders, experienced chicken farmers, development agents in animal production, and a veterinarian, to assess the availability and utilization practices of phytogenic feed additives. Both primary and secondary data were collected from various sources.

4.2.4. Conversion factor of livestock population (TLU)

Data on the livestock population in the sampled households were obtained from the interview of household heads during the survey. The number of livestock population was converted into tropical livestock unit (TLU) using the conversion factors of camel (1), cattle (0.7), sheep (0.1), goat (0.1), mules (0.7), horses (0.8), donkeys (0.5), and poultry (0.01) (Varvikko *et al.*, 1993).

4.2.5. Sampling procedure for phytogenic additives

The questionnaires addressed farmers' perceptions of phytogenic feed additive utilization and availability. Following an assessment of available phytogenic feed additive resources, the top five additives were selected for chemical analysis. These major phytogenic feed additives were selected based on availability and farmer preferences. Samples of these additives were collected from the study areas to determine their chemical composition.

4.2.6. Proximate chemical analysis for selected phytogenic additive

The identified top 5 phytogenic feed additives were selected based on rank results. The leaf part of neem, bitter-leaf and cabbage; the bulb part of garlic and the rhizome of ginger were collected. The samples were ground by using a hammer mill to pass through a 1 mm screen for chemical analysis. The samples of phytogenic feed additives were sent to Jimma University for chemical analysis. The DM content of feed samples was determined by drying them in an oven at 105°C overnight (AOAC, 2000). Ash was determined by the complete burning of the sample at 550°C for 5 hours in a muffle furnace. Nitrogen (N) was determined using the Kjeldjhal method and then the crude protein calculated as $N \times 6.25$ (AOAC, 1995). Crude fiber contents

were analyzed using AOAC (1990) method. Total phosphorus contents were determined by the Vanado-Molybdate method (AOAC, 2000). The metabolizable energy (ME) was estimated according to the equation proposed by Wiseman (2013); ME (kcal/kg) = 3951 + 54.40 fat-88.70 ash. Nitrogen-free extract (NFE) was calculated by the difference between organic matter and the sum of ash, CF, EE, and CP.

4.2.7. Survey data management and statistical analysis

The collected data from the survey of urban, peri-urban and rural areas were entered into *the Statistical Package* for Social Sciences (SPSS version 27) software. The normality of the data was tested using the Kolmogorov-Smirnov Test. Chi-square was employed for the association of such parameters between urban, peri-urban and rural. Descriptive statistics were used to analyze the mean values of the quantitative data means and percentages values. Means of quantitative data between urban, peri-urban and rural study areas were compared by employing analysis of variance (ANOVA). Means were separated using the tukey test at $p < 0.05$ significant level. The statistical model was used:

$$Y_{ij} = \mu + \alpha_i + \Sigma_{ij}$$

Where: y_{ij} is the response variable;

μ = is the overall mean

α = the effect of i^{th} study areas (urban, peri-urban and rural areas)

Σ_{ij} = random error

Priority index was employed using the following formula (Kosgey 2004).

index

$$= \frac{\Sigma (n \times \text{No. of HHs ranked 1st}) + (n - 1) \times \text{No. of HHs ranked 2nd} + \dots + 1 \times \text{No. of HHs ranked last}) \text{ for one factor}}{\Sigma (n \times \text{No. of HHs ranked 1st} + (n - 1) \times \text{No. of HHs ranked 2nd} + \dots + 1 \times \text{No. of HHs ranked last}) \text{ for all factors}}$$

Where; n: value given for the least ranked level (example if the least rank is 5th rank $n-1=4$, $n-$

$2=3$ and..... $n=1$)

4.3. Results and Discussion

4.3.1. Demographic characteristics of the respondents

Table 4.1 presents the demographic characteristics of households in the study areas. Age had a significant effect ($P < 0.05$) on involvement in chicken farming, with mean respondent ages of 31.25 years in urban areas, 37.63 years in peri-urban areas, and 43.33 years in rural areas. These values indicate that most participants fall within the active working-age group, which is typically associated with higher labor potential and greater adoption of improved livestock practices. Studies confirm that individuals aged 30-50 years constitute the dominant group in smallholder chicken production. For instance, FAO (2021) reported that younger and middle-aged farmers are more likely to engage in improved husbandry practices and chicken commercialization, while ILRI (2020) noted that households headed by younger adults tend to adopt formulated feeds, feed additives, and vaccination protocols. These findings reinforce the current results, suggesting that urban and peri-urban respondents whose mean ages fall within this productive range are well positioned to participate effectively in chicken farming and technology uptake.

The average family size across the study areas was 5.3 persons per household, with 3.83 in urban areas, 6.25 in peri-urban areas, and 5.68 in rural areas. This is comparable to the 5.7 persons reported by Addis and Malede (2014) in the Quara district. The significant differences ($P < 0.05$) in family size may reflect variations in family planning practices and socio-cultural norms across the production systems. Larger household sizes in peri-urban and rural areas may also translate into greater availability of family labor an important resource in traditional chicken production systems. Recent research supports this observation; the Ethiopian Socioeconomic Survey (CSA, 2019) found that larger rural families contribute more labor for livestock-related tasks, while (FAO, 2018) highlighted the crucial role of family labor, particularly women and children, in managing scavenging and semi-intensive chicken systems. These trends align with the present findings, indicating that larger household sizes may contribute positively to chicken management in rural and peri-urban settings.

Gender distribution also showed a high proportion of female household heads: 61.7% in urban areas, 67.7% in peri-urban areas, and 60% in rural areas. This confirms the central role of women in smallholder chicken production. Numerous recent studies demonstrate that primarily

women often manage chicken because it provides accessible income, improves household nutrition, and enhances women's decision-making autonomy. ILRI and CARE (2019) reported that women consistently dominate chicken ownership across East Africa, while the Africa Chicken Genetic Gains (Mulema, 2018) project found that women account for 65-70% of chicken keepers in Ethiopia. These findings strengthen the conclusion that women remain key actors in chicken production and should be prioritized in chicken development interventions.

Marital status also varied significantly ($P < 0.05$) among the study areas, with 83.3% of rural, 77.5% of peri-urban, and 81.7% of urban respondents being married. Differences in marital status can influence labor allocation, economic decisions, and level of responsibility within the household, which may further shape engagement in chicken farming.

Educational background likewise exhibited significant differences ($P < 0.05$) across the study areas. Illiteracy rates were highest in peri-urban (42.5%) and rural areas (43.3%), while only 3.3% of urban respondents were illiterate. Notably, 60% of urban households held a certificate or higher qualification, whereas none of the peri-urban or rural respondents had achieved such levels. This disparity suggests that urban households may have greater exposure to and understanding of modern chicken technologies, including phytogenic feed additives. This aligns with recent evidence indicating that education is a critical factor in technological adoption. IFPRI (2020) reported a strong positive association between farmers' education levels and their likelihood of adopting innovations such as improved feeds, feed additives, and vaccination strategies. Similarly, FAO (2021) noted that urban chicken producers with higher educational attainment are more receptive to new technologies and improved feeding systems. Therefore, the higher proportion of educated respondents in urban areas in this study likely contributes to greater openness toward technologies designed to enhance chicken performance.

Table 4.1. Demographic characteristics of the sampled households

Parameters	Study areas			X ²	Sig.
	Urban	Peri urban	Rural		
Age of HH heads (years)	31.25	37.63	43.33	148.97	***
Family size of HH heads (No.)	3.83	6.25	5.68	125.9	***
Sex of HH heads (%)					
Male	38.3	32.5	45.0	3.23	Ns
Female	61.7	67.5	55.0		
Marital status %					
Single	13.3	12.5	3.3	26.8	***
Married	81.7	77.5	83.3		
Widow	5.0	10.0	5.0		
Divorced	0.0	0.0	8.3		
Educational level (%)					
Illiterate	3.3	42.5	43.3	187.9	***
Elementary	26.7	21.3	29.2		
Secondary	3.3	17.5	19.2		
Preparatory	5	6.3	2.5		
Certificate and above	60	0.0	0.0		
Religious	1.7	12.5	5.8		

ns = non-significant, Sig.=significant value, X²= Chi-square, ***= significant at p< 0.001

4.3.2. Livestock holding and composition

The average number of livestock holding per household in the study area is shown in Table 4.2. The mean number of livestock per household in rural study areas was 4.13 heads of cattle, 0.61 heads of sheep, 0.12 heads of goats, 0.30 heads of donkey, 0.10 heads of mule, and 0.28 heads of chicken. In the peri-urban study area, the mean number of livestock per household was 3.85 heads of cattle, 0.67 heads of sheep, 0.13 heads of goats, 0.78 heads of donkey, 0.12 heads of mule, and 0.26 heads of chicken. However, livestock producers in urban study areas primarily raised cattle and chickens, with an average of 0.19 heads of cattle and 14.71 heads of chicken per household. In terms of Tropical Livestock Units (TLU), the average livestock ownership was 8.75, which differed significantly ($P < 0.05$) across study areas. However, urban study areas had a significantly higher average chicken keeping rate (14.71, $P < 0.001$) compared to peri-urban (0.26) and rural areas (0.28). Chicken accounted for the largest portion of the total livestock number in the sampled households in urban areas. This is due to population growth, rising individual consumption, and the expansion of hotels, which have led to a higher demand for chicken meat and eggs in urban areas compared to peri-urban and rural study areas. This

indicates that chickens are also a good option for self-employment for those who cannot access government jobs, or even for side-by-side business.

Table 4.2. The mean of livestock composition per household in the study areas in terms of TLU

Livestock type	Total livestock in TLU				
	Urban	Peri-urban	Rural	SEM	P-value
Cattle	0.19 ^b	3.85 ^a	4.13 ^a	0.042	***
Goat	-	0.13 ^a	0.12 ^a	0.004	Ns
Sheep	-	0.67 ^a	0.61 ^a	0.011	Ns
Donkey	-	0.78 ^a	0.30 ^b	0.010	***
Mule	-	0.12 ^a	0.10 ^a	0.004	Ns
Chicken	14.71 ^a	0.26 ^b	0.28 ^b	0.213	***

ns = non-significant, ***= significant at $p < 0.001$

4.3.3. Type of additives and purpose of feeding for Chickens

Table 4.3 presents the types of feed additives used in the study areas. In urban areas, 59.3% of respondents used a combination of antibiotics and phytogenic feed additives, while 35.1% used only antibiotics, and 5.6% used only phytogenic. Additionally, 4.3% of respondents in peri-urban areas reported using both phytogenic and antibiotic feed additives. In contrast, the majority of peri-urban and rural respondents primarily relied on phytogenic feed additives, with 91.5% and 87.3% of respondents using them, respectively. In peri-urban areas, 4.3% of respondents used only antibiotics, while in rural areas, 12.7% used both phytogenic and antibiotic feed additives. This suggests that the predominance of phytogenic additive use in peri-urban and rural areas may reflect limited access to veterinary pharmaceuticals, while the emphasis on treatment over performance enhancement suggests a reactive rather than preventive approach to chicken health management.

The majority of respondents in urban areas (70.4%) used feed additives for improving chicken productivity, egg quality, growth, and disease treatment. In contrast, only 4.3% and 6.3% of peri-urban and rural respondents, respectively, reported using phytogenic feed additives for these purposes. Urban respondents were less likely to use feed additives for chicken treatments (9.3%) compared to peri-urban (95.7%) and rural (92.1%) respondents. A small percentage of urban respondents (7.4% and 3.7%, respectively) reported using feed additives for egg production and quality improvement. This was significantly lower than the 1.6% of rural

respondents who used feed additives for this purpose. A significant proportion of respondents in all study areas reported never using phytogetic feed additives: 41.7% in urban areas, 43.8% in peri-urban areas, and 47.5% in rural areas. The primary reason mentioned for non-use was a lack of knowledge about phytogetic feed additives, their utilization practices, and their importance. Despite the local availability of phytogetic feed sources (e.g., spices), farmers primarily cultivate these for household consumption or market sale, not for inclusion in chicken diets. This limits the use of natural feed additives in rural and peri-urban settings.

Table 4.3. Types and Purpose of feeding the feed additives in study areas

Type of feed additives supplementation		Urban		Peri-urban		Rural		X ²
		N	%	N	%	N	%	
Did you provide any feed additives for your chickens?	Yes	108	90	47	58.8	63	52.5	43.17***
	No	12	10	33	41.2	57	47.5	
Did you provide phytogetic feed additives for your chicken?	Yes	70	58.3	45	56.2	63	52.5	0.84 ^{ns}
	No	50	41.7	35	43.8	57	47.5	
Antibiotics		38	35.1	2	4.3	-	-	154.11***
Phytogetic feed additives		6	5.6	43	91.5	55	87.3	
Both phytogetic and antibiotics feed additives		64	59.3	2	4.2	8	12.7	
Purpose of offering feed additives for chicken								
Egg production improvement		8	7.4	-	-	-	-	156***
Egg quality improvement		4	3.7	-	-	-	-	
For treatment (medicine)		10	9.3	45	95.7	58	92.1	
For production, quality, growth, and treatment		76	70.4	2	4.3	4	6.3	
Egg production and quality improvement		8	7.4	-	-	1	1.6	
Increase growth		2	1.9	-	-	-	-	

N= frequency, X²=chi square, ***=significant p≤ 0.001, **=p≤ 0.01

4.3.4. Sources of chicken feed additives in study areas

Figure 4.1 illustrates the sources of chicken feed additives across urban, peri-urban, and rural study areas. Backyard sources were the most common in rural (72.2%) and peri-urban areas (22.2%), while urban respondents used both backyard (39.5%) and commercial (36.3%) sources. The relatively lower reliance on backyard sources in peri-urban areas, compared to rural areas, may reflect land limitations or better access to markets, enabling the use of commercial inputs. In contrast, the high dependence on backyard sources in rural areas underscores the importance of on-farm cultivation of phytogetic feed additives in these communities. This trend may be partly

attributed to the reduced availability of wild plant resources, especially medicinal plants and herbs, due to deforestation and lack of conservation efforts. These findings contrast with earlier studies by Regassa (2013) and Fenetahun *et al.* (2017), who reported that 70% and 50% of medicinal plants, respectively, were collected from wild habitats. The discrepancy likely reflects recent declines in wild plant availability in the current study area, possibly due to ongoing environmental degradation and overharvesting.

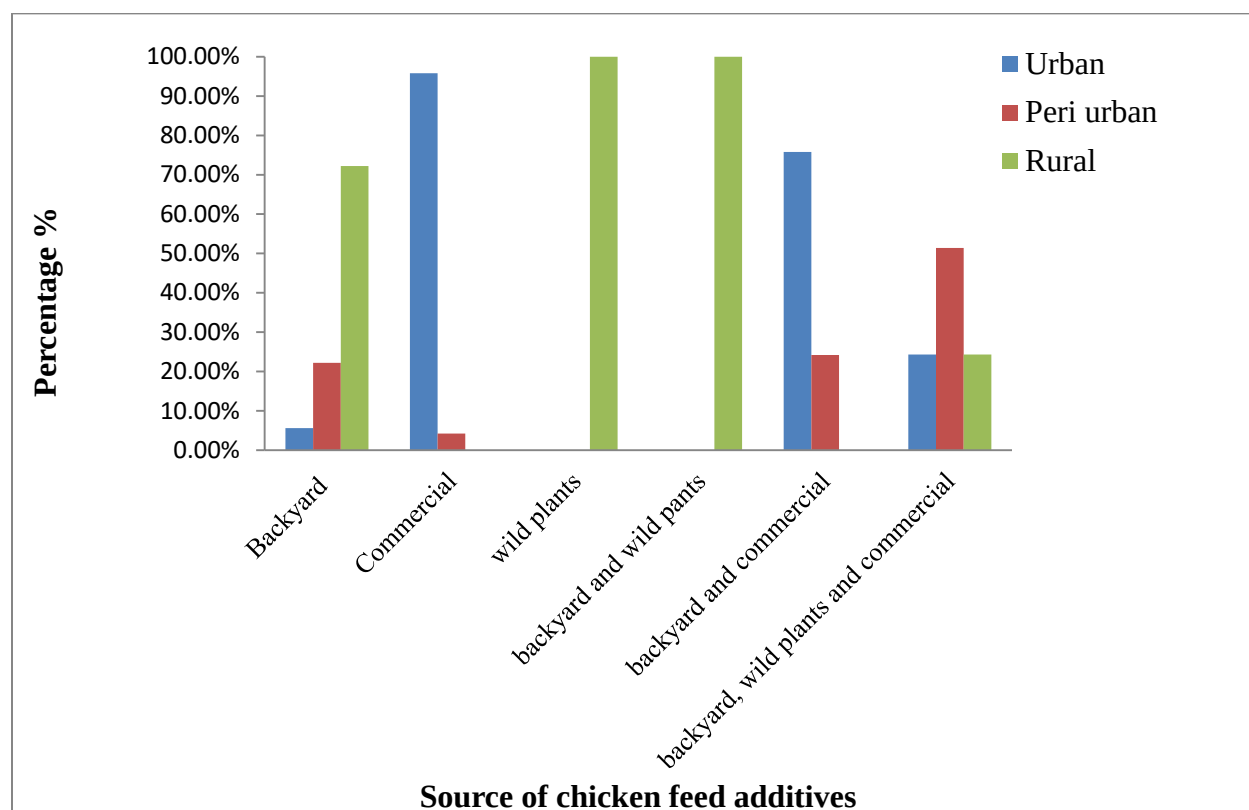


Figure 4.1. Different sources of chicken feed additives in the study areas

4.3.5. Availability of phytogetic feed additives in study areas

Table 4.4 presents the major phytogetic feed additives available in the study areas. The most commonly available additives included neem, bitter-leaf, garlic, cabbage, Ethiopian kale, hot red pepper, fenugreek, ginger, lime, black cumin, and chili, while other additives were available to a lesser extent. In urban areas, most phytogetic additives, except for neem, Ethiopian justicia, bitter-leaf, and Ethiopian basil were purchased from the market, with both availability and affordability influencing their usage and ranking.

Neem was the top-ranked additive in urban and rural areas and ranked fourth in peri-urban areas, largely due to its year-round availability in green form. Ginger, bitter-leaf, garlic, and cabbage were also highly ranked in both urban and peri-urban settings. In contrast, rosemary, Ethiopian thyme, and white mustard were unavailable in peri-urban and rural areas.

Spices such as fenugreek, black cumin, hot chili pepper, and garden cress were preferred for their long shelf life, which made them suitable for use during the dry season. However, preservation techniques for more perishable phyto-genic feed additives were not commonly adopted. This reflects a knowledge and infrastructure gap noted by several studies (Getahun *et al.*, 2025). Garden cress (*Lepidium sativum*), which is highly valued for its medicinal properties in peri-urban and rural areas, was reported as scarce due to limited distribution and the impact of herbicide use on its growth in crop fields. The current result aligns with Gebremedhin *et al.* (2022) research on the decline of traditional medicinal herbs caused by agricultural intensification. Such ecological pressures can reduce biodiversity and the local availability of plants used in ethno-veterinary practices.

4.3.6. Utilization practices and preferences of phyto-genic feed additives for chickens

Table 4.5 presents the most preferred phyto-genic feed additives in the study areas, including neem, garlic, bitter-leaf, cabbage, ginger, Ethiopian justicia, white mustard, garden cress, and others. Farmers ranked these additives based on their perceived effectiveness in enhancing chicken production, product quality, and health, reflecting the criteria they prioritize in their actual usage. Bitter-leaf ranked 1st in rural areas, 2nd in urban areas and 3rd in peri-urban areas. It was primarily used to treat chickens and improve egg quality. Neem was ranked 1st for egg production, quality improvement, and health care in urban and 2nd in rural areas. Farmers primarily used neem to treat sick chickens. The high ranking of bitter-leaf across all systems may indicate its widespread availability and established role in traditional chicken healthcare. Similarly, the consistent use of neem for disease treatment suggests strong ethno-veterinary knowledge and trust in its antimicrobial properties. Garlic ranked 2nd overall and was commonly used to enhance chicken performance, with specific rankings of 3rd in urban, 2nd in peri-urban, and 3rd in rural areas. The preference for these additives is attributed to their ready availability, affordability, and ease of application. These findings highlight the role of local knowledge and accessible resources in shaping feeding practices among smallholder chicken producers.

Table 4.4. The available phytogenic feed additive resources in the study areas

Local Name	Phytogenic feed additives		Urban		Peri-Urban		Rural		Overall	
	English Name	Scientific name	Index	Rank	Index	Rank	Index	Rank	Index	Rank
Neem	Neem tree	<i>Azadirachta indica</i>	0.0708	1	0.086	4	0.124	2	0.09	1
Zingible	Ginger	<i>Zingiber officinale</i>	0.069	2	0.087	3	0.049	11	0.056	9
Girawa	Bitter leaf	<i>Vernonia amygdalina</i>	0.068	3	0.11	1	0.135	1	0.088	2
Nech shinkurt	Garlic	<i>Allium sativum</i>	0.067	4	0.105	2	0.073	3	0.072	3
Tiql gomen	Cabbage	<i>Brassica oleracea</i>	0.063	5	0.065	5	0.055	10	0.071	4
Gomen	Ethiopian kale	<i>Brassica carinata</i>	0.06	6	0.055	10	0.0657	7	0.07	5
Qariya	chili	<i>Capsicum annuum L</i>	0.059	7	0.044	13	0.017	16	0.051	11
Abish	Fenugreek	<i>Trigenella foenum-graecum</i>	0.057	8	0.026	16	0.004	20	0.062	8
Berbera	Hot chili pepper	<i>Capsicum frutescens</i>	0.0568	9	0.06	6	0.066	6	0.068	6
Tiqur azimud	Black cumin	<i>Nigella sativa</i>	0.056	10	0.049	12	0.056	8	0.051	12
Lomi	Lime	<i>Citrus aurantiifolia</i>	0.055	11	0.05	11	0.0697	4	0.053	10
Tena adam	Fringed rue	<i>Ruta chalepensis</i>	0.053	12	0.044	13	0.039	13	0.05	13
Tomato	Tomato	<i>Lycopersicon esculentum</i>	0.05	13	0.057	9	0.0678	5	0.063	7
Qey shinkurt	Onion	<i>Allium cepa</i>	0.049	14	0.034	15	0.029	14	0.038	14
Feto	Garden cress	<i>Lepidium sativum</i>	0.043	15	0.059	7	0.047	12	0.028	15
Simiza	Ethiopian justicia	<i>Justicia schimperiana</i>	0.041	16	0.012	17	0.0177	15	0.025	16
Endod	African soapberry	<i>Phytolacca dodecandra</i>	-	-	-	-	0.009	18	0.014	19
Tosign	Ethiopian thyme	<i>Thymus schimperi</i>	0.0275	17	-	-	-	-	0.024	17
Sinafich	White mustard	<i>Sinapis alba</i>	0.027	18	-	-	-	-	0.015	18
Yetbs Qtel	Rosemary	<i>Rosmarinus officinalis</i>	0.025	19	-	-	-	-	0.007	20
Damakase	Ethiopian basil	<i>Ocimum urticifolium</i>	0.0008	20	0.057	8	0.0555	9	0.005	21
Eret	Ethiopian aloe	<i>Aloe adigratana</i>	-	-	-	-	0.0045	19	0.0012	23
Serkabeba	African senna	<i>Senna didymobotrya</i>	-	-	-	-	0.0128	17	0.0017	22
Nech bahir zaf	Eucalyptus	<i>Eucalyptus globulus</i>	-	-	-	-	0.0033	21	0.0006	24

Table 4.5. Utilization practice and preference of phytogenic feed additives ranked by respondents

Phytogenic feed additives		Urban		Peri-urban		Rural		Overall	
English name	Scientific name	Index	Rank	Index	Rank	Index	Rank	Index	Rank
Neem tree	<i>Azadirachta indica</i>	0.27	1	0.157	1	0.125	2	0.1	1
Bitter leaf	<i>Vernonia amygdalina</i>	0.16	2	0.142	3	0.130	1	0.086	3
Garlic	<i>Allium sativum</i>	0.14	3	0.148	2	0.100	3	0.0865	2
Cabbage	<i>Brassica oleracea</i>	0.13	4	0.117	4	0.057	8	0.08	4
Ginger	<i>Zingiber officinale</i>	0.07	5	0.116	5	0.081	4	0.075	5
White mustard	<i>Sinapis alba</i>	0.06	6	-	-	-	-	0.062	8
Garden cress	<i>Lepidium sativum</i>	0.05	7	0.104	6	0.073	6	0.057	9
Black cumin	<i>Nigella sativa</i>	0.045	8	0.025	11	0.043	11	0.042	11
Chili	<i>Capsicum annuum L</i>	0.03	9	0.036	8	0.013	17	0.0386	14
Hot chili pepper	<i>Capsicum frutescens</i>	0.016	10	0.035	9	0.019	15	0.0387	13
Onion	<i>Allium cepa</i>	0.015	11	0.009	13	0.032	13	0.072	6
Tomato	<i>Lycopersicon esculentum</i>	0.01	12	0.01	12	0.020	14	0.041	12
Ethiopian justicia	<i>Justicia schimperiana</i>	-	-	0.088	7	0.066	7	0.066	7
Ethiopian basil	<i>Ocimum urticifolium</i>	-	-	0.026	10	0.011	18	0.052	10
Eucalyptus	<i>Eucalyptus globulus</i>	-	-	-	-	0.014	16	0.017	17
African soapberry	<i>Phytolacca dodecandra</i>	-	-	-	-	0.036	12	0.0168	18
African senna	<i>Senna didymobotrya</i>	-	-	-	-	0.075	5	0.033	15
Fringed rue	<i>Ruta chalepensis</i>	-	-	-	-	0.050	10	0.024	16
Ethiopian aloe	<i>Aloe adigratana</i>	-	-	-	-	0.055	9	0.014	19

4.3.7. Perception of chicken producers towards phytogetic feed additives

Table 4.6 presents chicken producers' awareness and perceptions of phytogetic feed additives across the study areas. The majority of respondents reported using phytogetic feed additives primarily for treating sick birds. Awareness of their medicinal use was highest in rural (58.3%), followed by peri-urban areas (47.5%) and urban areas (40%). A smaller proportion (21.7%) of rural respondents also recognized their role in disease prevention.

However, knowledge of the benefits of these additives in enhancing production traits, such as egg quality and growth, was considerably lower. Only 7.5% and 5% of peri-urban respondents, and 2.5% and 1.7% of rural respondents, were aware of these roles. In urban areas, 19.2% and 6.6% of respondents reported using phytogetic feed additives to improve product quality and productivity, respectively. Furthermore, 5% of urban farmers viewed these additives as viable alternatives to antibiotics. This finding is consistent with Abdelli *et al.* (2021), who reported that although phytogetic feed additives (PFAs) hold great potential in poultry production, their adoption for performance enhancement remains limited due to inadequate awareness, insufficient dosing knowledge, and poor technical application. Similarly, poultry farmers in Africa often use plant-based remedies mainly for disease treatment rather than for growth promotion.

Anecdotal evidence from some farmers suggested that feeding neem leaves to older hens resulted in thicker eggshells and more pigmented yolks, potentially linked to the calcium and carotenoid content of neem. Despite some level of awareness, substantial knowledge gaps persist: about 34.2% of urban, 40% of peri-urban, and 37.5% of rural respondents had limited or no awareness of the broader applications of phytogetic additives beyond treatment. This underscores the need for targeted awareness campaigns and farmer training, particularly in rural areas, to promote the informed use of phytogetic feed additives for both health and productivity improvement in chickens.

Table 4.6. Respondants' perceptions regarding the use of phytogenic feed additives in chicken production

Parameters	Description of phytogenic feed additives importance for chicken	Study areas						X ²
		Urban		Peri-urban		Rural		
		N	%	N	%	N	(%)	
Knowledge	Awareness of product quality improvement	23	19.2	6	7.5	3	2.5	85.47**
	Awareness of production improvement	8	6.6	4	5.0	2	1.7	
	Aware that used as chicken treatments	48	40	38	47.5	70	58.3	
	No aware of phytogenic feed additives	41	34.2	32	40	45	37.5	
Attitude	Used as a good alternative to antibiotics	6	5.0	-	-	-	-	78.56**
	Aware that can be used as a prevention	-	-	-	-	26	21.7	
	Lack of perception	114	95	-	-	94	78.3	

N =frequency, X²= chi-square, **=significant at p<0.01

4.3.8. Phytogenic feed additives utilization techniques and its parts used

Group discussions across production systems showed broad farmer agreement on preparing and using phytogenic feed additives (Table 4.7). Farmers typically crushed or chopped plant materials, soaked them in water, and provided the infusion as drinking water for chickens. This aqueous method likely enhances the extraction of polar bioactive compounds such as flavonoids, alkaloids, tannins, and saponins known for their antimicrobial and immune-stimulating effects. In rural areas, plants such as garden cress (*Lepidium sativum*), Ethiopian justicia, Ethiopian aloe, eucalyptus, bitter leaf, and garlic were commonly used to manage diseases like Newcastle disease. These remedies were often mixed with local feeds such as Injera Fitfit to improve palatability. Such practices reflect well-established ethno-veterinary knowledge tailored to local chicken health challenges.

Leaves were the most commonly used plant parts, followed by seeds. This agrees with Nigussie *et al.* (2018), who reported that leaves are preferred for their year-round availability, ease of processing, and higher secondary metabolite content. In contrast, studies from Uganda (Atagal, 2015) and Ethiopia (Birhane *et al.*, 2011; Mengistu *et al.*, 2017) found roots to be more commonly used. These differences likely reflect regional variations in plant availability, cultural practices, or perceived efficacy.

Table 4.7. List of phytogenic feed additives identified processing methods in the study areas

English Name	Plant scientific Name	Part used	Method of Processing	Utilization Techniques of Phytogenic Feed Additives	Role Phytogenic feed Additives for Chicken in Study Areas
Neem Tree	<i>Azadirachta indica</i>	Leaf	Chopped and crushed, as it is	Crushed and Chopped neem leaf soaked with water then the juice mixed with injera and other feed or hinging neem leaf in the chicken house for direct fed	Disease treatment and prevention, egg yolk and shell improvement
Garlic	<i>Allium sativum</i>	Bulb	Chopped and crushed	Added into chicken drinking water or mixed with <i>injera fitfit</i> or other feed	Disease treatment
Ginger	<i>Zingiber officinale</i>	Rhizome	Crushed and chopped	Added into chicken drinking water	Disease treatment
Bitter leaf	<i>Vernonia amygdalina</i>	Leaf	Crushed, rubbed and as it is	Crushed and rubbed Bitter-leaf leaf by soaking with water then the juice mixed with <i>injera</i> and other feed	Disease treatment
Fenugreek	<i>Trigenella foenum-graecum</i>	Seed	Grinded and crushed	Mixed with other feed and added into drinking water	Disease treatment
Chili	<i>Capsicum annum</i>	Fruit	Chopped, as it is	Direct to fed or the chopped fruit soaked with water then added into drinking water	Disease treatment
Rosmary	<i>Rosmarinus officinalis</i>	Leaf	Rubbed and as it is	The rubbed leaf of <i>Rosmarinus officinalis</i> direct fed or mixed with other feed	Disease treatment
Garden cress	<i>Lepidium sativum</i>	Seed	Ground and crushed	Ground and crushed garden cress mixed with <i>injera fitfit</i> or added into drinking water	Disease treatment and prevention
Fringed rue	<i>Ruta chalepensis</i>	Leaves and young stem	Crushed, soaked, as it is	Crushed part of Fringed rue added into drinking water	Disease treatment and prevention
Hot chili pepper	<i>Capsicum annum L.</i>	Fruit pulp	Grinded	Hot chili pepper powder mixed with oil and then added to other feed or chicken drinking water	Disease treatment

Table 4.8. List of phytogenic feed additives identified processing methods in the study areas (*continued*)

Ethiopian kale	<i>Brassica carinata</i>	Leaf and seed	Crushed, chopped and grinded	The powder mixed with feed or water which was given to sick chicken and chopping leaf used as it is for chicken feed	Disease treatment and egg yolk improvement
Lime	<i>Citrus aurantiifolia</i>	The liquid in the fruit	Sliced	Slicing then Squeezing it to produce juice and added it to chicken drinking water	Disease Treatment
Cabbage	<i>Brassica oleracea</i>	Leaf	Chopped and chopped	Chopping it and as it is to provide it for chicken	Disease treatment and egg yolk improvement
White mustard	<i>Sinapis alba</i>	Seed	Crushed and grinding	The powder mixed with feed or water which was given to sick chicken	Disease treatment
Ethiopian basil	<i>Ocimum urticifolium</i>	Leaf	Rubbed, soaked	rubbed the leaf and add water to produce leaf juice then added to drinking water and mixed with <i>injera</i>	Disease Treatment
Ethiopian justicia	<i>Justicia schimperiana</i>	Leaf	Chopped and rubbed, soaked	Chopped leaf and rubbed with water then added into drinking water or mixed with <i>injerafift</i>	Disease Treatment
Eucalyptus	<i>Eucalyptus globulus</i>	Leaf	Crushed, chopped and rubbed	Processed leaf soaked with water then added to the chicken drinking water or leaf juice mixed with <i>injerafift</i>	Disease Treatment
African soapberry	<i>Phytolacca dodecandra</i>	Leaf	Crushed	The crushed leaf is soaked with water and then filter the leaf juice and added to the drinking water	Disease treatment
African senna	<i>Senna didymobotrya</i>	Leaf	Chopped and rubbed, soaked	The chopped leaf rubbed with water then filter the leaf juice and added to the drinking water	Disease treatment
Ethiopian aloe	<i>Aloe adigratana</i>	Leaf	Chopped and crushed	The jelly juice of Ethiopian aloe is added into drinking water or mixed with <i>injera</i>	Disease treatment

4.3.9. Phytogenic feed additive processing techniques and feeding frequency

Table 4.8 presents the various methods used to process phytogenic feed additives across the study areas, showing significant differences ($P < 0.05$) among study areas. The most common methods included crushing, chopping, soaking, and grinding. Crushing was the predominant technique in urban (41.4%) and peri-urban areas (48.9%), whereas rural farmers most frequently used a combination of chopping and crushing (34.9%). These methods likely improve the bioavailability of active compounds by increasing the surface area and facilitating extraction. Soaking, used by 12–14% of respondents across all areas, likely aids in extracting water-soluble phytochemicals such as saponins, flavonoids, and alkaloids. Grinding was limited to urban areas (14.3%) likely due to better access to milling equipment while 10% of urban respondents administered the additives raw, without processing.

Feeding frequency also varied significantly across study areas ($P < 0.05$). In peri-urban and rural areas, the majority of respondents (80% and 68.3%, respectively) administered phytogenic feed additives only when chickens were sick, reflecting a therapeutic approach. Conversely, urban producers reported more preventive and regular use. 28.6% administered additives once daily after feeding, and 11.4% reported feeding them three times daily. Additionally, 31.7% of rural respondents used the additives proactively during nearby disease outbreaks.

These findings suggest that while traditional knowledge of processing is widespread, consistent preventive application is less common. This may reflect gaps in awareness about the long-term benefits of routine supplementation with phytogenic feed additives.

Table 4.9. Processing techniques and feeding frequency of phytogenic feed additives

Processing techniques	Urban		Peri-urban		Rural		X ²
	N	%	N	%	N	%	
Chopping	14	20.0	16	35.6	6	9.5	92.58***
Soaking	10	14.3	6	13.3	8	12	
As it is	7	10.0	1	2.2	1	1.6	
Gridding	10	14.3	-	-	-	-	
Crushing	29	41.4	22	48.9	16	25.4	
Chopped and rubbed	-	-	-	-	8	12.7%	
Sliced and rubbed	-	-	-	-	2	3.2%	
Chopped and crushed	-	-	-	-	22	34.9%	
Feeding frequency							
One time per day	20	28.6	-	-	-	-	157***
Two times per day	4	5.7	-	-	-	-	
Three times per day	8	11.4	-	-	-	-	
Some times	12	17.1	-	-	-	-	
Once a week	4	5.7	-	-	-	-	
Three times a week	14	20.0	9	20	-	-	
When chickens become sick	8	11.4	36	80	43	68.3	
Disease occurrences in neighbor farm	-	-	-	-	20	31.7	

N =frequency, X²=chi square, ***=significant at P ≤ 0.001

4.3.10. Constraints to the utilization of phytogenic feed additives

Table 4.9 presents the key constraints affecting the use of phytogenic feed additives across the study areas, with statistically significant differences observed among study areas (P < 0.05). The most frequently reported constraint was a lack of knowledge about the role and application of phytogenic feed additives, mentioned by 34.2% of respondents in urban areas, 40% in peri-urban areas, and 37.5% in rural areas. This widespread knowledge gap suggests that most farmers, particularly in peri-urban and rural settings, remain unaware of the full range of benefits such as improved egg quality, growth performance, and disease resistance that these natural additives can offer.

Despite some extension efforts promote leafy greens as nutritional supplements; there is limited information or using spices and medicinal plants in chicken feed. This indicates a gap between general nutrition education and the targeted promotion of phytogenic alternatives.

In urban areas, 18.3% of respondents also reported the high cost of certain spices such as ginger, garlic, or fenugreek as a major barrier to their consistent use. This reflects potential issues in the supply chain or pricing of value-added natural products within urban markets. Other

constraints noted (though to a lesser extent) included seasonal availability, labor-intensive preparation, and concerns over dosage or potential side effects.

These findings underscore the need for targeted educational campaigns, improved access to affordable phytogetic inputs, and practical training on preparation and safe administration. Addressing these constraints is essential to support the broader adoption of phytogetic feed additives, especially as alternatives to antibiotics in smallholder chicken systems.

Table 4.10. The major constraint of phytogetic feed additives utilization in the study areas

Phytogetic additive utilization constraints	Urban		Peri-urban		Rural		X ²
	N	%	N	%	N	%	
Lack of knowledge about the level of inclusion	35	29.2	28	35	36	30	44.39***
High prices of some types of spices additives	22	18.3	-	-	-	-	
Lack of extension worker advice	22	18.3	20	25	39	32.5	
Lack of knowledge about the importance	41	34.2	32	40	45	37.5	

N= frequency, X² = chi square, ***=significant P ≤ 0.001

4.3.11. Chemical composition of major phytogetic feed additives

Table 4.10 presents the chemical compositions of the top five ranked phytogetic feed additives. The current study found that neem contained 90.62% DM, 18.11% ash, 3.40% EE, 7.54% CF, 16.78% CP, 0.095 mg/g phosphorus (P), and 44.79% NFE. These values are lower than those reported by Bonsu *et al.* (2012) for CP, CF, EE, ash, moisture, and NFE, but higher for ash, CP, DM, and EE. Additionally, the ash, DM, EE, and CF contents differed from the values reported by Ubua *et al.* (2019). These variations in chemical composition likely result from factors such as the type of neem tree, leaf age, geographic location, season of harvest, soil type, and processing methods.

The ash content of bitter-leaf in the current study (16.29%) was notably higher than the 9.56% reported by Asaolu *et al.* (2012) for bitter leaf. Ash content is an important indicator of mineral element presence. The protein content of ginger in this study (9.68% CP) aligns with findings by Dolle (2020), but is higher than the values reported by Otunola *et al.* (2010) (8.54% CP) and Onimawo *et al.* (2019) (8.91% CP). Garlic contains 90.78% DM, 20.07% ash, 3.46% EE, 7.89% CF, 11.38% CP, and 0.079% P. Cabbage contains 87.3% DM, 18.95% ash, 3.53% EE, 8.08% CF, 12.26% CP, and 0.079% P.

Table 4.11. Chemical composition of the first five ranked phytogenic feed additives

Phytogenic feed additives	<i>Neem</i>	<i>Garlic</i>	<i>Cabbage</i>	<i>Bitter-leaf</i>	<i>Ginger</i>
Parameters					
DM	90.62	90.78	87.3	93.28	89.87
Ash	18.11	20.07	18.95	16.29	15.85
EE	3.4	3.46	3.53	3.6	3.66
CF	7.54	7.89	8.08	8.01	7.81
CP	16.78	11.38	12.26	22.62	11.08
P (mg/g)	0.095	0.079	0.081	0.096	0.088
OM	81.89	79.93	81.05	83.71	84.15
NFE	44.79	47.98	44.48	42.76	51.47
ME (Kcal/kg DM)	2728.27	2620.53	2653.18	2771.72	2810.68

4.4. Conclusions

The survey found that farmers commonly use chicken feed additives sourced from backyard and wild plants and rarely use commercial antibiotics. In peri-urban and rural areas, awareness of using phytogenic feed additives (plant-based additives) to improve chicken performance beyond just health benefits is limited. Farmers tend to select phytogenic feed additives based on their perceived effectiveness in improving chicken health, production, and product quality. Based on farmers' rankings across different study areas, the top three most preferred phytogenic feed additives were neem, bitter-leaf, and garlic. Bitter-leaf emerged as the most consistently valued plant, ranking 1st in rural areas and among the top three in urban and peri-urban settings. These plants were primarily selected for their perceived ability to improve chicken health and support overall chicken productivity. Key challenges faced by producers include a lack of knowledge about proper usage, limited guidance from extension workers, and the high cost of some spice additives. To enhance the utilization of locally available medicinal plants, herbs, and spices, several recommendations are proposed; the incorporation of phytogenic feed additives into packaged forms at regional and national levels, for instance through the Ministry of Agriculture; the provision of training for chicken producers on proper usage via agricultural officers and development agents; the promotion of awareness regarding the potential of phytogenic feed additives as alternatives to antibiotics; and the conduct of further research to assess their effects on chicken performance.

CHAPTER 5: EFFECT OF NEEM (*AZADIRACHTA INDICA*), BITTER-LEAF (*VERNONIA AMYGDALINA*) LEAVE MEALS AND GARLIC (*ALLIUM SATIVUM*) POWDER ON PERFORMANCE AND EGG QUALITY OF LAYER CHICKENS



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Effect of neem (*Azadirachta indica*), girawa (*Vernonia amygdalina*) leaves meal and garlic (*Allium sativum*) on performance and egg qualities of layer chickens

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ABSTRACT

A study was conducted to evaluate the efficacy of neem, bitter-leaf leaves meal, and garlic powder for layer hens on performances and egg quality parameters. A total of 128 Bovans brown layer hens with uniform body weight (BW) were randomly distributed to 4 treatments, each with 4 replications in a completely randomized design (CRD). The treatments consisted of a commercial diet without additives (T1), and the remaining groups received commercial diet with neem leaf meal (T2), bitter-leaf leaf meal (T3), and garlic powder (T4) at 2.5% level of inclusion. Performance parameters such as hen day egg production, egg weight, feed intake, and internal and external egg qualities were evaluated for the laying hens. The results showed that the highest feed intake was determined in T2 (117.77g/hen) and T4 (117.12g/hen), while the lowest was determined in T3 (108.20g/hen) compared to T1 (110.51g/hen). T2 had the highest ($P<0.05$) hen-day egg production (92.45%), followed by T4 (89.78%), and the least hen-day egg production was recorded in T3 (83.86%) compared to T1 (87.4%). The egg mass was highest for T2, while T1 and T3 had the lowest. No significant difference was observed in egg weight, shell thickness, shell weight, albumin height, albumin weight, yolk weight, yolk height, and yolk

diameter. However, egg length and yolk color were significantly ($P<0.05$) higher in T2. Therefore, it was concluded that neem leaf meal can be recommended in this study for layer performance and egg qualities.

Keywords: Egg quality, Layer performance, Phytogenic feed additive

5.1. Introduction

Chicken eggs are good sources of protein with high bioavailability to meet the requirements of human food (Abera and Geta, 2014). The major constraint on egg production is the high cost of conventional feeding materials, especially the primary energy and protein sources (Thirumalaisamy *et al.*, 2016). The laying hen producers are searching for nonconventional feed ingredients that may be cheaper, such as leaf meals of ethno-medicinal plants (Ndou, 2018). The restriction of antibiotic usage in chicken diets is the main driving force that has caused the surge in the use of phytogenic feed additives (Wati *et al.*, 2015). Consequently, there is an urgent need to improve birds' productivity and enhance their health using natural feed additives (El-Saadany *et al.*, 2022; El-Sabrout *et al.*, 2022). Any safe compounds derived from plants that have the potential to aid the immune system, boost stress resistance, and return physiology to normal should be given top consideration when selecting phytogenic feed additives. By reducing the impact of environmental stress on chickens, the addition of polyphenols, vitamins, minerals, and other herbal ingredients in feed has demonstrated a significant increase in health (Abd El-Hack *et al.*, 2020). The additives with proven pharmacological value without affecting the nutritive values improved the poultry production performances (Olgun *et al.*, 2021).

Herbal and medicinal plants are a new class of growth promoters and in recent years, these feed additives have gained extensive attention in the feed industry (Gao *et al.*, 2023). Realizing this, a number of herb and medicinal plants have been identified for their use as feed additives, including neem, garlic and bitter-leaf, which in turn may improve the performance of layers. Some green additives have been studied as alternatives in order to maximize the production performance of chicken in diets. Compared with synthetic antibiotics or inorganic chemicals, these plant derived products have proven to be less toxic and residue free and are thought to be ideal feed additives in food animal production (Dalal and Kosti, 2018).

Furthermore, some studies suggest that feeding hens diets with natural additives like garlic, can help them lay super eggs (Damaziak *et al.*, 2017; Mirbod *et al.*, 2017; Asrat *et al.*, 2018). However, in Ethiopia, limited research has been done to evaluate the effect of phytogetic plants as alternative natural feed additives in layer hen rations on production performance and egg quality. Therefore, this study aims to evaluate the effect of selected phytogetic feed additives on layer chicken production performance and egg qualities.

5.2. Materials and Methods

5.2.1. Description of the study area

The experiment was conducted at Bahir Dar University Poultry Farm in Zenzelma campus, which is located 565 km west of Addis Ababa at an altitude of 1911 meter above sea level, 11° 37' 18"N latitude, and 37° 27'47" E longitude. The mean annual rainfall is 1445 mm. The mean minimum and maximum temperatures during the experimental period were 18.5°C and 30.28°C, respectively.

5.2.2. Phytogetic feed additives collection and preparation

Garlic was purchased from the local market in Bahir Dar city. Fresh bitter-leaf (*Vernonia amygdalina*) and neem (*Azadirachta indica*) leaves were harvested in Woreb Kebele and Maraki Kebele, respectively, both located in Bahir Dar city. To prepare the garlic, the whole part of fresh garlic was chopped into small pieces and spread on a plate, using plastic shade netting to dry under the sun. The dried garlic was then ground into a fine powder using a 2 mm sieve and stored in an airtight polyethylene bag until needed. For the leaves, fresh bitter-leaf and neem leaves were sparsely spread on a plastic mat at room temperature for 8 days, allowing them to dry until crispy and dirt was then handpicked. The leaves were turned regularly to ensure even drying and prevent decay, maintaining their greenish color. Once dry, the crispy leaves were hammer milled to pass through a 2 mm sieve, and then stored in airtight plastic sack to prevent moisture absorption. These powdered leaves were subsequently used as supplements in commercial diets for layer chickens. The processed leaves and garlic powder were manually

mixed with the commercial diet. A small amount (0.625 kg) of each additive was added per 25 kg of feed, and then blended properly to prepare homogenous mixtures.

5.2.3. Experimental design and treatments layout

The experiment was conducted using a completely randomized design (CRD), consisting of four treatments, each replicated four times. The layout of the experimental treatments is shown in Table 5.1 as follows:

Table 5.1. Layout of experimental treatments

Treatments	Layouts
T1	Commercial diet with no additives
T2	Commercial diet + 2.5% Neem
T3	Commercial diet + 2.5% Bitter-leaf
T4	Commercial diet + 2.5% Garlic

5.2.4. Nutrient composition of experimental diets

The nutrient content of the commercial diet and treatments were analyzed at two different laboratories, Bahir Dar and Jimma Universities (Table 5.2). The following parameters were measured in representative samples: dry matter (DM), crude protein (CP) and total ash were analyzed at Bahir Dar University; and the rest, ether extracts (EE), crude fiber (CF), and phosphorus (P) were analyzed at Jimma University. Nitrogen was determined using the Kjeldahl procedure, and CP was calculated by multiplying nitrogen content by 6.25 (AOAC, 2000). Metabolizable energy (ME) values were calculated indirectly from the EE, CF, and ash using the following formula, adapted from Wiseman (2013):

$$\text{ME (Kcal/kg DM)} = 3951 + 54.4 \text{ EE} - 88.7 \text{ CF} - 40.8 \text{ Ash}$$

Table 5.2. The nutrient content of experimental diets (% for DM and % DM for others)

Ingredients	Parameters						
	DM	CP	CF	EE	P	Ash	ME (Kcal/kg DM)
Commercial diet (T1)	92.43	18.34	4.07	6.00	0.4400	5.20	3533.04
<i>Azadirachta indica</i>	90.62	16.78	7.54	3.40	0.0095	18.1	2728.68
<i>Vernonia amygdalina</i>	93.28	22.62	8.01	3.60	0.0096	16.3	2771.31
<i>Allium sativum</i>	90.78	11.38	7.89	3.46	0.0079	20.1	2619.30
Treatments							
T2	94.69	18.76	4.26	6.080	0.450	5.65	3552.91
T3	94.76	18.91	4.27	6.090	0.449	5.61	3552.17
T4	94.70	18.62	4.27	6.086	0.451	5.70	3555.83

T1=commercial diet without additives; **T2**=2.5% neem leaf meal+commercial diet; **T3**=2.5% Bitter-leaf leaf meal+commercial diet; **T4**=2.5% garlic+commercial diet; DM: Dry matter, CP: Crude protein, CF: Crude Fiber, EE: Ether extract, P: Phosphorus, Ash: Total ash, ME: Metabolizable energy

5.2.5. Management of experimental chickens

The experimental house was divided into 16 separate pens with an area of 2 m². The floor was covered by *teff* straw with a thickness of about 5 cm, used as a bedding material. Before the placement of the chickens in the experimental house, the experimental pens, watering, and feeding troughs were thoroughly cleaned, disinfected, and sprayed against external parasites. A total of 128 Bovans brown layers at 26 weeks of age were obtained from Bahir Dar University chicken Farm. They were randomly distributed to each pen, with 8 hens per replication and a total of 32 hens per treatment. Chickens were adapted to experimental diets for 7 days before the commencement of actual data collection. The feed was offered in a group per pen or replication twice a day at 08:00 and 17:00 hours throughout the experimental period. Feed was offered in hanging feeders, which were suspended considering accessible to the chickens, and water was provided in a waterier placed at the center of the pens. Clean and fresh water was available all the time, and the experiment lasted for 90 days. Vitamins were given to the chickens, and the wet litter was replaced with clean and dry litter.

The daily temperature (T^oC) and relative humidity (RH) were recorded throughout the experimental period using a digital thermo-hygrometer. This instrument was hung at the center of the pens inside the experimental layer house to record the maximum and minimum values of temperature and RH. The average temperature humidity index (THI) was calculated weekly

using the equation below Ravagnolo *et al.* (2000): $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$.

Where T = temperature (°C) and RH = relative humidity (%).

5.2.6. Data collection

The feed offered and refused were recorded daily for each replicate. Feed intake was determined as the difference between the feed offered and refused.

$$\text{Feed Intake (g /hen)} = \frac{\text{feed offered} - \text{feed refused}}{\text{number of hens in replication}}$$

The initial body weight of layer hens was individually measured at the beginning (26 weeks) and found to be 1.73 ± 0.05 kg/chicken, while the final body weight was recorded at the end of the experimental periods (38 weeks). Eggs were collected twice a day from each pen at 10:00 a.m. and 17:00 p.m. The sum of the two collections, along with the number of hens alive on each day in each pen, was recorded. The rate of egg lay was expressed as the average percentage of hen-day and hen-housed egg production based on the average values from each replicate based on the method of Hunton (1995) as follows:

$$\% \text{ Hen} - \text{day egg production} = \frac{\text{number of eggs collected per day}}{\text{number of hens present that day}} \times 100$$

$$\% \text{ Hen} - \text{housed egg production} = \frac{\text{number of eggs collected per day}}{\text{Number of hens originally housed}} \times 100$$

Eggs collected daily were weighed immediately after collection. The average egg weight was determined by dividing the total weight of daily collected eggs by the number of eggs laid in each replicate. The mortality of chickens was calculated as the initial total chickens minus the final total chickens divided by the initial total chickens multiplied by 100%. The average egg mass per hen per day was calculated using the formula described by North and Bell (1990):

$$\text{Egg mass (g/hen/day)} = \frac{\text{no. of eggs per day} \times \text{average egg weight}}{\text{number of hens}}$$

$$FCR = \frac{\text{Feed consumed (gram/ hen/day)}}{\text{egg mass (gram/ hen/day)}}$$

The egg quality parameters include albumen qualities (albumen height, albumen diameter, and albumen weight), yolk quality (yolk height, yolk weight, yolk diameter, yolk color, and yolk index), shell weight and thickness, and Haugh Unit Score (HUS). For the measurement, 12 eggs per treatment (3 eggs per replication) were taken randomly, and the average was computed for egg quality parameters once a week throughout the experimental periods. The sample eggs were individually weighed, coded, broken, placed on the glass plate, and drawn gently. The shells of the broken eggs were separated from their shell membrane, air dried, and weighed. The measurement of shell thickness was carried out with a digital caliper at three points on the egg shell (air cell, equator, and sharp end).

Yolk of each sample egg was separated by water bottle (*highland*) from the white; yolk height and diameter were measured using a tripod micrometer and digital caliper, respectively. Then, the yolk was thoroughly mixed and a sample droplet from each egg was placed on a Petri dish. The Roche color fan consisting of a series of fifteen colored plastic strips was used as a reference to determine yolk color, with 1 rated as very pale yellow and 15 as deep reddish orange. Yolk index was also computed using the following formula:

$$\text{Yolk index} = \frac{\text{Yolk Height}}{\text{Yolk Diameter}}$$

The average Huagh Unit value for each replicate was calculated by using the formula given by Haugh (1937).

$$\text{Huagh Unit (HU)} = 100 \times \log (H - 1.7W^{0.37} + 7.6)$$

Where, H= albumen height (mm), W= weight of egg (g)

5.2.7. Statistical analysis

The data were analyzed using the General Linear Model (GLM) procedure in SAS version 9.4 (SAS, 2013, Cary, NC). Differences between treatments were separated using tukey tests, and the means were considered significant at $P < 0.05$. The model used for statistical analysis was:

$$Y_{ij} = \mu + T_i + e_{ij},$$

Where;

Y_{ij} = the response variable (e.g., feed intake, FCR, egg production, and egg qualities) measured for each treatment i , μ = the overall mean, T_i = the effect of treatment i , and e_{ij} = the random error associated with each observation.

5.3. Results and Discussion

5.3.1. Productive performances

The effects of dietary supplementation with phytogenic plants on feed intake, egg production, egg mass, feed conversion ratio, final and initial body weight are presented in Table 5.3. Phytogenic feed additives significantly ($P < 0.05$) increased the daily feed intake, hen-day egg production, and egg mass of the layer hens. The hens in T2 groups had significantly higher egg mass, feed intake, and hen-day egg production relative to T1. The current study is supported by Omer *et al.* (2019), who found that incorporating garlic powder, onion powder, and a mixture of them into laying hen diets significantly improved feed conversion, egg mass, and egg production. The observed increase in egg production and egg mass could be attributed to the positive influence of phytogenic plants on the efficiency of converting digested nutrients into egg formation. In contrast to the observation by Asrat *et al.* (2018), the inclusion of 1, 2, and 3% garlic powder in diets had no effect on hen-day egg production, egg mass, or feed conversion ratio with the control treatment group.

The egg weight and final body weight of the hen were not significantly ($P > 0.05$) affected by the dietary treatments. This result is in line with a previous study by Asrat *et al.* (2018), which also showed no improvement in egg weight and final body weight with the addition of garlic

powder in white leghorn hens. The daily feed intake increased significantly ($P < 0.05$) with the inclusion of neem and garlic feed additives. These results agreed with Khatun *et al.* (2013) and Hasan *et al.* (2016), who found that hens fed neem had higher feed consumption. Similarly, a study conducted by Haque *et al.* (2018) also found that hens provided with a combination of 2% neem and niyshinda leaf displayed the highest feed intake. These findings indicate that neem and other additives may have a stimulating effect on hens' appetite, resulting in increased feed consumption. However, hens fed diet supplemented with bitter-leaf (T3) significantly reduced feed intake as compared with other supplemented groups. This low feed intake might be due to the high content of alkaloids, saponins, tannin, and glycosides in bitter-leaf, which result in decreased absorption and utilization of nutrients by inhibiting metabolic or digestive enzymes (Corthout *et al.*, 1992; Mohammed and Zakariya, 2012; Li *et al.*, 2023).

Table 5.3. Effect of Neem, Bitter-leaf leaves meal and Garlic powder on performance of layer hens

Parameters	Treatments				SEM	p-value
	T1	T2	T3	T4		
Feed intake (g/hen/d)	110.51 ^b	117.77 ^a	108.20 ^b	117.12 ^a	1.18	0.000
Initial BW (kg)	1.72	1.74	1.72	1.73	0.04	0.87
Final BW (kg)	1.98	1.86	1.86	1.83	0.13	0.69
Hen day egg production (%)	87.40 ^b	92.45 ^a	83.86 ^c	89.78 ^{ab}	3.50	0.03
Hen Housed Egg Production (%)	85.03	87.99	82.53	87.74	4.38	0.17
Egg weight (g)	58.76	59.20	58.02	58.83	0.92	0.64
Egg mass (g)	51.34 ^b	54.44 ^a	49.8 ^b	52.76 ^{ab}	2.05	0.02
Feed Conversion Ratio	2.15	2.15	2.22	2.22	0.04	0.320
Mortality (%)	3.13	6.25	3.13	3.13	1.5	0.87

T1=commercial diet without additives; **T2**=2.5% neem leaf meal+commercial diet; **T3**=2.5% Bitter-leaf leaf meal+commercial diet; **T4**=2.5% garlic+commercial diet. ^{a-c} Means with in a row with different superscripts are significantly differ ($P < 0.05$ SEM = Standard error of mean)

Figure 1 indicates the trend of egg production in the experimental layer hens. The results of egg production in T1, T2, T3, and T4 were significantly different ($P < 0.05$) from each other. Chickens fed with T2 exhibited significantly higher egg production than T1 and T3. This might be a variation in the type of active compounds, like flavonoid and others, in the treatment, which positively enhanced egg production by facilitating digestion and nutrient absorption. This result was supported by Bratte and Isikwenu (2015), who showed that supplementing neem leaf meal at a level of 7.5% showed a significant increase in hen-day egg production. Igugo *et al.* (2022) also found that laying hens fed a 2.5% combination of moringa and neem leaf meals had increased

egg production intensity along with feed intake. The egg production of layer hens in T3 appeared to be below as compared with T1. This reduction in egg production might be associated with the high content of secondary metabolites in *Vernonia amygdalina*. On the other hand, egg production of in the T2 and T4 supplemented groups showed almost a similar trend throughout the experiment. The egg production rate of layer chickens in T2 supplemented groups appeared to be at its maximum after week 6 of the experimental period. The main reason for the higher production rate of the T2 supplemented group of layer hens after week 6 than earlier suggests that it might have taken some time for the layer hen or their gastrointestinal system to well acclimatize to the experimental treatment in this study.

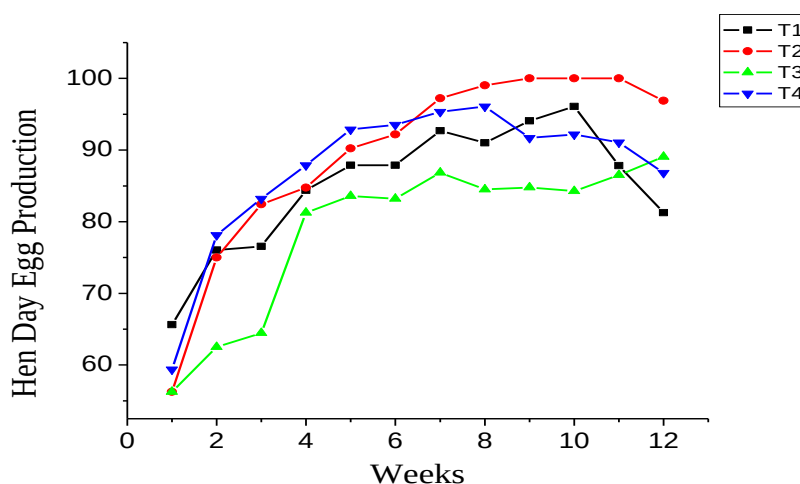


Figure 5.1. Egg production of layer chicken fed on commercial diet supplemented with *Azadirachta indica*, *Vernonia amygdalina* leaves meal and *Allium sativum* powder; **T1**=commercial diet without additives; **T2**=2.5% neem leaf meal+commercial diet; **T3**=2.5% Bitter-leaf leaf meal+ commercial diet; **T4**=2.5% garlic+commercial diet

5.3.2. Effect of phytogetic feed additives on egg quality parameters

Table 5.4 indicates the dietary inclusion of experimental additives that led to significant ($P < 0.05$) differences in egg length, albumin diameter, and yolk color compared with control group. There were significant differences in egg length on layer received (T2, and T4) compared with T1. T3 has the highest albumin diameter, which is significant ($P < 0.05$) compared with T2. This shows that as the albumen diameter increases, the quality decreases, and the albumen appears watery. The egg yolk color score was significantly increased by the addition of neem and bitter-leaf to laying hen diets compared with the control and garlic groups. T2 was shown to have the highest

value of yolk color among phytogetic feed additives due to carotenoids. Similarly, feeding T3 supplements to the chicken improved their absorption of the feed's beta-carotene. Therefore, changes in color values, which increase the yolk color fun (YCF) score from the experimental treatments in the current study, are compatible with differences in the amount of carotenoid components in the diets (Kljak *et al.*, 2021). It is a possible that these phytogetic feed additives may have stimulated the hepatic secretion of egg yolk precursors, which protects hepatocytes from oxidative stress and there by improves yolk formation and ovulation.

However, no significant differences were recognized in shell thickness, egg shell weight, egg width, egg weight, yolk (weight, diameter, height, and index), Hough unit, and albumin height by phytogetic feed additive supplementation ($P > 0.05$). This indicated that neem, bitter-leaf leaves and garlic powder additives did not significantly affect the quality of the eggs. The current study was in line with the finding of Asfaw *et al.* (2022), who noted the non-significant effect of chat (*Catha edulis*) leaves on shell thickness, albumen height, Haugh unit, and yolk (height, diameter, and index). Likewise, Tessema *et al.* (2023) reported no significant ($P > 0.05$) difference in the shell thickness, shell weight, albumen height, yolk (weight, height, diameter, index), and haugh unit by supplementation of the *ruta graveolen*, coriander and basil seed extracts in white leghorn. A similar result was reported by Damaziak *et al.* (2017), who found that the inclusion of garlic and onion in the hen diet was beneficial, as it increased egg weight, yolk weight, and the yolk-to-egg weight ratio, and improved albumen quality as expressed by Haugh units. But contrary to the current study, inclusion of 2.0 g/kg fermented garlic in the diet of laying hens significantly increased albumin height and haugh unit in subsequent tests of eggs as reported by Hossain *et al.* (2016). These differences might be due to the use of different garlic sources, method of garlic powder preparation, and age of layers used in the experiments.

Table 5. 4. Egg quality parameters of layer chickens fed on diets with Neem, Garlic and Bitter-leaf as phytogetic feed additives

Parameters	Treatments				SEM	P-value
	T1	T2	T3	T4		
Sample egg weight (g)	61.05	65.3	61.075	62.782	1.66	0.079
Egg length (mm)	55.46 ^b	57.19 ^a	56.23 ^{ab}	57.83 ^a	0.58	0.007
Egg width (mm)	44.03	44.72	44.15	44.84	0.55	0.397
Egg Shell Weight (g)	7.25	7.86	7.42	7.32	0.23	0.085
Albumen Weight (g)	36.05	39.01	35.85	36.84	0.58	0.122
Yolk Weight (g)	17.3	17.9	17.3	18.1	0.46	0.203
Albumen Height (mm)	8.1	8.7	7.7	8.1	0.57	0.379
Albumen diameter (mm)	83.5 ^{ab}	80.2 ^b	88.2 ^a	84.6 ^{ab}	2.42	0.041
Yolk Height (mm)	14.8	15.4	15.5	15.5	0.35	0.170
Egg Shell Thickness (mm)	0.34	0.37	0.42	0.35	0.03	0.145
Yolk Color (RSP)	4.0 ^c	6.8 ^a	4.8 ^b	4.0 ^c	0.38	0.000
Yolk Diameter (mm)	40.5	40.2	40.8	40.2	0.52	0.626
Haugh unit (%)	89.93	92.06	87.45	89.19	2.98	0.508
Yolk index	0.37	0.38	0.38	0.39	0.97	0.187

T1=commercial diet without additives; T2=2.5% neem leaf meal+commercial diet; T3=2.5% Bitter-leaf leaf meal + commercial diet; T4=2.5% garlic+commercial diet; ^{a-c} row means with different superscripts are significantly different at ($p < 0.05$), RSP =Roche Scale Point, SEM = Standard error of mean

5.3.3. Relationship of egg production and THI

The correlation indicates that increasing temperature humidity index (THI) caused a significant ($p < 0.05$) decrease in egg production (Table 5.5). In this study, the calculated THI ranged from 64.97 to 67.82, with an average of 66.1. The study revealed that a negative correlation existed between average egg production and THI for treatments T1, T2, and T3; while no significant ($p > 0.05$) correlation was observed in the T4 group. These relationships between average egg production and THI were highly negative (-0.713), (-0.843), and (-0.840) for T1, T2, and T3, respectively. The negative value points out that egg production decreases with increasing THI values. The results showed a significant decrease in egg production for the T2 and T3 groups as a negative effect of high THI. Interestingly, the addition of garlic had no significant ($p > 0.05$) effect of THI on the egg production performance.

Table 5.5. Relationship between total number of egg production and temperature humidity index (THI) of experimental layer chickens

Week	T ^o c		RH		THI	Average egg production per week			
	Max.	Min.	Max.	Min.		T1	T2	T3	T4
1	33.00	19.96	59.63	50.5	67.82	74.6	71.1	65.9	73.8
2	32.86	18.87	58.57	50.71	67.31	76.8	82.1	69.8	83
3	29.86	18.89	71.25	51.25	66.10	82.4	84.4	80.9	87.5
4	31.19	19.2	65.86	48.57	66.77	87.5	90.2	84.8	92.4
5	31.73	19.08	62.5	48.75	66.93	86.7	89.5	84.5	93.1
6	31.71	18.19	64.43	48.86	66.57	92.9	97.3	85.7	94
7	29.73	19.05	72.25	50.88	66.11	91.8	98.8	86.9	97.3
8	28.54	17.61	85.71	49.57	65.05	93.3	99.4	88.7	93.4
9	28.93	17.66	86.75	42.13	65.22	94.5	100	92.9	91.7
10	27.77	18.34	86.14	44.43	65.03	95.5	100	88.6	92
11	29.05	17.81	88.5	44.25	65.34	87.9	100	87.9	91.2
12	28.83	17.13	90.71	45.43	64.97	85.7	98.2	89.7	88.3
Average	30.27	18.48	74.36	47.94	66.1	87.5	92.6	83.86	89.8
Pearson						-0.713	-.843	-0.840	-0.54
Sig. (2 tailed)						0.009	0.001	0.001	0.069
Type of correlation						negative	negative	Negative	negative

T1=commercial diet without additives; T2=2.5% neem leaf meal+commercial diet; T3=2.5% Bitter-leaf leaf meal+commercial diet; T4=2.5% garlic+commercial diet

5.4. Conclusions

The present study revealed that the inclusion of neem leaf meal significantly improved egg length, albumin diameter, yolk color, and egg production compared to the control group. The supplementation of garlic can improve feed intake, and egg length of the laying hens. Neem leaf meal can be used in feeding layers because it has no adverse effect on performance and can improve egg production and egg quality. This finding suggests that the supplements of neem leaf meal and garlic can enhance laying hen performance. However, the 2.5% inclusion of bitter-leaf leaf meal in the layer diet has inhibited the layer's performance. Finally, further research is needed below the 2.5% supplementation level in layer hens' diets to determine if a better result can be achieved with the use of bitter-leaf leaf meal.

CHAPTER 6: HAEMATOBIOCHEMICAL INDICES AND GUT BACTERIAL LOAD IN LAYER HENS FED DIETS CONTAINING LEAF MEALS OF NEEM AND BITTER LEAF AND GARLIC POWDER

ABSTRACT

This study evaluated the effects of neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*), and garlic (*Allium sativum*) powder on the haematological, serum biochemical, and gastrointestinal bacterial counts in layer hens. A total of 128 layer hens were randomly assigned to four dietary treatments: a control diet (T1) with no additives; neem leaf meal (T2); bitter-leaf leaf meal (T3); and garlic powder (T4), each at 2.5% inclusion to the control diet. Each treatment included 4 replicates, with 8 hens per replicate (32 hens per treatment), and the study lasted for 12 weeks following a Completely Randomized Design (CRD). Blood samples were collected for haematological and serum biochemical analysis. At the end of the study, two chickens from each replicate were slaughtered for bacterial load analysis. The results showed significant effects of dietary treatments on haematological parameters. Packed cell volume (PCV) differed significantly ($P < 0.01$), with the highest value in T2 (35%) and the lowest in T1 (17.43%). Hemoglobin concentration and red blood cell (RBC) count also differed significantly ($P < 0.01$), with the highest values in T2 (9.22 g/dl and $2.61 \times 10^6/\text{mm}^3$, respectively) and the lowest in T1. Lymphocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) showed no significant differences ($P > 0.05$). Serum biochemical indices were not significantly affected ($P > 0.05$). Bacterial counts in various gut segments were significantly lower in treated groups compared to control ($P < 0.01$), with reductions in *E. coli* and *Salmonella* counts and increased *Lactobacillus* counts. In conclusion, the inclusion of 2.5% neem, bitter-leaf, and garlic powder improved haematological health and gut microbiota without adverse effects on blood or serum biochemical profiles.

Keywords: Bacterial load, Haematobiochemical, Layer hens, Phytogenic feed additives

6.1. Introduction

Chicken production is popular among small- and medium-scale farmers due to its high turnover and quick return on investment (Phiri *et al.*, 2023). However, diseases and feed costs have reduced profits (Fasina *et al.*, 2012). Improvements in management practices, such as maintaining chicken health and controlling disease, have boosted productivity (Habte *et al.*, 2017). The health and growth of laying hens, particularly their digestive system, are vital for productivity, highlighting the importance of gut-supportive diets for optimal productivity (Adedokun and Olojede, 2019).

Antibiotics were historically used as growth promoters, but rising concerns about antimicrobial resistance and food safety have led to their ban in many countries (Castanon, 2007). This has sparked interest in phytogetic as alternatives, driven by concerns about antibiotic resistance and consumer demand for drug-free food (Mahlake *et al.*, 2022).

Studies now focus on plant-based feed additives such as spices and herbs to enhance chicken health (Durmic and Blache, 2012). These additives, rich in bioactive compounds, have antibacterial, antifungal, antioxidant, and functional properties. Bitter-leaf (*Vernonia amygdalina*) and garlic (*Allium sativum*) are known for their medicinal and nutritional benefits (Alem, 2024). Bitter-leaf has demonstrated antibacterial, antiparasitic, and antioxidant effects, improving growth through enhanced gastrointestinal enzyme activity and feed conversion (Adetoro, 2021). Neem (*Azadirachta indica*) also promotes growth and feed efficiency in hens, owing to its antibacterial and hepatoprotective properties (Dhana, 2015). Neem leaf meal improves chicken meat and egg pigmentation and stimulates immune responses (Shahrajabian *et al.*, 2022), with no adverse health effects noted when included in layer diets (Pliego *et al.*, 2022).

The haematological system is key to evaluating chicken health (Mishra *et al.*, 2016), highlighting the importance of assessing the safety and nutritional potential of feed. Gut health is crucial to chicken performance (Diaz Carrasco *et al.*, 2019), as medications and toxic compounds can affect the gastrointestinal tract (GIT) and haematological and biochemical parameters (Tavangar *et al.*, 2021). While studies have examined the individual effects of neem, bitter-leaf, and garlic on chicken health, limited research exists on their comparative impact on haematobiochemical parameters and gastrointestinal bacterial counts in layer hens. The effects of these plants inclusions in chicken diets remain underexplored, and their influence on blood markers and gut microflora is not well documented. This study aims to evaluate the effects of

neem, bitter-leaf, and garlic powder inclusions on the health and microbiological balance of layer hens, contributing to more sustainable chicken management.

6.2. Materials and Methods

6.2.1. Study site

The experiment was conducted at the Bahir Dar University chicken farm, located on the Zenzelma campus, as described in Subheading 5.2.1

6.2.2. Collection and preparation of phytogenic feed additives

The collection and preparation of phytogenic feed additives were described in detail in Subheading 5.2.2.

6.2.3. Feeding and experimental treatments

All chickens received a commercial diet throughout the experiment, and the treatment groups were described in Subheading 5.2.3.

6.2.4. Experimental chickens and management

For this study, a total of 128 Bovans Brown layers weighing 1.73 ± 0.05 kg/chicken were selected from Bahir Dar University Chicken Farm. Four dietary treatments were used, and each treatment had 32 chickens. Each group, which was repeated 4 times in a completely randomized design (CRD), consisted of 8 chickens per replicate. The layers were acclimatized to the experimental diets for seven days and then fed for 90 days. Each replicate was housed in 2 m² pens with wire-mesh partitions on deep litter bedding covered with 5 cm of *teff* straw. Before the start of the experiment, watering and feeding troughs were thoroughly cleaned and disinfected. The experimental pens were sprayed against external parasites. A measured amount of feed was offered twice daily at 08:00 and 17:00 hours to the layers in each pen, with water available *ad libitum*.

The daily temperature (T°C) and relative humidity (RH) were recorded throughout the experimental period using a digital thermo-hygrometer. This instrument was hung at the center of the pens inside the experimental layer house to record the maximum and minimum values of temperature and RH. The average temperature humidity index (THI) was calculated weekly using the equation below (Ravagnolo *et al.*, 2000):

$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$. Where T = temperature (°C) and RH = relative humidity (%). The average THI is presented in (Figure 6.1).

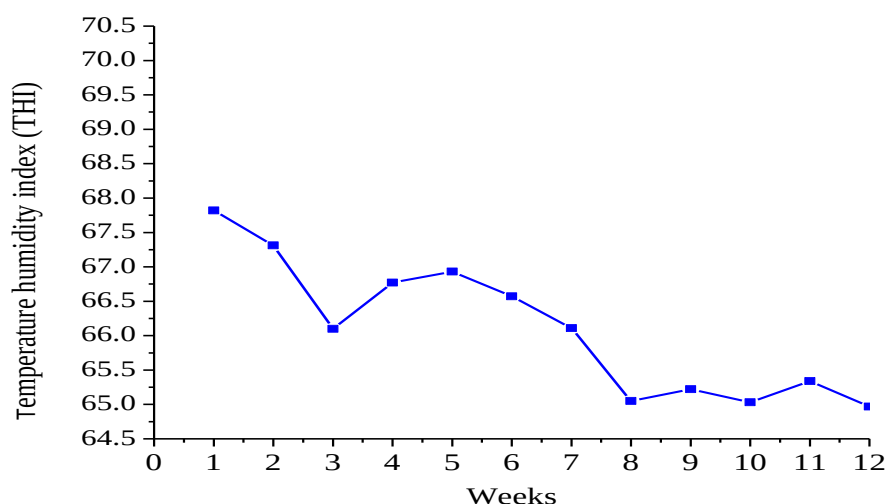


Figure 6.1. Weekly Temperature Humidity Index throughout the experiment

6.2.5. Blood sample collection and analysis

At the end of the 12-week feeding trial, 8 hens were randomly selected from each treatment group. Blood samples were collected from the overnight-fasted hens via the wing vein using a 5 ml syringe fitted with a 22-gauge sterile hypodermic needle. Blood samples were collected using disposable specimen tubes containing EDTA (ethylene diamine tetraacetic acid) as an anti-coagulant for haematological analysis. The blood sample from each layer hen was separated into two labeled bottles for haematological and serum biochemical tests. The bottles were then immediately transferred to the laboratory. An automatic haematology analyzer, Hemax330, determined red blood cell (RBC) count, haemoglobin (Hb) content, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Packed cell volume (PCV) was determined according to

(Kelly, 1979) using the microhaematocrit method. Serum biochemical traits, including total protein, albumin, triglyceride, total cholesterol count, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were analyzed by an automated chemistry analyzer (Weiss and Wardrop, 2011). Globulin values were calculated by subtraction of albumin from serum total protein (Doumas *et al.*, 1985).

6.2.6. Total and selected bacterial counts in gut of layer hens

At the end of the experiment, two hens per replicate (eight hens from each treatment group) were slaughtered. This allowed for the determination of total bacterial counts in the gastrointestinal tract (GIT) and the count of selected bacteria in the ceca. Digesta samples were collected from the crop, gizzard and small intestine (SI), ceca, and feces at cloaca. The samples were aseptically transferred to sterile sampling tubes and transported immediately for microbial analysis in the laboratory.

Samples were diluted in phosphate-buffered saline (10^{-1} to 10^{-10}) for bacterial counts. Each 1 g sample was weighed and suspended in 9 mL of buffered peptone water solution. The suspension was then subjected to serial dilution ranging from 10^{-1} to 10^{-10} . 0.1 mL of each diluted sample was spread on plate count agar selective media for counting total bacteria. For gut bacteria quantification, diluted samples were seeded on freshly prepared bacterial growth media for quantitation of particular gut flora. *E. coli* colonies were enumerated on Eosin Methylene Blue (EMB) Agar incubated aerobically at 37°C for 24 hours. *Lactobacilli* counts were determined on deMan-Rogosa-shape (MRS) agar incubated anaerobically at 37°C for 72 h. *Salmonella* colonies were enumerated using a colorimetric reaction on Xylose Lysine Deoxycholate (XLD) Agar. Samples were incubated on XLD Agar at 37°C for 48 hours.

6.2.7. Statistical analysis

The data for all measured variables were analyzed using a completely randomized design with the General Linear Model procedure in the Statistical Analysis System (SAS, 2013) (version 9.4; SAS Institute Inc., Cary, NC). Tukey test was used to determine mean differences at $P < 0.05$.

The model used for statistical analysis was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y_{ij} = the observed value of each of the response variable;

μ = overall mean; T_i = observed effect of the i^{th} dietary treatment, and, and e_{ij} = random error

6.3. Results and Discussion

6.3.1. Haematological parameters of layer hens

The haematological profile of laying hens fed diets supplemented with neem, bitter-leaf, and garlic showed significant effects on certain parameters (Table 6.1). The incorporation of neem, bitter-leaf and garlic in the layer diet enhanced ($P < 0.01$) the blood concentration of red blood cell count (RBC), hemoglobin (Hb), and packed cell volume (PCV). Similar findings were made by Oluwafemi *et al.* (2021) who reported that broiler chickens given different inclusion levels of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) oil mixture had significantly higher ($P < 0.05$) RBC, PCV, and Hb levels compared to the control group. In contrast, Odoh and Bratte (2015), who stated that adding neem leaf meal at different levels of inclusions, had non-significant effect on RBC, Hb, and PCV counts when compared to the control group.

Hens fed neem, bitter-leaf, and garlic supplements had significantly higher PCV values (26–35) compared to the control group (17.43) ($P < 0.01$). This result corroborated with the findings of Handique *et al.* (2019) who found higher PCV values in garlic treated groups as compared to control. Contrary to the present results, Sufi (2019) stated that there was no significant effect ($P > 0.05$) on the differential count of PCV in broiler chickens supplemented with neem (*Azadirachta indica*) and bitter-leaf (*Vernonia amygdalina*) leaves extracts in their feed. The PCV values in the supplemented groups were within the normal range (22.00-35.00%) as reported by Bounous and Stedman (2000) and Aksu and Ozsoy (2010), suggesting an improvement in blood quality. PCV is a key indicator of anemia and dehydration, with values below 22% and above 42% indicates anemia and dehydration respectively (Joshua *et al.*, 2022).

RBC count was significantly higher ($P < 0.01$) in hens fed neem leaf meal (T2) and bitter-leaf leaf meal (T3) compared to the control group (T1). The RBC values in this study were found in the normal range ($2.2\text{--}4.0 \times 10^6/\text{mm}^3$) reported for healthy broiler chickens (Ubua *et al.*, 2018; Joshua *et al.*, 2022) but lower than those found by Olumide and Odunowo (2019) for

broiler chickens fed garlic-supplemented diets ($3.06\text{-}3.96 \times 10^3/\text{mm}^3$). Hemoglobin (Hb) concentration was significantly higher ($P < 0.01$) in the supplemented groups compared to the control group. The Hb concentrations were also comparable to those found by Unigwe and Igwe (2022), who observed Hb levels ranging from 7.23 to 10.27 g/dl in hens fed garlic and ginger powder meals. However, contradictory findings were reported by Alam *et al.* (2015) and Nodu *et al.* (2016), who found that neem supplementation did not affect Hb concentration in broilers. Inconsistent findings were also reported by Zeryehun *et al.* (2017), who found no significant difference in Hb levels in White Leghorn layer chickens fed 1, 2, and 3% garlic powder for 90 days. Hemoglobin concentrations reflect the efficiency of oxygen and nutrient transport in hens. PCV, RBC, and Hb are crucial indicators of hen health and nutrition, as changes in blood constituents can reveal metabolic status and feed quality.

There were no significant differences ($P > 0.05$) observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and lymphocyte counts across the groups. This result is consistent with the findings of Prakash *et al.* (2022), who reported no significant differences in the hematological parameters (MCV, MCH, and MCHC) of Japanese quail provided with neem leaf extract at different levels. The current result is also similar to the findings of Odoh and Bratte (2015), who reported that varying levels of neem leaf meal inclusion in layer diets had no significant ($P > 0.05$) effect on MCV, MCH, MCHC, and plasma proteins.

Table 6.1. Haematological indices of laying hens as affected by the supplementation of *Azadirachta indica*, *Vernonia amygdalina* leaves meal and *Allium sativum* powder Meal.

Indices	Treatments				SEM	P-value	Ref. value
	T1	T2	T3	T4			
PCV (%)	17.43 ^b	35 ^a	31.2 ^a	26.68 ^{ab}	0.51	0.002	22-35 ¹
RBC ($10^6/\text{mm}^3$)	1.3 ^b	2.61 ^a	2.27 ^a	1.87 ^{ab}	0.04	0.0059	2.2-4.0 ²
Hb (g/dl)	4.8 ^b	9.22 ^a	8.68 ^a	7.67 ^a	0.13	0.0013	7-13 ¹
MCV (fl)	134.3	134.5	138.5	143.95	0.41	0.1208	90-140 ¹
MCH (pg)	37.3	35.48	39.2	42.2	0.32	0.3009	33-47 ¹
MCHC (g/dl)	27.33	26.32	28.2	29.13	0.14	0.4143	26-35 ¹
Lymphocytes (%)	92.8	88.80	75.20	90.76	0.76	0.1520	-

T1=commercial diet without additives; T2=2.5% *Azadirachta indica* leaf meal+commercial diet; T3=2.5% *Vernonia amygdalina* leaf meal+ commercial diet; T4=2.5% *Allium sativum* + commercial diet; ^{a, b} Means within a row with different superscript letters are significantly different, ($P < 0.05$); SEM=Standard error of the mean

¹Bounous and Stedman, 2000; ² Ubua *et al.*, 2018 and Joshua *et al.* 2022

6.3.2. Serum biochemistry parameters of layer chickens

The effects of dietary inclusion of neem leaf meal, bitter-leaf leaf meal, and garlic on serum chemistry parameters in laying hens are presented in Table 6.2. The results show no significant differences ($P > 0.05$) among the dietary treatments, indicating that these interventions did not adversely affect blood chemistry profiles. This finding is consistent with Odoh and Bratte (2015), who also reported no significant effects of neem leaf meal on serum biochemistry in layer hens. In contrast, Oluwafemi *et al.* (2021) observed significant changes ($P < 0.05$) in serum biochemical parameters such as albumin, globulin, and total protein in broiler chickens fed varying levels of a ginger and garlic oil mixture.

Total cholesterol levels in this study ranged from 80.07 to 139.47 mg/dL, which fall within the standard avian reference range (87-192 mg/dL; Meluzzi *et al.*, 1992), suggesting that the dietary treatments did not disrupt lipid metabolism. However, these values were slightly lower than those reported by Khawaja *et al.* (2013), who observed levels between 130.70 and 138.80 mg/dL in layer chickens.

Total protein concentrations in the current study ranged from 4.2 to 5.5 g/dL, falling within the normal reference range of 3.5–6 g/dL (Campbell and Ellis, 2013). This is supported by Ojambati *et al.* (2019), who reported total protein levels in chickens ranging from 4.25 to 5.53 g/dL. However, these values are lower than observed by Zeryehun *et al.* (2017), who reported levels between 6.16 and 9.37 g/dL in layer hens fed garlic powder. Such differences may be attributed to variations in bird type, feed composition, or environmental conditions.

Albumin levels ranged from 1.53 to 2.2 g/dL, while globulin levels ranged from 2.7 to 3.4 g/dL. These values are within or near the expected ranges for chicken 1.2-2.5 g/dL for albumin and 1.5-3.5 g/dL for globulin (Campbell and Ellis, 2013). Albumin helps maintain blood osmotic pressure and transports various molecules, whereas globulin supports immune function. The balance observed between these proteins suggests a stable physiological status across all treatments.

Triglyceride levels ranged from 86.43 to 152.4 mg/dL. Notably, levels reached 115.43 mg/dL in T3 and 152.4 mg/dL in T4 both higher than in T1 but remained within the standard reference range of 45.7-172 mg/dL (Meluzzi *et al.*, 1992). Despite this elevation, the highest values were observed in the supplemented groups, suggesting that the increased triglycerides

may reflect a targeted modulation of lipid metabolism by the phytochemical compounds rather than indicating an adverse effect. Furthermore, the absence of significant differences among treatment groups suggests that overall lipid metabolism and energy storage were not negatively affected.

HDL and LDL levels ranged from 80.7 to 101.1 mg/dL and 15 to 17 mg/dL, respectively. These values fall within normal reference ranges for healthy chickens (HDL: 60-140 mg/dL; LDL: 15-50 mg/dL; Mitruka and Rawnsley, 1997) are consistent with findings from Ghasemi *et al.* (2010) and Saki *et al.* (2014), who reported no significant effects of phytochemical feed additives on serum lipoprotein profiles.

In instant, the serum biochemical parameters indicate that dietary inclusion of neem, bitter-leaf leaf meal, and garlic did not compromise the metabolic health of laying hens. Layers fed these diets showed similar levels of total protein, albumin, globulin, triglycerides, cholesterol, HDL, and LDL compared to those on the control diet. These comparable profiles suggest that such dietary supplements can be safely incorporated without adversely affecting serum biochemistry or overall nutritional status.

Table 6. 2. Serum biochemical indices of laying chickens as affected by the supplementation of *Azadirachta indica*, *Vernonia amygdalina* leaves meal and *Allium sativum* powder Meal.

Indices	Treatments				SEM	P-value	Ref. value
	T1	T2	T3	T4			
Total protein (g/dl)	5.3	5.5	4.2	4.5	0.07	0.388	3.5-6 ¹
Albumin (g/dl)	2.20	2.11	1.54	1.53	0.07	0.815	1.2-2.5 ¹
Globulin (g/dl)	3.1	3.4	2.7	3.0	0.08	0.895	1.5-3.5 ¹
Cholesterol (mg/dl)	135.5	137.0	80.1	139.5	3.06	0.262	87-192 ²
Triglyceride (mg/dl)	96.65	86.43	115.43	152.4	3.31	0.330	45.7-172 ²
HDL (mg/dl)	91.2	101.1	80.7	97.7	0.82	0.123	60-140 ³
LDL (mg/dl)	17.25	15.00	16.75	12.75	0.96	0.367	15-50 ³

T1=commercial diet without additives; *T2*=2.5% *Azadirachta indica* leaf meal+commercial diet; *T3*=2.5% *Vernonia amygdalina* leaf meal+ commercial diet; *T4*=2.5% *Allium sativum* + commercial diet, SEM=Standard error of the mean

¹campbell and Ellis, 2013; ²Meluzzi *et al.*, 1992; ³Mitruka and Rawnsley 1997

6.3.3. Total bacterial counts in gut of layer hens

There was a significant difference ($P < 0.01$) in total bacterial populations across the GIT segments (Table 6.3). The bacterial load in the crop was reduced with neem, bitter-leaf, and garlic supplementation. The phytochemical feed additives, which significantly lowered bacterial populations compared to the control diet, and improved gut health in the supplemented groups,

indicating their antimicrobial properties. This finding aligns with similar results reported for other phytogetic feed additives (Murugesan *et al.*, 2015).

The total bacterial count in the gizzard was significantly lower ($P < 0.05$) in treatments T2 and T4 compared to the control group, indicating a possible inhibitory effect of neem and garlic supplementation, respectively (Table 6.3), however, no significant difference ($P > 0.05$) was observed with T3 supplementation. The total bacterial population in the small intestine was significantly ($P < 0.05$) reduced by treatments T2, T3, and T4 compared to T1. Phytogetic additives may inhibit microbial growth possibly by interfering with electrostatic forces or transcriptional mechanisms (Murugesan *et al.*, 2015).

Layers in the supplemental treatment groups had a significantly lower total bacterial count in the ceca ($P < 0.05$) compared to the control group. The ceca play a crucial role in the fermentation process and nutrient absorption. The decrease in bacterial counts is likely due to the antimicrobial properties of the phytochemicals present in the phytogetic plants. The overall reduction in gut bacterial load from these phytogetic feed additives (PFAs) may lead to immune system-sparing effects, allowing more energy to be directed toward improved performance.

Dietary phytogetic supplements also significantly ($P < 0.05$) reduced bacterial populations in hens' feces compared to the control diet. This finding is consistent with a study by Sakthi *et al.* (2017), who revealed that layers fed tulsi, garlic, ginger, fenugreek, and curcumin, exhibited significantly lower fecal colony-forming units. Similarly, Odoh and Bratte (2015) demonstrated the antibacterial effect of neem leaf meal, which reduced fecal bacterial counts in Shikka Brown layers compared to the control group. Moreover, Al-Obaidi *et al.* (2019) reported a decrease in the total fecal bacterial count, including coliforms, among layer hens supplemented with parsley (*Petroselinum sativum*). So this suggests that phytogetic products have the potential to reduce pathogenic populations, thereby improving gut health.

Table 6.3. The influence of *Azadirachta indica*, *Vernonia amygdalina* leaves meal and *Allium sativum* powder on bacterial populations (log cfu/g) in the GIT digesta

Parameters	Dietary Treatments				SEM	P-value
	T1	T2	T3	T4		
Crop	6.00 ^a	4.03 ^b	4.34 ^b	4.23 ^b	0.06	0.002
Gizzard	5.00 ^a	3.54 ^b	4.19 ^{ab}	3.5 ^b	0.04	0.002
Small Intestine	7.00 ^a	3.50 ^b	4.71 ^b	3.98 ^b	0.10	0.000
Ceca	10.5 ^a	7.32 ^b	7.17 ^b	6.85 ^b	0.11	0.000
Feces	6.53 ^a	4.12 ^b	3.66 ^b	3.73 ^b	0.08	0.000

T1=commercial diet without additives; **T2**=2.5% *Azadirachta indica* leaf meal+commercial diet; **T3**=2.5% *Vernonia amygdalina* leaf meal+ commercial diet; **T4**=2.5% *Allium sativum* + commercial diet; ^{a, b} row means with different superscripts are significantly different at ($p < 0.05$), SEM = Standard error of mean

Lactobacillus counts were consistently higher in all supplemented groups compared to the control (Table 6.4). The highest levels of *E. coli* (2.91 log cfu/g) and *Salmonella* (2.01 log cfu/g) were found in the control group (T1), indicating lower immunity in layers. The lowest *E. coli* colony count was observed in T2 (1.79 log cfu/g) and T4 (1.78 log cfu/g), while the lowest *Salmonella* count was found in T3 (0.64 log cfu/g) and T4 (0.65 log cfu/g). This result suggests that the addition of those additives to the diet reduces the number of pathogenic bacteria in the ceca and improves gut health. This finding is consistent with a study by Sakthi *et al.* (2017), which reported that tulsi, garlic, ginger, fenugreek, and curcumin effectively reduce pathogenic bacterial loads and possess significant antimicrobial activity.

The lowest *Lactobacillus* colony count was found in the control group (T1) (3.5 log cfu/g), while the highest counts were observed in T2 (4.9 log cfu/g) and T4 (4.91 log cfu/g). This result aligns with the findings of Tesfaye *et al.* (2023), who reported a higher number of *Lactobacilli* and a lower *E. coli* count in the intestinal content of chickens fed lemongrass, peppermint, and rosemary leaf powder as inclusion. Similarly, Chowdhury *et al.* (2023) found that incorporating phytogetic feed additives into chicken diets increased *Lactobacillus* counts and decreased *coliform*, *E. coli*, and *Salmonella* counts compared to the control group. Moreover, the addition of 2.0% mint leaf in broiler diets significantly reduced *Salmonella* and *E. coli* counts in the ceca compared to the control group and improved the overall health status of the broilers (Akter and Asaduzzaman, 2023).

Table 6.4. Cecal bacterial counts of layer hens supplemented with *Azadirachta indica*, *Vernonia amygdalina* leaves meal and *Allium sativum* powder.

Bacterial counts (log cfu/g)	Dietary Treatments				SEM	P-value
	T1	T2	T3	T4		
Total bacterial count	10.5 ^a	7.32 ^b	7.17 ^b	6.85 ^b	0.11	0.000
<i>Lactobacillus</i>	3.51 ^b	4.90 ^a	4.70 ^{ab}	4.91 ^a	0.20	0.018
<i>Escherichia coli</i>	2.91 ^a	1.78 ^b	2.21 ^{ab}	1.79 ^b	0.16	0.014
<i>Salmonella</i>	2.01 ^a	0.86 ^{ab}	0.64 ^b	0.65 ^b	0.20	0.025

T1=commercial diet without additives; **T2**=2.5% *Azadirachta indica* leaf meal+commercial diet; **T3**=2.5% *Vernonia amygdalina* leaf meal+ commercial diet; **T4**=2.5% *Allium sativum* + commercial diet; ^{a,b} row means with different superscripts are significantly different at ($p < 0.05$), SEM = Standard error of mean

6.4. Conclusions

The study on the haematological profile and gut health of laying hens supplemented with neem, bitter-leaf, and garlic improved key haematological parameters, including RBC, Hb, and PCV counts with no significant effect and with reduced bacterial load in the gastrointestinal tract. Thus, this finding suggests that phytogetic feed additives are a natural alternative to enhance the health and productivity of laying hens.

CHAPTER 7: GROWTH PERFORMANCE, CARCASS TRAITS, BLOOD INDICES, AND CECAL BACTERIAL LOAD OF KOEKOEK CHICKENS FED DIETS CONTAINING NEEM, BITTER LEAF, AND GARLIC POWDER

ABSTRACT

This study evaluated the effect of dietary supplementation with phytogenic feed additives: neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*), and garlic (*Allium sativum*) on growth performance, carcass traits, haematobiochemical indices, and cecal microbial load in Koekoek chickens. A total of 192 chicks were randomly allocated to four dietary treatments in a completely randomized design (CRD): a basal control diet (T1) and three experimental diets supplemented with neem leaf meal (T2), bitter-leaf leaf meal (T3) and garlic powder (T4) at a 2.5% level of inclusion for each. Result demonstrated that the chickens fed phytogenic-supplemented diets (T3) exhibited significantly higher ($P < 0.05$) average daily body weight gain, feed conversion efficiency and final body weight compared to T1. Carcass trait analysis revealed significant enhancement ($P < 0.05$) in slaughter weight, dressing weight, different cut parts, and liver, though other parts did not differ significantly. Hematological profile indicated a reduction in white blood cell counts in T1. Serum biochemical assays revealed significant differences ($P < 0.05$) in total protein, globulin, creatinine, glucose, and high-density lipoprotein levels. The elevated total protein levels observed in T2 indicate improved protein metabolism. Additionally, cecal microbiota analysis showed a pronounced increase ($P < 0.01$) in *lactobacillus* counts, coupled with significant suppression of pathogenic bacterial (*E. coli* and *salmonella*), underscoring the antimicrobial efficiency of phytogenic compounds. In conclusion, dietary incorporation of neem, bitter-leaf, and garlic at 2.5% improved growth performance, feed efficiency and gut microbiota without adverse effects on health.

Keywords: Blood profiles, carcass traits, gut bacteria, koekoek chicken performances, phytogenic feed additives

7.1. Introduction

Poultry farming is a vital component of global food systems, providing affordable meat and eggs to a large population (FAO, 2017). It contributes significantly to alleviating protein deficiencies, improving food security, and generating income, particularly for vulnerable communities. In Ethiopia, chicken production plays an essential role in rural livelihoods, with more than 57 million chickens reported nationwide (CSA, 2021). Chickens account for approximately 98% of Africa's poultry population and are considered efficient sources of animal protein due to their rapid growth, high productivity, and excellent feed conversion efficiency (Erdaw and Beyene, 2022).

The rising global demand for safe, high-quality poultry products, coupled with concerns over antimicrobial resistance, has led to increased scrutiny of antibiotic growth promoters (AGPs). Historically used to enhance broiler performance, AGPs are now being phased out or banned in many countries due to their contribution to antibiotic resistance in both animals and humans. This shift has prompted research into safer, more sustainable alternatives such as probiotics, prebiotics, and phytogenic feed additives (Golowczyc and Gomez-Zavaglia, 2024; Wickramasuriya *et al.*, 2024).

Phytogenic feed additives derived from herbs, spices, and plant extracts are gaining attention for their antioxidant, immunomodulatory, antimicrobial, and digestive-stimulant properties (Omar *et al.*, 2016). Among these, neem (*Azadirachta indica*), bitter-leaf (*Vernonia amygdalina*), and garlic (*Allium sativum*) are widely recognized for their pharmacological activities and have a long history of use in traditional medicine and ethnoveterinary practices. In northwest Amhara, these plants are commonly used as natural remedies and feed supplements. Garlic has demonstrated hepatoprotective, antimicrobial, and growth-promoting effects in poultry (Karangiya *et al.*, 2016; Sangilimadan *et al.*, 2019). Neem possesses antibacterial, antifungal, antiviral, and antiparasitic properties (Jawad *et al.*, 2014), while bitter-leaf has been reported to improve feed utilization and immune response, potentially due to its strong antioxidant compounds (Olorunleke *et al.*, 2015; Offor, 2014). Unlike antibiotics, which have supported poultry growth but raised concerns over microbial resistance and residues, these natural additives offer safer, and sustainable alternatives (Abou-Jaoudeh *et al.*, 2024).

Despite their potential, limited research exists on these additives in Ethiopia. Given the risks of antibiotic overuse, exploring phytogetic feed additives alternatives could provide sustainable solutions for chicken production (Mehdi *et al.*, 2018). Therefore, this study was conducted to evaluate the effects of neem leaf meal, bitter-leaf leaf meal, and garlic powder as dietary supplements on growth performance, carcass traits, hematobiochemical parameters, and cecal microbial populations in Koekoek chickens.

7.2. Materials and Methods

7.2.1. Description of the study area

This experiment was conducted at the chicken farm of Bahir Dar University, Zenzelma campus. Zenzelma is located 565 km Southwest of Addis Ababa, at coordinates 11°37'18.5"N and 37°27'47.3"E, with an altitude of 1911 meters above sea level. The mean daily temperature during the experimental period ranged between 15°C and 27°C.

7.2.2. Collection, preparation and chemical analysis of treatment diets

Fresh neem (*Azadirachta indica*) and bitter-leaf (*Vernonia amygdalina*) leaves were harvested from Maraki and Worb kebeles in Bahir Dar city, respectively. The leaves were separated from their stems and shade-dried to preserve bioactive compounds, with regular turning to prevent mold and ensure even drying. Once crisp, they were hand-cleaned, ground using a hammer mill, and sieved through a 2 mm mesh. The resulting leaf meals were stored in airtight polyethylene bags.

Fresh garlic was sourced from the local market of Bahir Dar, chopped into small pieces, shade-dried, ground to a fine powder using a 2 mm sieve, and stored in airtight packaging. Neem leaf meal, bitter-leaf leaf meal, and garlic powder were manually incorporated into the basal diet at 2.5% of the chickens' daily feed intake. Their proximate composition dry matter (DM), crude protein, crude fat, crude fiber, ash, calcium (Ca), and phosphorus (P) and some secondary metabolite content are presented in Table 7.1. Analyses of crude fat, crude fiber, Ca, P, and secondary metabolites were conducted at Jimma University using standard methods, while the

remaining proximate analyses were performed at the Animal Nutrition Laboratory of Bahir Dar University.

Tannins were measured using the Vanillin-HCl method (Maxson and Rooney, 1972) with 1 g of sample extracted in 1% HCl-methanol and absorbance read at 500 nm using a D-catechin standard. Total flavonoids were analyzed following Chang *et al.* (2002) and Goyeneche *et al.* (2015) with 2 g of methanol-extracted sample, reacting with sodium nitrite, aluminum chloride, and sodium hydroxide, and measured at 510 nm using catechin as a standard. Total phenolics were determined according to Woldegiorgis *et al.* (2014) and Kuyu *et al.* (2016), by reacting methanol extracts with Folin–Ciocalteu reagent and sodium carbonate, incubating at 37°C, and reading at 765 nm using gallic acid. Alkaloids were quantified based on Harborne (1973) through acetic acid-ethanol extraction, ammonium hydroxide precipitation, and gravimetric analysis.

Feed rations were formulated according to National Research Council (NRC, 1994) recommendations using WinFeed 2.8 software. All experimental chickens received a starter diet containing 22% crude protein (CP) and 2800 kcal metabolizable energy (ME)/kg, followed by a finisher diet with 20% CP and 3000 kcal ME/kg. The ME content (kcal/kg DM) of the treatments was calculated following Wiseman (2013).

$$\text{ME (kcal/kg DM)} = 3951 + 54.4\text{EE} - 88.7\text{CF} - 40.8\text{Ash}$$

Table 7.1. Ingredients and chemical composition of the four dietary treatments for koekoek chickens

Feed Ingredients	Starter (3 rd to 5 th weeks)				Finisher (6 th to 13 th weeks)			
	T1	T2	T3	T4	T1	T2	T3	T4
Maize	48	48	48	48	59	59	59	59
Soybean Meal	32	32	32	32	26	26	26	26
Noug Cake	6	6	6	6	5	5	5	5
Wheat Bran	6	6	6	6	5	5	5	5
Bone Meal	2	2	2	2	1.5	1.5	1.5	1.5
Salt	1	1	1	1	0.5	0.5	0.5	0.5
Vitamin Premix	1.2	1.2	1.2	1.2	1	1	1	1
L-Lysine	1	1	1	1	0.6	0.6	0.6	0.6
DL-Methionine	0.8	0.8	0.8	0.8	0.4	0.4	0.4	0.4
Di-calcium Phosphate	2	2	2	2	1	1	1	1
Total	100	100	100	100	100	100	100	100
Inclusion levels (%)	0	2.5 NLM	2.5 GLM	2.5 GP	0	2.5 NLM	2.5 GLM	2.5 GP
Nutrient Composition								
CP (% DM)	21.9	22.3	22.4	22.1	20.1	20.5	20.6	20.3
ME (Kcal/Kg)	2828.2	2823.9	2814.5	2805	3038.9	3059.5	3038.9	3053.8
CF (% DM)	9.5	9.7	9.7	9.7	9.8	9.9	9.8	9.7
Ca (% DM)	1.14	1.15	1.16	1.15	1.0	1.01	1.02	1.01
P (% DM)	0.81	0.82	0.81	0.82	0.73	0.73	0.73	0.74
Bioactive compounds	Alkaloids		Tannin		Total Phenolic		Total Flavonoids	
Neem (<i>Azadirachta indica</i>)	1.6		0.39		0.21		0.070	
Bitter-leaf (<i>Vernonia amygdalina</i>)	0.8		0.26		0.27		0.072	
Garlic (<i>Allium sativum</i>)	0.4		0.023		0.02		0.001	

NLM: neem leaf meal, GLM: bitter-leaf leaf meal, GP: garlic powder; T1= control diet without additives; T2= treatment diet containing neem leaf meal at 2.5%; T3= treatment diet containing grawa leaf meal at 2.5%; T4= treatment diet containing garlic powder meal at 2.5%.

7.2.3. Management of the experimental chickens

The experiment was conducted using 224 day-old Koekoek chicks. The chicks were vaccinated against Newcastle disease, infectious bursal disease (Gumboro), and fowl pox, following the recommended vaccination schedule. Initially, the chicks were housed in the brooder house for two weeks and provided with a formulated starter ration. During this period, small quantities of measured phytogenic feed additives were included in their diet to help them adapt to the experimental feed.

After the adaptation period, the chicks were individually weighed on a digital balance, and 192, fifteen-day-old chicks were selected for the experiment. These chicks were then divided into four treatment groups, each consisting of 48 chicks, and randomly assigned to one of four treatment diets. Each treatment group was further subdivided into four replications, with 12 chicks per replication. The dietary treatments were: T1: Basal diet (no additive); T2: Basal diet + 2.5% neem leaf meal; T3: Basal diet + 2.5% bitter-leaf leaf meal; and T4: Basal diet + 2.5% garlic powder.

The basal diet was formulated according to the specifications of the National Research Council (NRC, 1994) to meet the nutritional requirements for broiler chickens. Each replication was provided with the necessary brooding facilities, including a 200-watt heat lamp. The chicks were housed in 2m² wire mesh-partitioned pens, with each pen equipped with a feeder and waterer. The pens were thoroughly cleaned and disinfected using a 37% formalin solution prior to the chicks' arrival and the floors were covered with approximately 8 cm depth of wood shavings.

The experimental feed was provided twice per day at 08:00 and 17:00 hours, and water was available *ad libitum*. Environmental conditions and other relevant factors were monitored throughout the experiment, which lasted for 13 weeks with brooding times. Biosecurity measures were strictly adhered to during the entire experiment.

7.2.4. Feed intake and body weight

The experimental chicks were fed ad libitum with a weighed amount of feed. The feed refusal was collected, weighed and subtracted from the weight of the feed offered to determine feed intake. The body weight of the experimental chicken was taken at weekly intervals using digital balance. Average daily body weight gain and feed conversion ratio were calculated with following formulas.

$$\text{Feed intake (g/bird)} = \frac{\text{Total feed offered} - \text{leftover of feed}}{\text{Number of chicks}}$$

$$\text{Average Body weight gain(g)} = \frac{\text{Final body weight (g)} - \text{Initial body weight(g)}}{\text{Number of chickens}}$$

$$\text{Feed conversion ratio} = \frac{\text{Average feed intake (g)}}{\text{Average body weight gain(g)}}$$

The mortality of chickens was recorded throughout the experimental period, and mortality percentage (MP) was calculated as:

$$\text{Mortality Percentage (MP)} = \frac{\text{number of dead chicks}}{\text{Number of total chicks}} \times 100$$

7.2.5. Dressing percentage and carcass characteristics

At the end of the feeding trial, 2 chickens from each replication (8 chickens per treatment) were selected randomly. The chickens were fasted overnight before slaughter. They were individually weighed and euthanized by severing the jugular vein to ensure complete bleeding. The processing included scalding, plucking, eviscerating, and cutting. The dressing percentage was calculated as the dressed carcass weight relative to the slaughter weight, expressed as a percentage. Slaughter weight is the weight of the chicken immediately before slaughter; while dressed carcass weight is the weight of whole carcass with skin, neck and giblets (heart, liver, and gizzard) without blood, feather, head, feet, and internal organs.

7.2.6. Blood analysis and cecal bacterial counts

7.2.6.1. Blood analysis

At the end of the experiment, blood samples were collected by a qualified veterinarian from the wing vein of two randomly selected chickens per replication, resulting in a total of eight chickens per treatment. Samples were drawn using a sterile 22-gauge disposable syringe.

For hematological analysis, 2 mL of blood was collected from each chicken and immediately transferred into a collection tube containing an anticoagulant. The following hematological parameters were analyzed: hemoglobin (Hb), packed cell volume (PCV), red blood cell count, total white blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These parameters were measured using the ADVIA 560 automatic hematology analyzer.

For biochemical analysis, 3 mL of blood was collected from each chicken and transferred into a sterilized test tube. The samples were then centrifuged at 5,000 rpm for 5 minutes. The resulting serum was decanted and transported on ice to a laboratory for analysis using a biochemical analyzer. The following serum biochemical parameters were evaluated: total protein, uric acid, albumin, creatinine, alkaline phosphatase (ALP), glucose, triglycerides, HDL, LDL, and cholesterol. Both hematological and biochemical analyses were conducted at Tibebe Ghion Referral Hospital, Bahir Dar, Ethiopia.

7.2.6.2. Caecal bacterial count

Cecal digesta samples were aseptically collected post-slaughter from two chickens per replication (8 samples per treatment) for bacterial analysis. These samples were obtained from chickens slaughtered for the purpose of carcass trait analysis. The caeca were ligated, and the contents were carefully hand-stripped into sterile plastic bags. In this study, total bacteria, *Escherichia coli*, *Salmonella spp.*, and *Lactobacillus spp.* were enumerated from the caecal digesta samples.

A bacterial dilution culture was prepared by adding 1 mL of the sample to 9 mL of sterile blank. Subsequently, 250 μ L of the diluted culture was spread on selective agar plates. Serial dilutions were performed from 10^{-1} to 10^{-10} , with 0.1 mL of each diluted sample spread on the respective agar media for the enumeration of specific microbes.

Total bacteria were counted using Plate Count Agar, while *E. coli* colonies were enumerated on Eosin Methylene Blue (EMB) Agar, incubated aerobically at 37°C for 24 hours. *Lactobacillus* spp. was counted on MRS Agar, incubated anaerobically at 37°C for 72 hours. *Salmonella* spp. was enumerated using a colorimetric reaction on Xylose Lysine Deoxycholate (XLD) Agar, incubated at 37°C for 48 hours.

7.2.7. Statistical Analysis

All collected data were subjected to analysis of variance (ANOVA) in a completely randomized design using the general linear model procedure of SAS software version 9.4 (SAS, 2013, Cary, NC) with a model consisting of treatments. The tukey test method was used to identify treatment means that significantly differed from one another.

7.3. Results and Discussion

7.3.1. Growth performances

The effects of dietary phytogetic feed additives on the performance parameters of Koekoek broiler chicken are shown in Table 7.2. Significant differences ($P < 0.05$) were observed in final body weight (FBW), average daily body weight gain (ADBWG), and feed conversion ratio (FCR). Chickens in T3 had the greater FBW than that of T1. This result suggests that some phytogetic feed additives may enhance growth performance by improving feed utilization. The increased body weight gain may be attributed to the growth-promoting and antimicrobial properties of bitter-leaf leaves, which likely reduced the microbial load in the birds and improved feed efficiency. This finding seems to align with the findings of Rafeeq *et al.* (2023), who reported that the dietary inclusion of different phytogetic feed additives, improved body weight gain in chickens. Similarly, Shaheed (2021) reported that supplementation with 2% lemongrass leaf powder enhanced body weight gain. This benefit is likely due to the antimicrobial and antioxidant properties of phytogetic compounds, which are known to improve digestive enzyme secretion and enhance feed digestion (Alagawany *et al.*, 2021; Madkour *et al.*, 2021; Sugiharto, 2020). However, in the current study, significant improvement in body weight

gain was not observed with the addition of neem leaf meal and garlic as compared to the control despite a noticeable numerical difference.

FCR was also significantly better (lower) in T3 compared to T1. The improved FCR in T3 may be due to the antimicrobial and antioxidant properties of phytochemical compounds, which support digestion and nutrient absorption. This is supported by the presence of bioactive compounds such as flavonoids and alkaloids (Table 7.1). This finding is consistent with those of Tokofai *et al.* (2020), who reported improvements in FBW, ADBWG, and FCR in birds fed giraw (*Vernonia amygdalina*) at inclusion levels of 1%, 2%, and 3%. However, there were no significant differences ($P > 0.05$) among treatments in feed intake, initial body weight, and mortality. This aligns with the findings of Abdurahman (2016), who reported no significant differences in feed intake between supplemented with bitter-leaf and control diets in Koekoek chickens. In contrast, Oleforuh-Okoleh *et al.* (2015b) found increased feed intake in broilers supplemented with ginger and garlic, which differs from the present results.

Table 7.2. Growth performances of Koekoek chickens fed Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal, and Garlic (*Allium sativum*) powder meal.

Performance parameters	Treatment				SEM	P-value
	T1	T2	T3	T4		
Feed intake g/day/chicken)	56.7	62.7	60.2	63	0.28	0.151
Initial body weight (g/chicken)	93.5	92.8	91.5	92.8	0.23	0.917
Final body weight (g/chicken)	1413 ^b	1589 ^{ab}	1722.5 ^a	1617.5 ^{ab}	9.63	0.017
Body weight gain (g/day/chicken)	16.3 ^b	18.5 ^{ab}	20.1 ^a	18.8 ^{ab}	0.27	0.015
Feed conversion ratio	3.5 ^a	3.4 ^{ab}	3 ^b	3.4 ^{ab}	0.02	0.040
Mortality (%)	6.3	0.0	4.2	4.2	1.07	0.214

T1= control diet without additives; T2= treatment diet containing neem leaf meal at 2.5%; T3= treatment diet containing grawa leaf meal at 2.5%; T4= treatment diet containing garlic powder meal at 2.5%; SEM= Standard error of the mean. ^{a,b} Row means with different superscript letters differ significantly at $p < 0.05$.

Figure 7.1 illustrates the trend in daily body weight gain (DBWG) across different weeks of the experimental period. Starting from the 3rd week, chickens fed the T4 diet generally showed higher body weight gain compared to the other dietary treatments. However, the weight gains in the T2 and T4 groups were comparable throughout the experimental period. Chicks on T4 exhibited the highest DBWG ($p < 0.05$) until week 11, followed by T2, while T1 consistently produced the lowest gains. After week 11, T3 surpassed T4 in DBWG. No significant differences were observed among T2, T3, and T4 throughout the experimental period, though DBWG in T2 and T4 gradually declined as the study progressed. Chickens fed the control (T1) diet showed

relatively lower weekly weight gains after the 5th week compared to the other groups. Overall, the mean weekly body weight gain increased from the 2nd to the 13th week.

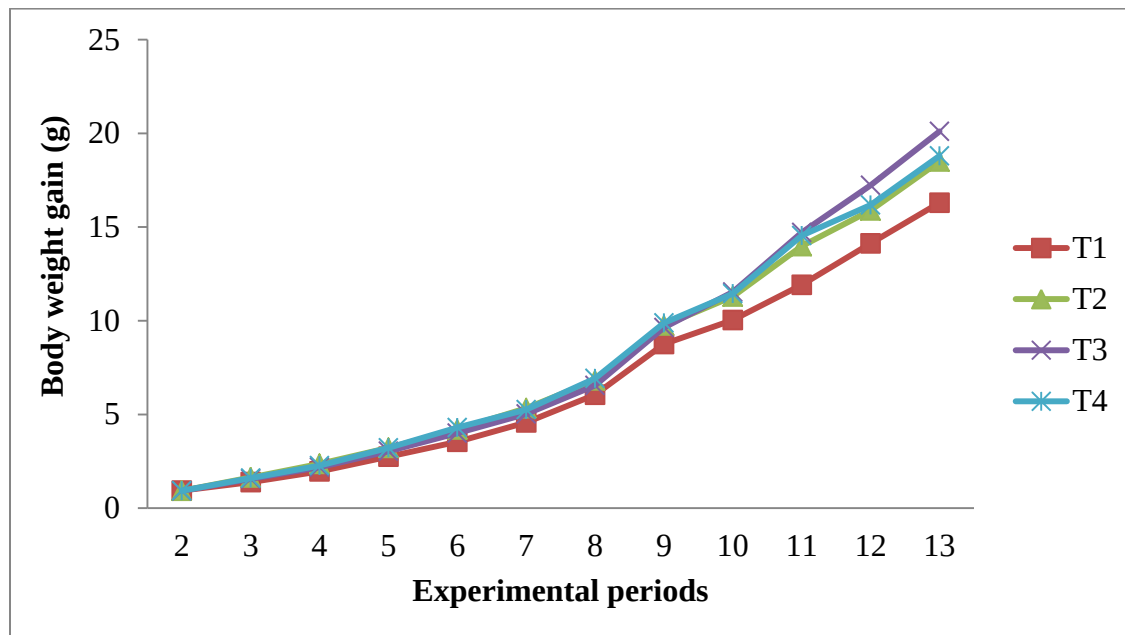


Figure 7.1. Weekly weight gain of koekoek chickens fed diet supplemented with Neem, Bitter-leaf and Garlic powders. T1 = control diet; T2 = 2.5% Neem leaf meal in diet; T3 = 2.5% Bitter-leaf leaf meal in diet; T4 = 2.5% Garlic powder in diet.

7.3.2. Carcass components

There were significant differences in several carcass traits among the treatment groups (Table 7.3). Slaughter weight was significantly higher in T3 and T4, compared to T1. Similarly, dressed weight was lower in T1 than the other treatments ($P < 0.01$). For individual carcass components, breast weight was significantly higher in T3 compared to T1 ($P < 0.01$). Thigh and drumstick weights were significantly greater in T2, T3, and T4 compared to T1 ($P < 0.01$). Back weight was significantly higher in T2 and T4 compared to T1. This suggests that the basal diet in T1 was less effective in promoting gain of different carcass cuts. The improvements observed in T3 and T4 may indicate a potential role of bitter-leaf and garlic in supporting muscle development, possibly through antimicrobial or nutrient enhancing mechanisms. These findings align with those of Hassan *et al.* (2024), who reported that dietary inclusion of thyme, ginger, and their mixture significantly increased carcass weight and breast muscle yield in broilers.

Similarly, Kairalla *et al.* (2022) demonstrated increased breast and thigh muscle weights in broilers fed garlic powder.

The liver weight in T2 was significantly higher ($P < 0.05$) than in T1. This increase may suggest enhanced metabolic activity, possibly influenced by bioactive compounds. No significant differences were observed in wing, skin, neck, heart, gizzard weights, or dressing yield (%) among the treatment groups ($P > 0.05$). This finding contrast with those of Rastad (2020), who reported significant increases in organ weights in broilers supplemented with 1% garlic powder and probiotics. Likewise, Al-Massad *et al.* (2018) observed significant organ weight changes in broilers fed diets containing 2.57 kg/ton of garlic powder.

Table 7.3. Effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal, and Garlic (*Allium sativum*) powder meal on carcass traits of Koekoek chicken

Carcass traits	Treatments				SEM	P-value
	T1	T2	T3	T4		
Slaughter weight (g)	1375 ^b	1580 ^{ab}	1685 ^a	1595 ^a	9.2	0.006
Dressed weight (g)	774.5 ^b	895 ^a	946 ^a	917 ^a	5.2	0.004
Dressing yield (%)	56.33	56.65	56.14	57.49	0.10	0.869
Breast (g)	239.5 ^c	259.5 ^{bc}	307.5 ^a	293 ^{ab}	2.05	0.001
Thigh(g)	123.5 ^b	141.5 ^a	142.5 ^a	144 ^a	0.63	<0.001
Drumstick (g)	127.5 ^b	153 ^a	154 ^a	153.5 ^a	1.43	<0.001
Back (g)	108.5 ^b	134.5 ^a	128 ^{ab}	132.5 ^a	0.89	0.018
Wing (g)	46	56.5	59	56.5	0.44	0.143
Skin (g)	80	92	98	92.5	0.53	0.065
Neck (g)	50	57	62.5	57.5	0.41	0.183
Heart (g)	10.5	9.5	13	11	0.11	0.158
Liver (g)	27.5 ^b	37.5 ^a	35.5 ^{ab}	33.5 ^{ab}	0.65	0.024
Gizzard (g)	45	48.5	40.5	49.5	0.32	0.173

^{a,b} Row means with different superscript letters differ significantly at ($p < 0.05$). SME =standard error of mean; T1= control diet without additives; T2= treatment diet containing neem leaf meal at 2.5%; T3= treatment diet containing grawa leaf meal at 2.5%; T4= treatment diet containing garlic powder meal at 2.5%.

7.3.3. Haematobiochemical parameters

The effects of phytogenic feed additives on hematological and serum biochemical parameters in Koekoek chickens are presented in Table 7.4. Significant differences ($P < 0.05$) were observed in white blood cell (WBC) count, total protein (TP), albumin, creatinine, high-density lipoprotein (HDL), and glucose levels. WBC counts were lower for T1 than other treatments ($P < 0.01$). The elevated WBC counts in Koekoek chickens fed additives suggest enhanced immune status and disease resistance, likely due to the immune-modulatory properties of the phytogenic feed additives. This finding aligns with Eid and Iraqi (2014), who reported increased WBC counts following garlic supplementation. Similar results were reported by Rajput and Arain (2023), who observed elevated WBC counts in broilers fed diets containing *Eucalyptus globulus* leaves. These findings support the idea that the increased WBC counts in birds receiving phytogenic feed additives are likely due to their immune-enhancing properties, which may improve overall health and productivity. In contrast, the current results differ from those of Girma *et al.* (2021), who reported lower WBC counts in chickens fed 1% neem leaf meal. Additionally, Wang *et al.* (2024) highlighted the anti-inflammatory and immune-regulatory roles of plant-derived feed additives. Conversely, Mohiti-Asli and Ghanaatparast-Rashti (2017) found no significant change in WBC counts in broilers supplemented with essential oils from oregano, anise, and citrus peel.

No significant differences were found in red blood cell (RBC), hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) ($P > 0.05$). All values remained within normal physiological ranges, indicating that the dietary treatments did not adversely affect erythropoiesis or oxygen-carrying capacity. MCHC, MCH, and MCV are important markers for diagnosing anemia and evaluating bone marrow function (Aletor and Egberongbe, 1992). The RBC counts observed ($2.3\text{--}2.5 \times 10^6/\text{mm}^3$) fall within the healthy range reported by Ubua *et al.* (2018) and Joshua *et al.* (2022) for broilers ($2.2\text{--}4.0 \times 10^6/\text{mm}^3$), suggesting adequate hemoglobin production and oxygen transport. These findings align with Alagbe (2024), who found no significant changes in haematological profiles of chickens fed *Aristolochia indica* leaf meal. In contrast, Ansari *et al.* (2012) reported significantly increased

PCV, RBC, and HGB levels in broilers supplemented with up to 2.5 g/kg of dried *Azadirachta indica* leaf meal.

Total protein (TP) was higher in T2 than T4. Albumin levels were significantly higher ($P < 0.05$) T2 than T1 and T4. These increases in T2 may indicate enhanced protein metabolism and liver function, possibly due to bioactive compounds that improve nutrient absorption. These findings are consistent with those of Ismail *et al.* (2021), who reported elevated serum protein levels in broilers supplemented with garlic powder, likely due to enhanced gut health and improved amino acid utilization. Creatinine levels differed significantly ($P < 0.01$), with T2 and T4 showing higher values compared to T1. Although all values remained within the normal reference range (0.2-0.64 mg/dL), the increase may indicate higher muscle metabolism or nutrient turnover in the supplemented groups. HDL levels were significantly higher ($P < 0.05$) in T3 than T1. An increase in HDL is generally considered beneficial and may reflect improved lipid metabolism induced by the phytogetic compounds. Glucose levels was in the order of $T4 > T3 > T1$ while the value for T2 was similar with T3 and T4. Although the glucose level in T4 slightly exceeded the reference range (120-220 mg/dL), the elevated concentrations may suggest improved energy availability, possibly resulting from increased feed efficiency or metabolic stimulation due to the phytogetic additives. Talpur and Ikhwanuddin (2013) also reported similar effects with neem (*Azadirachta indica*) leaf meal supplementation at 2 g/kg.

No significant differences ($P > 0.05$) were observed in triglycerides, cholesterol, globulin, uric acid, alkaline phosphatase (ALP), low-density lipoprotein (LDL), or other markers of organ function, indicating that the phytogetic feed additives did not cause any adverse metabolic or renal effects.

Table 7.4. Hematological and Some serum biochemical parameters in Koekoek chickens fed diets containing Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal, and Garlic (*Allium sativum*) powder meal.

Hematology Parameters	Treatment						
	T1	T2	T3	T4	SEM	P-value	Ref. Value
WBC (10 ³ / mm ³)	42.6 ^b	59.9 ^a	53.4 ^a	57 ^a	0.62	0.001	12-30 ¹
RBC (10 ⁶ / mm ³)	2.3	2.5	2.3	2.4	0.02	0.394	2.2- 4.0 ²
HGB (g/dL)	17.3	19.4	18.1	17.6	0.09	0.055	7-13 ¹
PCV (%)	26.3	29.8	27.4	27.1	0.17	0.156	22-35 ¹
MCV (fL)	118	122.2	123.5	124.2	0.38	0.396	90-140 ¹
MCH (pg)	76.2	79.4	81.2	82.1	0.3	0.190	33-47 ¹
MCHC (g/dL)	64.6	65.4	65.8	66.2	0.1	0.476	26-35 ¹
Serum chemistry							
Triglycerides (mg/dl)	106.7	88.3	94.3	81.7	4.9	0.363	45.7-172 ³
Cholesterol (mg/dl)	145	123.3	121.7	111	6.2	0.302	87-192 ³
TP (g/dl)	4 ^{ab}	4.6 ^a	4.2 ^{ab}	3.5 ^b	0.04	0.007	3.5-6 ⁴
Albumin (g/dl)	1.23 ^b	2.1 ^a	1.37 ^{ab}	1 ^b	0.15	0.017	1.2-2.5 ⁴
Globulin (g/dl)	2.77	2.6	2.8	2.47	0.08	0.405	1.5-3.5 ⁴
Uric Acids (mg/dl)	4.7	5.1	5.05	5.95	0.06	0.253	4-12 ⁴
Creatinine (mg/dl)	0.12 ^b	0.44 ^a	0.28 ^{ab}	0.4 ^a	0.01	0.003	0.2-0.6 ⁴
ALP (μ/L)	737	847	898	983	17.4	0.385	200-700 ⁵
LDL (mg/dl)	30	37	48.5	30	1.05	0.238	15-50 ⁵
HDL (mg/dl)	40 ^b	45 ^{ab}	55 ^a	50 ^{ab}	2.08	0.031	30-90 ⁵
Glucose (mg/dl)	211 ^c	248.5 ^{ab}	240 ^b	253.5 ^a	1.45	0.001	120–220 ⁵

^{a-c} means within a row with different superscripts differ ($P < 0.05$); SME = standard error of mean; T1= control diet without additives; T2= treatment diet containing neem leaf meal at 2.5%; T3= treatment diet containing grawa leaf meal at 2.5%; T4= treatment diet containing garlic powder meal at 2.5%.

¹Bounous and Stedman, 2000; ²Ubua *et al.*, 2018 and Joshua *et al.* 2022; ³Meluzzi *et al.*, 1992; ⁴Campbell and Ellis, 2013; ⁵Mitruka and Rawnsley 1997

7.3.4. Cecal bacterial load

The caecal bacterial loads of *E. coli*, *Salmonella*, *Lactobacilli*, and total bacterial counts are presented in Table 7.5. *E. coli* colony counts was higher for T1 than other treatments ($P < 0.05$). This reduction in *E. coli* aligns with the findings of Rashid *et al.* (2019), who reported decreased populations in broilers supplemented with blends of garlic, peppermint, cinnamon, black cumin, and green tea likely due to the antibacterial properties of compounds such as allicin, carvacrol, cinnamaldehyde, cuminaldehyde, and polyphenols.

A similar pattern like that of *E. coli* was observed for *Salmonella*, with counts being higher for T1 than other treatments. These results are consistent with Akter and Asaduzzaman (2023), who found that 2.0% mint leaf supplementation reduced both *Salmonella* and *E. coli*, and with Ali *et al.* (2021), who reported similar reductions using neem leaf meal. The consistent

suppression of these pathogens highlights the broad-spectrum antimicrobial effects of phytogetic feed additives. Conversely, *Lactobacilli* counts was lower for T1 than other treatments ($P<0.05$). These findings correspond with those of Alagawany *et al.* (2021), who noted increased *Lactobacillus* counts and reduced pathogen levels following lemongrass essential oil supplementation. The antimicrobial activity of phytochemicals such as tannins, flavonoids, and phenolic acids is thought to contribute to these effects by disrupting bacterial membranes and inhibiting pathogen growth (Alagawany *et al.*, 2021; Manafi *et al.*, 2016). Additionally, the observed improvements in gut microbiota may be linked to the antioxidant properties of bioactive plant compounds, which enhance the birds' antioxidant status (Rababah *et al.*, 2004). In contrast, no significant differences ($P>0.05$) were observed in total bacterial counts among the dietary treatments. This contrasts with Reis *et al.* (2018), who reported reduced total counts in broilers supplemented with essential oil blends including carvacrol, thymol, and cinnamaldehyde. The lack of change in total counts in the present study suggests that the supplements selectively modulated specific microbial populations without disrupting the overall gut microbial balance and diversity.

Table 7.5. Effect of neem, bitter-leaf leaves meal and garlic powder inclusion on cecal bacterial counts (log cfu/ml) in Koekoek chickens.

Bacterial load	Treatments				SEM	P-value
	T1	T2	T3	T4		
<i>E. coli</i>	3.94 ^a	3.73 ^b	3.74 ^b	3.71 ^b	0.030	<.0001
<i>Salmonella</i>	3.92 ^a	3.73 ^b	3.72 ^b	3.71 ^b	0.034	0.0012
<i>Lactobacillus</i>	3.89 ^b	3.95 ^a	3.99 ^a	3.98 ^a	0.010	0.0017
Total bacterial	3.97	3.82	3.89	3.82	0.020	0.0712

^{a b} means within a row with different superscripts differ ($P<0.05$); SEM = standard error of mean; T1= control diet without additives; T2= treatment diet containing neem leaf meal at 2.5%; T3= treatment diet containing grawa leaf meal at 2.5%; T4= treatment diet containing garlic powder meal at 2.5%.

7.4. Conclusion

This study shows that dietary supplementation with phytogetic feed additives; neem, bitter-leaf, and garlic improve growth performance, carcass traits, and gut health in Koekoek chickens. Chickens receiving bitter-leaf (T3) showed the most significant improvements in body weight gain, feed efficiency, and microbial balance, with higher beneficial *Lactobacillus* counts and reduced *E. coli* and *Salmonella* loads. Enhanced liver weight and total protein levels in supplemented groups also suggest improved protein metabolism. These findings highlight the potential of phytogetic feed additives, especially bitter-leaf, as sustainable alternatives to antibiotics in chicken production. Further studies are recommended to optimize dosages and assess the long-term effects particularly of bitter-leaf (*Vernonia amygdalina*) on growth performance, health, and carcass traits. Furthermore, analysis of the main bioactive compounds in neem, bitter-leaf, and garlic as phytogetic feed additives is warranted.

CHAPTER 8: GENERAL CONCLUSION AND RECOMMENDATIONS

8.1. Conclusion

Many rural and peri-urban farmers are unaware of the benefits of phytogetic feed additives for improving chicken performance and use them mainly for health-related purposes. Although the most preferred additives among farmers were neem, bitter-leaf, and garlic, challenges such as limited knowledge, lack of extension support, and the high cost of some additives were reported. Neem leaf meal significantly improved egg quality parameters including egg length, albumin diameter, and yolk color as well as overall egg production, without causing adverse effects. Garlic supplementation also enhanced feed intake and egg length. In contrast, a 2.5% inclusion of bitter-leaf leaf meal reduced laying performance. Supplementation with neem, bitter-leaf, and garlic improved haematological parameters such as red blood cell count, hemoglobin concentration, and packed cell volume, indicating better blood quality and general health. These additives also reduced harmful bacteria (*E. coli* and *Salmonella*) and increased beneficial *Lactobacillus* counts in the gastrointestinal tract, contributing to improved gut health.

In Koekoek chickens, dietary supplementation with neem, bitter-leaf, and garlic enhanced growth performance, carcass traits, and gut health. Bitter-leaf (T3) produced significantly higher average daily body weight gain (ADBWG), final body weight (FBW), and better feed conversion efficiency (FCR) compared to the control group. All additives demonstrated antimicrobial properties, improved protein metabolism, and supported overall health and production efficiency. In conclusion, neem and garlic improved egg production, feed intake, and haematological parameters in layer hens, while bitter-leaf was the most effective additive for enhancing growth performance and feed efficiency in Koekoek chickens. All three phytogetic additives showed strong antimicrobial effects, highlighting their potential as natural alternatives to antibiotics in poultry production.

8.2. Recommendations

The adoption of phytogenic feed additives should be promoted by incorporating locally available options such as neem, bitter-leaf (girawa), and garlic into chicken feeding programs to enhance health, productivity, and gut microbiota. Farmers should receive practical training on the benefits, proper usage, and appropriate dosages of these additives as natural alternatives to antibiotics. The use of neem leaf meal (T2) should be encouraged to improve egg production, feed intake, and blood health in layer hens, while supplementation with bitter-leaf leaf meal (T3) is recommended to enhance growth performance, carcass traits, and feed efficiency in growing chickens. Further research is needed to optimize dosages and assess the long-term effects of bitter-leaf and other additives on growth, health, and carcass characteristics. Additionally, analyzing the main bioactive compounds in neem, bitter-leaf, and garlic will help clarify their functional roles, and nationwide studies are recommended to assess the availability of phytogenic feed additives.

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APPENDICES

Questionnaire

Section 1: General Information

- 1.1. Date: _____
- 1.2. Region: _____
- 1.3. Zone and District: _____
- 1.4. Kebele Name: _____
- 1.5. Householder's Name: _____
- 1.6. Sex: _____
- 1.7. Religion:
- a) Orthodox c) Protestant
- b) Muslim d) other (please specify): _____
- 1.8. Educational Status (circle one):
- | | |
|-----------------------------------|--------------------------------------|
| 1. Illiterate | 5. Preparatory School (Grades 11–12) |
| 2. Can read and write | 6. Certificate and above |
| 3. Elementary School (Grades 1–8) | 7. Religious education |
| 4. Secondary School (Grades 9–10) | |

Section 2: Household Characteristics

- 2.1. Marital Status:
- | | |
|------------|-------------|
| 1. Married | 3. Widowed |
| 2. Single | 4. Divorced |
- 2.3. Household Size: _____
- Age: _____
- 2.3. Livestock composition:

Species	Total animals (TLU)	Rank based on income generate
Cattle		
Goats		
Sheep		
Donkey		
Chicken		
Horse		
Honey bee colony		
Other (specify)		

- 2.4. How many years of experience do you have in chicken farming?

2.4.1. Chicken Production System (select one):

- a) Extensive c) Intensive
- b) Semi-intensive d) other (specify): _____

- 2.4.2. Which production system do you plan to use in the future?

Why? _____

- 2.4.3. What is your main production objective for keeping chickens?
- For income
 - For home consumption
 - For both income and consumption
 - for religious or cultural purposes
 - other (specify): _____

Section 3: Use and Availability of Feed Additives

3.1. Are feed additive resources available in your area? 1. Yes 2. No

3.2. Sources of feed additives:

- Homegrown
- Purchased (commercial)
- Wild plants
- Other (specify): _____

3.3. Do you currently provide any feed additives to your Chickens? 1. Yes 2. No

3.3.1. If yes, what types of feed additives do you use? _____

3.3.2. What is your purpose for using feed additives?

- To improve production
- To improve quality
- For treatment (medicinal use)
- All of the above
- other (specify) _____

3.4. Do you use commercial feed additives? 1. Yes 2. No

3.4.1. If yes, for what purpose?

- Improve meat and egg yield
- Improve meat and egg quality
- For disease treatment
- other (specify) _____

3.4.2. Which types of antibiotic feed additives do you use?

3.5. Which group of Chickens receives the most feed additives?

- Layers
- Broilers
- Chicks
- other (specify) _____

3.6. What are the specific benefits for these groups? _____

3.7. Have you used phytogetic (plant-based) feed additives? 1. Yes 2. No

3.7.1. If yes, for what purpose?

- To improve egg production
- To improve egg quality
- For treatment
- Multipurpose
- other (specify) _____

3.7.2. If used for treatment, for what types of diseases?

3.7.3. If using natural additives, what is your basis for dosage?

Chicken type	additives type	Quantify per day per Chickens
Layers		
Broilers		
Cockers		
Broody hens		

3.8. List the phytogetic feed additives you use (from most to least important):

S. no.	Additives Type	Priority
1		
2		
3		

3.9. Which part of the plant do you use?

- Leaf
- Seed

- b) Bark
- c) Stem
- d) Flower
- f) Bud
- g) Husk
- h) other (specify): _____

3.10. In what form do you feed the additives?

- a) Powdered
- b) Fresh (as-is)
- c) Other (specify): _____

3.11. How often do you feed additives?

- a) Once per day
- b) Twice per day
- c) Three times per day
- d) other (specify): _____

3.12. Amount used (grams per bird) and method (mixed in feed or water)?

3.13. Besides health recovery, have you noticed other benefits (e.g., weight gain, egg production)?

3.14. When do you mostly use natural additives?

- a) Summer
- b) Winter

Why? _____

3.15. How many phytogetic plant species are available in your area?

3.16. What are the sources of these plants?

3.17. Have you ever purchased natural feed additives? 1. Yes 2. No

3.17.1. If yes, list the type and price:

Name of phytogetic feed additive	Price/g/birr

3.18. Which option is more cost-effective?

- a) Feeding with natural additives
- b) Feeding without additives
- c) Other (specify) _____

3.19. Primary use of phytogetic plants:

- a) As feed additives
- b) For income
- c) For human food
- d) other (specify) _____

3.20. Processing methods used:

- a) Grinding
- b) Roasting
- c) Soaking
- d) other (specify) _____

3.21. Do you have prior knowledge of phytogetic feed additives?

- a) Yes, aware they can be used in chicken feed
- b) Yes, aware they can replace antibiotics
- c) No, not aware

3.22. In your opinion, are PFAs a good alternative to antibiotics?

- A. strongly agree
- B. Agree
- D. Disagree
- E. Strongly disagree

C. Neutral

3.23. Do you believe PFAs can be used to prevent diseases in chickens?

1. Yes 2. No

3.24. If you don't use available phytogenic additives, why not?

3.25. What special characteristics do phytogenic additives have for Chicken?

3.26. What are the main challenges in using phytogenic additives?

3.27. Do you require training or support on phytogenic additives? 1. Yes 2. No

3.28. If yes, which areas? a) How to use phytogenic additives b) Proper feeding levels c) Other (specify): _____

3.29. General comments on the use of phytogenic additives in your area:

Appendix Tables

Table A 1. ANOVA results regarding effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on layer hens' production performances

Statistics	IBW	FBW	HDEP	HHEP	EM	FCR	FI	MP	AEW
DF	3	3	3	3	3	3	3	3	3
Sum of Squares	0.002	0.052	305.543	224.955	130.852	0.369	273.343	29.3	2.918
Mean Square	0.001	0.017	101.848	74.985	43.617	0.123	91.114	9.766	0.973
Tukey HSD (0.05)	0.87	0.678	0.498	0.228	0.462	0.941	0.980	0.903	0.589
F-value	0.236	0.493	4.156	1.957	5.173	4.102	15.513	0.231	0.579
P-value	0.87	0.694	0.031	0.174	0.016	0.32	0.0001	0.873	0.64

DF: degree of freedom, IBW: Initial Body Weight, FBW: Final Body Weight, HDEP: hen day egg production, HHEP: Hen housed egg production, EM: Egg mass, FCR: Feed conversion ratio, FI: Feed intake, MP: mortality percentage, AEW: Average egg weight

Table A 2. ANOVA results regarding effect of neem, giraw and garlic on egg quality parameters

Statistics	DF	Sum of Squares	Mean Square	Tukey HSD (0.05)	F-value	P-value
egg weight	3	48.004	16.001	0.101	2.899	0.079
egg length	3	13.131	4.377	0.071	6.502	0.007
egg width	3	1.952	0.651	0.487	1.074	0.397
egg shell wt	3	0.888	0.296	0.087	2.799	0.085
shell thickness	3	0.014	0.005	0.167	2.165	0.145
Abumen heights	3	2.196	0.732	0.311	1.121	0.379
albumen diameter	3	132.212	44.071	0.258	3.767	0.041
Yolk Weights	3	2.267	0.756	0.277	1.788	0.203
Yolk heights	3	1.462	0.487	0.217	1.987	0.17
Yolk diameter	3	0.994	0.331	0.666	0.601	0.626
Yolk color	3	20.25	6.75	0.254	23.143	0.000
Hough unit	3	43.697	14.566	0.443	0.819	0.508
Yolk index	3	10.828	3.609	0.190	1.88	0.187
Albumin weight	3	25.281	8.427	0.203	1.868	0.189

DF: degree of freedom

Table A 3. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the haematological parameters of Layer Chickens

Statistics	DF	Sum of Squares	Mean Square	Tukey HSD (0.05)	F-value	P-value
LYM %	3	760.992	253.664	0.160	2.113	0.152
RBC 10 ¹² /L	3	3.805	1.268	0.122	6.901	0.006
HGB(g/dl)	3	46.562	15.521	0.328	10.143	0.001
MCHC g/dl	3	16.427	5.476	0.355	1.029	0.414
MCH pg	3	99.052	33.017	0.266	1.363	0.301
MCV (fL)	3	245.147	81.716	0.146	2.38	0.121
HCT %	3	687.806	229.269	0.155	8.681	0.002
TG mg/dL	3	10124.55	3374.85	0.317	1.265	0.33
TCHO mg/dL	3	9864.837	3288.279	0.319	1.513	0.261
HDL mg/dL	3	966.695	322.232	0.117	2.358	0.123
LDL mg/DL	3	0.005	0.002	0.846	0.271	0.845
Albumin g/L	3	1.084	0.361	0.867	0.198	0.895
Total protein g/L	3	4.483	1.494	0.427	1.098	0.388
Globulin g/L	3	1.542	0.514	0.880	0.314	0.815

Table A 4. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the Total Bacterial Load in Different Gut Section of Layer Chickens

statistics	Crop	Gizzard	S. intestine	Ceca	feces at cloca
DF	3	3	3	3	3
Sum of Squares	9.916	5.917	28.861	34.978	22.172
Mean Square	3.305	1.972	9.62	11.659	7.391
Tukey HSD (0.05)	0.880	0.187	0.126	0.840	0.548
F-value	9.346	9.758	19.368	17.923	33.143
P-value	0.002	0.002	0.000	0.000	0.000

DF: degree of freedom; S. intestine: Small intestine

Table A 5. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the growth performances of Koekoek chickens

Statistics	IBW g/chicken	FBW g/chicken	ABWG g/day/chicken	FI g/day/chicken	FCR	Mortality
DF	3	3	3	3	3	3
Sum of squares	8.25	198246	30.272	100.457	0.657	82.465
Mean square	2.75	66082	10.091	33.486	0.219	27.488
Tukey HSD (0.05)	0.898	0.388	0.372	0.172	0.736	0.174
F Value	0.167	5.065	5.269	2.122	3.794	1.727
P Value	0.917	0.017	0.015	0.151	0.04	0.214

Table A 6. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the heamatological parametes of koekoek chickens

Statistics	WBC	RBC	HGB	PCV	MCV	MCH	MCHC
Df	3	3	3	3	3	3	3
Sum of Squares	516.037	0.137	8.136	20.271	68.655	61.146	4.268
Mean Square	172.012	0.046	2.712	6.757	22.885	20.382	1.423
Tukey HSD (0.05)	0.141	0.353	0.053	0.136	0.398	0.183	0.444
F-value	16.518	1.129	3.905	2.279	1.121	2.02	0.914
P-value	0.001	0.394	0.055	0.156	0.396	0.19	0.476

Table A 7. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the serum chemistry parameters of koekoek chickens

Statistics	ALP	TP	Cr	LDL	Glu	UA	Chlo	Trig	HDL	Alb	Glob
Df	3	3	3	3	3	3	3	3	3	3	3
Sum of Squares	144944.3	2.196	0.19	686.063	3249.75	2.535	1828.917	1016.469	375	0.23	2.029
Mean Square	48314.75	0.732	0.063	228.688	1083.25	0.845	609.639	338.823	125	0.077	0.676
Tukey HSD (0.05)	0.367	0.096	0.102	0.274	0.357	0.219	0.256	0.324	0.144	0.458	0.095
F-value	1.153	8.674	11.269	1.732	89.34	1.653	1.439	1.221	5	1.095	6.341
P-value	0.385	0.007	0.003	0.238	0	0.253	0.302	0.363	0.031	0.405	0.017

Table A 8. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the carcass characteristics of koekoek chickens

Statistics	SW	DW	DY	B	TH	DS	Ba	Wi	Sk	Ne	He	Li	Gz
DF	3	3	3	3	3	3	3	3	3	3	3	3	3
Sum of squares	20587 5	68164.8	2.91 5	11522. 8	1114.8	6350	1698.8	301. 5	518.06	237. 8	19.5	168	149.0 6
Mean square	68625	22721.6	0.97 2	3841	371.6	2116.7	566.3	100. 5	172.69	79.3	6.5	56	49.69
Tukey HSD (0.05)													
F Value	6.923	7.59	0.23 5	10.01	11.45	17.151	4.99	2.4	3.61	2.07	2.26 1	5.46	2.14
P Value	0.006	0.004	0.86 9	0.001	<0.001	<0.001	0.018	0.14 3	0.065	0.18 3	0.15 8	0.02 4	0.173

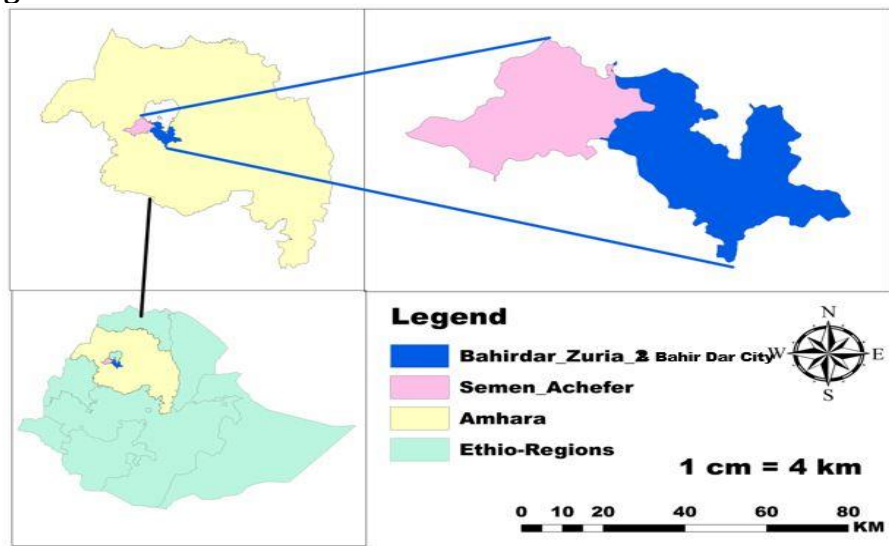
DF: degree of freedom; Sw: slaughter weight; DW: dressed weight; DY: dressing yield; B: breast; TH: thigh; DS: drumstick, Ba: back; Wi: wing; sk: skin; Ne: neck; He: Heart; Li: liver; Gz: Gizzard

Table A 9. ANOVA results for the effect of Neem (*Azadirachta indica*), Bitter-leaf (*Vernonia amygdalina*) Leaves Meal and Garlic (*Allium sativum*) on the cecal bacterial load of koekoek chickens

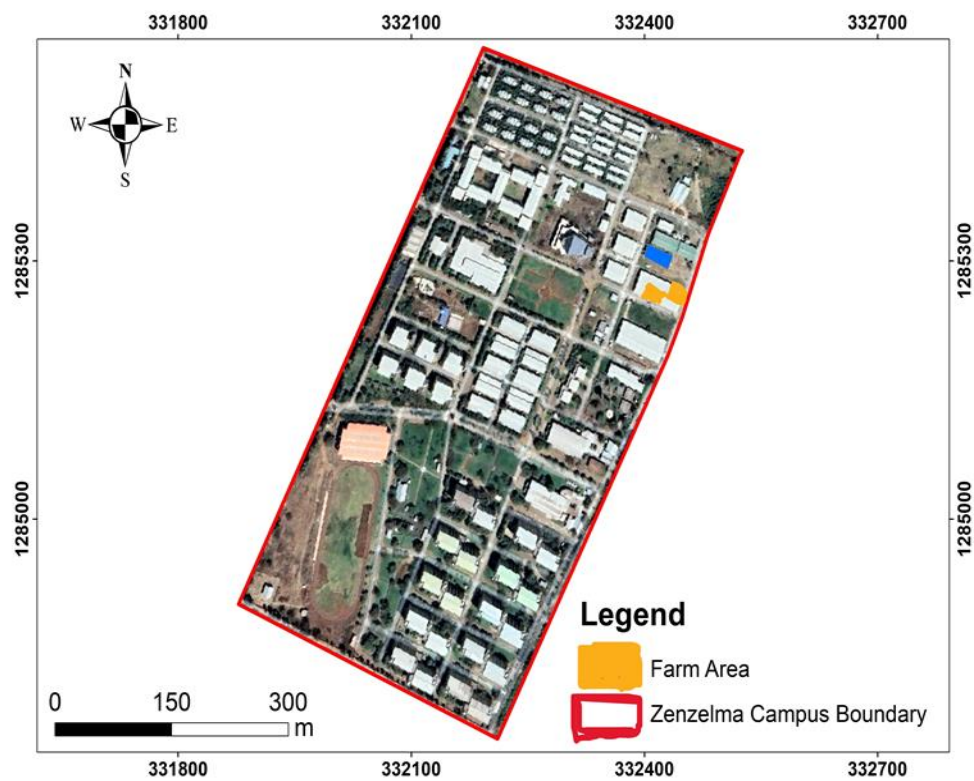
Statistics	Lactobacillus	<i>E. coli</i>	Salmonella	Total Bacteria
DF	3	3	3	3
Sum of Squares	0.025	0.136	0.116	0.058
Mean Square	0.008	0.045	0.039	0.019
Tukey HSD (0.05)	0.445	0.823	0.091	0.936
F-value	9.525	22.345	10.419	3.028
P-value	0.0017	<.0001	0.0012	0.0712

DF: degree of freedom

Appendix Figures



Appendix Figures 1: Map of study areas for survey work



Appendix Figures 2. Map of experimental site



Appendix Figures 3. Photos taken during interviews, farm observation and focus group discussion with chicken producers



Appendix Figures 4. Preparation of experimental pens



Appendix Figures 5. Measured egg quality parameters



Appendix Figures 6: Collection of Blood Samples and analysis



Appendix Figures 7. Measured the body weight of Keokeok broiler chickens



Appendix Figures 8. Measurement of carcass traits of Keokeok fed phytogenic feed additives



Solution
preparations



Solution mixed with samples



Diluted samples were seeded



samples
were incubated



Cultured bacteria



Bacterial colony count

Appendix Figures 9. Identifying and counting of gut bacterial load of layer and broiler chickens fed selected phytogenic feed additives

BIOGRAPHICAL SKETCH

Minchle Yigrem Gedamu was born on May 29, 1994 in Bahir Dar Zuria District, Amhara Region, Ethiopia. He completed his elementary education at Yemoshit Elementary School and his secondary education at Tiss Abay Fuafuatie high School. He then attended his preparatory education at Ghion Preparatory School at Bahir Dar city in June 2014. He joined in Debre Markos University in September 2014. He graduated with a B.Sc. degree in Animal Science in July 2016. Following his graduation, he took and passed the lecturer qualifying exam administered by the Ministry of Education at Debre Markos University in 2016. He then joined once again the School of Graduate Studies (SGS) at Debre Markos University in 2016 to pursue his MSc degree. He graduated with a M.Sc. degree in Animal Nutrition and Feed Processing in September 2018. After completing his M.Sc., he worked as a Lecturer at Salale University for one year. He was then transferred to Debark University in August 2019 and served as a Lecturer. In December 2020, he started his PhD. study and published 2 articles in peer-reviewed journals and the other two are under review. He also presented his works in 2 international conferences. Now he is a candidate for the Degree of Doctor of Philosophy (PhD) in Animal Nutrition at the College of Agriculture and Environmental Sciences, Bahir Dar University.