



**EVALUATING THE ALTERNATIVE USE OF PHYTOGENICS AS FEED
ADDITIVES: EFFECTS ON GROWTH PERFORMANCE AND IMMUNE
RESPONSE IN NEWCASTLE DISEASE-VACCINATED BROILER CHICKENS**

**DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY
HEALTH
MASTER OF SCIENCE IN POULTRY HEALTH AND MANAGEMENT**

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**BY
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” has been submitted by **ROBEL GIRMA** for presentation with my approval as academic advisor.

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ACRONYMS AND ABBREVIATIONS

ADWG	Average daily weight gain
AGPs	Antibiotic growth promoters
ANOVA	Analysis of Variance
BW	Body weight
BWG	Body weight gain
CVMA	College of Veterinary Medicine and Agriculture
ELISA	Enzyme-linked immunosorbent assay
FCR	Feed conversion ratio
FI	Feed intake
g	gram
IB	Infectious Bronchitis
IBD	Infectious bursal disease
ID.vet	Innovative diagnostic veterinary
IgA	Immunoglobulin A
kg	Kilogram
LGO	Lemongrass oil
ND	Newcastle Disease
NVI	National Veterinary Institute
PFA	PFA
PLC	Private limited company
SEM	Standard Error of the Mean
USDA	United States Department of Agriculture

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ABSTRACT

Background: Phytogetic feed additives (PFAs) a group of medicinal plants and their essential oils—are increasingly used in poultry sector due to their biologically active compounds that promote animal health, enhance immune function, stimulate growth, boost productivity and reduce mortality. This study aimed to evaluate the effects of dietary PFAs on the growth performance and immunological response of broiler chickens to Newcastle disease (ND).

Methods: Experimental study was conducted on a total of 200 Cobb 500 chicks. The chicks were randomly assigned to six groups, with each group comprising three replicates of 10 chicks. To establish baseline immunological data, 20 chicks were bled before the experiment. One treatment group (G4) received only basal diet, while the remaining groups (G1, G2, G3, G5, and G6) were supplemented with 2% of specific phytoGENICS: thyme (*Thymus vulgaris*, G1 and G6), lemongrass (*Cymbopogon schoenanthus*, G2), and rosemary (*Rosmarinus officinalis*, G3). Additionally, G5 received a commercially available vitamin supplement commonly used by medium-scale poultry farms. Throughout the 42-day study period, mortality, feed conversion ratio (FCR), body weight gain (BWG), and feed intake were recorded. Serological tests were conducted on three representative chickens per replication to assess antibody responses by ELISA test.

Results: The findings of this study demonstrated that the inclusion of PFAs to the diet had no significant effect on overall feed intake. Body weight (BW), BWG, and average daily weight gain (ADWG) did not differ significantly among groups during the starter and grower stages. However, by day 42, G2 and G5 exhibited significantly greater final BW, overall BWG, and ADWG ($p < 0.01$) compared to the other groups. Over the study duration, G5 chickens achieved the best FCR, followed by G2 and G4. Serological results showed that on day 42 of the trial, the G3 and G5 chickens had a higher antibody titer against the ND vaccination. These findings suggested that adding 2% of this PFA leaf powder to broiler diets had no detrimental effects on feed consumption, while lemongrass inclusion enhances growth performance (BW and ADWG) and FCR similarly to commercially available vitamins. However, the inclusion of these dietary PFAs did not

significantly influence immune organs development. Rosemary inclusion into the diet and supplementing vitamins improved the immune status of broiler chickens against ND.

Conclusion: The selected herbs, particularly lemongrass and rosemary, may be used as effective natural alternatives to synthetic supplements by improving growth performance and immune response in broiler chickens. Further research is needed to fully elucidate their potential as substitutes for antibiotic growth promoters in commercial poultry production.

Key words; broilers; immune response; growth performance; PFAs

1. INTRODUCTION

1.1 Background

In the meat-producing agricultural sector, poultry farming is a rapidly expanding business due to the need for protein sources to feed the world's expanding population. The producers are working to create innovative animal production techniques in order to meet the demand for food derived from animals (Ramesh *et al.*, 2024).

Due to their beneficial effects on both the health of the birds and production performance, poultry farmers have been using antibiotic growth promoters (AGPs) extensively for the past few decades (Jing *et al.*, 2024). Long-term use of antibiotics to boost chicken growth resulted in decreased levels of good endogenous bacteria, the emergence of drug-resistant pathogenic microorganisms, and the buildup of antibiotic residues in the environment and animal products, endangering the health of consumers (Youcef *et al.*, 2018). These circumstances compelled the global prohibition of the use of AGPs as feed additives for animals raised for food and spurred the hunt for safer substitute materials (Feiyang *et al.*, 2021).

Increased feed prices, the ban on using AGPs, and public awareness of food safety presented challenges for the global animal feed sector. As a result, poultry nutritionists become interested in natural feed additives as a substitute for AGPs and natural ingredients (Burel, 2012). PFAs (PFAs) are among the many natural feed additives available for chicken feed that are extensively supported and accepted by consumers. PFAs are naturally occurring heterogeneous groups that are suitable non-antibiotic growth promoters, less toxic, and residue-free. They are obtained from plants, PFAs, fruit, spices, and their essential oil, and are employed as feed additives in the production of meat animals (Murugesan *et al.*, 2015).

Several researchers have reported that adding phytogenics and their extract to chickens can increase their appetite, improve nutrient digestibility, boost growth, improve

gastrointestinal morphology and physiological functions, have antioxidant qualities, help treat specific diseases, and have immune-modulating effects (Abdelli *et al.*, 2021). Additionally, the active components in PFAs boost the performance of chickens by promoting the production of digestive enzymes, which improves feed digestion and nutrient absorption and raises the feed conversion ratio in poultry (Alloui *et al.*, 2014).

PFA addition in animal feed has recently been shown by several researchers to improve the immune response and protect the gastrointestinal system from outside stimuli. Because of their beneficial effects on controlling the immunological response of broiler chickens to certain viral vaccinations, their phytochemicals are regarded as the most promising feed additives (Biswas *et al.*, 2024).

Boosting hens' diets for optimal use and boosting their health particularly with feed derived from phytonics are two recommended ways to increase their output. Poultry nutritionists have recently become interested in the PFAs, a relatively new class of poultry feed additives (Reham *et al.*, 2018). In order to improve the immune response against illness and to better prevent disease challenges, phytonics are useful in disease prevention and control techniques. Phytonic essential oil supplementation greatly increases the viral antibody titer in broiler chickens. In order to encourage the use of PFAs as a substitute source to enhance chicken production performance, it is required to broaden the perspective on them (Mohamed *et al.*, 2022).

1.2 Research Problems Statement

The use of phytonic in poultry production is expected to continue growing, as evidenced by a recent study by Engida *et al.* (2023) on the effect of PFAs on broilers' growth performance and immunity against infectious bursal disease (IBD) (Gumboro disease) indicated selected phytonic in Cobb 500 broiler chicks with 1% inclusion rate of the diet indicated that supplementation of lemongrass as well as thyme leaf powder as PFAs improved the growth of the chickens. Feeding peppermint, lemongrass, and thyme as PFAs in the broilers' diet improved feed conversion ratio (FCR), while

supplementation of rosemary and thyme developed the highest antibody titer against the IBD vaccine.

The appealing effect of supplementing broilers with PFAs was linked to improved growth and weight gain performance as well as increased immunological response, maybe as a result of its immune-stimulant qualities to lower the likelihood of vaccine failures (Shah *et al.*, 2021). These plants have potential health benefits and may be useful ingredients in the production of pure substances. For broiler chickens, they could be developed as substitute feed additives (Leitzmann, 2016). But in Ethiopia, little research has been done on their impacts (Engida *et al.*, 2023).

Therefore, this study further investigated the effects of selected phytogetic additives namely lemongrass, thyme, and rosemary leaves based on the performance parameters and immunological response achieved by Engida *et al.* (2023) and incorporated at an optimized inclusion rate of 2% in the diet focusing on their influence on growth performance parameters (including weight gain, average daily gain, and feed conversion ratio), as well as on the development of immune organs and the enhancement of immune response to the Newcastle disease (ND) virus. Additionally, this study compared the impact of these PFAs with that of commercially available vitamin supplements commonly used by medium-scale commercial farmers, addressing a research gap not explored by Engida *et al.* (2023).

1.3 Objectives

1.3.1. General objective

- To assess the impact of selected phytogetic plants—lemongrass, rosemary and thyme—at optimized inclusion ratios in poultry diet as alternative feed additives in enhancing growth performance, immune organ development, and immune response, compared to the commercially used commercial vitamin supplements by medium-scale poultry farmers.

1.3.2 Specific objectives

- To assess the effects of selected phytochemicals (lemongrass, rosemary and thyme) on growth performance (weight gain, average daily gain and feed conversion ratio) of broiler chicks.
- To evaluate the effects of selected phytochemicals (lemongrass, rosemary and thyme) on immune organ development and immune response against Newcastle disease vaccinated broiler chicks.

2. LITRATURE REVIEW

2.1 Phytogenic Feed Additive

PFAs (PFAs) are emerging as crucial components in livestock nutrition, enhancing feed quality, animal health, and the overall quality of animal products. These additives can be categorized into four main types: Zootechnical, which bolster immunity and growth; Technological, which preserve feed and reduce toxins; Sensory, which improve flavor and aroma; and Nutritional, which supply essential nutrients such as vitamins and minerals (Karásková *et al.*, 2015). Often, phytogenic additives exhibit multiple benefits, positioning them as versatile alternatives to conventional feed components. The significance of PFAs has surged, particularly after the European Union's restrictions on antibiotic growth promoters (AGPs). An ideal alternative to AGPs should replicate their positive effects; optimize animal performance while enhancing nutrient availability (Windisch *et al.*, 2008).

Natural herbal and spice-derived products are particularly promising, as they can significantly improve digestion, nutrient absorption, and overall health, while also reducing disease incidence. To be effective, these alternatives must be safe for both animals and humans, easy to handle and store, and cost-efficient. Dry powder formulations of phytogenics, for instance, are preferred for their practicality, including low inclusion rates and extended shelf life (Lillehoj *et al.*, 2018).

2.2 Mode of Action of PFAs

Medicinal plants play a significant role in modern medicine, particularly in developing new drugs that enhance animal health. Among these, PFAs (PFAs) are gaining attention for their various benefits when incorporated into poultry diets. These natural additives are known for their antimicrobial, antioxidative, anticoccidial, and immunogenic properties, contributing to improved overall health and productivity in birds (Madhupriya *et al.*, 2018).

PFAs not only enhance the palatability of feed but also protect it from oxidative damage, thereby improving nutrient digestibility and absorption. They support gut health by reducing harmful bacterial loads and minimizing the production of detrimental fermentation byproducts such as ammonia. Additionally, certain phytochemical compounds may stimulate the production of intestinal mucus, further promoting a healthy gut environment. The effectiveness of PFAs is attributed to their composition, which includes both primary nutrients like carbohydrates, fats, and proteins, and secondary metabolites that have antibiotic and antioxidant properties (Madhupriya *et al.*, 2018).

Phytochemical substances (PFAs) are also gaining attention for their potential antimicrobial properties, though the exact mechanisms underlying these effects remain unclear (Windisch *et al.*, 2008). Research suggests several ways PFAs may exert their beneficial actions against pathogens. These mechanisms include disrupting the cellular membranes of harmful microorganisms, altering the surface characteristics of these cells to reduce their virulence, and enhancing immune responses, particularly through the activation of various immune cells. Key proposals regarding PFAs' antimicrobial action highlight their role in protecting intestinal mucosa from colonization by harmful bacteria while simultaneously encouraging the growth of beneficial microorganisms like lactobacilli and bifidobacteria (Windisch *et al.*, 2008; Bhavsar *et al.*, 2022). This dual action could play a vital role in maintaining gut health and enhancing the overall immune response. The stimulation of immune cells such as lymphocytes, macrophages, and natural killer cells further emphasizes the potential of PFAs in supporting the body's defense mechanisms against infections (Windisch *et al.*, 2008; Bhavsar *et al.*, 2022).

2.3 PFAs as Performance Enhancer in Chicken

The utilization of PFAs in poultry farming has been shown to significantly enhance the growth performance of chickens. These additives improve feed utilization and digestion, positively affect the microbiota in the gastrointestinal tract, and enhance various performance metrics such as feed intake, feed conversion ratios, and carcass quality (Karásková *et al.*, 2015). Additionally, PFAs offer immunomodulatory and antioxidant benefits, creating a holistic improvement in poultry health and productivity. In developed

nations, the demand for poultry meat, particularly broiler chicken, is rising due to its favorable nutritional profile and affordability. Research has demonstrated that a variety of phytogetic additives can stimulate pancreatic and intestinal enzyme secretion, which in turn boosts the production of bile and lipase (Fascina *et al.*, 2012). This biochemical enhancement leads to better nutrient absorption, thereby optimizing feed efficiency and overall growth in chickens (Kralik *et al.*, 2013).

Lemongrass (*Cymbopogon citratus*) is increasingly recognized as a beneficial feed additive for poultry, particularly in enhancing production performance. Its rich composition of flavonoids, phenolic compounds, terpenoids, and essential oils, such as citral and geraniol, contributes to its various health benefits (Shah *et al.*, 2011). These components are linked to biological activities that include antibacterial, antifungal, and antioxidant properties, making lemongrass a promising substitute for traditional antibiotics in broiler diets (Cross *et al.*, 2007).

Research highlights the effectiveness of lemongrass oil in promoting growth among broiler chickens. Studies have demonstrated that incorporating lemongrass into poultry diets can lead to increases in feed intake and body weight gain, outperforming control groups not receiving the additive. This not only supports the overall health of the birds but also reduces feed costs, contributing positively to the economic aspects of poultry production. The potential of lemongrass as a natural growth promoter has been explored in various studies, solidifying its role as a valuable dietary supplement (Mohammed and Hassen, 2023).

Phytogenics, particularly rosemary (*Rosmarinus officinalis*), have emerged as vital growth promoters and alternatives to antibiotics in animal husbandry over recent decades. Their complex blend of bioactive components shows promise in enhancing both production and health in livestock, especially poultry. Rosemary's antimicrobial and antioxidant properties are primarily attributed to its phenolic terpenes, rosmarole, and rosmarinic acid, which contribute to improved oxidative stability in poultry meat and exhibit antimicrobial effects that can benefit animal health (Windisch *et al.*, 2008). These additives also improve gut microbiota by decreasing harmful pathogens while promoting

beneficial bacteria, which boosts immunity and resistance to digestive diseases. Studies have shown that the incorporation of rosemary in poultry diets can lead to significant improvements in nutrition, production performance, and overall health (Bhavsar *et al.*, 2022).

Thyme, along with other herbs, plays a significant role in enhancing poultry health and performance through the inclusion of essential oils and plant extracts in their diets. Thyme essential oils contribute to improving digestibility, fostering a balanced gut microbiome, and stimulating the production of digestive enzymes, thus positively impacting growth in poultry. Additionally, thyme exhibits antibacterial, anticoccidial, and antifungal properties, which collectively promote the overall health of broiler chickens. These findings underscore the importance of PFAs in achieving optimal poultry growth and health (Cross *et al.*, 2007).

2.4 Immune Boosting and Anti-Inflammatory Effects of Phytonics

Phytonic oils, also known as plant-derived essential oils, play a significant role in enhancing the immune system of birds. These oils stimulate the production of immunoglobulins, boost lymphocyte activity, and increase the release of interferon- γ , which are crucial components of the avian immune response (Cross *et al.*, 2007). Studies have shown that incorporating PFAs (PFAs) into poultry diets can lead to notable improvements in immune function, particularly through the support of the intestinal lymphatic system (Abdel-Wareth and Lohakare, 2014).

Key findings demonstrate that specific oils, such as thyme and ginger, can enhance the immunological profiles of chickens by elevating antibody levels, particularly immunoglobulin A (IgA). For instance, the inclusion of thyme oil in poultry feed has been linked to significantly increased IgA levels (Krishan and Narang, 2014). Moreover, the active compounds in PFAs are recognized for their anti-inflammatory properties, which help reduce inflammation and alleviate pain by inhibiting the production of inflammatory prostaglandins.

Recent research has focused on the application of plant-derived essential oils, particularly PFAs, alongside vaccination efforts for various poultry diseases, including infectious bronchitis, Newcastle disease, avian influenza, and Gumboro disease. These studies aim to evaluate how essential oils can potentially bolster the immune response in vaccinated birds, thereby improving overall vaccine effectiveness (Barbour *et al.*, 2011; Faramarzi *et al.*, 2013).

Findings from these studies indicate that the incorporation of essential oils significantly enhances both cell-mediated and humoral immune responses. This dual activation of the immune system may lead to better protection against the targeted diseases, suggesting that essential oils could serve as a valuable adjunct to conventional vaccination strategies. The work of researchers such as Barbour *et al.*, (2011) and Faramarzi *et al.*, (2013) highlights the potential benefits of integrating these natural compounds into poultry health management.

2.5 Effects of Phytochemicals on Feed Digestibility

Studies indicate that the incorporation of PFAs (PFAs) like thyme, rosemary, cinnamon, and sage in broiler diets can significantly enhance nutrient digestibility. Studies also shown improvements in both dry matter and starch digestibility, as well as total tract dry matter and crude protein retention (Hernández *et al.*, 2004). Notably, blends of essential oils from sources such as oregano and citrus have been associated with better fat digestibility in broilers, suggesting that PFAs play a crucial role in optimizing nutrient absorption (Mountzouris *et al.*, 2010).

The mechanisms behind these benefits are linked to the stimulation of digestive secretions and increased enzyme activities, which include trypsin, amylase, and lipase (Williams and Losa, 2001; Jang *et al.*, 2002). PFAs also promote the secretion of intestinal mucus, aiding pathogen resistance and maintaining a balanced gut microbiome. Additionally, dietary inclusion of these additives can lead to structural improvements in the gut, such as increased villus height and crypt depth, which are indicators of enhanced gut health and nutrient absorption efficiency (Jamroz *et al.*, 2006).

2.6 Phylogenics of interest as poultry feed additives

The increasing reliance on synthetic and semi-synthetic antibiotics as growth promoters in poultry has raised concerns about their negative impacts, prompting a shift towards natural alternatives (Lavinia *et al.*, 2009). Recent studies suggest that herb and herbal products can serve as effective replacements due to their inherent antimicrobial, anti-inflammatory, and growth-enhancing properties. This transition highlights the importance of exploring natural solutions to improve poultry health and production efficiency (Lavinia *et al.*, 2009).

Key benefits of incorporating herbs and spices into poultry diets include enhanced digestion, improved immune function, and overall better health outcomes for broilers. Active compounds in these natural products can stimulate endogenous enzymes, leading to improved nutrient absorption (Ghazalah and Ali, 2008). Additionally, these herbs possess antioxidative and antimicrobial properties that contribute positively to the birds' health and performance. The use of these natural alternatives not only supports growth but also addresses concerns related to antibiotic residues in poultry products (Izunobi, 2002).

The global rise in awareness regarding drug-resistant microbes and the adverse effects of antimicrobial agents in animal products has prompted a search for alternative approaches to maintain gut health and enhance immunity in livestock (Oni *et al.*, 2024). This situation has led to the exploration of new feed options that can replace or complement traditional antibiotics, particularly in poultry farming. One promising avenue is the incorporation of beneficial herbs into poultry diets, which can serve as a natural substitute for antibiotic growth promoters (Oni *et al.*, 2024).

Studies indicate that various common herbs, such as lemongrass, rosemary, and thyme, can significantly improve the health and productivity of broiler chickens. These herbs not only enhance the flavor of the feed, thereby increasing consumption and growth rates, but they also stimulate the secretion of digestive enzymes and improve gastrointestinal motility. Additionally, these herbs possess antimicrobial, antiviral, and anti-parasitic

properties, along with benefits that support immune function, reduce inflammation, and offer antioxidant effects (Cengiz *et al.*, 2016).

2.6.1 Lemongrass (*Cymbopogon citratus*)

Lemongrass, or 'teji sar' in Amharic, is a perennial grass from the Poaceae family, commonly found across Africa, Europe, and Asia. This tall, aromatic grass is recognized for its diverse phytochemical components, including steroids, alkaloids, and flavonoids. These compounds contribute to its various health benefits, such as antibacterial, antifungal, and antioxidant properties. Lemongrass oil is particularly notable for its medicinal uses, including treatments for intestinal worms and anemia, as well as applications in the production of pesticides, perfumes, and cosmetics (Shah *et al.*, 2011). Lemongrass is increasingly recognized for its health benefits and medicinal properties, particularly in the poultry industry. It is often used as a feed additive to enhance growth performance in broilers, offering a natural alternative to antibiotics (Peter and Babu, 2012). Research highlights its effectiveness in promoting growth and reducing feed costs, making it an appealing option for poultry producers looking to improve efficiency and animal health (Mukhtar *et al.*, 2013).

Studies have demonstrated the positive impact of lemongrass and its secondary metabolites on broiler performance. These natural compounds not only stimulate growth but also provide essential nutrients, such as vitamin C, and possess antifungal properties that help control pathogens (Smith, 2002). Furthermore, lemongrass extracts have been linked to improved cholesterol levels and overall antioxidant status, contributing to better health outcomes in poultry. Trials indicate that broilers receiving lemongrass oil (LGO) in their diets showed significantly higher feed intake and body weight gain compared to control groups, underscoring its potential as an effective dietary supplement (Mukhtar *et al.*, 2013).

2.6.2 Rosemary (*Rosmarinus officinalis*)

Rosemary, referred to as 'yetbis kitel' in Amharic, is a significant aromatic plant in Ethiopia, valued for its culinary and medicinal applications. Belonging to the Lamiaceae family, this herb is rich in antioxidant compounds, including phenolic acids and flavonoids, making it a popular choice in food processing. The essential oil derived from rosemary leaves contains key active ingredients such as camphor, alpha-pinene, and cineole, which contribute to its various health benefits (Ghazalah and Ali, 2008).

The beneficial properties of rosemary extend beyond culinary uses; it serves as a natural feed additive in animal nutrition due to its antimicrobial, antioxidant, antiviral, anti-inflammatory, and anti-carcinogenic effects (Al-Kassie *et al.*, 2008; Genena *et al.*, 2008; Al-Kassie *et al.*, 2011; Abo Ghanima *et al.*, 2020). Research indicates that incorporating rosemary into poultry diets can delay rancidity in products and enhance overall feed conversion ratios (Al-Kassie, 2008), nutrient digestibility, and immunological performance. Additionally, it positively influences carcass quality and improves critical hematological and biochemical parameters in broiler chickens (ELnaggar *et al.*, 2016).

Research indicates that incorporating rosemary into the diets of chickens can lead to a notable decrease in bacterial counts in both the small and large intestines (Al-Kassie *et al.*, 2011). Specifically, a 1% rosemary supplementation has been linked to enhanced microbiological health, potentially increasing the presence of beneficial bacteria while reducing harmful pathogens. This change is believed to bolster the birds' immune systems, enhance their resistance to gut diseases, and improve nutrient digestibility, thereby promoting overall health and productivity (Attia, 2018).

Further studies reveal that even lower concentrations, such as 0.25% rosemary leaf meal, can yield significant benefits in poultry production. This inclusion not only enhances the digestibility of protein and minerals but also acts as a natural antioxidant and immune booster (ELnaggar *et al.*, 2016; Attia, 2018). Additionally, higher inclusion levels of rosemary have been shown to positively affect the weights of key lymphoid organs,

which play critical roles in immune function, thereby supporting better growth and health outcomes in broilers (Yesilbag *et al.*, 2014).

2.6.3 Thyme (*Thymus vulgaris*)

Thyme, or 'tosinyi' in Amharic, is a notable medicinal plant from the Lamiaceae family, recognized for its low-growing shrub characteristics and widespread use in the poultry industry as a natural growth promoter. This plant serves as an effective alternative to synthetic additives, boosting poultry health and productivity globally (Alcicek *et al.*, 2004). Research indicates that thyme contains key bioactive compounds, primarily thymol and carvacrol, which are known for their potent antioxidant, antibacterial, and antiseptic properties (Mitsch *et al.*, 2004).

The health benefits of thyme extend significantly to poultry, with numerous studies highlighting its anticoccidial, antifungal, and antibacterial effects. These properties stem from thyme's phenolic compounds, which not only inhibit microbial growth but also promote better overall health in birds (Cao *et al.*, 2010). Additionally, thyme essential oils have been shown to enhance broiler growth rates, improve feed digestibility, and positively influence intestinal microflora, leading to increased nutrient absorption and utilization in poultry diets (Cross *et al.*, 2007; Brenes and Roura, 2010).

Thyme essential oil has been recognized for its significant advantages in broiler diets, particularly regarding nutrient utilization and overall poultry health (Gumus *et al.*, 2017). Research indicates that incorporating thyme essential oil at a concentration of 100 mg/kg can lead to notable improvements in body weight gain, feed conversion ratios, and overall livability in broilers (Mitsch *et al.*, 2004). Additionally, it enhances appetite and feed intake while promoting the secretion of digestive enzymes and strengthening the immune system, ultimately boosting production profitability (Zhu *et al.*, 2014).

Key findings highlight thyme's capacity to improve growth rates and optimize performance metrics in poultry. Studies show that administering thyme extract in drinking water can lead to better slaughter weights and a healthier gut microbiome,

evidenced by reduced levels of harmful bacteria and increased beneficial lactic acid bacteria. Beyond its nutritional benefits, thyme exhibits various medicinal properties, including pain relief and digestive support, as well as antiseptic qualities that contribute to overall poultry health (Zhu *et al.*, 2014).

3. MATERIALS AND METHODS

3.1 Study Area Description

The experimental study was conducted at a rented private poultry house in Bishoftu city, Ethiopia, using unsexed broiler chicks from March to May 2025. To evaluate the immune enhancing effects of the selected phytonics, antibody titer determination was carried out at the National Veterinary Institute (NVI).

Bishoftu city, located in the East Shewa Zone of the Oromia Region, lies in the central high lands of Ethiopia, approximately 47 km Southeast of Addis Ababa. The city is positioned at 8^o45'N latitude and 38^o59'E longitude, at an altitude of 1,920 meters above sea level. It receives an average annual rainfall of 1150 mm, with 84% during the main rainy season (June to September) and the remaining during the short rainy season (March to May). The mean annual minimum and maximum temperatures are 12.3^oC and 28^oC, respectively, with a mean relative humidity is 61.3% (CSA, 2018).

3.2 Study Design and Sampling Methods

3.2.1 Preparation of experimental herb powders

The experimental PFAs included lemongrass (*Cymbopogon schoenanthus*), rosemary (*Rosemarinus officinalis*), and thyme (*Thymus vulgaris*) green leaves were purchased from Selale region from local farmers, Bishoftu local market and Debersina highland irrigation farm, respectively. Specimens were transported to Addis Ababa University (AAU), College of Natural Science herbarium for future reference, and taxonomically identified and authenticated by a botanist with voucher specimen numbers TB001, TB002, and TB003, respectively.

Preparation: after washing the PFAs, the leaves were separated from the stem, laid out on a plastic sheet, and left to dry at room temperature. The leaves were made into powder in the pharmacology lab of the college of veterinary medicine and agriculture, Addis Ababa

University. Before being used, the dried leaves were ground into a powder and kept in plastic containers.

3.2.2 Experimental setup and animal management

For this experimental trial, a day-old 200 birds (including 20 for baseline maternal antibody study) were purchased and grouped into 6 groups of three replicates (10 chicks per pen; 10*3*6).

A total of 200 day-old broiler chicks of Cobb-500 breed were purchased from Alema farms, Bishoftu (Ethiopia) and birds were vaccinated with VECTOR-IMMUNE ND (HVT Vector vaccine with F protein of ND) for Mareks and Newcastle disease (ND), NOVAMUNE (immune- complex live virus coated with antibody) for IBD, VITABRONE for live Infectious bronchitis Mass type and IBIRD for live Infectious bronchitis Variant type all four vaccines are from (Ceva, France) at the hatchery on day 1. Blood collection was performed from 20 randomly selected chicks on day one of their age directly from their heart and transferred it into the non-heparinized tubes (Legese *et al.*, 2022). The remaining chicks (n = 180) were weighed and randomly assigned into six different treatment groups with three replicates based on a completely random design. Chicks were reared in a wire-meshed wood partitioned deep litter floor housing system (appendix 8.3 showing house preparation and chick placement (1.20 m × 1 m)) for 42 days of the experimental period. Electric power and charcoal stove used as a source of heat and light according to the recommendation of the broiler management guide (Anonymous, 2018).

All experimental chickens, except those in group 6, were vaccinated against ND. Throughout the study, the chicks were fed commercial broiler feed in three phases: starter (days 1-15), grower (days 16-30), and finisher (days 31-42), following the recommendation of the feed supplier (Alema Koudijs Feed PLC, Ethiopia). The control group were fed only commercial broiler feed (basal diet), while the five treatment groups were fed a basal diet plus 2% of one of the four PFAs prepared in powdered form. Additionally, one treatment group was raised under conditions commonly used by

medium-scale farmers, incorporating synthetic feed additives, namely “Neobro,” which contains multivitamins (widely used by small scale farmers for boosting growth and immunity of broiler chickens, manufactured by Medion, Indonesian company). The prepared treatment herbs were manually and homogeneously mixed with the broiler diet (Nielsen, 2010), and all diets were provided in mash form. Water and weighed feed were provided ad libitum to the chicks throughout the experiment. Standard biosecurity protocols were strictly followed throughout the experiment (USDA, 2014; SAPA, 2022). The experimental setup is presented as follows:

1. Group-1: Commercial feed + thyme leaf powder (2% ration) + vaccinated
2. Group-2: Commercial feed + lemongrass leaf powder (2% ration) + vaccinated
3. Group-3: Commercial feed + rosemary leaf powder (2% ration) + vaccinated
4. Group-4: Commercial feed (without any supplements) + vaccinated
5. Group-5: Commercial feed + other supplements (vitamins and amino acids) + vaccinated (conventional production-mostly practiced by farms)
6. Group-6: Commercial feed + thyme leaf powder (2% ration) + unvaccinated

3.2.3 Assessing immune enhancement and production performance of selected phytonics (lemongrass, rosemary and thyme)

Production performance evaluation

The growth performance of broiler chicken fed with selected PFAs has been assessed. A recent study on the effects of PFAs on broilers' body weight gain reported superior performance of the selected phytonics (Engida *et al.*, 2023). Therefore, this study further investigates the optimal inclusion ratios of these PFAs in the same breed (broilers) diets, assessing their impact on weight gain, average daily gain and feed conversion ratio.

Immune organ development and immune enhancing activity evaluation

Evaluation of immune organ development and immune enhancing activity of PFAs inclusion in broiler chicken feed has also been done. A recent study on the effects of PFAs on broiler immunity against IBD reported superior performance of the selected phytonics (Engida *et al.*, 2023). Hence, this study further investigates optimal inclusion

ratios of these feed additives in broiler diets, assessing their impact on the immune organ development and immune response of chicks vaccinated with Newcastle disease (ND).

3.3 Data Collection

3.3.1 Feed intakes

Feed intake (FI) was calculated as the difference between feed offered and feed leftover for each replication for 42 days (Engida *et al.*, 2023).

3.3.2 Body weight, body weight gain and average daily weight gain

At the beginning of the experiment, all chicks were weighed, followed by weekly measurements and assessments at the end of each growth phase (starter, grower, and finisher) to determine the body weight (BW) in relation to respective treatment groups. Body weight gain (BWG) was determined by subtracting the initial weight from each successive BW for each replication during the experimental time. Average daily weight gain (ADWG) was calculated by dividing the total BWG by the number of days the chicken stayed on the farm (42 days) (Engida *et al.*, 2023).

3.3.2 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the ratio of total consumed feed (gm) to total BW gain (gm) (Al-Beitawi and El-Ghousein, 2010). At the same time, cumulative FI was computed at the end of the experiment.

3.3.3 Immune response measurement and immune organ development

Blood collection was performed on 20 randomly selected chicks on day 0 (D0) before vaccination to obtain baseline immune data (Legese *et al.*, 2022). All chickens, except those in Group 6 (G6), were vaccinated against Newcastle disease (ND) following the recommended dose and schedule of the product on days 12 and 24 (CEVAC@New L, France). To assess post-vaccination antibody titer, 3 ml of blood samples were collected from the wing vein of three 3 randomly selected chicks per group at 42 days of age using a disposable 3 mL syringe with a 22-gauge needle. The collected blood sample was kept

undisturbed to clot for 30 minutes, then centrifuged at 1500 rpm for 10 minutes to separate the serum. The harvested serum samples were transferred into labeled cryovials and stored at -20°C until serological analysis was conducted at the National Veterinary Institute (NVI, Ethiopia).

The indirect ELISA test (ID screen[®] ND indirect, ID.vet, France) was performed according to the manufacturer's instructions to quantify ND-specific antibodies in chicken serum. The antibody titer was computed as log₁₀ values following the instruction provided by the manufacturer of the kit (Tamir *et al.*, 2017). The test was considered valid if the mean optical density (OD) value of the positive control exceeded 0.3 and the ratio of the mean OD values for positive and negative controls was greater than 3 (Tamir *et al.*, 2017). At 42 days, five chickens from each group were randomly selected, and live weight, spleen weight, thymus weight and bursa of fabricius weight were recorded to compute the absolute weight and relative weights of immune organs (Abd El-Latif *et al.*, 2013).

2.4 Data Analysis

The raw data were stored in Microsoft Excel for data management. All data pertaining to measured parameters were analyzed using the R tools (R project, 2020). Group (treatment) means were compared by one-way ANOVA. Significant differences among the treatments' effects were separately analyzed with Tukey multiple comparison test. Significance mean differences between treatments were considered at $p < 0.05$.

2.5. Ethical Approval

All procedures related to animal handling and their routine manipulations were carried out according to animal care guidelines and protocols approved by the institutional animal ethics committee of the College of Veterinary Medicine and Agriculture (VM/ERC/04/71/17/2025) (Appendix 8.5: attached ethical clearance certificate).

4. RESULTS

4.1 Feed Intake

The findings showed that feed intake (FI) remained consistent across all six groups throughout the growth phases. During the starter phase, G3 had the lowest FI, while G5 had the highest; however, the differences were not statistically significant. In the grower phase, FI ranged from 93.57 g (G1) and 102.13 g (G5), and in the finisher phase, G6 exhibited the highest FI while G4 had the lowest-yet again the differences were not statistically significant (Table 1).

Overall, total feed intake over the entire growth period and across all phases showed no statistically significant difference among groups (p-values all > 0.05) (Table 1). These findings indicate that the inclusion of PFA did not influence FI in broiler chickens.

Table 1. The effect of inclusion of treatment PFAs on feed intake of broiler chickens during the starter, grower, and finisher phases

Group		Feed intake of the treatment group (g/bird)						SEM	P-value
Parameter									
Growth phase	Age (days)	G1	G2	G3	G4	G5	G6		
Starter	1-15	32.8	34.49	32.35	33.86	35.8	34.85	1.43	0.9747
Grower	11-30	93.57	99.57	100.01	95.23	102.13	101.65	2.323	0.868
Finisher	31-42	157.04	161.93	153.82	147.91	156.41	163.30	2.251	0.406
Overall	1-42	89.71	94.14	91.22	88.36	93.95	95.41	3.315	0.980

SEM: Standard Error of Mean, g: Gram, G1: Commercial feed +2%thyme + vaccinated, G2: Commercial feed +2%Lemmon grass + vaccinated, G3: Commercial feed +2%Rosemary + vaccinated, G4: Commercial feed +no supplement + vaccinated, G5: Commercial feed +with supplement + vaccinated, G6: Commercial feed +2%thyme +unvaccinated

4.2 Evaluation of Effect of Phytogetic Feed Additives on Body Weight Gain Performance

The study evaluated the impact of different PFA in groups (G1–G6) on the body weight (BW), body weight gain (BWG) and average daily weight gain (ADWG) of broiler chickens across three growth phases—starter, grower, and finisher—with different feed types (Table 2).

At the beginning of the experiment, initial body weights across all groups were similar, ranging from 40 to 43.33 g, with no significant differences observed ($p = 0.224$). This indicates that all groups started with comparable initial body weights. During the starter phase, BW increased in all groups, with G4 exhibiting the highest BWG and ADWG. However, these differences were not statistically significant (p -values > 0.05) (Table 2), suggesting that the PFA had no measurable effect on growth during the starter phase.

Similarly, in the grower phase, BW continued to increase, G2 and G5 showing relatively higher BWG and ADWG. However, the differences remained statistically insignificant ($p > 0.05$) (Table 2), suggesting that PFAs did not significantly influence growth in the grower phase too.

Significant differences emerged during the finisher phase. Final body weights ranging from lower BW observed in G3 and highest in G5. Both BWG and ADWG were highest in G5, with statistically significant differences among groups for BW ($p = 0.000$), BWG ($p = 0.014$), and ADWG ($p = 0.014$) (Table 2). These results indicate that the PFAs used in G5 notably improved growth performance during the finisher phase.

Considering the entire growth period, G5 showed the highest overall WG and ADWG, followed by G2 and G4, with significant differences compared to other groups ($p = 0.000$). This suggests that the PFA treatment in G5 had a positive effect on the total growth performance of broiler chickens, followed by G2.

Table 2. Effect of phytochemicals inclusion into broilers diet on body weight gain and average daily weight gain during starter, grower, and finisher phases

Growth phase	Measured parameter	Weight of the experimental group in gram						SEM	P-value
		G1	G2	G3	G4	G5	G6		
Starter Phase	IBW	40	43.33	40	41.11	40	40.55	0.50	0.224
	BW	293.33	310.66	318.66	349.66	324	306	6.57	0.213
	BWG	253.33	264.67	278.67	308.27	284	265.5	6.56	0.222
Grower phase	ADWG	16.89	17.84	18.58	20.55	18.93	17.70	0.43	0.221
	BW	981	1086.66	990	1074.33	1085.66	989	16.4	0.127
	BWG	687.7	776.01	671.34	724.67	761.67	683	16.2	0.301
Finisher Phase	ADWG	52.9	59.69	51.64	55.74	58.59	52.54	1.24	0.301
	BW	2027.14 _b	2190.23 ^a	1899.04 ^c	2110 ^{ab}	2245.23 ^a	2086.42 ^b	22.2	0.000
	BWG	1046.14 _b	1103.58 ^{ab}	909.05 ^c	1035.67 ^b	1159.58 ^a	1094.43 ^{ab}	21.1	0.014
Overall	ADWG	78.83 ^{ab}	78.83 ^{ab}	64.93 ^c	73.98 ^b	82.83 ^a	78.39 ^{ab}	1.50	0.014
	BWG	1987.14 _b	2147.21 ^a	1859.04 ^c	2068.61 ^{ab}	2205.2 ^a	2045.91 ^b	22.2	0.000
	ADWG	47.31 ^c	51.12 ^a	44.26 ^d	49.25 ^b	52.51 ^a	48.71 ^b	0.52	0.000

IBW: Initial body weight, BWG: Body weight gain, BW: Body weight, ADWG: Average daily weight gain SEM: Standard Error of Mean, g: Gram, G1: Commercial feed +2%thyme + vaccinated, G2: Commercial feed +2%Lemmon grass + vaccinated, G3: Commercial feed +2%Rosemary + vaccinated, G4: Commercial feed +no supplement + vaccinated, G5: Commercial feed +with supplement + vaccinated, G6: Commercial feed +2%thyme +un vaccinated

4.3 Evaluation of Effects of Phytochemicals on Feed Conversion Ratio

The feed conversion ratio (FCR) was measured across three different feed types and growth phases: starter (days 1–15), grower (days 11–30), finisher (days 31–42), and overall (days 1–42) (Table 3). Six treatment groups (G1 to G6) received different PFAs.

During the starter phase, no significant differences on FCR were observed among the groups ($p = 0.269$), indicating similar feed conversion efficiency (FCE). In the grower phase, FCR values varied from 1.76 (G2) to 2.05 (G6), with no significant differences among groups ($p = 0.573$), suggesting consistent FCE across treatments.

In the finisher phase, FCR differences among groups were statistically significant ($p = 0.024$), suggesting that the PFA treatment in G5 may have improved FCE compared to other groups. The overall FCR varied from 1.83 (G5) to 2.12 (G3), with significant differences ($p = 0.000$), indicating that certain PFA treatments resulted in better FCE over the entire growth period.

While no significant differences were observed in the starter and grower phases, the inclusion of PFA had a significant effect on the FCR during the finisher phase and overall growth period. These results suggest that specific PFA can enhance FCE in broiler chickens, particularly in the later stages of growth.

Table 3. Effect of inclusion of selected phytochemicals into broiler diet on a feed conversion ratio during the starter, grower, and finisher phases

Group		Feed conversion ratio of the treatment group						SEM	P-value
Growth phase	Age (days)	G1	G2	G3	G4	G5	G6		
Starter	1-15	2.00	1.97	1.85	1.71	2.02	2.15	0.055	0.269
Grower	11-30	1.81	1.76	1.96	1.79	1.79	2.05	0.053	0.573
Finisher	31-42	2.22 ^{ab}	2.07 ^{bc}	2.50 ^a	2.07 ^{bc}	1.91 ^c	2.17 ^{ab}	0.050	0.024
Overall	1-42	1.96 ^{ab}	1.88 ^b	2.12 ^a	1.84 ^b	1.83 ^b	2.018 ^a	0.022	0.000

SEM: Standard Error of Mean, g: Gram, G1: Commercial feed +2%thyme + vaccinated, G2: Commercial feed +2%Lemmon grass + vaccinated, G3: Commercial feed +2%Rosemary + vaccinated, G4: Commercial feed +no supplement + vaccinated, G5: Commercial feed +with supplement + vaccinated, G6: Commercial feed +2%thyme +un vaccinated.

4.4 Effect of Phytochemicals Inclusion into Broiler Diet on Immune Response

4.4.1. Immune organ development

This study assessed the effects of selected PFA on the development of immune organs (Table 4), considering both absolute weight and relative weight in relation to the live body weight of the broiler chickens. Initially, no significant differences in live body weights were observed among the treatment groups.

Additionally, there were no significant changes recorded in the absolute weight or relative weight of the spleen (%). The weight of the spleen varied from 1.98 g (G1) and 2.54 g (G4), while its percentage relative to body weights varied between 0.085% (G2) and

0.108% (G6). However, absolute thymus weights showed statistically significant differences among groups ($p = 0.013$), with G2 (7.12 g) and G5 (7.54 g) exhibiting notably higher thymus weights. Despite this, relative thymus weight (%) did not differ significantly ($p = 0.156$), suggesting that its relative weight was influenced by variations in chicken's live body weight. In contrast, the development of bursa of Fabricius showed no significant differences in either absolute weight or relative weight (%) among the groups ($p = 0.845$ and $p = 0.727$, respectively) (Table 4).

Overall, the inclusion of different PFA did not significantly affect live body weight or the development of the spleen and bursa of Fabricius. However, the observed increase in thymus weight in some treatment groups (G2 [lemongrass] and G4 [conventional vitamin supplement]) was significantly increased, suggesting a potential immunomodulatory effect of certain PFA on thymus development in broiler chickens.

Table 4. The effect of inclusion of selected phytochemicals into broilers diet on immune organ development

Group Parameter	Immune organ development between treatment groups (LW, AW in gram and relative weight in %)						SEM	P-value
	G1	G2	G3	G4	G5	G6		
Immune Organs								
Live weight (g)	2220	2410	2340	2410	2564	2170	41.519	0.885
Spleen	1.98	2.04	2.24	2.54	2.30	2.38	0.104	0.704
Thymus	5.33 ^c	7.12 ^a	6.80 ^{ab}	5.62 ^c	7.54 ^a	6.60 ^b	0.200	0.013
Bursa	1.02	1.00	1.06	1.10	1.26	1.26	0.071	0.845
Spleen %	0.089	0.085	0.096	0.106	0.090	0.108	0.004	0.679
Thymus %	0.253	0.299	0.290	0.236	0.296	0.307	0.181	0.156
Bursa %	0.045	0.041	0.045	0.046	0.049	0.058	0.029	0.727

LW, Live weight of chickens in grams; AW, Absolute weight in grams, relative weight in (%) SEM: Standard Error of Mean, g: Gram, G1: Commercial feed +2%thyme + vaccinated, G2: Commercial feed +2%Lemmon grass + vaccinated, G3: Commercial feed +2%Rosemary + vaccinated, G4: Commercial feed +no supplement + vaccinated, G5: Commercial feed +with supplement + vaccinated, G6: Commercial feed +2%thyme +un vaccinated

4.4.2. Antibody titer

The current study evaluated the impact of experimental phytogetic herbs on the immunity of broiler chickens using a suggested ND vaccination protocol. A serological test using antibody titers from representative chicken serum samples was used to identify the experimental herb that exhibited an immunological response to the ND vaccination during the trial. Figure 1 shows how the experimental herbs affected the ND (log₁₀) immune responses. The day-old chicks' serum samples had an average ND antibody titer of (log₁₀) 3.95.

Chickens fed different treatments had statistically significant differences in mean antibody titers ($p < 0.05$), according to the study. Significant variations in antibody titers across the groups were noted at D42 ($p = 0.006$). The titers varied from 2.536 (G6) to 4.138 (G5), with the vitamin-supplemented G5 group exhibiting the highest titer, suggesting a more robust immune response than the other groups. This was followed by the 3.386 (G3) group using rosemary leaf as their food source.

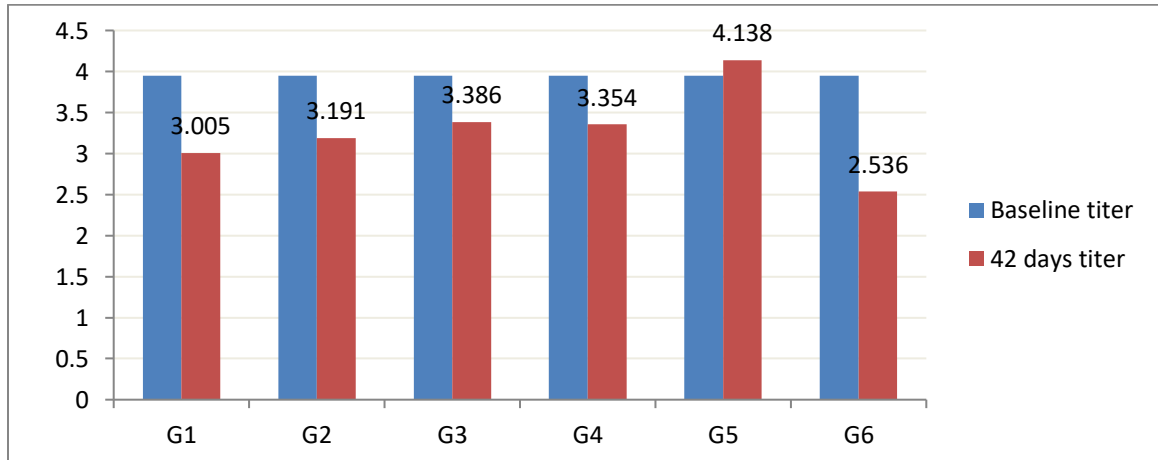


Figure 1. Mean antibody titer (Geometric mean titer) of broiler chickens treated with different herbs against ND vaccine during day 0 and 42.

G1: Commercial feed + 2%thyme + vaccinated, G2: Commercial feed + 2%Lemmon grass + vaccinated, G3: Commercial feed + 2%Rosemary + vaccinated, G4: Commercial feed + no supplement + vaccinated, G5: Commercial feed +with supplement (“Neobro” vitamin) + vaccinated, G6: Commercial feed +2%thyme + unvaccinated

Table 5. The effect of treatment PFAs on Mean Antibody titer of broiler chickens vaccinated with NDV

Group Parameters	Mean antibody titer of treatment groups against ND vaccine						SEM	P- value
	G1	G2	G3	G4	G5	G6		
Age (Days)								
D 0	3.95	3.95	3.95	3.95	3.95	3.95	-	-
D 42	3.005 ^{bc}	3.191 ^{bc}	3.386 ^{ab}	3.354 ^{ab}	4.138 ^a	2.536 ^c	0.124	0.006

D0, baseline (day 0); D42, Day 42 (at the end of the experiment); G1: Commercial feed +2%thyme + vaccinated, G2: Commercial feed +2%Lemmon grass + vaccinated, G3: Commercial feed +2%Rosemary + vaccinated, G4: Commercial feed +no supplement + vaccinated, G5: Commercial feed +with supplement + vaccinated, G6: Commercial feed +2%thyme +un vaccinated

5. DISCUSSION

5.1 Feed Intake

The results of the present study indicate that the addition of PFAs did not significantly affect overall feed intake across all groups, as evidenced by the lack of statistically significant differences. These findings are consistent with several previous studies that reported no effect of herbal supplementation on the broiler chicks' feed intake (Sangani *et al.*, 2014; Soltani *et al.*, 2016; Criste *et al.*, 2017; Hasan and Awad, 2017; Ri *et al.*, 2017). Furthermore, the current study supports the findings of Engida *et al.* (2023), which showed no significant difference in feed intake on selected PFAs (Lemongrass, rosemary and thyme) at a 1% inclusion rate compared to the control group. Similarly, earlier research (Alcicek *et al.*, 2003; Golestan, 2010; Mountzouris *et al.*, 2011) has reported that PFAs supplementation does not influence the feed intake.

On the other hand, the findings of this study contradict earlier research indicating that broiler chickens fed these herbs had higher feed intake (Ghazalah and Ali, 2008; Ampode and Mendoza, 2022; Sugiharto and Ayasan, 2023). Similarly, studies by Windisch *et al.* (2008) and Alhadj *et al.* (2017) reported that PFAs reduced feed intake while maintaining largely unchanged BWG or final body weight, thereby improving FCR. Additionally, this result contrasts with the findings of Fallah and Mirzaei (2016), which showed that thyme supplementation at 5g/kg feed increased feed intake in broilers. Likewise, Shariatmadari and Ahmadi (2025) observed that broiler chickens' feed intake was increased with 5g/kg rosemary powder inclusion compared to the control group. This discrepancy may stem from differences in inclusion rates, as the current study utilized a 2% inclusion level, whereas studies reporting increase feed intake often used slightly lower inclusion levels. The observed difference could also be attributed to the antibacterial and antioxidative properties of bioactive compounds in these herbs, which improve feed palatability, stimulate appetite, promote digestive enzymes secretion, and facilitate intestinal absorption in broilers (Windisch *et al.*, 2008; Yang *et al.*, 2015).

5.2 Body Weight Gain and Average Daily Weight Gain

The present study demonstrated that, by the end of the study period, no statistically significant difference were observed on BW, BWG, and ADWG of broiler chickens that had received PFAs in the starter and grower phase, whereas there was a significant difference in BW, BWG, and ADWG in the finisher phase which indicate the inclusion of phyto-genic feed additive have a significant effect on the growth of the broiler chickens in the late stage of the growth phase. The current results contradict Engida *et al.* (2023) on the starter phase, not only on the group of PFAs that provide higher performance in the broiler chicks, but also on the fact that there was no significant difference in BW, BWG, and ADWG. Engida *et al.* (2023) reported that during the initial phase, lemongrass and thyme provided better growth performance compared to the other group while the current study found that broiler chicks supplemented with vitamins and rosemary provided the best performance. On the other hand, these results align with Engida *et al.* (2023) on the grower phase in which PFAs that provide better performance in the broiler chicks. According to Engida *et al.* (2023), lemongrass provides higher growth performance compared to the other group, whereas the rosemary group achieved lower growth performance.

In the final phase, these results are consistent with Engida *et al.* (2023), who found a significant difference between groups, with higher BW, BWG, and ADWG in the groups fed lemongrass and thyme and lower BW, BWG, and ADWG in the groups fed rosemary leaf powder. Additionally, even though the growth parameters were higher in the lemongrass and thyme group compared to the non-supplemented and rosemary group, they were still significantly lower than those of the G5 broiler chicks supplemented with vitamins.

In addition to the above comparison with Engida *et al.* (2023) in the contrary, to the reports, which suggest dietary thyme, lemongrass and rosemary as feed additives to either increase or decrease performance parameters, there are also other studies, indicating no statistically significant difference to have occurred in the body weights and daily body

weight gain of broiler chickens given of thyme (Saleh *et al.*, 2018) and rosemary (Abd El-Latif *et al.*, 2013). Similarly, other reports have also indicated no effect to have been achieved with adding rosemary as feed additives on body weight (Franciosini *et al.*, 2016), daily body weight gain (Franciosini *et al.*, 2016; Norouzi *et al.*, 2015) in broiler chickens in the late phase of growth stage.

This result was in the line of (Tiwari *et al.*, 2018), where he noted that chicks fed on diet with supplemented lemongrass recorded significantly body weight gain and average body weight gain in the last phase of the growth of the broiler chickens, This improvement is may be attributed to the increase in total feed consumption, the active compounds, antimicrobial and antioxidant activities of the lemongrass.

PFA's are useful for improving digestion because they help keep the microbiota in the intestines in balance and encourage the release of internal digestive enzymes, which improves poultry growth in general (Winiarska-Mieczan *et al.*, 2023; Yilmaz and Gul, 2024). Also, according to Vlaicu *et al.* (2023), phytochemicals contains active ingredients that boost the activity of digestive enzymes in chicken, improving overall performance. Previous studies by Raya *et al.* (2014) suggested that increased BWG and better nutrient digestibility may be connected to the improved FCR.

Phytochemical plants have been demonstrated to improve poultry performance. Most of its advantageous properties are ascribed to the chemicals found in their essential oil. These substances have a variety of interactions with the immunological and digestive systems of the chickens. These compounds can increase the secretion of digestive enzymes, which helps break down nutrients (Baptiste *et al.*, 2020).

5.3 Feed Conversion Ratio

According to the current study, broiler chickens that received PFA's during the starter and grower phases did not exhibit a statistically significant difference in feed conversion ratio. However, there was a significant difference in the FCR during the finisher and

overall phase, indicating that the addition of PFAs had a significant impact on the broiler chickens' growth during the later stages of the growth phase.

As compared to the findings of Engida *et al.* (2023), the PFAs that provide better FCR differ in the starter phase, where better FCR was observed in the lemongrass and thyme group in Engida *et al.* (2023), while the rosemary group provides higher FCR in the current study, even though there was no statistical difference between groups at the starter and grower phase. Additionally, in the grower and overall phase, both studies demonstrate a better FCR for the group fed on lemongrass; however, in the final phase, both studies demonstrate a statistically significant difference, with the current study showing a better FCR for the lemongrass and vitamin-supplemented groups, while Engida *et al.* (2023) found no statistically significant difference between the selected PFAs for the current study. Similarly, Mukhtar *et al.* (2012) reported that supplementation of 0.5%, 1% and 1.5% lemongrass is significantly improved in feed conversion ratio for broilers compared with control group in the overall growth phase.

The present results obtained are contrary to the finding of Sariozkan *et al.* (2016) who reported the feed conversion ratio were not affected by lemongrass supplementation. Thayalini *et al.* (2011) also reported that supplementation of 2% lemongrass leaf to diet did not affect the feed conversion ratio in broilers. Similarly, supplementation of 1.5% and 3% lemongrass leaf did not affect the feed conversion ratio of quail; whereas Mmereole (2010) reported that 1% lemongrass leaf supplementation to broiler diet improved the feed conversion ratio.

The FCR was not improved with rosemary group in broiler chickens, Abd El-Latif *et al.* (2013), who found that feed consumption and feed conversion ratio were increased in rosemary groups at 100 and 200 mg inclusion in the diet which wasn't observed in the current study. They have found out that a difference is formed between groups as to feed consumption in the study of Al-Kassie *et al.* (2011), where they added rosemary to broiler rations and the highest live weight gain with 1% rosemary and 0.5% rosemary groups. In the same way they have determine the conversion rate of feeds were improved.

In contrary, Franciosini *et al.* (2016) study with broiler rations they determined that 2 gr/kg rosemary additives do not have any effect on body weight and feed conversion ratio. In addition, this study agrees with Jakubowska & Karamucki, (2021) who used the thyme flowers as growth promoter in diets of Japanese quails. They found that the level of thyme flowers (1 g/kg of diet) improved FCR.

Khaksar *et al.* (2012) explained that the increases in live body weights of Japanese quail were significantly positive after using thyme. They noted also that the feed consumption decreased while feed conversion ratio was enhanced as a result of including this phytogetic feed additive in the basic diet. Moreover, Dehghani *et al.* (2018) observed that adding 400 ppm of thyme essential oils in quail's diet has a significant reduction in feed consumption and the same time, feed conversion ratio significantly enhanced ($P < 0.05$) in the same treatment. However, some other authors have not noticed the positive or negative impact of these essential oils on poultry production performance (Hoffman-Pennesi & Wu, 2010; Mehdipour *et al.*, 2014 and Montazeri *et al.*, 2014). Lee *et al.* (2003) further, indicated that the used of 200 ppm thymol in the diet did not affect the body weight gain, feed intake and feed efficiency of female broilers.

PFAs play an effective role as enhancers for digestion, maintaining the balance of the microbial ecosystem of the intestine and also stimulating the secretion of internal digestive enzymes, thus improving growth and FCR performance in poultry in general (Lovkova *et al.*, 2001; Williams & Losa, 2001 and Cross *et al.*, 2007).

5.4 Immune Organ Development

In the present study, no significant effects of PFAs were observed on the relative weight of lymphoid organs. However, significant variation was observed between groups on the absolute weight of the thymus, which could be highly influenced by differences in the live weight of the bird. Ghazalah and Ali (2008) also reported a reduction in relative thymus weight with 2% rosemary leaf inclusion in the chicken diet, without any effect on spleen and thymus weight. Similarly, Abd El-Latif *et al.* (2013) found negative results for spleen and bursa of Fabricius.

Conversely, Akosile *et al.* (2023) reported an increase in the relative weight of the bursa of Fabricius with a 1% PFAs inclusion in broiler diets. However, the present study found no significant variation among groups fed on different PFAs. These findings suggest that PFAs may exert either positive or negative effects on lymphoid organ development, and potentially influencing immune modulation. Further research is needed to better understand the interactions between PFAs and immune organ development to optimize their application in poultry nutrition.

5.5 Antibody Titer

Poultry experts are increasingly focused on strengthening the immune system, improving overall health, and enhancing production performance without relying on antibiotics. Previous studies showed that phytogenic feed additives (PFAs) can boost immune responses, reduce pathogenic microorganisms, balance intestinal microbial flora, enhance disease resistance, and improve feed digestibility, absorption, and nutrient availability (Zhu *et al.*, 2014; Abdelli *et al.*, 2021; Wakjira *et al.*, 2021). Feeding PFAs has also been shown to support nutrient utilization, strengthen immune function under stressful conditions, and promote growth in poultry (Jameel *et al.*, 2014).

The current study revealed statistically significant differences in antibody titers among the groups at 42 days ($p = 0.006$). Specifically, higher mean antibody titers were observed in groups supplemented with vitamin additives and rosemary, aligning with the findings of Engida *et al.* (2023). However, this study contrasts with prior findings regarding lemongrass and thyme supplementation, which yielded lower mean antibody titers.

These results are consistent with studies by Abdulkarimi (2011), Mansoub & Myandoab (2011), and Toghyani *et al.* (2011), which reported that thyme supplementation did not produce a statistically significant impact on broilers' humoral immune response to ND vaccination. The variability in thyme's effects on immunity may stem from differences in dosage, preparation methods, and vaccination protocols. In contrast, Hosseini *et al.* (2013) found that ground thyme inclusion improved broiler immune responses to sheep

red blood cells, demonstrating that thyme may act as both an immune stimulator and a post-vaccination reaction mitigator.

Similarly, studies by El-Latif *et al.* (2013) reported increased plasma antibody titers against ND and IB following supplementation with rosemary and garlic essential oils. Khaligh *et al.* (2011) observed a positive effect of a mixture of garlic, thyme leaf, cinnamon, rosemary leaves, and anise on ND antibody titers after a 33-day production cycle. However, the same study noted a negative effect at day 42 compared to the control group. Akosile *et al.* (2023) also reported increased plasma antibody titers against ND in broilers supplemented with 0.5% rosemary, though no improvement was observed at 1%.

Additionally, Alhajj *et al.* (2015) and Mandey & Sompie (2021) found that broiler chickens supplemented with Chinese star anise exhibited enhanced antibody titers and improved performance, supporting its potential as a natural immunomodulator. In the present study, vaccinated groups supplemented with lemongrass demonstrated significantly higher antibody titers compared to the unvaccinated control group (G6), suggesting a potential role for lemongrass in modulating humoral immune responses. This immunostimulatory effect may be linked to the activation and proliferation of lymphocytes or the stimulation of macrophages and T-helper lymphocytes (Haque *et al.*, 2018).

The antiviral properties of PFAs are attributed to their ability to inhibit viral replication, disrupt viral structures, and enhance immune responses (Tazi *et al.*, 2024). Additionally, their antioxidant effects help mitigate ND virus-induced oxidative stress, improving poultry health and reducing mortality rates (Tang *et al.*, 2024). Their antimicrobial and immunomodulatory properties also support gut health and reduce secondary infections (Boukhatem *et al.*, 2014; Bhakta & Das, 2021). These findings demonstrate that natural feed additives can influence broiler humoral and cellular immunity. However, further research is necessary to determine the most effective application of individual PFAs and their potential synergistic effects in commercial poultry production.

6. CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates that all the tested PFAs can be safely incorporated into the diets of Cobb 500 broiler chicks at a level of 2% without adversely affecting feed intake. Notably, lemongrass supplementation exhibited the potential to enhance growth performance to a level comparable with that achieved by commercially available vitamin supplements, indicating its viability as a natural and effective alternative to synthetic vitamins in broiler nutrition. Furthermore, broilers receiving rosemary leaf powder supplementation showed significantly higher antibody titers against ND vaccine compared to those supplemented with commercial vitamins, suggesting an immunostimulatory effect that may enhance vaccine efficacy and overall bird health. Although the differences in overall growth performance and immune response between the phytogetic-supplemented groups and the vitamin control were statistically significant, the observed trends indicate that PFAs can contribute positively to both productivity and health parameters. These findings align with the broader objective of reducing reliance on synthetic additives and antibiotics, supporting the advancement of more sustainable and natural poultry production.

In light of these promising yet preliminary results, it is strongly recommended that future research should focus on:

- Conducting extended studies in commercial production settings to assess the long-term effects of PFAs on immunological competence, disease resistance and growth performance.
- Systematic dose-response experiments to determine the optimal inclusion levels that maximize effectiveness without compromising bird health or performance.
- Further investigations into the economic feasibility of incorporating PFAs compared to commercially available vitamin supplements.

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8. APPENDICES

Appendix 1. Pictures showing preparation of herbs for the experiment

A) Drying phytonics .



B) Chopping of lemongrass and preparing it for grinding



Appendix 2. Pictures showing house preparation for chicken placement



Appendix 3. Day 0 and Day 42 serum collection



Appendix 4. Feed preparation and adding PFAs into the feed



Appendix 5. Ethical Clearance certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/71/17/2025

Name of Applicant: **Robel Girma** (DVM, MSc student)

Address: Microbiology, Parasitology and poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Evaluating the alternative use of phylogenetics as a feed additive in broiler chickens: effect on growth performance and immune response in Newcastle disease vaccinated chickens*

Date of application: **December, 2024**
Nature of the project: **Experimental trial**
Target animal species: **Broiler chicken**
Number of animals involved: **200**
Study area: **Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Robel Girma.


Professor Getachew Terefe (DVM, PhD)
Chairman

[Signature]
Signature

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