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Availability and Utilization Practices of Organic Acid Feed Additives in Commercial Chicken Production System and Their Effects on Production and Economic Performance of Bovans Brawn Layers and Cobb500 Broilers

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BAHIR DAR UNIVERSITY
College of Agriculture and Environmental Sciences,
Department of Animal Sciences

**Availability and Utilization Practices of Organic Acid Feed Additives in
Commercial Chicken Production System and Their Effects on
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Cobb500 Broilers**

PhD Dissertation

By
Endalkachew Birilie Mintesnot

December, 2025
Bahir Dar, Ethiopia

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By
Endalkachew Birilie Mintesnot

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

Advisory Board members:

- 1. Major supervisor: Prof. Yeshambel Mekuriaw (PhD, BDU)**
- 2. Co-supervisor: Dr. Getachew Animut (Associate Professor, ATI)**

December, 2025
Bahir Dar, Ethiopia

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DECLARATION

This is to certify that the thesis entitled “**Availability and Utilization Practices of Organic Acid Feed Additives in Commercial Chicken Production System and Their Effects on Production and Economic Performance of Bovans Brawn Layers and Cobb500 Broilers**”, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy/ PhD in Animal Nutrition to the Department of Graduate Council of Animal Sciences, Graduate Program of College of Agriculture and Environmental Sciences, 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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Place: Bahir Dar, Ethiopia

APPROVAL OF DISSERTATION

I hereby certify that I have supervised, read, and evaluated this dissertation titled **“Availability and Utilization Practices of Organic Acid Feed Additives in Commercial Chicken Production System and Their Effects on Production and Economic Performance of Bovans Brawn Layers and Cobb500 Broilers”** by Endalkachew Birilie prepared under my guidance. I recommend the thesis/dissertation be submitted for oral defense.

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APPROVAL OF DISSERTATION

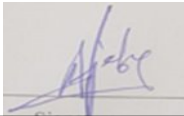
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Department of Animal Sciences

As members of the board of examiners, we examined this dissertation entitled **“Availability and Utilization Practices of Organic Acid Feed Additives in Commercial Chicken Production System and Their Effects on Production and Economic Performance of Bovans Brawn Layers and Cobb500 Broilers”** by Endalkachew Birilie. We hereby certify that the dissertation is accepted for fulfilling the requirements for the award of “Doctor of Philosophy in Animal Nutrition.

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DEDICATION

I dedicate this dissertation to my family, my wife Agerie Felek, my children, Barkot Endalkachew, Dagmawi Endalkachew, Liyu Endalkachew, and Amiran Endalkachew, and my wonderful extended family.

ABBREVIATIONS AND ACRONYMS

ADG	Average Daily Gain
AFP	Abdominal Fat Percentage
AFW	Abdominal Fat Weight
AGPs	Antibiotics Growth Promoters
AMR	Antimicrobial Resistance
BCR	Benefit Cost Ratio
CP	Crude Protein
CRD	Completely Randomized Design
CSA	Central Statistical Agency
DCP	Dressed Carcass Percentage
DCW	Dressed Carcass Weight
DFI	Daily Feed Intake
DM	Dry Matter
EFSA	European Food Safety Authority
EM	Egg Mass
EU	European Union
EW	Egg Weight
FAO	Food And Agricultural Organization Of The United Nation
FBW	Final Body Weigh
FCR	Feed Conversion Ratio
GIT	Gastrointestinal Tract
GLM	General Linear Model
GP	Giblets Percentage
GW	Giblets Weight
HDEP	Hen-Day Egg Production
HU	Haugh Unit
LI	Large Intestine
ND	New Castle Disease

ABBREVIATIONS AND ACKRONYMS (*Continued*)

NP	Net Profit
NPM	Net Profit Margin
NRC	National Research Council
OA	Organic Acid
OTC	Oxytetracycline
PP	Profitability Percentage
REE	Relative Economic Efficiency
RH	Relative Humidity
SAS	Statistical Analysis System
SI	Small Intestine
SR	Survival Rate
SW	Slaughter Weight
TC	Total Cost
TFC	Total Fixed Cost
THI	Temperature Humidity Index
TR	Total Return
TVC	Total Variable Cost
USD	United States Dollar
WHO	World Health Organization

Availability and Utilization Practices of Organic Acid Feed Additives in Commercial Chicken Production System and Their Effects on Production and Economic Performance of Bovans Brawn Layers and Cobb500 Broilers

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ABSTRACT

The aim of this dissertation was to assess the availability and utilization of organic acid feed additives in commercial chicken production and their effects on the production and economic performance of Bovans Brown layers and Cobb500 broilers. In the survey study, an assessment was conducted on the availability and utilization of organic acids (OAs) in urban and peri-urban commercial chicken farms of Bahir Dar and Gondar cities in Northwest Ethiopia. A cross-sectional study using a semi-structured questionnaire survey and direct observation on the availability and utilization of OAs as feed additives was employed with a total of 211 commercial chicken farms. Quantitative data were analyzed using descriptive statistics, while qualitative data were analyzed thematically to support quantitative findings. The survey results reveal that OAs are not currently utilized in commercial chicken production within the study areas. All participant (100%) indicated a lack of knowledge about OAs as feed additives, highlighting a complete lack of information among commercial chicken producers. The second study elucidated the potential of dietary citric and malic acid supplementation on growth performance and economic efficiency by replacing conventional antibiotics in Cobb500 broiler chickens. This study used a completely randomized design (CRD) consisting of five treatments with four replicates each. A total of 280 one-day-old chicks were randomly assigned to 5 treatment groups, consisting of 56 chicks per treatment, and further replicated four times with 14 chicks per replicate. The experimental treatments were a basal diet without additives (T1), 60 mg/kg oxytetracycline (T2), 16 g/kg citric acid (T3), 8 g/kg malic acid (T4), and a mixture of 8 g/kg citric acid and 4 g/kg malic acid (T5). Dose-level antibiotics and OAs were determined based on previous studies.

The results revealed that the average daily weight gain of chicks was significantly ($P < 0.05$) different among the treatments during the starter and grower phases. The T3, T4 and T5 groups presented better (62.09, 61.98, and 64.30 g) average weight gain than the T1 group (59.58 g), whereas the mixed OAs mixture showed comparable results to the antibiotic group (64.06 g) in terms of daily weight gain during the overall experimental period. Broilers receiving citric and malic acids showed improved feed conversion ratios. Diets containing a citric and malic acid mixture produced the highest dressed carcass yield ($P < 0.05$), which was comparable to that of the antibiotic group. Mixed acid group achieved superior relative economic efficiency. The third experiment described the effects of dietary citric and malic acid supplementation on laying performance, egg quality traits, and economic efficiency of commercial Bovans Brown layer hens. This study used a completely randomized design. A total of 260 25-week-old layer hens weighing an average initial body weight of 1609 ± 18.5 g were obtained from the poultry farm of Bahir Dar University and randomly assigned to 5 treatment groups, each with 4 replicates. Experimental layer hens were acclimated to the experimental diets for 7 days and were fed for 120 days. The treatments consisted of basal diets without any additive (T1), with 30 mg of oxytetracycline (T2), 16 g of citric acid (T3), 8 g of malic acid (T4), and a combination of 8 g of citric acid and 4 g of malic acid (T5) per kg of the basal diet. The inclusion levels of additives were selected based on the published literature. The results revealed a significantly higher difference ($P < 0.001$) in hen-day egg production, egg quality traits, and FCR. Layer hens fed OA diets improved the hen-day egg production by 85.51% to 86.54% compared to T1 (77.75%) and T2 (80.07%). Overall, T5 exhibited better performance in terms of FCR, albumin weight, egg mass, total return, and net profit. T5 improved profitability by 13.26% and 11.82% and REE by 16.83% and 17.40% more than T1 and T2, respectively. In conclusion, dietary supplementation with a mixture of 8 g/kg citric acid and 4 g/kg malic acid effective alternative strategy to replace antibiotic feed additives, in broilers, and layer chickens. Therefore, the incorporation of this mixture into broiler and layer production systems is recommended.

Keywords: Antibiotics, Citric acid, Economic efficiency, Malic acid, Mixed organic acids

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Background and Justification

Poultry production is crucial for global food systems, contributing to food security, economic development, and nutritional well-being, making it the most commonly farmed species globally (Mottet & Tempio, 2017). The global demand for poultry meat and eggs is increasing rapidly, driven by population growth, urbanization, and rising incomes, primarily in developing nations (FAO, 2020). Chickens, being the most numerous poultry species, are a primary source of meat and egg production, providing a substantial share of animal protein for human consumption worldwide, representing 89 and 92 percent of chicken meat and eggs, respectively (Erdaw, 2022). Chicken offers a highly efficient means of converting feed into high-quality human food, making it a crucial component in meeting the growing demand for animal-sourced food sources. Furthermore, they are a preferred source of animal protein over other meats because of their high levels of protein and calcium, as well as their lower levels of fat, cholesterol, and calories (Köse & Öztürk, 2017; Birhanu *et al.*, 2023). Moreover, their rapid growth rate and short production cycles allow for quick turnover and consistent supply, which is critical in ensuring food availability, particularly in regions where other protein sources may be limited or less sustainable (Birhanu *et al.*, 2023). Since they provide almost all the necessary nutrients, including energy, fatty acids, protein, vitamins, and minerals, in proportionate amounts, chicken meat and eggs are essential chicken products for human nutrition.

In low- and middle-income nations, like Ethiopia, smallholder poultry farming in particular has been found to play a major role in improving household food security and lowering poverty by supplying both income and nutrient-rich animal-source food (Birhanu *et al.*, 2023; Tirfie, 2023). In Ethiopian poultry production, chickens are the most dominant species and one of the major subsectors of livestock production, with the total population at the country level estimated to be about 57 million, of which 78.85, 12.02, and 9.11 percent of the total poultry were reported to be indigenous, hybrid, and exotic, respectively (CSA, 2021). It contributes to poverty alleviation and socio-economic inclusion of vulnerable

groups such as the urban poor, women, the disabled, orphans, and the unemployed to provide them with a decent livelihood (Shapiro *et al.*, 2015), and it plays an important part in family nutrition (Sime, 2022).

The Government of Ethiopia aims to meet the rising national demand for chicken meat and eggs while contributing to poverty and malnutrition reduction (Woldegiorgiss *et al.*, 2016). Although improved broiler and layer breeds have been introduced since the 1950s and perform well under intensive systems (Weyuma *et al.*, 2015), their contribution to the economy remains limited due to major constraints particularly the shortage and high cost of quality feed (Milkias, 2016; Alemneh & Getabalew, 2019). Commercial feed represents 75 to 80% of production costs (Negash, 2022), and essential ingredients such as maize, soybean meal, and premixes are expensive and insufficient in supply (Woldegiorgiss *et al.*, 2016). To meet increasing poultry product demand, improvements in feed quality, efficiency, and nutrient utilization are essential, as commercial poultry production requires high feed efficiency for profitability and sustainability (Yadav & Jha, 2019). The strategic use of feed additives can support these goals by enhancing feed quality and overall productivity (Haq *et al.*, 2017; Aikpitanyi *et al.*, 2019).

Antibiotics have long been used as feed enhancers and growth promoters in livestock production (Polycarpo *et al.*, 2017; Markowiak & Śliżewska, 2018); while in many developing countries, including Ethiopia, antibiotics are routinely incorporated into poultry feed to enhance growth and overall productivity (Woldegiorgiss *et al.*, 2017; Mezene *et al.*, 2020). Specifically, tetracyclines, penicillins, and sulfonamides are among the most commonly used antibiotic groups in Ethiopian poultry production (Mezan *et al.*, 2020; Ayalew *et al.*, 2022). With the country's growing demand for chicken products, this reliance on antibiotics is expected to continue, unless alternative solutions like OA like citric and malic acid feed additives have been introduced and evaluated in the chicken production. However, concerns over antibiotic resistance and residue accumulation in poultry products have led to increasingly strict regulations, and in many regions including the European Union the use of antibiotics as growth promoters has been banned (Agbai *et al.*, 2020). The withdrawal of these additives can negatively affect growth performance and gastrointestinal function unless effective alternatives are adopted (Pirgozliev *et al.*, 2019), posing challenges

for meeting rising global demand for poultry products. Consequently, the identification of safe and effective substitutes is essential for sustaining productivity and flock health. Several alternatives are being explored, including organic acids (OAs), probiotics, prebiotics, medicinal plant extracts, and exogenous enzymes (Adhikari *et al.*, 2020).

Organic acids have gained particular attention due to their strong antimicrobial activity against pathogenic bacteria (Ji *et al.*, 2023) and their potential to serve as natural, safe replacements for antibiotics without contributing to antimicrobial resistance (Waghmare *et al.*, 2025). They have been shown to improve growth performance, gut health, and overall productivity in commercial poultry production (Khan *et al.*, 2022). However, results for organic acids more broadly remain inconsistent due to differences in physicochemical properties of OAs, dosage, modes of action, diet composition and environmental conditions (Hajati, 2018; Filho *et al.*, 2022). Organic acids like citric and malic acids are commonly added to poultry feed to prevent intestinal diseases, enhance immunity, improve nutrient digestibility, and support growth (Yadav & Jha, 2019). They are considered promising alternatives to antibiotic growth promoters (AGPs) for enhancing performance and health in broilers (Kim *et al.*, 2015). Supplementation with OAs has also been reported to improve economic and productive efficiency, including live body weight, laying persistence, and overall profitability in layer production (AM *et al.*, 2017; Soltan, 2008). A review of eight studies on citric acid (5 to 60 g/kg feed) found that dietary inclusion generally increased body weight gain and feed efficiency in broilers, although feed intake tended to decrease (Kim *et al.*, 2015). Similarly, OAs can reduce gut pH, suppress pathogens such as *Salmonella*, and control molds when included at 0.5 to 3 kg/ton of feed, while malic acid at 0.5 to 2% improves gut microbial balance and weight gain (Hajati, 2018). Citric acid is commonly included at 4 to 30 g/kg, with 16 to 30 g/kg optimizing growth, feed intake, nutrient digestibility, and health (Biggs *et al.*, 2007; Nourmohammadi & Khosravinia, 2015; Katoch *et al.*, 2021; Makofane *et al.*, 2022; Sharifuzzaman *et al.*, 2024; Attia *et al.*, 2024), whereas malic acid is typically added at 5 to 10 g/kg, with 8 g/kg producing the most consistent improvements in feed conversion, daily gain, antioxidant capacity, immune function, and meat quality in broilers and layers (Bhatt & Sharma, 2012; Qiu *et al.*, 2022).

Despite these promising findings, research gaps remain regarding the combined use of citric and malic acids in broiler and layer diets. While their individual effects are well-documented, studies exploring potential synergistic effects are limited. Furthermore, there is a lack of information on the availability, use, and effects of organic acid feed additives in the Ethiopian commercial chicken production. With the country's growing demand for chicken products, this reliance on antibiotics is expected to continue, unless alternative solutions like OA like citric and malic acid feed additives have been introduced and evaluated in the chicken production. Therefore, assessing the availability of OAs and understanding current utilization practices among commercial chicken farmers, and evaluation of the effect of OAs supplements on biological performances and economic viability of commercial broiler and layer chickens are essential to identify gaps and promote OAs adoption for the free or reduced antibiotics chicken production in Ethiopia.

1.2. Statement of the Problem

Chicken production is an essential component of the Ethiopian food system, providing a relatively low-cost source of high-quality animal protein. However, productivity in commercial chicken production remains constrained by feed-related challenges, including poor feed quality, inconsistent supply, and high costs (Habte *et al.*, 2017). To enhance production efficiency, AGPs have historically been used, but their continued use poses risks of antimicrobial resistance, residues in poultry products, and environmental pollution have led to bans or restrictions in many regions (Xiao *et al.*, 2021). Banning of AGPs from poultry diets is also affecting the productivity performance of chickens (Pirgozliev *et al.*, 2019). For these reasons, it is necessary to find alternative feed additives which are able to improve production and economic performance of chickens' without antibiotic free feed additive has become more urgent such as dietary OAs.

Globally, organic acids such as citric and malic acids have shown promise as alternatives to antibiotics. Several studies have reported that OAs feed additive improve nutrient digestibility, maintain gut health, and enhance overall performance without the need for AGPs in poultry (Toschi *et al.*, 2024; Waghmare *et al.*, 2025). Moreover, combining two or more OAs like citric acid and malic acid may generate synergistic effects that improve

digestion, nutrient absorption, and microbial balance, ultimately enhancing broiler and layer performance (Ji *et al.*, 2023). Despite these benefits, there is a lack of documented information on the availability, awareness, and utilization of organic acids in Ethiopian commercial chicken production context. Additionally, lack of comprehensive studies have been conducted in Ethiopia to evaluate the effects of citric and malic acids, alone and in combination, on the production and economic performance of commercial layer and broiler chickens.

Therefore, assessment on OAs availability and utilization practices in the commercial chicken production, and animal feeding trial experiments were proved a greater understanding of the potential impacts of OAs supplementations in chicken production. Furthermore, investigations provide valuable information to support strategies for replacing antibiotics use in chicken feed with application of dietary OAs. Hence, the aim of this study was to assess and generates usable baseline information about the availability of OAs in Ethiopia, and subsequently, evaluates the effect of citric and malic acid on production and economic performance of commercial layer and broiler chickens. This assessment would provide valuable baseline data to support strategies for replacing antibiotics with OAs, fostering sustainable poultry production. This dissertation was then conducted based on the following research objectives.

1.3. Research Objectives of the Study

1.3.1. General objective

The general objective of this dissertation was to assess the availability and utilization practices of organic acids feed additive and evaluation the effect of citric and malic acid on production and economic performance of commercial layer and broiler chickens with the following specific objectives.

1.3.2. Specific objectives

This dissertation had the following specific objectives:

- To assess organic acids availability and its utilization practice in urban and peri-urban commercial poultry farms of Bahir Dar and Gondar Cities of Amhara Region, Northwest Ethiopia.
- To evaluate the potential of dietary citric and malic acid supplementation on biological performance and economic efficiency by replacing conventional antibiotics in Cobb500 broiler chickens.
- To evaluate the effect of dietary citric and malic acid supplementation on laying performance, egg quality traits, and economic efficiency of commercial layer hens

1.4. Organization of the Dissertation

This dissertation thesis is structured into seven chapters (Fig.1.1), which cover different issues regarding the availability and utilization practices of OAs in Ethiopian commercial chicken production. Chapter 1 provides background of the study, a problem statement, and objectives of the study, Chapter 2 reviews Ethiopian chicken production, feed sources, antibiotics, and OAs' impact on health, growth, egg production, and economic efficacy, while chapter 3 details materials and methods used in survey assessment study and animal feeding trials.

Chapters 4, 5, and 6 present findings of specific studies (study 1, 2 & 3): Chapter 4 (Study 1): assessment of OA availability and its utilization practice in urban and peri-urban commercial chicken farms of Bahir Dar city and Gondar town: exploring options for antibiotic replacement. Chapter 5 (Paper 2) investigates the potential of dietary citric and malic acid supplementation on biological performance and economic efficiency by replacing conventional antibiotics in Cobb500 broiler chickens, while chapter 6 (Paper 3) presents the effect of dietary citric and malic acid supplementation on laying performance, egg quality traits, and economic efficiency of commercial layer hens. Chapter 7 highlights the overall summary, conclusions, and recommendations for future research directions based on the entire study.

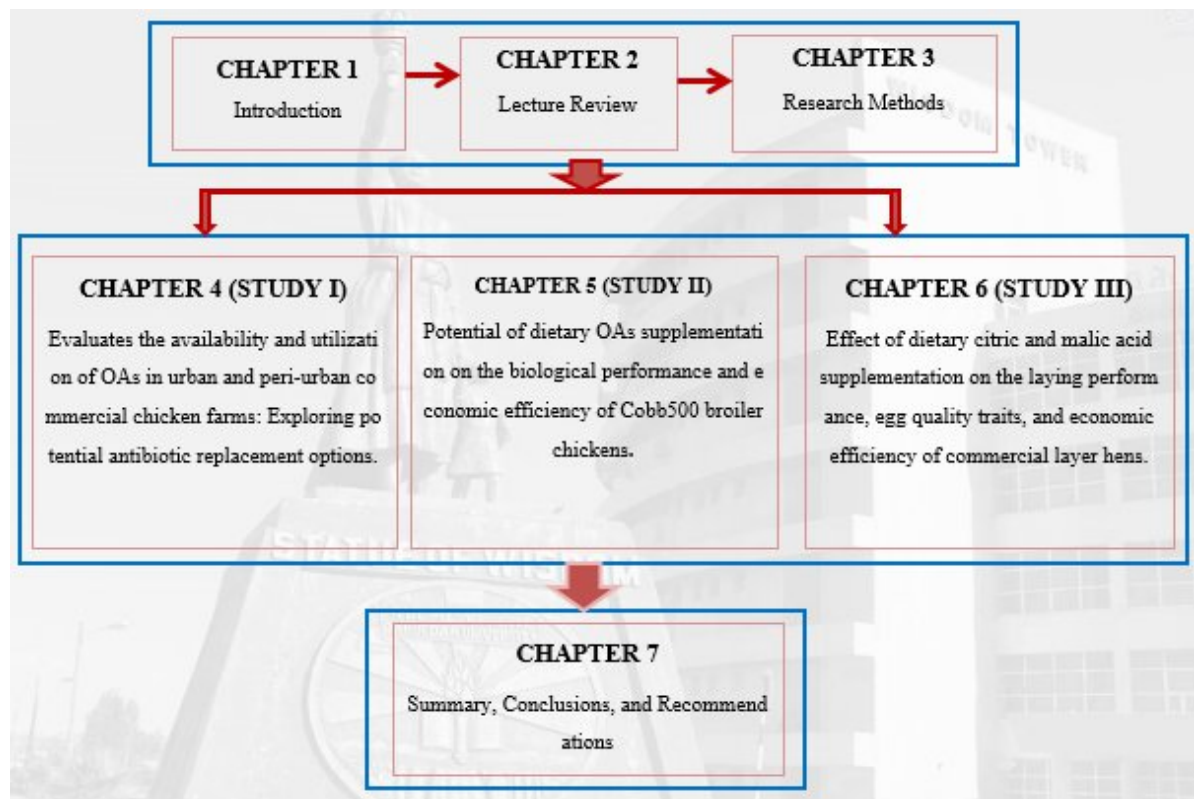


Figure 1:1 Organization of the dissertation

CHAPTER 2: LITERATURE REVIEW

2.1. Overview of Chicken Production in Ethiopia

Chicken production is an important agricultural activity in Ethiopia. The country has huge number of chickens' population, it holds about 60% of the total chicken population of East Africa (Fulas *et al.*, 2018). In Ethiopian poultry production, chickens are the most dominant species and one of the major subsectors of livestock production, with the total population at the country level estimated to be about 57 million, of which 78.85, 12.02, and 9.11 percent of the total poultry were reported to be indigenous, hybrid, and exotic, respectively (CSA, 2021). Chicken production in Ethiopia is the fast-growing agricultural sub-sector, with demand driven by population growth, rising incomes and urbanization (Urgesa, 2023; Getiso, 2024). In advance of other domestic animals, chicken have a short generation interval and higher feed conversion efficiency, thus providing a cheap source of animal protein. Chicken meat is the most palatable, easily digestible, contains essential amino acids required for human beings, and eggs are richly endowed with nutrients (Birhanu *et al.*, 2023).

In Ethiopia, chicken production contributes significantly to food security, job creation, and income generation, especially for women and rural households (Sebho, 2016; Urgesa, 2023; Getiso, 2024), and the chicken husbandry is mostly carried out by women and children, which can play a key role in household labor productivity and gender empowerment (Habte *et al.*, 2017). Thus, if food self-sufficiency is to be achieved and combat malnutrition in developing countries, particularly in Ethiopia, there is a need to give due attention to chicken production (Yitbarek *et al.*, 2016). Indigenous chickens are being the dominant, estimated to 78.85 percent of the total population, the rest 12.02 and 9.11 percent were hybrid, and exotic chicken breeds, respectively (CSA, 2021), and about 95.86% of the total national poultry products (eggs and meat) are contributed by indigenous chickens kept under village management system while the remaining 1.35% is obtained from intensively kept exotic breed of chickens and 2.79% are obtained from hybrids (Milkias, 2016). Chicken production systems in Ethiopia is differentiated based on diverse

criteria like breed, flock size, management practices, purpose of production, level of commercialization, and location. The categorization usually identifies three major systems: village or traditional or backyard, semi-intensive, and modern or intensive (Habte *et al.*, 2017).

2.1.1. Village poultry production system

Village poultry production system also known as traditional or free-range backyard chicken production system (Moredaa & Mesekel, 2016). It accounts for 78.85% of Ethiopia's 57 million poultry population (CSA, 2021). In this production system indigenous chickens are well-adapted to local conditions but have lower productivity compared to exotic breeds (Sebho, 2016; Urgesa, 2023). It is characterized by a low level of inputs and output (Moredaa & Mesekel, 2016), and chickens are mostly depending on locally available feed material as supplement with low health services and other management practices (Birhanu *et al.*, 2023). In Ethiopia, the village chicken production system is dominated by indigenous chickens, which are well-adapted to local environments and require low input, making chicken accessible even to the poorest families (Chaimiso, 2018).

However, the village chicken production contributes 99.2 percent of national chicken meat production with an annual output of 72,300 metric tons of meat, making, which has an important part of the national economy in general and the rural economy in particular (Sime, 2022); it is not profitable due to its lack of market orientation (Sime, 2022). Moreover, chicken production in this system is characterized by the production of a small number of low-yielding chickens (40–60 eggs/hen per year), an average flock size of 7–10 mature birds per household and with little or no additional inputs for feeding and health care, except provision of shelter during the night time. Hatching and brooding are entirely natural, by broody hens, but there is often very high chick mortality, of 25–88% (Habte *et al.*, 2017).

The eggs and meat produced in this way is insufficient to supply the increased demand for eggs and poultry meat in metropolitan areas (Sime, 2022). In order to catch up with the huge demand for chicken products, the sector is shifting from traditional, extensive scavenging systems to more semi-intensive and intensive commercial systems, but the majority of chickens are still kept under traditional management (Urgesa, 2023).

Furthermore, the high cost and limited availability of quality feeds are significant barriers, among other challenges, to scaling up commercial operations (Negash, 2022).

2.1.2. Semi- intensive poultry production system

Semi-intensive systems are the combination of the extensive and intensive systems in which birds are housed in a certain area and can access the shelter (Teyib *et al.*, 2020). This chicken production system applies some level of production inputs, such as protein and energy feed supplementations as a backup for the scavenging feed resource base; minimal health care and vaccination; and construction of a house, feeder, drinker and nesting boxes from locally available materials (Habte *et al.*, 2017). Mostly these systems concentrate on exotic; dual-purpose chicken breeds, with the potential to lay about 150–200 eggs/bird per year. Depending on the available scavenging feed resources in the area, the flock size under this system ranges from 10–50 chickens (Habte *et al.*, 2017; Woldegiorgiss *et al.*, 2017).

2.1.3. Intensive chicken production system

This type of chicken production system is the modern production systems apply standard inputs such as feed, breed, health care, housing etc. Moreover, chickens kept in this system are highly productive hybrid chickens developed for commercial production (Habte *et al.*, 2017). It is market oriented and the main objective of production is to get better profit and chicken breed used is specialized improved layer or broiler chicken. Commercial chicken production in Ethiopia is classified into three main systems based on size of the flock, imputes and biosecurity: large-scale farms have over 10,000 birds, medium-scale farms have 1,000 to 10,000 birds, and small-scale farms have 50 to 1,000 birds (Woldegiorgiss *et al.*, 2017).

2.2. Cobb500 Broiler Chicken Productions

Broiler production, such as Cobb500 breed, represents one of the most economic and easiest means of bridging the supply demand gap of animal protein, due to their rapid

growth rate and superior feed conversion ratio (Gadzama, 2024). The efficiency of broiler rearing is important in addressing food security concerns, especially in the developing countries where resources are typically scarce. Broiler chickens like Cobb500 have a significant value due to their ability to grow quickly and produce much meat within a short production period (Cobb-Vantress 2021). They also known as meat chickens and they are floor-raised on litter such as wood shavings, peanut shells, and rice hulls, indoors in climate-controlled housing.

Broiler chickens turn feed to food over faster than other animals, and for that reason, they are convenient in addressing immediate needs for protein. Under modern farming methods, meat chickens reared indoors reach slaughter weight at 5 to 9 weeks of age. Moreover, Cobb 500 broilers are designed specifically to grow quickly and produce a lot of breast meat (Cobb-Vantress, 2021). Faster weight gain and more meat production per unit of feed consumed are made possible by this genetic selection. A nine-week-old Cobb 500 broiler chicken averages over 9 pounds in body weight. At nine weeks, a hen will average around 7 pounds and a rooster will weigh around 12 pounds, having a nine-pound average. A commercial broiler farmer in Ethiopia receives the birds from the hatchery at one day old.

2.2.1. Nutrient requirement of Cobb500 broiler chickens

Nutrient requirement as the amount of nutrients needed by broilers to maintain their activities, maximize growth, feed utilization efficiency, and optimize fat accumulation (Gadzama, 2024). However, meeting the nutrient requirements of Cobb500 broiler is essential for maximizing growth performance and minimizing health problems, nutrient supply should be in cost-effective way to meet their requirements without excess. These requirements vary depending on the age, stage of production, and environmental conditions (NRC, 1994). Moreover, diets for broilers are formulated to provide a balanced array of nutrients that are essential for health and efficient production (Madhupriya *et al.*, 2018; Engida *et al.*, 2023; Khidr & El-Baz, 2024).

Chicken nutritionists formulate feed to maximize profitability and optimize carcass yields, particularly for broiler chickens, which require high protein for rapid growth, carcass composition, and finished product cost. According to National Research Council (NRC,

1994), the recommended dietary protein levels for the broiler chickens during the starter (0-3weeks), grower (3-5weeks) and finisher phases (6-8weeks) is 23%, 20% and 18 %, respectively for optimal growth and maximum productivity. From the nutrients requirement of broilers chickens, energy constitutes the major entity, as it required in large quantity, kcal ME/kg DM from 0-8 weeks of age, than the other nutrients the diet should contains adequate levels of amino acids, vitamins, and minerals (NRC, 1994). According to Cobb500 broiler chicken performance and nutrition supplement guideline (2022), the recommended crude protein level is 21-22, 19-20, 18-19, 17-18 and 17-18 percent crude protein, and 2900, 2950, 3050, 3100, and 3150 Kcal/kg Metabolizable energy recommended from 0-12, 13-28, 29-39, 40-49 and > 50 days of growth period, respectively. The chicken gastrointestinal tract (GIT) plays a vital role in nutrient digestion and absorption, which should be have a healthy gut environment for optimal growth and feed efficiency (Vera-Álava *et al.*, 2023). Organic acids, such as acetic, propionic, butyric, lactic, citric, and malic acids, are usually used as feed additives in broiler diets to promote gut health and also nutrient utilization.

2.2.2. Feed intake and efficiency of Cobb500 broiler chickens

Feed intake is the major factor that influences body weight gain and feed efficiency (Table 2.1). The feed intake of birds can be affected by age, reproductive stage, ambient temperature, metabolic energy, energy to protein ratio, a feed structure, feeding space, the health of the chicks, water quantity and quality and housing system (Cobb-Vantress 2022; Abdollahi *et al.*, 2018). Similarly, FCR is a crucial indicator of broiler production efficiency, and enhancing it can significantly decrease feed costs. The FCR also an indicator for a reduction in the time taken to reach market weight and decreased maintenance costs. To improve feed intake and FCR, several feed additives like OAs has been utilized in commercial broiler industries. Moreover, the mode of action of OAs in broiler chickens enhances gut health, nutrient utilization, and feed conversion efficiency by promoting healthier gut environment.

Table 2:1 Body weight and cumulative feed consumption for broiler chickens

Age (Wks.)	Male		Female	
	BW (g)	Cumulative FI (g)	BW (g)	Cumulative FI (g)
0	40	0	40	0
1	170	150	165	145
2	450	480	420	460
3	865	1120	780	1030
4	1410	2020	1250	1825
5	2250	3200	1750	2830
6	2700	4500	2300	4020
7	3350	6000	2800	5400
8	3900	7400	3300	6800
9	4400	8800	3700	8200

Source: (Cobb-Vantress, 2022)

2.3. Bovans Brown Layer Chickens

Bovans Brown is a brown feathered, brown egg layer which can meet the expectations of a variety of egg producers with different objectives (Fulas *et al.*, 2018). Bovans brown birds thrive in cage, aviary, and free-range systems and are adaptable to a number of environments and also, maintain excellent health, resulting in good livability and are easy to manage. Moreover, Bovans Brown laying chickens show better productivity and lay nice dark brown eggs with good shell quality (<https://www.bovans.com/en/>). Bovans Brown layer chickens are the bird of choice for today's egg farmers who expect high egg numbers and a forgiving bird essential ingredient to keeping business profitable. It is not only performs well for the egg producer with traditional production facilities but is very docile making it's the perfect bird for alternative production methods as well (Fulas *et al.*, 2018), and the most dominant egg laying chicken breed in Bahir Dar town (Litigebew *et al.*, 2021).

2.3.2. Feed intake and efficiency of Bovans Brawn layer chickens

There are a number of factors that influence voluntary feed intake (discussed in the section on feed intake). From Week 18, hens start to enter their laying period, reaching peak of lay around 32 weeks of age, and typically maintaining egg production until 65-68 weeks of age.

Feed intake will increase to a steady level of 100-105 grams per day and hen body weight will reach a mature level of 1700-1800 grams. Bovans Brown chickens weigh around 1920g at 16 weeks and 2005 g at 100 weeks. Their average chicken FI and FCR is 114 g/day and 2.19 kg/kg, respectively (<https://www.bovans.com/en/>). Furthermore, improving the FCR in laying hens can lower feed costs and boost the economic viability of egg production. As feed additives, OAs can be given to layer hen diets to support intestinal health, nutrient utilization, and egg quality by exerting different modes of action to achieve their beneficial effects. However, research is needed to determine the optimal combination and dosage of OAs to maximize their beneficial effects in laying hens under various production conditions.

2.3.1. Nutrient requirements of egg laying Bovans Brown chickens

The aim of layer diets is to optimize egg production (in terms of egg numbers, egg size or egg mass), provide the nutrition required to safeguard health and maintain the desired body weight. As presented in Table 2.2, chickens are given relatively high levels of energy, protein and the vitamins and minerals for the starter period. Satisfying the requirements for individual nutrients of the laying hens is crucial to maximize egg production, hen health, and egg quality. The requirements vary by age, breed, level of production, and environment (NRC, 1994).

Table 2:2 Typical nutrient levels for layer diets

Nutrients	Units	Up to 32wks	32 - 44wks	44 - 55wks	>55wks
Metabolizable Energy	Kcal/kg	2770-2860	2725-2860	2675-2860	2550-2825
Crude Protein	%	19.80	17.50	17.00	16.00
Lysine	%	1.02	0.93	0.89	0.83
Methionine	%	0.51	0.46	0.41	0.38
Linoleic acid	%	1.10	1.60	1.60	1.60
Calcium	%	4.40	4.25	4.50	4.75
Av-Phosphorous	%	0.48	0.40	0.36	0.35

Source: <https://www.bovans.com/en/>

2.4. Major Chicken Feed Resources in Ethiopia

Chicken production in Ethiopia faces constraints due to availability, quality, and cost of feed, which is primarily composed of conventional and non-conventional feed resources, as the country lacks self-sufficiency in cereal grains. Scavenging is the major feed resource search in village chicken production system (Salo *et al.*, 2016). But the non-conventional source feed could not be suitable for the improved chicken breeds which are designed for the better productivity and demanding more nutrient feedstuff. Feed source also obtained from household waste and cereal grains of maize and sorghum were the most dominant supplementary. This supplement is happening occasionally when there is surplus grain.

In small and large-scale chicken production systems, the major feed source come from cereal grains such as, maize, rice, wheat, sorghum, mixture of wheat and maize, mixture of sorghum and maize (Kibreab *et al.*, 2016; Salo *et al.*, 2016), that constitute a large proportion (>50%) of poultry diets and they are contribute mainly dietary source of carbohydrates and to some extent proteins. Moreover, maize is the popular cereal used in combination with soybean meal, noug seed cake and wheat short to formulate compounded chicken feed (Negash, 2022). The commercial feeds are the major feed type for urban/peri-urban commercial production system. In response to the growth interest of chicken production, nowadays, commercial feed processors have been increasing in the country. While, the problem is feed price and quality issue are also a concern for the commercial feed in the country (Alemneh & Getabalew, 2019). The commercial feed quality might not be maintained because of the ingredients difference from place to place of production area, processing type and so on (Negash, 2020).

Sustainable availability of chicken feed is crucial for efficient poultry production in Ethiopian commercial farming systems, which requiring regular availability of high-quality ingredients and balanced complete feed. The quality of mixed feed used is generally poor and most formulations available do not have vitamin or mineral premixes (Negash, 2022). Locally obtained feed ingredients like cereal grains are primarily used for human consumption, and industrial by-products, oilseed cakes, and meat and bone meal become higher price increments, which affects the availability of quality feed that affects chicken health, productivity, and economic viability of poultry farms. As alternative interventions,

feed additives have long been part of feed and play substantial roles in success of chicken production (Ragaa *et al.*, 2016). Feed additives are substances added to chicken diets to improve the quality of feed and enhance the growth performance, health, and productivity of chickens. Moreover, supplementation of additives in to chicken diets help to mitigate the challenges posed by suboptimal feed ingredients, environmental stressors, and disease pressures, thereby contributing to sustainable and profitable commercial chicken production (Abd *et al.*, 2022; Khidr & El-Baz, 2024). Antibiotics have long been utilized as feed enhancers and growth promoters in the livestock production (Polycarpo *et al.*, 2017; Markowiak & Śliżewska, 2018). However, with increasing concerns about antibiotic resistance and consumer demands for antibiotic-free production, alternative strategies for maintaining gut health and optimizing performance are crucial, such as OAs (Rehman *et al.*, 2019; Waghmare *et al.*, 2025).

2.5. Role and Limitations of Antibiotics Feed Additive in Chicken Industry

Since the 1940s, antibiotics have been added to poultry feed primarily for disease prevention and growth promotion. Tetracyclines, such as oxytetracycline, have been shown to enhance production and economic performance in both broiler and egg-laying poultry at levels of 20–500 mg/kg of complete feed. The European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ) has issued guidelines for the use of antimicrobial substances in animal feed, including tetracyclines. For broilers, the recommended oxytetracycline range is 50 to 100 mg/kg of feed, while for layer birds, 20 to 50 mg/kg of feed is recommended; these levels support promotion performances while minimizing the risk of cross-contamination in non-target feeds (Koutsoumanis *et al.*, 2021).

Traditionally, dosing was based on recommended AGP inclusion ranges, production goals, bird age, environmental conditions, feed type, and economic considerations. Their inclusion at sub-therapeutic levels in feed improves feed efficiency, weight gain, and reduces mortality by modulating pathogenic bacteria in the GIT (Gadde *et al.*, 2017; Markowiak & Śliżewska, 2018). Antibiotics revolutionized poultry farming by enabling more intensive and productive farming systems. However, growing concerns over antibiotic resistance have prompted recent research and regulatory trends to focus on alternative

strategies for poultry production. Moreover, the widespread and indiscriminate use of antibiotics has generated numerous limitations and problems. The most critical problem is the emergence of AMR bacteria, which is a severe global public health threat (WHO, 2017). Such resistant pathogens can spread from animals to humans through direct contact, the food chain, or environmental routes, rendering antibiotics ineffective for the control of infections (Van Boeckel *et al.*, 2015; Agbai *et al.*, 2020).

Antibiotic residues in chicken meat and egg products can affect consumer safety and acceptance. Furthermore, the environmental impact of antibiotic use, including water and soil contamination, is another concern. Consequently, the use of antibiotics has been restricted and prohibited in animal feed using as growth promoters in most countries (Agbai *et al.*, 2020). The increased concerns on AMR consumers' call for antibiotic-free chicken production, non-antibiotic alternatives to enhance gut health and optimize performance are necessary (Waghmare *et al.*, 2025). Moreover, these limitations have driven research and industry efforts to identify and adopt safe and effective alternatives to antibiotics in animal nutrition (Van Boeckel *et al.*, 2015). In response, researchers are currently investigating various feed additives like organic acids, prebiotics, probiotics, medicinal plant extracts, and exogenous enzymes as a substitute for antibiotics (Adhikari *et al.*, 2020). Among these, OAs have been found to be promising feed additives in chicken production with diverse benefits and a contribution to sustainable and efficient commercial chicken production (Ji *et al.*, 2023). Also, OAs have attracted great interest because they have antimicrobial activity against pathogenic bacteria and because these compounds can lead to a reduction in pH in the GIT, which has the potential to increase the use of nutrients in chicken diets (Kim *et al.*, 2015).

2.6. Role of Organic Acids in Poultry Production

Organic acids are carboxylic acids with the general structure $R-COOH$, naturally occurring in plant and animal tissue. They are also produced by microbial fermentation in the hindgut of animals. Organic acids can be classified by their chemical structure, chain length (short-chain fatty acids, SCFAs; medium-chain fatty acids, MCFAs), and the number of carboxyl groups (monocarboxylic, dicarboxylic, tricarboxylic). They have gained

increasing attention in poultry production as potential substitutes for AGPs, driven by an increasing concern about antimicrobial resistance (Waghmare *et al.*, 2025). Organic acids like formic, acetic, propionic, butyric, lactic, citric, malic and fumaric acids are commonly used in poultry diets for their antimicrobial activity, gut health modulation, and improved nutrient utilization. These acids exert their beneficial effects primarily through antimicrobial activity, gut health modulation, and improved nutrient utilization, leading to enhanced growth performance and overall well-being in poultry. Despite their huge potential, OAs in poultry production face limitations due to dosage, buffering capacity of feed ingredients, and gut microbiota composition, which necessitating further investigation for synergistic effects of combined OAs.

Organic acids have long been used as additive for the reduction in food deterioration and prolonging the shelf life of food commodities, especially perishable food ingredients (Khan *et al.*, 2022). In animal nutrition, OAs has been used as feed preservatives for decades reducing the growth of molds and bacteria (Khan and Iqbal, 2016; Khan *et al.*, 2022). Organic acids have been a major focus of attention in poultry production as alternative strategies to antibiotics due to their antimicrobial property and ability to promote poultry health and performance (Chukwudi *et al.*, 2024; Du *et al.*, 2024). Moreover, these acids are approved by most developed nations for use in animal diets as a safe strategy for animal feed additives (El Baaboua *et al.*, 2018). Furthermore, they have been preferred by companies as a green feed additive because of their three-free properties (pollution-free, drug resistance-free and residual-free) and a potential alternative to AGPs (Dittoe *et al.*, 2018).

2.6.1. Mechanism of action of organic acid feed additives in poultry production

Organic acids used as feed additives in poultry primarily function by lowering the pH of both feed and the gastrointestinal tract. This acidic environment disrupts bacterial cell membranes, alters intracellular pH balance, and inhibits the growth of acid-sensitive pathogenic bacteria, while promoting a more favorable gut microbiota composition (Pham *et al.*, 2020; Wang *et al.*, 2021; Waghmare *et al.*, 2025). Their antimicrobial activity in feed and drinking water is further enhanced by reducing buffering capacity and limiting

microbial proliferation (Dai *et al.*, 2021). Due to their physicochemical properties, short-chain acids (C1-C7) are the most commonly used in poultry production (Kim *et al.*, 2015; Haq *et al.*, 2017). These actions collectively contribute to improved gut conditions that support bird health and productivity.

Beyond their direct antimicrobial effects, OAs beneficially influence intestinal physiology by modulating the microbial community, strengthening mucosal integrity, and enhancing digestive function (Dai *et al.*, 2021). Dietary supplementation with OAs has been shown to improve growth performance (Palamidi *et al.*, 2016; Long *et al.*, 2018; Galli *et al.*, 2021; Wang *et al.*, 2021), boost immune and antioxidant responses (Abudabos *et al.*, 2017), and support intestinal morphology and nutrient absorption (Xu *et al.*, 2018; Yang *et al.*, 2019; Stamilla *et al.*, 2020). Despite their recognized benefits in both broiler and layer production, inconsistent findings across studies arising from differences in OA type, concentration, formulation, combination effects and diet composition highlight the need for further research to optimize their application and clarify variation in outcomes (Hajati, 2018; Filho *et al.*, 2022).

2.6.2. Effects of organic acids on poultry health and performance

Organic acids have been widely recognized as effective feed additives for improving gut health, immune function, and production efficiency in poultry. Their primary mode of action involves lowering the pH of the gastrointestinal tract, thereby creating an unfavorable environment for pathogenic bacteria such as *E. coli* and *Salmonella*, while promoting the growth of beneficial microorganisms including *Lactobacillus* and *Bifidobacterium* spp. (Kim *et al.*, 2015; Haq *et al.*, 2017; Steyl, 2019). This microbial shift enhances digestive capacity, reduces toxin production, and minimizes microbial competition for nutrients, resulting in improved digestion and nutrient absorption (Vera-Álava *et al.*, 2023; Chukwudi *et al.*, 2024). OAs also improve intestinal morphology by increasing villus height, width, and surface area in the duodenum, jejunum, and ileum, thereby strengthening gut integrity and function (Du *et al.*, 2023; Haq *et al.*, 2017). Additionally, dietary acidification reduces subclinical infections, lowers ammonia and other growth-inhibiting metabolites, and

contributes to better immune responses through modulation of gut-associated lymphoid tissues and attenuation of oxidative stress (Khan *et al.*, 2022).

Beyond their impacts on gut health and immunity, OAs consistently enhance nutrient utilization and growth performance in broilers and layers. Numerous studies have reported improvements in body weight gain, feed conversion ratio, nutrient digestibility, and overall feed efficiency following organic acid supplementation (Khan & Javid Iqbal, 2016; Waseem *et al.*, 2016; Nguyen & Kim, 2020; Gharib-Naseri *et al.*, 2021). Their effects have been attributed to improved gut microbial ecology, enhanced enzyme activity, and increased nutrient retention (Hajati, 2018; Yadav *et al.*, 2016). Blends of formic, phosphoric, lactic, tartaric, citric, and malic acids have shown particular efficacy, with inclusion levels such as 0.15% significantly increasing body weight compared to non-supplemented controls (Hashemi *et al.*, 2014). Moreover, OAs have demonstrated growth-enhancing and mortality-reducing effects without compromising carcass yield (Brzoska *et al.*, 2013) and have been associated with improved blood and liver biochemical parameters in layers (Fouladi *et al.*, 2018). Collectively, these findings indicate that organic acid supplementation not only improves productivity but also reduces reliance on antibiotics and veterinary treatments, promoting more sustainable and economically viable poultry production systems (Rahman *et al.*, 2008; Abudabos *et al.*, 2017; Abbas *et al.*, 2022).

2.6.3. Antimicrobial and antifungal properties of organic acids

Organic acids are effective antimicrobial agents and can control the development of pathogenic fungi, bacteria, and protozoa in chicken production. Due to this property, they are very beneficial in poultry feed quality maintenance and disease avoidance (Hajati, 2018; Du *et al.*, 2023; Kiliç *et al.*, 2024). This is due to the capacity to decrease pH in the feed and GI tract by the organic acid likely dependent on the pH conditions of the GIT and pKa values of the organic acid used (Yadav *et al.*, 2016). Furthermore, in associations between OAs of different pKa ensure microbial action to a greater extent in the intestinal tract, as the pH changes throughout the GIT segments such as pH values of 3.5 to 4.0 in the crop and proventriculus; 6.4 in the duodenum, 6.6 in the jejunum and 7.2 in the ileum (Silva *et al.*, 2022). Moreover, the supplementation of dietary OAs improved growth performance,

intestinal morphology and barrier function, which might be attributed to the changes of intestinal microbiota, particularly the enrichment of SCFAs-producing bacteria (Dai *et al.*, 2021). This is due to the capacity to decrease pH in the feed and GIT by the OA likely dependent on the pH conditions of the GIT and pKa values of the organic acid used (Yadav *et al.*, 2016).

Dietary OAs exert growth inhibiting effects on stomach and gut microbes through pH reduction and anion and proton effects in the microbial cell. Growth rates of many microbes like *Cl. Perfringens*, *E. coli* or *Salmonella* spp. are reduced below pH 5, while acid tolerant microbes are unharmed. Optimum pH for *Escherichia coli*, *Lactobacillus* spp., *Salmonella* spp., *Campylobacter jejuni* growth are 6-8, 5.4-6.4, 6.8-7.2, and 6.8-7.2, respectively; and acids used as feed additives have pK values between 3 and 5, and are categorized as being of intermediate strength (Hajati, 2018). In previous research, OAs enhance gut health by ensuring a balance in gut microbiota, reducing pathogenic bacteria colonization, and improving intestinal morphology, thereby enhancing growth and feed efficiency (Waghmare *et al.*, 2025), and economic efficiency (Mahfuz, *et al.*, 2021) of broiler birds.

2.6.4. Impact of organic acids on egg quality and laying performance

In laying hens, OA supplementation has been reported to improve the egg production rate, eggshell quality, and reduce the *Salmonella* contamination of eggs (Rowghani *et al.*, 2019; Gong *et al.*, 2021). Recent studies have also shown that OA supplementation improves egg quality characteristics, antioxidant status and reduces peroxidation of lipids in the egg yolks, leading to an improved egg quality (El-Deek *et al.*, 2018). The enhanced egg quality is a result of the improved gut health and nutrient absorption caused by OAs (Kilinc *et al.*, 2024). The pH reduction in GIT in chickens increase gastric proteolysis, amino acid digestibility and form complex with Ca, P, Mg, Zn etc. resulting in an improved mineral digestibility (Haq *et al.*, 2017; Fouladi *et al.*, 2018). In laying hens, OA supplementation has been associated with increased egg production, improved eggshell quality, and better feed efficiency (Abbas *et al.*, 2022), and improved gut health and nutrient utilization, which can be contribute to sustained laying performance, especially under stressful conditions.

Organic acids feed additives have been reported to decrease the population of harmful bacteria and yield of poisoning metabolites, increase digestibility of peptides and mineral micronutrients and also serve as substrates in the intermediary metabolism (Fouladi *et al.*, 2018). Moreover, OA supplementation improves live body weight, persistence of lay, daily egg mass output, food conversion ratio, egg shell quality, blood serum protein, and calcium concentration during the period of 54-70 week of hens age, which might be the variable effect of OAs inclusion of laying hen diets may be confounded by variations in gut flora and environmental condition (Soltan, 2008; Dahiya *et al.*, 2016). Inclusions of dietary organic acids to layer chicken diets has showed a positive result on the most important performance parameters such as decline the pathogenic population bacteria and improved yield and quality of eggs traits improve blood and liver biochemical parameters, other (Fouladi *et al.*, 2018).

2.6.5. Effect of organic acids on economic viability of poultry production

From the economic perspective, reduced disease incidence, improved production performance, and improved feed efficiency associated with OA supplementation can result in increased profitability to chicken producers, and comprehensive cost-benefit analysis has demonstrated a favorable return on investment under various systems of chicken production (Khan *et al.*, 2017). Improved feed efficiency and reduced disease incidence which translate to improved production performance in chicken production that contributes to improve the profitability of chicken farmers' financial benefits. Organic acids reduce antibiotic use, lowering medication and withdrawal expenses (Gharib-Naseri *et al.*, 2021). Finally, OAs can contribute in providing a safer chicken egg and meat production by reducing *Salmonella* occurrence, residue problems and hence achieving improved food safety and environmental sustainability through reduced ammonia emissions in chicken farms with more positive economic return of chicken enterprise.

2.6.6. Effect of citric acid in poultry production

Citric acid, a naturally occurring tricarboxylic acid, is widely used as a safe and effective feed additive in poultry nutrition due to its multifaceted physiological and antimicrobial effects. It is a low cost, but efficient and effective OA, accepted in the European Union and other countries like Brazil, with no restriction on its food nationwide (Silva *et al.*, 2022). The primary mode of action of citric acid directly in the feed, preventing bacterial deterioration and pathogenic bacteria in the GIT as involves lowering the pH and creates an acidic environment with a pH between 3.5 and 4.0 favoring the development of lactobacillus (Chowdhury *et al.*, 2009), thereby enhancing digestive enzyme activities and inhibiting acid-sensitive pathogens such as *Salmonella*, *Escherichia coli*, and *Clostridium perfringens* (Islam *et al.*, 2016). Consequently, this acid increases protein and fiber digestibility, improving weight gain, feed conversion efficiency and improves mineral availability like Ca, P, AND Zn, through chelation, which enhancing calcium and phosphorus absorption, bone mineralization, and eggshell quality (Omar & Al-Rahmani, 2015). Citric acid reduces need for available phosphorus by making it more accessible due to the chemical reaction dissolution of phosphorus sources through citric acid, which contributes for economic advantage for feed processors considered during ration formulation (Sharifuzzaman *et al.*, 2020). Additional benefits includes modulation of gut microbiota by reducing the intestinal, cecal and gizzard pH in chickens, citric acid support of immune function through reduced pathogen load can compensate the performance in broiler chickens by mitigating about 10% of the energy and protein level in the diet and decreased metabolic stress (Alizadeh & Rodriguez-Lecompte, 2019; Adil & Magray, 2019; Sharifuzzaman *et al.*, 2020). Citric acid is the final common oxidative pathway for carbohydrates, fats and amino acids, therefore the most important metabolic pathway in providing energy to the body (Akram, 2014).

Citric acid enhances mineral absorption (especially calcium, phosphorus, and magnesium) by chelating minerals and maintaining acidic gut conditions. In broilers, citric acid supplementation improves growth performance and feed efficiency (Khan *et al.*, 2020). It improved body weight gain, feed conversion ratio, nutrient digestibility, gut morphology, and carcass traits in broilers (Omar & Al-Rahmani, 2015; Rajput *et al.*, 2016; Potočnik *et*

al., 2017; Al-Ramamneh & Al-Shdefat, 2018). In layers, citric acid supplementation has been associated with improved eggshell quality and production performance in egg laying hens (Mohamed *et al.*, 2016). It enhances egg production, total egg mass, eggshell strength, feed efficiency, bone integrity, and overall gut health, thereby supporting sustained laying performance across the production cycle (Soltan, 2009; Park *et al.*, 2016). Depending on production goals, diet composition, and cost-effective approaches, the inclusion dose-level of citric acid in poultry diets range from 4 to 30 g/kg (Nourmohammadi *et al.*, 2015). However, studies consistently report that inclusion rates between 16 to 30 g/kg optimize growth performance, feed intake, nutrient utilization, and improves the health indicators in both broilers and layers (Biggs *et al.*, 2007; Katoch *et al.*, 2021; Makofane *et al.*, 2022; Sharifuzzaman *et al.*, 2024; Attia *et al.*, 2024).

2.6.7. Effect of malic acid in poultry production

Malic acid is a naturally occurring C4-dicarboxylic acid found in fruits such as apples and a key intermediate of the tricarboxylic acid (Krebs) cycle, making it a multifunctional additive in poultry nutrition (Ghasemi *et al.*, 2015; Hajati & Kohram, 2018; Shabani *et al.*, 2019). Malic acid is a mild-testing acid and has widely used in food industry (Chi *et al.*, 2016), which allows its applications in animal feed to improve the performance of poultry birds and the quality of their products (Silva *et al.*, 2022), and it have no a pollutant or residue effect (Ren *et al.*, 2020). Its primary action involves lowering the pH of feed and the upper gastrointestinal tract, which enhances digestive enzyme activity like particularly pepsin and creates an unfavorable environment for acid-sensitive pathogens including *Salmonella*, *Escherichia coli*, and *Clostridium perfringens* (Ghasemi *et al.*, 2015). Moreover, malic acid's involvement in the Krebs cycle may enhance cellular energy availability within intestinal tissues, promoting mucosal repair and reducing the energy burden associated with immune activation. Malic acid inclusion in poultry diets has been shown to improve growth performance, gut health, and antioxidant status (Eid *et al.*, 2017). It also enhances feed conversion and supports immune function.

Malic acid functions as a strong acidifier with notable antimicrobial effects. Its role as a Krebs cycle intermediate allows it to directly support cellular energy metabolism,

particularly in rapidly dividing intestinal epithelial cells, thereby contributing to improved gut integrity (Ghasemi, *et al.*, 2015). By helping maintain a balanced gut microbiota, malic acid enhances the digestibility and absorption of proteins, amino acids, fats, and minerals such as calcium and phosphorus. This improved nutrient use supports better growth performance, stronger gut integrity, and enhanced bone mineralization (Rajput *et al.*, 2016; Shabani *et al.*, 2019). Together, these effects lead to increased body weight gain, improved feed conversion ratio (FCR), better nutrient utilization and gut morphology, as well as reduced pathogen load and mortality in broilers (Islam *et al.*, 2016; Potočnik *et al.*, 2017; Hajati & Kohram, 2018; Shabani *et al.*, 2019). Malic acid supplementation enhances egg production, total egg mass, eggshell strength, feed efficiency, bone health, and laying persistence in egg laying hens. Moreover, by optimizing nutrient absorption and maintaining robust gut health, malic acid supports the overall health and metabolic efficiency of laying hens. This often results in improved rates of egg production (hen-day or hen-housed egg production) and potentially an increase in average egg weight, leading to a higher total egg mass (Rezaei, *et al.*, 2013). Incorporating malic acid at levels from 0.5% to 2% boosts weight gain in broilers and production efficiency of chickens (Hajati, 2018). The optimal concentration is between 0.5% and 1% (Bhatt & Sharma, 2012), with 0.8% being the most advantageous, leading to enhanced antioxidant capacity, immune response, feed intake, daily gain, meat quality, hen-day egg production, and reduced mortality without adverse effects on poultry birds (Qiu *et al.*, 2022). Malic acid also has a strong acidifying and antimicrobial effect, and its direct role as an intermediate in the Krebs cycle suggests a potential direct contribution to cellular energy metabolism, especially for rapidly dividing intestinal epithelial cells, supporting gut integrity (Ghasemi *et al.*, 2015). Malic acid also a strong acidifier and antimicrobial effect, and its direct role as an intermediate in the Krebs cycle suggests a potential for direct contribution to cellular energy metabolism, especially for rapidly dividing intestinal epithelial cells, supporting gut integrity (Ghasemi, *et al.*, 2015).

2.6.6. Organic acid mixtures in poultry nutrition

The combination of OAs has also been shown to be beneficial in commercial poultry production. Combining two and more than OAs into blends can harness synergistic effects, providing broader and more sustained impacts throughout the GIT of chickens (Waghmare *et al.*, 2025). Organic acid mixtures exert multifaceted effects through direct antimicrobial activity, modulation of gut microbiota, improved nutrient digestibility, and indirect immune support. By lowering GIT pH and disrupting bacterial cell membranes, OA mixtures inhibit pathogenic bacteria while promoting beneficial microbiota (Ma *et al.*, 2021). Moreover, mixture of OAs supplementation enhances body weight gain, feed conversion ratio, carcass quality, and survivability in broilers; while, it improves egg production, egg mass, eggshell quality, feed efficiency, and laying persistence in layers hens (Islam *et al.*, 2016; Ma *et al.*, 2021).

Based on previous studies, mixture of OAs (lactic, fumaric, citric, and formic) in broiler feed reduced salmonella in crop and cecum (Picker *et al.*, 2012). The association between 5% acetic, 12% formic and 4% propionic acids, replace anticoccidial and antimicrobial, was beneficial in maintaining gut health in broiler chickens and control of *Salmonella enteritidis* with improving growth performance (Calaca *et al.*, 2019). Mixture of L-malic and L-lactic acids (0.8%) improved broiler growth performance and feed efficiency, likely by enhancing immunity, antioxidant status, and hepatic lipid metabolism (Qiu *et al.*, 2023). According to Colvara (2018), a mixture of propionic, lactic, and formic acids in the diets of Japanese quails challenged with *Salmonella enteritidis* resulted in reduced bacterial excretion and improved egg quality, without negatively affecting overall performance, in with quails receiving the acid blend produced eggs with greater weight, a higher albumen percentage, and better Haugh unit scores. The OAs blend lowers gut pH, inhibits pathogens, supports beneficial microbiota, and improves nutrient absorption, enhancing performance. Citric acid acts as a strong acidifier and mineral chelator, while malic acid supports acidification and cellular energy. Together, they may optimize nutrient use, strengthen gut barrier function, and offer a natural alternative to AGPs with production and economic benefits.

2.6.7. Availability and industrial application of organic acids in Ethiopia

Organic acids have diverse industrial applications in Ethiopia, particularly in the food processing, beverage, pharmaceutical, textile, leather and chemical sectors. Demand for these acids is high, and many are imported (Alemayehu *et al.*, 2024), China is being the leading source country for Ethiopian OAs, followed by India, Germany, United States, Turkey, and other. Based on the Ethiopian Customs Authority, four years (2021 to 2024) average import data shows that China accounted for 88.32% of Ethiopia's total imports of organic acid, followed by India (4.08%), Turkey (2.29%), the United States (1.21%) and Germany (1.21%), and the rest 2.29 % imported from other countries. The common organic acids including formic, acetic, propionic, butyric, citric, lactic, fumaric, tartaric, and gluconic acids, play essential roles across multiple Ethiopian industries (Table 2.3). They are used as preservatives, chelating agents, pH regulators, exfoliants, and dyeing agents, and they also serve as solvents and precursors in various chemical processes. In addition, these organic acids are employed in laboratory analyses and water treatment applications, contributing to improved product quality, safety, and sustainability across several industrial sectors in Ethiopia.

Table 2:3 Type and utilization purpose of organic acid in Ethiopian industries

Common Name	Molecular Name (IUPAC)	Industries	Industrial Functions
Formic Acid	Methanoic acid	Food and Beverages	Used as preservative agents in the food and soft and alcohol drink products. It used to balance sweetness, enhance flavors, and create appealing sensory products.
		Textile and Leather	It used for textile dyeing and leather tanning
		Detergent	Used as raw materials in the detergent producing industries
Acetic Acid	Ethanoic acid	Food and Beverages	Used to preservative and flavoring agent in vinegar industries. Strong antimicrobial properties.
		Pharmaceutical	Preservative agent and used as ingredient for skin anti-infections cream preparations.
		Plastic	It used as an biodegradation agent of biodegradable plastics production
		Textile	It used as pH adjuster in textile dyeing and printing processes
		Chemical Industry	It used as solvents in various chemical processes including cleaning products
Propionic Acid	Propionic acid	Food industry	Fungal inhibitor used as a preservative agent and mold inhibitor in bakery products to extend shelf life, prevent foodborne illnesses, and maintain quality
		Plastic industry	Used as an biodegradation agent of biodegradable plastics production
		Pharmaceutical	It used as antifungal agent in drug processing industries.
Butyric Acid	Botanic acid	Food industry	Botanic acid in pure, salts and esters used as flavoring agent food, beverages and other processed soft drinks it is impart a buttery or cheese-like taste and aroma.
		Plastic industries	It uses to in process of various products of plastics such as plastic bags, plastics bottles, plastic buckets, packaging plastic materials, plastic glasses and etc.
Fumaric Acid	(2E)-But-2-enedioic acid	Food and Beverages	Used as preservative and to regulate pH in food, beverages and confectionery industries. It used in bakery, fruit juice, powder drinks mixes to enhance flavors and extend shelf life of products including animal meat.
		Pharmaceutical	Fumaric acids used in drag processing industries as ingredient.

Table 2:3 Type and utilization purpose of organic acid in Ethiopian industries (*continued*)

Malic Acid	2-Hydroxybutanedioic acid	Food and Beverages	Used as food and beverage additives as acidity regulator and flavor enhancers (acidulantes) in most apple-flavored products. Malic acid is typically used to enhance tartness and flavor of fruit juice, carbonated soft drinks.
		Pharmaceutical	It used as an active pharmaceutical ingredient, it is used as an ingredient to synthesize certain drugs
		Cosmetics	It used as pH-regulating agent in cleaning and personal care products. It is used to control and adjust pH level in cosmetic formulations,
		Plastic	As a dicarboxylic acid, malic acid widely used by plastic industries as a monomer for the synthesis of special polymers, like biodegradable plastics that are ecofriendly.
		Cement industries	It is used is utilized as a cement additive to lower solidification time and increase the strength of concrete.
Citric Acid	2-Hydroxy-1,2,3-propanetricarboxylic acid	Food and Beverages	Used as a preservative agents in raw materials and processed products preservative by inhibiting microbial growth and extending their shelf life and ensuring safety flavoring agent cleaning agent and pH adjuster in the processed products. Used as flavor enhancers in soft drinks, candies, and fruit juices. Used as chelating agents in food industries, which can bind to metal ions, preventing discoloration, rancidity, and unwanted reactions in foods which helps maintain color, flavor, and stability, especially in processed fruits and vegetables, and used for water treatments.
		Pharmaceuticals	To maintain pH stability of drug solutions and suspensions. Used as chelating agent, which used to remove heavy metals from the body in cases of metal poisoning (chelation therapy)
		Chemical	Cleaning products to remove hard water stains, rust, and other deposits
		Textile	Used as pH adjusters in textile dyeing and printing processes, and used as a crosslinking agent to improve the wrinkle resistance and dimensional stability of textiles during finishing
		Cosmetics	It Used to adjust the pH of cosmetic formulations in the cosmetics and personal care products to be compatible with skin and hair. It used to bind to metal ions in cosmetic formulations to prevent discoloration, maintain stability, and enhance the effectiveness of other ingredients

Table 2:3 Type and utilization purpose of organic acid in Ethiopian industries (*continued*)

Lactic Acid	2-Hydroxypropanoic acid	Food and Beverages	For preservative and pH regulator in food and drinking products, used for preservation and flavor in meat processing, and contributes to flavor and texture in milk processing
		Pharmaceutical	It used in to improve the solubility of poorly water-soluble drugs
		Cosmetics	Used to adjust the pH of cosmetic formulations to be compatible with skin and hair of cosmetics and personal care products
Tartaric Acid	2,3-dihydroxybutanedioic acid	Food and Beverages	Used as baking powder to react the baking soda to produce carbon dioxide, making cakes and pastries rise, and it used as acidulant to enhance the flavor and color profiles of various fruit-flavored products in juice processing industries.
		Pharmaceutical	It used as a chelating, it binds to metal ions, which can be useful for stabilizing drug compounds agent, and it used to improve the solubility of poorly water-soluble drugs,
		Cosmetic	It used in the cosmetics industries as pH-regulator and exfoliating cosmetic products
		Leather	It used for leather tanning in the leather processing industry
		Metal and Glass	It is widely use in metal industries to binds to metal ions to clean and polish metal surfaces of copper, aluminum, and iron, and improve electroplating quality; and in silvering of mirrors.
Gluconic Acid	D-Gluconic acid	Food and Beverages	Used in food and beverage industries for stabilization and as acidulates, it used to adjust pH, prevent bitterness, and provide minerals.
		Cosmetic	It used in cosmetic industries as pH regulator in cosmetic product.
		Pharmaceutical	It used in electrolyte solutions for formulation of parenteral nutrition, which used as a source of additives for essential mineral nutrients to treat mineral deficiencies.

CHAPTER 3: GENERAL MATERIALS AND METHODS

The materials and methods presented in this section are general, and the detailed methodologies are available within each of the manuscripts embedded herein.

3.1. Description of the Study Area

The survey assessment and observation on organic acids (OAs) availability, and its utilization practice in the commercial chicken farms was conducted in the urban and peri-urban commercial chicken farms of Bahir Dar and Gondar cities, while the two feeding trial (broiler and layer chickens) were conducted at the poultry farm of Bahir Dar University, Zenzelma Campus, Bahir Dar, Ethiopia.

3.2. Survey Design and Sampling Procedures

A cross-sectional survey design was employed to evaluate the availability and utilization of OA feed additives among commercial chicken farmers. All commercial chicken farmers who reared at least 50 commercial chickens on their farms were considered part of the target study population. A total of six, two sub-cities and one peri-urban area in Bahir Dar and three sub-cities in Gondar, were purposively selected. Then, a total of 211 commercial chicken farmers were selected for: 115 from Bahir Dar and 96 from Gondar. The sample size for both study areas was determined using Yamane's (1976) formula at a 5% precision level and a 95% confidence interval:

$$n = \frac{N}{1 + N(e)^2}$$

Where: n = the desired sample size, N = the population size, and e = the desired level of statistical precision (0.05).

3.3. Experimental Chickens and Management

For the second study (Broiler Experiment), a total of 280 one-day-old Cobb500 broiler chicks were obtained from ELRE private farm in Debre-Zeit, Ethiopia. The chicks were randomly assigned to 5 treatment groups, consisting of 56 chicks per treatment, and further replicated 4 times with 14 chicks per replicate. For the third study (Layer Experiment), a total of 260 layer hens obtained from poultry farm of Bahir Dar University. Layer chickens with an average body weight of $1609.28 \pm 18.45\text{g}$ were randomly assigned to 5 treatments, consisting of 52 chicks per treatment, and 13 layers chickens per replicate. Mash-type commercial broiler feed was purchased from Albar Trading Plc., Addis Ababa, Ethiopia. While a mash-type commercial layer feed was purchased from MAS animal feed factory, Bahir Dar, Ethiopia. Citric and malic acids were purchased from a local supplier (Global Chemicals Plc.), Bahir Dar, Ethiopia and oxytetracycline was obtained from a veterinary drug supplier in Bahir Dar, Ethiopia.

For both broiler and layer feeding evaluations, five experimental treatments were applied: a basal diet without supplementation (T1); a diet with oxytetracycline at 60 mg/kg for broilers and 30 mg/kg for layers (T2); a diet with 16 g/kg citric acid (T3); a diet with 8 g/kg malic acid (T4); and a diet combining 8 g/kg citric acid and 4 g/kg malic acid (T5). Oxytetracycline and organic acid levels in this study were selected based on EFSA guidelines and previously reported effective dosages for poultry performance (Koutsoumanis *et al.*, 2021; Ali & Hassan, 2010; Bhatt & Sharma, 2012; Qiu *et al.*, 2022).

3.4. Data collection

3.4.1. Survey data collection

A semi-structured questionnaire was designed and administered to collect quantitative and qualitative data from commercial chicken farmers. A total of six focus group discussions were conducted, three in Bahir Dar and three in Gondar, involving 8 to 12 participants in each discussion, all of whom were well-educated and experienced commercial chicken producers. In addition, for acquiring in-depth information about the

availability and utilization practices of OAs feed additives, key informant interviews were held with animal science experts, feed industry managers, animal nutritionists and importers/exporters. Furthermore, direct market observation was employed to record the type, sources, and possible availability of OAs in Ethiopia. Secondary data was gathered through desk reviews of Ethiopian Customs Authority and Ethiopian Agricultural Authority national reports, regulatory documents, websites, and import and export databases. Additionally, direct market observation was conducted to assess the availability of OAs in Ethiopia.

3.4.2. On-station research data collection

3.4.2.1. Evaluation of dietary organic acids supplementation on broiler chicken performances

The experimental chickens were weighed individually on the first day of the commencement of the experiment (40.517 ± 0.414), and then the live weight of the broilers was measured weekly every morning at 6:00 AM before the feed was offered via an electronic balance with a 40 kg weighing capacity and an accuracy of 0.001. Data on daily amounts of offered and refused feed were collected throughout the experimental period. Then, the average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and survival rate (SR) of broiler chickens were calculated by using standard formulas. At the end of the feeding trial, 8 broilers per treatment were randomly selected for carcass evaluation.

3.4.2.2. Effect of organic acids on egg-laying hen performances

The experimental layer hens were weighed before the commencement of the actual experiment and the average was calculated and recorded as initial weight, and at the end of the experiment the final weight was measured. Then, the average body weight change for each replicate was calculated by subtracting the average initial weight from the average final weight and dividing by the number of layers. Feed intake was calculated as a difference of

feed offered and refused. Then mean daily feed intake was calculated by dividing daily feed intake by the number of hens multiplied by the experimental period. By using different standard formulas and data records, the laying rate, hen-day egg production (HDEP), the average egg weight (AEW), average egg mass (EM), and FCR was obtained. The external and internal egg quality evaluations were conducted in every two weeks.

3.4.2.3. Economic evaluations of organic acids in broiler and layer chicken production

Partial budget analysis was performed to evaluate the economic viability of organic acids. The economic parameters were calculated by analyzing the commercial carcass weight of a single broiler chicken and the egg production performance of a single-layer hen. The total fixed cost (TFC) includes experimental house preparations and equipment costs, while the total variable cost (TVC) includes management, feed and feed additives costs.

Total return (TR) was calculated from the sale price of one kilogram of carcass weight (Study 2) and sale price of single egg (Study 3) in the local market. The net profit (NP) was calculated by subtracting TC from TR. The benefit-cost ratio (BCR) was estimated as the ratio of TR to TC, and the profitability percentage (P.P.) was the percentage of NP divided by TC. The net profit margin (NPM) was calculated by dividing the NP by the TR, and the obtained result was multiplied by 100. The added costs were calculated as the ratio of the additive cost divided by the TC and TVC multiplied by 100. Similarly, the ratio of the EE of the OA groups to that of the control groups was used to determine the relative economic efficiency (REE) of each dietary supplement of OAs.

3.5. Data analysis

For all the survey data collected from the assessment of OAs availability and its utilization practice in commercial poultry production (Chapter 4), a descriptive statistic, mean, percentage values and tabular presentations were employed. For the second and third studies, the collected data were subjected to one-way ANOVA using the general linear model (GLM) procedure of SAS (Version 9.2, 2002). Significant differences between treatment means were determined via Tukey's HSD test at a significance level of $P < 0.05$.

The model used for statistical analysis for Chapter 5 and Chapter 6 was: $Y_{ij} = \mu + T_i + e_{ij}$, where, Y_{ij} is the response variable, μ is the overall mean, T_i is the treatment effect (of the i th treatment), and e_{ij} is the random error.

CHAPTER 4: ASSESSMENT OF ORGANIC ACIDS AVAILABILITY, AND ITS UTILIZATION PRACTICE IN URBAN AND PERI-URBAN COMMERCIAL POULTRY FARMS OF BAHIR DAR AND GONDAR CITIES, NORTHWEST ETHIOPIA

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Abstract

The use of antibiotics in livestock production, particularly in poultry, has been increasingly restricted due to concerns over antimicrobial resistance, creating a need for alternative feed additives. This study assessed the availability of organic acids (OAs) and their utilization in commercial chicken farms in the cities of Bahir Dar and Gondar, Northwest Ethiopia. A cross-sectional survey design was employed, using both quantitative and qualitative approaches. Data were collected from 211 commercial chicken farms through a semi-structured questionnaire and direct observations, complemented by focus group discussions, key informant interviews, and a review of secondary data. Quantitative data were analyzed using descriptive statistics, while qualitative data were thematically analyzed to support the quantitative findings. The results show that all surveyed commercial chicken producers were unaware of OA feed additives and therefore did not use them, despite the availability of several OAs for industrial purposes in Ethiopia. These findings highlight a substantial need for information among poultry farmers regarding the role, benefits, and application of OA feed additives. Therefore, strengthening extension services and training is essential to improve awareness, encourage adoption, and enhance poultry production.

Keywords: Antibiotics, Chickens, Feed additives, Organic acids, Utilization practice

4.1. Introduction

Poultry is the most widely kept species and plays a vital role in meeting global food demands. Chickens, the most dominant poultry species, are a primary source of meat and egg production, providing a substantial share of animal protein for human consumption worldwide, representing 89% of chicken meat and 92% of chicken eggs (Erdaw, 2022). Chicken production is among the major subsectors of the livestock industry in Ethiopia, which has significantly contributed to reducing malnutrition, poverty alleviation, and encouraging economic progress in poor households in Ethiopia (Tirfie, 2023). The Ethiopian Ministry of Agriculture has planned to increase commercial egg production from 189 million in 2019/20 to 568 million by 2029/30 at 20% per annum and to increase poultry meat production from 30,000 to 2,415,000 tonnes over the same period (Fekadu *et al.*, 2022). This rapid expansion of commercial poultry production will create a greater demand for quality and sufficient feed supplies, requiring improvements in chicken health, feed quality, and nutrient use efficiency. However, among the most important challenges facing Ethiopia's poultry sector is the availability of inexpensive and good-quality feed (Woldegiorgiss *et al.*, 2016; Negash, 2022).

To meet the huge demand for chicken products, chicken production and productivity should be improved by improving feed quality, nutrient utilization, and efficiency. High production rates and feed conversion efficiency may be achieved using specific feed additives to improve feed quality, enhance nutrient utilization, and enhance overall performance in poultry (Aikpitanyi *et al.*, 2019). For many years, antibiotic feed additives have been widely used in poultry production (Yadav *et al.*, 2016; Abd, 2024), and these substances have shown promise in reducing pathogenic bacteria, improving gut microbiota, and enhancing health, growth performance, and feed efficiency in broilers and layer chickens (Gadde *et al.*, 2017; Markowiak & Śliżewska, 2018). However, owing to concerns over antibiotic resistance, several regions, including the European Union in 2006 (Europa, 2005) and the United States in 2017 (AccessScience, 2018), have banned the use of antibiotics as growth promoters (AGPs) in poultry feed. In response of banning AGPs, researchers have explored alternatives to antibiotics, such as probiotics, prebiotics, enzymes, phytogenics, and OAs, all of which have shown potential as replacements (AM *et al.*, 2017).

Among these, OAs have gained significant attention due to their antibacterial properties and nutritional benefits (Hajati, 2018), and approved by most EU countries for use of OAs and their salts as safe feed additives in animal nutrition (Sheikh Adil *et al.*, 2010).

Organic acids, naturally occurring compounds with the structure R-COOH (Ji *et al.*, 2023), are widely used as preservatives in food and feed owing to their antimicrobial properties. Beyond their role as feed additives, they can be used as multiple purposes in industries, including food, chemicals, agriculture, cosmetics, and cleaning, which are common practices in Ethiopia (Alemayehu *et al.*, 2024). In poultry, short-chain fatty acids (C1-C7), which consist of simple monocarboxylic acids such as formic, acetic, propionic, and butyric acids, and other carboxylic acids such as lactic, malic, tartaric, fumaric, and citric acids, and their salts, are commonly used (Haq *et al.*, 2017). Studies have shown that supplementing broiler diets with OAs improves growth performance, health, and economic efficiency (Kim *et al.*, 2015; AM *et al.*, 2017), whereas in layer hens, it supports increased body weight, sustained egg production, and better economic returns (Soltan, 2008). They are added to feed or water to prevent intestinal diseases, enhance immunity, improve nutrient digestibility, and feed efficiency, and promote chicken growth (Al-Kuhla *et al.*, 2023).

In developing countries, such as Ethiopia, antibiotics are commonly added to chicken feed to enhance productivity and growth; however, the weak enforcement of regulations has led to widespread misuse among commercial chicken farmers aiming to maintain production levels (Woldegiorgiss *et al.*, 2017; Mezene *et al.*, 2020). With the country's growing demand for chicken products, this reliance on antibiotics is expected to continue unless alternative solutions, such as OA feed additives, are introduced and evaluated in poultry nutrition. Although OAs have various industrial applications and potential benefits in poultry nutrition, there is limited information on their availability and farmers' utilization practices as antibiotic alternatives in chicken production. Assessing the availability of OAs and understanding current utilization practices among chicken farmers is essential to identify gaps and promote their adoption for free or reduced-antibiotic chicken production systems in the country. This assessment provides valuable baseline data to support future research and development activities for replacing antibiotics with OAs, fostering sustainable poultry production. Therefore, this study aimed to evaluate the

availability and utilization practices of OAs in urban and peri-urban commercial poultry farms in Northwest Ethiopia.

4.2. Materials and Methods

4.2.1. Description of study areas

A survey study was conducted from December 2022 to February 2023 to assess the availability of organic acids and their utilization practices in urban and peri-urban commercial poultry farms in the cities of Bahir Dar and Gondar in the Amhara Region, Northwest Ethiopia. The two study sites for the field survey assessment were selected based on chicken production potential (CSA, 2021). Bahir Dar is the capital city of the Amhara National Regional State, located 565 km northwest of Addis Ababa at geographical coordinates of 11°38'N latitude and 37°10'E longitude and an average elevation of 1,830 m above sea level. It receives an average annual rainfall ranging from 850 mm to 1,250 mm and experiences average daily temperatures between 10°C and 32°C. Administratively, Bahir Dar is divided into six sub-city administrations and 13 peri-urban kebeles. Gondar Town is located in the Amhara Regional State, in the Northwestern highlands of Ethiopia, 727 km from Addis Ababa and 120 km from the main city of the Amhara Region (Bahir Dar City). Gondar city is located at the geographical coordinates of 12° 36' N latitude and 37° 28' E longitude, with an average elevation of 2133 m above sea level and a total area of 192.3 km². It receives an average annual rainfall of approximately 104.26 mm, with daily temperatures ranging from 13.50°C to 21.40°C. The town serves as the administrative center of the Central Gondar Zone and is administratively organized into six sub-cities and eleven peri-urban kebeles.

4.2.2. Study designs

A cross-sectional survey design was employed to evaluate the availability and utilization of OA feed additives among commercial chicken farmers. The study used both

quantitative and qualitative approach, incorporating household surveys, focus group discussions, key informant interviews, and a review of secondary data.

4.2.3. Study population and sampling procedure

All commercial chicken farmers who reared at least 50 commercial chickens on their farms were considered part of the target study population. Lists of commercial operations were obtained from the Livestock and Fishery Development Offices in both study areas. Based on this information, three study locations, two sub-cities and one peri-urban area, in Bahir Dar and three sub-cities in Gondar were purposively selected. Farms were then categorized according to Woldegiorgiss et al. (2017) based on flock size: large-scale poultry farms have more than 10,000 birds, medium-scale farms house 1,000 to 10,000 birds, and small-scale farms hold 50 to 1,000 birds. A total of 211 commercial chicken farmers were selected for: 115 from Bahir Dar and 96 from Gondar. The sample size for both study areas was determined using Yamane's (1976) formula at a 5% precision level and a 95% confidence interval.

$$n = \frac{N}{1 + N(e)^2}$$

Where, n = the desired sample size,

N = the population size,

e = the desired level of statistical precision (0.05).

4.2.3. Data collection techniques

Primary data were collected using semi-structured questionnaires. The questionnaire captured information about household characteristics, chicken farm characteristics and management practices; trends in antibiotic utilization practices, knowledge of antibiotic resistance and antibiotic alternative utilization practices, and chicken farmers' information on OAs were included in the questionnaire. To enrich the data, direct observations were made in commercial chicken farms regarding the types of feed additives used and the constraints related to chicken production. Additionally, qualitative data were collected through focus group discussions and key informant interviews to gain insights into OA

availability and its current use. A total of six focus group discussions were conducted, three in Bahir Dar and three in Gondar, involving 8 to 12 participants in each discussion, all of whom were well-educated and experienced commercial chicken producers. In addition, for acquiring in-depth information about the availability and utilization practices of OAs feed additives, key informant interviews were held with animal science experts, feed industry managers, animal nutritionists and importers/exporters. Secondary data were gathered through desk reviews of Ethiopian Customs Authority and Ethiopian Agricultural Authority reports, regulatory documents, websites, and import and export databases. Additionally, direct market observation was conducted to assess the availability of OAs in Ethiopia.

4.2.4. Statistical analysis

All information gathered from the survey and document assessments was organized, categorized, and coded in an MS Excel sheet. Descriptive statistics were used to analyze the questionnaire data using statistical software (SPSS v.16). Percentages, means, frequencies, and tabular presentations were employed to summarize the main outcome variables, including household characteristics; chicken farm characteristics and management practices; antibiotic utilization practices; and farmers' knowledge of the availability and use of OA feed additives in poultry production. Qualitative data from focus group discussions and key informant interviews were transcribed and analyzed thematically to support the quantitative findings. Data generated from the document review were then summarized and presented using percentages, tables, and graphs, highlighting key variables such as Ethiopia's OA import trends, types, and sources.

4.3. Results

4.3.1. Socioeconomic characteristics of farm respondents

The demographic characteristic of the respondents of this study is presented in Table 4.1. The current study revealed that majority of (74.4%) chicken farms managed by male, while only 25.5% of chicken farms were managed by female. On age demographics, 56.9%

respondents were between the ages of 31 and 45, followed by 35.1% and 8% respondents were 15 to 30 and of 46 to 60 years of age, respectively. About 38.4% of the respondents reported having completed a diploma level of education, followed by 22.3%, 19.7 and 17.5% having a degree and above, grades 1-8 and grades 9-12, respectively, while the rest other 2.4% who were unable to read or write, 66.8% of the respondents had 5 to 10 years of experience, and 25.6% had an experience of less than 5 years, while the rest smaller proportion (7.6%) had more than 10 years of experience. The majority (91.9%) of chicken farms in the study areas were managed by farm owners, while 8.1% were employed.

Table 4:1 Demographic characteristics of the respondents in the chicken farms

Variables	Bahir Dar (n=115)		Gondar (n=96)		Overall (n=211)	
	Freq.	%	Freq.	%	Freq.	%
Sex						
Male	83	72.2	74	77.1	157	74.4
Female	32	27.8	22	22.9	54	25.6
Age category						
15-30 years	41	35.7	33	34.4	66	35.1
31- 45 years	63	54.8	57	59.4	134	56.9
46-60 years	11	9.6	6	6.3	11	8.1
Education level						
Diploma (TVT)	28	24.3	19	19.8	47	22.3
Grade 9-12	43	37.4	38	39.6	81	38.4
Grade 1-8	19	16.5	18	18.8	37	17.5
Cannot write & read	21	18.3	20	20.8	41	19.4
Cannot write & read	4	3.5	1	1	5	2.4
Experience in chicken farming						
More than 10 years	13	11.3	3	3.1	16	7.6
5-10 years	79	68.7	62	64.6	141	66.8
Less than 5 years	23	20	31	32.3	54	25.6
Responsibility of the respondent in chicken farms						
Farm owner	102	88.7	92	95.8	194	91.9
Hired keeper	13	11.3	4	4.2	17	8.1

4.3.2. Characteristics and management of commercial chicken farms

The characteristics and management practices of the poultry farms are presented in Table 4.2. The study revealed that 100% of respondents used commercial exotic chicken breeds, with 63% using potential egg-laying, 36.5% dual-purpose, and 0.5% broiler breeds. Moreover, the current study revealed that 89.1% of the sampled farms had 50-1000 chickens and the remaining 10.9% had 1001-10,000 chickens as small- and medium-scale commercial chicken production systems, respectively. Regarding housing system, 71.1% of the respondents reared their chickens on deep-litter with concrete floor, while 28.9% used deep litter-without concrete floor. All respondents (100%) used commercial feed. Of them, 94.8% obtained feed from wholesalers and retailers, whereas 5.2% bought it directly from commercial feed factories. Nearly 74.4% of the respondents provided feed for their chickens two times a day, whereas the remaining 23.2% and 2.4% of the respondents provided feed for their chickens three and one times a day, respectively. However, 52.1% of the respondents had received training on feed quality and feeding strategy, whereas 47.9% had not been adequately trained. Similarly, only 42.7% of respondents had received training regarding biosecurity in chicken farms, while 57.3% had not received any training in this regard.

Table 4:2 Characteristics of the commercial chicken farms

Variables	Bahir Dar (n= 115)		Gondar (n= 96)		Overall (n= 211)	
	Freq.	%	Freq.	%	Freq.	%
Class of breed						
Exotic	115	100	96	100	211	100
Number of chickens						
50 to 1,000	97	84.3	91	94.8	188	89.1
1,001 to 10,000	18	15.7	5	5.2	23	10.9
Production type						
Layers	73	63.5	60	62.5	133	63
Broilers	1	0.9			1	0.5
Dual purpose	41	35.7	36	37.5	77	36.5
Housing type						
Deep-liter with concrete floor	86	74.8	64	66.7	150	71.1
Deep-liter without concrete floor	29	25.2	32	33.3	61	28.9
Type of feed offered to your chicken						
Commercial chicken feed	115	100	96	100	211	100
Source of feed for your chicken						
Feed industries	7	6.1	4	4.2	11	5.2
Wholesalers and retailers	108	93.9	92	95.8	200	94.8
Feeding frequency per day						
One times	2	1.7	3	3.1	5	2.4
Two times	86	74.8	71	74	157	74.4
Three times	27	23.5	22	22.9	49	23.2
Have you received any training on feed quality and feeding techniques?						
Yes	57	49.6	53	55.2	110	52.1
No	58	50.4	43	44.8	101	47.9
Do you have training related to biosecurity of chicken farm?						
Yes	63	54.8	27	28.1	90	42.7
No	52	45.2	69	71.9	121	57.3

4.3.3. The use of antibiotic in commercial chicken farms

The result of this assessment survey study revealed that all chicken farms (100%) used antibiotics for the purpose of treatment, prevention of disease, and growth promotion (Table 4.3). The most commonly used antibiotics were oxytetracycline 100%, Enrofloxacin 70.6%, Tylosin 43.6%, Sulfonamides + Trimethoprim 67.8%, and doxycycline 25.1% (Figure 4.1). These antibiotics are often utilized as individual agents and in combinations on commercial poultry farms for multifaceted goals, including treatment, prevention, and growth promotion, as shown in Figure 4.2. The dose level of antibiotics use was based on

prescribed by animal health professions 56.4% and based on leaflet information 37.9%, while the remaining 5.7% provided with assumption. More than 91.9% of the respondents administered antibiotics to their chickens without the supervision of veterinarians, while 8.1% farms assisted by animal health professionals. Moreover, 86.7 % of respondents did not have any information on antibiotic drug resistance, this absence of knowledge leads to the misuse of antibiotics in chicken production; consequently, the harmful impacts of antibiotic medicine resistance unlikely promoted and transferred to human with meat and egg and environment through faeces, litters and other waste disposals.

Table 4:3: Antibiotic utilization in commercial chicken farms

Variables	Bahir Dar (n= 115)		Gondar (n= 96)		Overall (n= 211)	
	Freq.	%	Freq.	%	Freq.	%
Do you use antibiotics for chicken health?						
Yes	115	100	96	100	211	100
Who recommend to the use antibiotics in your farm for intended use?						
Veterinary professionals	79	68.7	63	65.6	142	67.3
Friends that have chicken farms	15	13.0	12	12.5	27	12.8
Self-prescriptions	21	18.3	21	21.9	42	19.9
How determined the antibiotics dosage for use in chicken on your farms?						
As prescribed by animal health professions	66	57.4	53	55.2	119	56.4
Based on leaflet information	43	37.4	37	38.5	80	37.9
With assumption	6	5.2	6	6.3	12	5.7
Who administer antibiotics to your chicken?						
Animal health professionals	10	8.7	7	7.3	17	8.1
Self-administer	105	91.3	89	92.7	194	91.9
Do you have information about antibiotic drug resistance?						
Yes	16	13.9	12	12.5	28	13.3
No	99	86.1	84	87.5	183	86.7

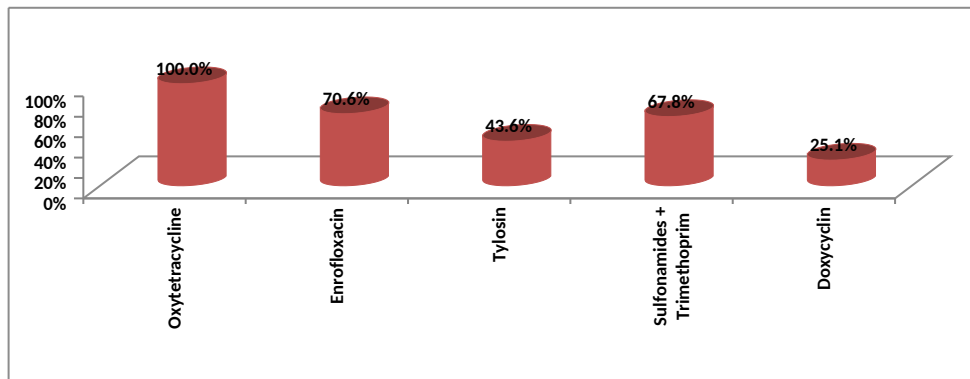


Figure 4:1 Types of antibiotics used in commercial chicken farms

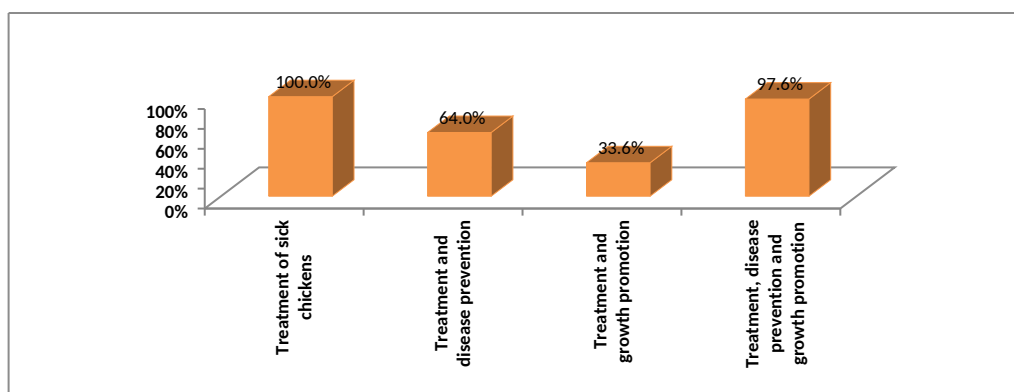


Figure 4:2 Purposes of antibiotics used in the commercial chicken farms

4.3.4. Utilization practices of antibiotic alternative in chicken farms

The utilization practice of antibiotic alternatives in the study area is presented in Table 4.8. The majority of the respondents (84.4%) revealed that different locally available medical plants, herbs and spices has been utilized for their chickens for the purpose of disease prevention, treatment of sick birds and to prompt production. Moreover, neem (*Azadirachta indica*), garlic (*Allium sativum*), grawa (*Vernonia amygdalina*), ginger (*Zingiber officinale*), and chile (*Capsicum annum*) has been frequently cited by 69.7, 58.3, 49.8, 43.6 and 37.4 percent of study participants, respectively, used for their chicken. Additionally, tena'adam (*Ruta chalpinesis*) and feto (*Lepidium sativum*) were indicated by 7.3 and 6.6 percent of respondents, while some of the study participant identified red onion (*Allium cepa*), and lemon (*Citrus Limon*) are among an option for antibiotics alternative for their chickens. These antibiotics alternatives provided to chickens as directly feeding (82.9%), mixing with feed (6.2%), adding in water (3.3%), and directly oral administration (1.9%). Poultry farmers' knowledge about these antibiotic alternatives was mostly from their own traditional knowledge (48.3%), followed by friends who have chicken farms (19.4%), extension officers (16.6%) and veterinary professionals (10.4%). All (100%) of the respondents, though, did not utilize OAs in their chicken farm, and no respondent was aware of dietary OA feed additives. Instead of OAs, different antibiotics widely used in poultry farms to improve productivity, improve growth rate, treat sick chickens, and prevent disease.

Table 4:4 Availability and Utilization practices of Antibiotic Alternative in chicken farms

Variables	Bahir Dar (n= 115)		Gondar (n= 96)		Overall (n= 211)	
	Freq.	%	Freq.	%	Freq.	%
Are there any local sources you use instead of antibiotics when your chickens get sick?						
Yes	101	87.8	77	80.2	178	84.4
No	14	12.2	19	19.8	33	15.6
If yes, what are those alternatives						
Neem (<i>Azadirachta indica</i>)	79	68.7	68	70.8	147	69.7
Grawa (<i>Vernonia amygdalina</i>)	31	27.0	20	20.8	51	24.2
Garlic (<i>Allium sativum</i>)	65	56.5	58	60.4	123	58.3
Red onion (<i>Allium cepa</i>)	2	1.7	4	4.2	6	5.28
Chile (<i>Capsicum annuum</i>)	17	14.8	16	16.7	33	15.6
Ginger (<i>Zingiber officinale</i>)	23	20.0	14	14.6	37	17.5
Feto (<i>Lepidium sativum</i>)	7	6.1	7	7.3	14	6.63
Tena'adam (<i>Ruta chalpinesis</i>)	3	2.6	6	6.3	9	7.27
Lemon (<i>Citrus limon</i>)	7	6.1	4	4.2	11	5.21
How to provide those alternatives for your chickens						
In feed	6	5.2	5	5.2	11	5.2
In water	4	3.5	3	3.1	7	3.3
Directly feeding	89	77.4	67	69.8	156	73.9
Dropping into mouth	2	1.7	2	2.1	4	1.9
For what purpose you provide those alternatives to your chickens?						
For treatments of sick chickens	102	88.7	76	79.2	178	84.4
For prevention of disease	67	58.3	51	53.1	118	55.9
For growth promotion	5	4.3	4	4.2	9	4.3
For maximized production	7	6.1	4	4.2	11	5.2
Prevention of cannibalism	32	27.8	29	30.2	61	28.9
Who recommend using those alternatives to your chickens?						
Indigenous knowledge	56	48.7	46	47.9	102	48.3
Friends that have chicken farm	17	14.8	10	10.4	27	12.8
Extension workers	15	13	13	13.5	28	13.3
Veterinary professionals	13	11.3	8	8.3	21	10
Do you use organic acid feed additives for your chicken?						
No	115	100	96	100	211	100
Why not giving organic acid feed additives for your chickens						
Lack of awareness	115	100	96	100	211	100

4.3.5. Availability of organic acids in Ethiopia

Different OAs are available in Ethiopia (Table 4.5). Among others, formic, acetic, propionic, butyric, citric, lactic, fumeric, tartaric and gluconic acids are potentially available as pure, salts and esters form. According to information gathered from Ethiopian Customs Authority, currently, organic acids are imported into the country by chemical importers for different purposes including preservative and flavoring agent in the food, oil, beverage, chemical, textile, plastics, cosmetics, pharmaceutical, and other industrial sectors.

Table 4:5 Type and import volume of organic acids in Ethiopia

Organic acids	Import Volume (Ton)			
	2021	2022	2023	2024
Formic acid	260.6	102.8	325.7	400.6
Acetic acid	124.7	458.4	403.9	509.7
Propionic acid, its salts and esters	0.8	0.5	1.6	2.0
Butyric acid, their salts and esters	36.2	29.7	60.7	77.9
Fumaric acid	63.3	206.0	19.1	37.5
Malic acid	1.1	5.1	1.2	4.0
Citric acid	736.3	1466.4	1532.7	1655.3
Lactic acid, their salts and esters	27.4	20.6	28.9	3.2
Tartaric acid	0.6	0.0	3.1	2.9
Gluconic acid, their salts and esters	217.1	657.2	1125.5	1406.9

Source: Ethiopian Customs Authority (2021 - 2024)

Based on the data obtained from Ethiopian Customs Authority, import volume of OAs between the years 2021 and 2024 in the country has shown increased trend (Figure 4.3). Furthermore, the information gathered from Ethiopian Custom Authority, China is being the leading source country for Ethiopian OAs, followed by India, Germany, United States, Turkey, and other countries (Table 4.6).

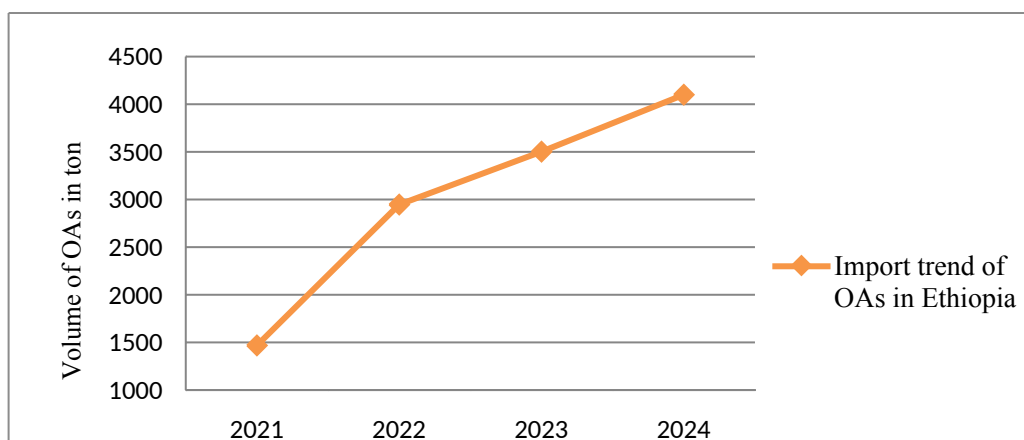


Figure 4:3 The Ethiopian import trend of organic acid from 2021 to 2024 per ton

Table 4:6 Sources of organic acids for Ethiopian local market

Country	Import volume (%) from 2021 to 2024			
	2021	2022	2023	2024
China	80.91	89.7	88.94	93.78
India	3.32	5.10	5.31	2.62
Germany	0.56	0.26	2.20	1.83
United States	1.02	1.6	0.92	0.18
Turkey	6.56	1.64	2.06	0.22
Other Countries'	7.84	1.74	0.58	1.41

Source: Ethiopian Customs Authority (2021-2024)

4.4. Discussion

4.4.1. Socioeconomic characteristics of respondents

Majorities of the commercial poultry farms operated in the study areas are owned by male farmers. Similar results by Tsegaye *et al.*, (2023) reported that 69.7% of the commercial farms are owned by males. In terms of age distribution, the majority of respondents were aged between 31- 45 years. The current result was similar to the report of Mezene *et al.*, (2020) who reported the majority of the producers were between 31 and 45 years old. Data on the educational attainment of respondents in the study area revealed that the majority of respondents completed a diploma level, followed by a degree or higher qualification. The highest involvement of this educational status might be due to the absence

of job opportunities in the government and private sectors that could create opportunities for graduated manpower in the country. Almost all commercial poultry farms were managed by the farm owners. This result is in line with (Mezene *et al.*, 2020) who reported that 88.9 % of poultry farming activities were carried out by the farm owners. Similarly, above half of the respondents had between 5 - and 10 years of experience in operating commercial poultry farming. On the contrary, Litigebew *et al* (2021) reported that about 66.7% of poultry farmers had less than one year of experience; on the other side, Mezene *et al.* (2020) reported that the majority of respondents had over 10 years of experience in poultry production.

4.4.2. Characteristics of commercial poultry farms

Under small and medium-sized production systems, all of the chicken farmers maintained exotic chicken breeds on their farms. However, the Bovans Brown breed predominated, as this breed is readily available and very desirable for egg production, and the majority of the commercial farms in the study areas were used for commercial egg production. Similar findings revealed by other researchers support the current finding. Bovans Brown, an egg layer, was the most popular exotic chicken breed in Bahir Dar City's small-scale production system (Litigebew *et al.*, 2021). In consistent with Mezene *et al.* (2020), in the current research locations, the majority of commercial chickens were housed in deep-litter with concrete floor sheds. All commercial chicken farms fed commercial feed for their chickens; just a few farmers bought directly from feed companies, while the majority bought from wholesalers and retailers. Similarly previous findings shows that majority of commercial chicken farmers proved commercial feed to their chicks (Tadesse *et al.*, 2017; Litigebew *et al.*, 2021), and the majority of the farmers offered feed for their chickens three times per day followed by two and one times per day, which is in line with Tadesse *et al.* (2017).

Majority of commercial chicken farmers did not receive any training on the quality and feeding strategies of chickens, while these knowledge gaps leads the chicken farmers to inefficient feeding practice and counted as a hindering factor for maximizing the overall production performance and profit efficiency of commercial chicken farms. Although,

biosecurity is one of the potential components for enhancing the productivity and health of commercial chicken production; disputed, the majority of respondents in the current study areas had not received any training about poultry farm biosecurity. In consistent with the current finding, Tsegaye *et al.* (2023) noted that above 68.8% of farm owners in East Showa and Arsi Zones have not gotten any training on the concept of biosecurity. This could be significant contribution for disease prevalence and spreads from one farm to another. Moreover, the majority of commercial poultry farm sheds built by the government did not take into account the proper recommended poultry houses (Litigebew *et al.*, 2021), due to lack of sufficient space chicken production sheds were constructed without or with minimal isolation pens prepared inside the main farms (Yitbarek *et al.*, 2016), this leads to the spread of infectious diseases that leads to the utilizations of antibiotics. Moreover, the prevalence of chicken diseases obviously known to raise the use of antibiotics for treatment and preventions purposes; consequently, the chicken farmers forced to frequent utilization of antibiotics in their chicken farms which leads and unfavorable conditions for the emergence of microbial resistant strains.

4.4.3. The use of antibiotics in commercial chicken farms

All commercial chicken producers employed antibiotics, both alone and in combination, to treat sick chickens, prevent disease, promote growth, and to maximize egg output. According to similar findings by Ragassa & Berhanu, (2023), the majority of respondents use antibiotics for sick animals, but some also use them to prevent infections in healthy animals and to promote growth or fattening. In central Ethiopia, all farms utilized one or more antibiotics for therapeutic purposes (100%), preventive purposes (56%), and to a lesser amount for growth promotion, according to the current study, which is consistent with the findings of Mezene *et al.* (2020). Among others, oxytetracycline, enrofloxacin, tylosin, sulfonamides + trimethoprim and doxycycline are the most frequently used antibiotics in chicken production, and more than half of the farmers administered these antibiotics to their chickens based on the recommendation of veterinary drug shops, while the remaining 32.7% chicken farmers were used without any professional prescription based on their friends and self-prescriptions. Similar report noted by Mezene *et al.* (2020), 61.9%

of the chicken farmers used antibiotics for their chickens based on veterinary professionals recommendation the remaining 38.1% used antibiotics without prescription based on their own and their friends past experiences. Although, the dose level of antibiotics use was based on prescribed by animal health professions and leaflet information, same are provided with assumption. Moreover, the majority of the chicken farmers administrated antibiotics to chickens operated by the farm owners or poultry herders which could be contributed the development of drug resistance bacteria. Similar to the current finding, a significant portion of farmers use antibiotics without proper guidance, often not adhering to recommended dosages or withdrawal periods, which increases the risk of antibiotic microbial (Gemedo *et al.*, 2020; Tufa *et al.*, 2023).

In the present study, only fewer chicken farmers had informed about the harmful impacts of antibiotic resistance, while the rest majorities had not any information. In line with the current finding, several studies reported that majority of chicken farmers in Ethiopia gave their hens antibiotics to promote growth and cure and prevent infections, while a widespread lack of knowledge among chicken farmers regarding antibiotic resistance (Gemedo *et al.*, 2020; Geta & Kibret, 2021; Woldegeorgis *et al.*, 2023). According to Tufa *et al.* (2023), an overwhelming 94% of livestock farmers, including those involved in poultry farming in Ethiopia, lack sufficient knowledge about antimicrobial usage, their residues, and resistance issues. This misuse of antibiotics could play a major role in fostering antibiotic resistance and leaving residues in meat and eggs, which may be transferred to humans (Mezene *et al.*, 2020). Such circumstances encourage the spread of resistant microorganisms that can be easily transmitted between food-producing animals and humans (Roth *et al.*, 2019). The study's results underscore the importance of raising awareness among chicken farmers, enabling them to acquire knowledge through hands-on experience, extension services, and training.

4.4.4. Utilization practices of antibiotics alternatives in commercial chicken farms

Medical plants, herbs, and spices have been used in the commercial chicken farms for the purpose of treatment, prevention, growth promotions and relief chickens' stress. Neem (*Azadirachta indica*), garlic (*Allium sativum*), grawa (*Vernonia amygdalina*), ginger

(*Zingiber officinale*), chile (*Capsicum annum*), lemon (*Citrus limon*) and others are among an option for antibiotics alternative which has being utilized by chicken farmers for the purpose of treatment, prevention, growth promotions, relief cannibalism. These alternatives were administered to chickens through direct feeding, with mixing feed and water, and dropping directly to mouth. Chicken farmers utilized these antibiotic substitutes from their indigenous knowledge, experience sharing from friends who had chicken farm, veterinary and development agents. Comparable results were reported by Lagu and Kayanja (2010), poultry farmers employ traditional medicine, including herbal drugs like Aloe vera and *Capsicum annum*, to combat diseases and parasites in their poultry. Moreover, certain herbs and spices have beneficial bioactivities for poultry production, including antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant properties, improving performance and product quality (Rafeeq *et al.*, 2022; Pashaei *et al.*, 2024). Neem leaf meal enhances egg-laying potential and quality in laying hens, while garlic meal increases feed intake and extends egg lay compared to the control group (Gedamu *et al.*, 2024).

This study identified a significant information gap among commercial chicken farmers regarding the use of OAs as additives to feed and an efficient alternative to AGPs. This absence of practices to implement OAs in small and medium-sized commercial chicken farms in the study areas was attributed to limited awareness regarding the availability and beneficial roles of OA addition to chicken health, nutrient utilization, and production performances. Conversely, commercial chicken farmers frequently utilize antibiotics for therapeutic, prophylaxis, and growth promotion purposes in their chickens. Alternate ways to AGPs are more akin to the economic realities of the developing nations, to increase growth and limit infections in commercial poultry industries need to be explored (Kousar *et al.*, 2021). Among others, OAs are one option to replace antibiotics in chicken diets (Markowiak & Śliżewska, 2018). Organic acids may be a potential substitute for AGPs in the Ethiopian chicken industry, but further research is needed to explore their use on health, nutrient utilization efficiency, and production and economic performance of broiler and layer chickens. Therefore, this research highlights the urgent necessity to change paradigms to more sustainable chicken farming strategies.

4.4.5. Availability of organic acids in Ethiopia

Ethiopia imports different types of organic acids such as formic, acetic, propionic, butyric, lactic, malic, citric, fumaric, and others. These OAs serve various roles in Ethiopia, primarily as preservatives and flavoring agents in the food industry. They help inhibit microbial growth, enhancing flavors, and regulating acidity, making them essential in food processing and preservation. Ethiopia imports organic acid from the US, Saudi Arabia, China, India, Germany, and Kenya, among other nations; for various uses, primarily in the food processing, pharmaceutical, beverage, and agriculture sectors (Alemayehu *et al.*, 2024). Based on the Ethiopian Customs Authority, four years (2021 to 2024) average import data shows that China accounted for 88.32% of Ethiopia's total imports of organic acid, followed by India (4.08%), Turkey (2.29%), the United States (1.21%) and Germany (1.21%), and the rest 2.29 % imported from other countries. According to Ethiopian Customs Authority, Ethiopia produces a total of 91.63, 11.25 and 25.5 kg of acetic acid in 2022, 2023 and 2024 for domestic use. Organic acids are utilized in the majority of industries in Ethiopia, including the chemical, textile, plastics, cosmetics, pharmaceutical, food, oil, and beverage industries, as chemical reagents, fungicides, microbicides, pH regulators, drug production ingredients, and food additives. Organic acids are also used in large quantities as solvents in cleaning, cosmetics, culinary, and agricultural sectors (Diba *et al.*, 2015; Alemayehu *et al.*, 2024). Among others, fumaric acid, acetic acid, citric, lactic, formic, tartaric, malic, and gluconic acid are the main OAs utilized in various industries in the country. Based on OAs import data's registered from 2021 to 2024 by Ethiopian Customs Authority, has shown that an increased trend of import volume for the last four years, and this could continue due to the food and beverage industrial growth of Ethiopia, the import quantity of OAs and sustainable supply in the domestic market has been rising steadily over the past few years. The continues growth of industrialization can increase the import volume and stimulate local production of OAs, which results in an increased sustainable supply of different OAs in the local market of the country, which could a wonderful opportunity to utilized in the commercial poultry industry, particularly concerning antibiotic reduction strategies.

4.5. Conclusion and Recommendations

The research revealed that commercial chicken producers in the surveyed areas do not utilize organic acid feed additives, with all farmers (100%) reporting a lack of awareness about these acids and their use as feed additives. Although organic acids such as butyric, propionic, fumaric, sorbic, lactic, malic, tartaric, and citric acids are available in Ethiopia for industrial purposes, the study highlights the necessity of raising awareness to educate farmers on the role and benefits of organic acids in chicken production. By sharing experiences, providing extension services, and offering training, farmers' understanding can be enhanced, encouraging the adoption of these additives and leading to safer, more efficient, and sustainable poultry farming practices

CHAPTER 5: POTENTIAL OF DIETARY CITRIC AND MALIC ACID SUPPLEMENTATION ON BIOLOGICAL PERFORMANCE AND ECONOMIC EFFICIENCY BY REPLACING CONVENTIONAL ANTIBIOTICS IN COBB500 BROILER CHICKENS

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Potential of Dietary Citric and Malic Acid Supplementation on Biological Performance and Economic Efficiency by Replacing Conventional Antibiotics in Cobb500 Broiler Chickens

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Abstract

This study aimed to evaluate the synergistic effects of dietary citric and malic acids supplementation on biological performance and economic efficacy by replacing conventional antibiotics in cobb500 broiler chickens. A total of 280 one-day-old chicks were randomly assigned to five dietary treatments with four replicates in a completely randomized design. The dietary treatments included a basal diet without any additive (T1), 60 mg/kg oxytetracycline (T2), 16 g/kg citric acid (T3), 8 g/kg malic acid (T4), and a mixture of 8 g/kg citric acid and 4 g/kg malic acid (T5). At the end of the feeding trial, eight broiler birds per treatment were slaughtered for carcass evaluation and gut morphology. The results showed that the average daily weight gain differed significantly ($P < 0.05$) among treatments during both the starter and grower phases. Chicks receiving organic acid

supplementation achieved higher gains (61.98–64.30 g) than the T1 group (59.58 g) and performed similarly to the T2 group (64.06 g) across all phases. Organic acid-supplemented groups also exhibited better FCR. The highest dressed carcass yields were observed in T2 and T5 ($P<0.05$), and T5 showed superior relative economic efficiency. Overall, the citric-malic acid mixture proved to be an effective strategy for antibiotic alternatives, enhancing growth performance, carcass traits, and economic efficiency in Cobb500 broilers.

Keywords: Broiler chickens, Carcass, Citric acid, Economic efficiency, Malic acid, Performance

5.1. Introduction

The global demand for animal products is predicted to increase, particularly in developing countries such as Ethiopia. The broiler industry is an animal industry that produces meat for human consumption in Ethiopia. However, the broiler industry faces severe challenges, including rising feed costs, disease outbreaks, and customer concerns regarding food safety and antibiotic use. To maintain a sustainable profit in the broiler chicken industry in the country, optimal growth, and feed conversion are essential requirements, which can be achieved via the use of feed additives (Yadav & Jha, 2019). In the poultry industry, antibiotics have been used for a long time in chicken feed to promote growth, enhance feed efficiency, and guard against bacterial infection (Polycarpo *et al.*, 2017; Markowiak & Ślizewska, 2018). However, due to public health concerns regarding the administration of AGPs in feed, the emergence of microorganisms resistant to antibiotics, and the possible accumulation of residues in meat and egg products, many countries have banned the use of AGPs in chicken feed (Agbai *et al.*, 2020). However, the prohibition of AGP use in the poultry industry could have significant impacts, particularly in light of the expanding population and the subsequent poultry product demand. In response, alternative feed additives such as organic acids (OAs) have been introduced and tested with promising findings on positive effects on chicken performance and health; as they could replace the conventional but unwanted antibiotics practices (Xue *et al.*, 2023; Waghmare *et al.*, 2025).

Organic acids have been utilized for decades in the feed industry to preserve feed and as a substitute for growth promoters to protect poultry birds from disease and enhance

performance (Kumar *et al.*, 2021). Previous studies have reported that OAs improved growth performance (Dai *et al.*, 2021), immune and antioxidant properties (Abudabos *et al.*, 2017), intestinal function (Yang *et al.*, 2019), regulation of intestinal microbiota, and economic efficiency (Mahfuz, *et al.*, 2021) of broiler chickens. However, in broiler production, OAs have shown promise as potential alternatives to antibiotics; there are factors such as their mode of action, dose level, tolerance of the intestinal microflora, chicken health, and diet composition, which could explain why earlier findings were inconsistent (Kumar *et al.*, 2021; Waghmare *et al.*, 2025).

Recent researches have demonstrated that combining OAs improve intestinal health, performance, and nutrient digestion and absorption (Mahfuz, *et al.*, 2021; Waghmare *et al.*, 2025) compared to applying them alone because of their synergetic benefits on. When it comes to a specific OAs, citric and malic acids have shown promise as potential substitutes for AGPs (Xue *et al.*, 2023); due to their ability to improve gut health by reducing the intestinal pH, inhibiting harmful bacteria, stimulating nutrient digestion, and improve the performance of broiler chickens (Sharifuzzaman *et al.*, 2024). Moreover, they are metabolic intermediates in the Krebs cycle, which may improve energy and growth efficiency (Kim *et al.*, 2015). Based on previous studies on broiler chickens, citric acid enhances gut health, nutrient digestion and utilization, growth, and overall well-being (Mamabolo *et al.*, 2023). Malic acid improved growth performance, feed conversion efficiency, immune response, and antioxidant status (Qiu *et al.*, 2023). Additionally, malic acid has antimicrobial properties (Ren *et al.*, 2020); and improves intestinal health, altering the gut microbiota and enhancing the intestinal structure, which can contribute to better nutrient absorption and overall health in broilers (Ren *et al.*, 2020; Dai *et al.*, 2021). Citric acid is added to poultry feed in amounts ranging from 4 to 30 g/kg, depending on production goals and cost factors (Nourmohammadi *et al.*, 2015). Studies have shown that concentrations between 16 and 30 g/kg improve the production performance and health of broiler and layer chickens (Biggs *et al.*, 2007; Katoch *et al.*, 2021; Makofane *et al.*, 2022; Sharifuzzaman *et al.*, 2024; Attia *et al.*, 2024). Incorporating malic acid in chicken diets at levels ranging from 0.5% to 2% boosts the weight gain and production efficiency of broilers and layers (Hajati 2018). The optimal concentration of malic acid in chicken nutrition is between 0.5% and 1%, with 0.8%

being the most advantageous, leading to enhanced effects on chicken production efficiency (Bhatt & Sharma, 2012; Qiu *et al.*, 2022).

While citric and malic acids exhibit beneficial effects when used separately; their effects can be limited by factors like dose, diet composition, and acid tolerance by gut microflora. Their combination might exert a synergistic effect, enhancing digestion efficiency, nutrient absorption, and gut microbial balance, which improves broiler performance. The benefits of both acids have been well documented, but there is a lack of information on their mixture. Moreover, there is no utilization practice of dietary OAs as alternative feed additives in Ethiopia's poultry industry, although they are available for other industrial uses in the form of flavoring and preservative compounds in the food, beverage, cosmetics, and pharmaceutical industries. In the current research, it was hypothesized that a diet supplemented with a mixture of citric and malic acid could have a better synergistic effect than their single applications on broiler performance. The result of this study could contribute to the chicken production of safer and more sustainable broiler meat for consumers. Therefore, this study aimed to evaluate the effects of dietary supplementation with citric and malic acids and their mixture on the growth performance, carcass characteristics, gut and organ traits, and economic efficiency of Cobb500 broiler chickens fed a commercially balanced diet.

5.2. Materials and Methods

5.2.1. Study areas

The study was carried out at the poultry farm of the Bahir Dar University Animal Farm at Zenzelma Campus, Bahir Dar, Ethiopia, for 56 d. throughout the experimental period, the internal temperature and relative humidity (RH) of the experimental house were measured every morning (6:00 AM) during the study period via a Digital Thermo hygrometer. The average maximum and minimum temperatures were 31.05 and 16.11C⁰, respectively, and RH of 75.39 and 37.34% were recorded. According to (Thom, 1959), the daily temperature–humidity index (THI) was calculated as $THI = (0.8 \times AT + (RH/100) \times (AT - 14.4) + 46.4)$, where T is the air temperature in degrees Celsius and RH is the relative

air humidity in percent. On this basis, an average mean THI of 70.40 was obtained (Figure 5.1).

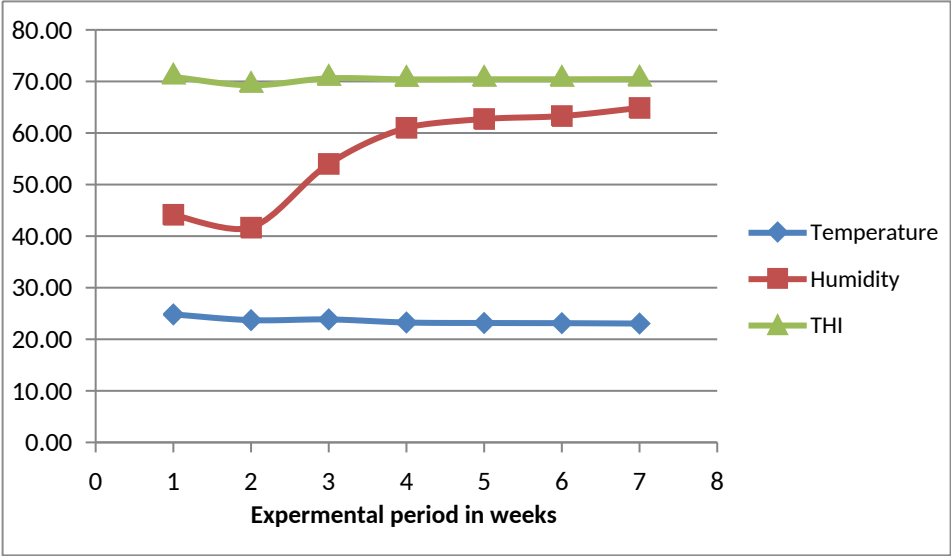


Figure 5:1 Average temperature, relative humidity, and temperature–humidity index of the experimental facility during the study period (56 d).

5.2.2. Experimental chickens and their management

The experiment was conducted with 280 one-day-old commercial broiler chicks (Cobb500 breed) in a roofed tin-shed house with two-sided wire mesh-covered openings. The house was partitioned into 20 equal compartments with an area of 1.26 m² using wire mesh, and the floor was covered with wood shavings at a depth of 5 cm as bedding material. The experimental house was cleaned and disinfected with tap water and 10% formalin before the chicks arrived. Equipment, such as a feeder and water trough, was cleaned, disinfected, fixed in each pen, and adjusted according to the progressive growth of the chicks. The chicks were purchased from the ELRE private farm in Debre-Zeit, Ethiopia. The chicks were first examined for external anatomical defects and then individually weighed using a sensitive balance to ensure homogeneity.

The temperature was maintained at 33°C in the brooding house during the first week by fixing a 200 W incandescent bulb. The temperature was gradually decreased by 2.5°C each week until the broilers were acclimatized to room temperature. The light was provided according to the methods of Aviagen (2018); 23 h light and 1 h dark for the first 7 d of age,

followed by 20 h light and 4 h darkness until slaughter. Chicks were vaccinated through drinking water against New Castle disease (ND) at the age of 7 days, with the Lasota strain at 21 and 42 days of age, and infectious bursal disease (Gumboro) at 12 and 23 days of age. Feed was offered *ad libitum*, and water was provided freely to the experimental birds. The feeding of the birds was based on their growth phases (Saleh *et al.*, 1997), starter phase (0 to 14 days), grower phase (15 to 42 days), and finisher phase (43 to 56 days). Strict biosecurity measures were implemented using a footpath at the entrance of the experimental house throughout the study period.

5.2.3. Experimental design and dietary treatments

The experiment was conducted using a completely randomized design (CRD) consisting of five treatments with four replications each. A total of 280 one-day-old unsexed Cobb500 broiler chicks were randomly assigned to five treatment groups, consisting of 56 chicks per treatment, and further replicated four times with 14 chicks per replicate. The experimental treatments included a basal diet without supplementation (T1), 60 mg/kg oxytetracycline (T2), 16 g/kg citric acid (T3), 8 g/kg malic acid (T4), and a mixture of 8 g/kg citric acid and 4 g/kg malic acid (T5). Antibiotic and organic acid treatments were selected based on EFSA guidelines and previously reported effective dosages for poultry performance (Koutsoumanis *et al.*, 2021; Ali & Hassan, 2010; Bhatt & Sharma, 2012; Qiu *et al.*, 2022).

5.2.4. Experimental diet

Commercial broiler mash-type feed was purchased from Albar Trading Plc., Addis Ababa, Ethiopia. Based on their availability in the local market, citric and malic acids were purchased from a local supplier in Bahir Dar (Global Chemicals Plc.), and oxytetracycline (OTC) was obtained from a veterinary drug supplier in Bahir Dar. The nutrient level of the purchased commercial diet was 22, 20, and 19% CP and 3000, 3150, and 3250 kcal ME/kg for starter, grower, and finisher diets of broiler birds, respectively, meeting the nutrient requirements of broiler birds set by the National Research Council (NRC, 1994).

The chemical compositions (% dry matter basis) of the commercial diets used during the different growth phases of the broiler chickens in this study were obtained from Albar Trading Plc., Addis Ababa, Ethiopia and are shown in Table 5.1.

Table 5:1 Chemical composition (% DM basis) of commercial broiler diets in different growth phases of broiler chicks

Parameters	Starter	Grower	Finisher
Crude protein (%)	22	20	19
Crude fat (%)	6.5	9	6.5
Crude fiber (%)	5.5	5.5	8
Metabolizable energy (kcal/kg)	3000	3150	3250
Calcium (%)	0.6	0.75	0.65
Moisture (%)	10	10	10

5.2.5. Data Collection and Observation

5.2.5.1. Feed intake

Data on daily amounts of offered and refused feed per pen were collected throughout the experimental period. Refused feed per pen was collected and measured the morning before a daily feed was offered. The feed intake was calculated as the difference between the feed offered and the refusal. The mean daily feed intake was calculated by dividing the daily feed intake by the number of birds multiplied by the experimental period.

5.2.5.2. Body weight measurement

The experimental chickens were weighed individually on the first day of the commencement of the experiment (40.517 ± 0.414), and then the live weight of the broilers was measured weekly every morning at 6:00 AM before the feed was offered via an electronic balance with a 40 kg weighing capacity and an accuracy of 0.001 g. The body weight gain of the experimental broiler chickens was calculated by subtracting the initial weight from the final weight. The average daily gain was calculated as the mean of the final body weight change divided by the number of experimental days.

5.2.5.3. Feed conversion ratio

The FCR was calculated by dividing the average daily feed intake by the average daily body weight gain per bird.

5.2.5.4. Survival rate

Dead chicks were recorded as they occurred during the entire experimental period, and on day 56, the mortality percentage was calculated by dividing the sum of dead birds by the number of stocked birds and multiplying by 100; the survival rate (SR) of birds was calculated by subtracting the value of the mortality percentage from 100.

5.2.5.5. Carcass characteristics

At the end of the feeding trial (56 days of age), two birds per replicate (eight per treatment) with live body weights nearest to each pen mean were randomly selected for carcass evaluation. These birds were subjected to feed withdrawal for 12 h, but drinking water was available, and then weighed before slaughtering. The birds were euthanized, de-feathered, and eviscerated manually. Dressed carcass weight was estimated after complete bleeding and removal of the head, shank, feather, and visceral contents. The cut carcass parts (thigh, drumstick, breast, back, wings, and neck), giblets (gizzard, liver, and heart), pancreas, small intestine, large intestine, ceca, and abdominal fat were weighed and expressed in grams. The percentages of dressed carcasses, giblets, and abdominal fat were calculated by dividing their weights by the slaughter weight and multiplying by 100. The lengths of the gastrointestinal parts (small intestine, large intestine, and ceca) were measured via a tap meter and expressed in centimeters. The gizzard thickness and width were measured via a digital caliper with an accuracy of 0.001 mm. Average data were recorded and expressed in millimeters (mm) for the width and thickness of the gizzard from two distinct equatorial sites and its thickest and thinnest sections.

5.2.5.6. Economic analysis

Partial budget analysis was performed to evaluate the economic viability of organic acids. Cost calculations were based on costs of one day-old chick, feed cost per kg, cost of organic acids, total feed cost, Oxytetracycline cost per broiler. Each economic parameter was estimated based on the commercial carcass weight of a single broiler bird. The estimation of total fixed cost (TFC) includes the cost of experimental house preparations and equipment depreciations. The total variable cost (TVC) is estimated from the costs of chick, management, feed, and feed additives. The total cost (TC) was estimated as the sum of the TFC and TVC. The total return (TR) was calculated from the sale price of one kilogram of chicken carcass weight in the local market and the returns from litter and feed bags per bird. The net profit (NP) was calculated by subtracting TC from TR. The benefit-cost ratio (BCR) was estimated as the ratio of TR to TC, and the profitability percentage (P.P.) was the percentage of NP divided by TC. The net profit margin (NPM) was calculated by dividing the NP by the TR, and the obtained result was multiplied by 100. The added costs were calculated as the ratio of the additive cost divided by the TC and TVC multiplied by 100. Similarly, the ratio of the economic efficiency of the organic acid groups (T3, T4, and T5) to that of the control groups (T1 and T2) was used to determine the relative economic efficiency (REE) of each dietary supplement of OAs.

5.2.6. Statistical analyses

The collected data were analyzed with analysis of variance (ANOVA) with the statistical program SAS (Version 9.2, 2002). Significant differences between treatment means were determined via Tukey's HSD test at a significance level of $p < 0.05$.

The statistical model was $Y_{ij} = \mu + T_i + e_{ij}$, where: Y_{ij} is the response variable, T_i is the treatment effect (of the i^{th} treatment) and μ is the overall mean, and e_{ij} is the random error.

5.3. Results

5.3.1. Growth performance evaluation

The effects of dietary OA supplementation on the growth performance of broiler chickens during the starter, grower, finisher, and overall experimental periods are presented in Table 5.2. Significant differences ($P<0.05$) in broiler chicken body weight and average daily weight gain were detected among the treatment groups during the starter phase. In the present study, the mixture of citric and malic acids and the antibiotic-fed groups presented the highest final body weights, whereas no significant differences were detected in the average daily gain or final body weight between T2 and T5. The citric acid (T3) group responded to intermediate performance in terms of final body weight and average daily weight gain. Compared with those broiler chicks' fed diets in the citric and malic acid mixture and antibiotic supplemented groups, the final body weight and average daily weight gain in the malic and untreated groups were significantly lower.

During the grower phase, significant differences ($P<0.001$) in broiler chickens' final body weight, average daily weight gain, and FCR across treatments were detected. The T1 group had the lowest final body weight. Antibiotic, citric acid, and mixed acid had the highest values and statistically comparable final body weights, whereas malic acid was intermediate. The untreated control group presented the lowest average daily weight gain, whereas the antibiotic and organic mixture groups presented the greatest and statistically similar average daily weight increases. Mixed acid group presented the most efficient FCR, which was significantly ($P<0.001$) better than all other treatment groups, while the untreated control group was lowest. Malic and antibiotic demonstrated statistically comparable intermediate FCRs.

During the finisher phase, there were significant differences ($P<0.001$) in the final body weights and FCRs of the broiler chickens among the treatments. The mixed organic acid and antibiotic groups presented the highest final body weight ($P<0.001$), followed by the citric and malic groups, whereas T1 presented the lowest body weight during the finisher phase. Among all the treatment groups, the combination of citric and malic acids (T5) had the most effective FCR, whereas the group that received no treatment (T1) had the least

effective FCR. The groups treated with antibiotics (T2), citric acid (T3), or malic acid (T4) presented intermediate final body weights and were statistically similar.

During the overall experimental period (0-56 days), significant ($P<0.05$) differences were observed in the final body weight, average daily gain, daily feed intake, FCR, and SR among the treatment groups. The untreated control group presented the lowest average daily gain and final body weight. In contrast, the antibiotic and mixed acid groups presented the highest final body weights and average daily gains. The citric and malic groups presented intermediate values for both parameters and were not significantly different from each other. The treatment group receiving a mixture of citric and malic acids had the lowest feed intake, whereas the antibiotic group shows the highest feed intake. In contrast to T1 group, which displayed the lowest efficient FCR, the mixed acid demonstrated the better FCR. With no statistical differences, the FCR values of the antibiotic, citric acid, and malic acid groups were intermediate. As expected, the SR of broiler chickens was significantly lower ($P<0.05$) in the untreated control group than in all other treatment groups. Compared with all treatment groups, the antibiotic, citric, and mixed acid groups presented similar and higher survival rates.

Table 5:2 Effects of supplementation with dietary citric and malic acids and their mixture on the growth performance of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM	P-value
IBW	40.53 ^a	40.52 ^a	40.52 ^a	40.50 ^a	40.53 ^a	0.41	1.000
Starter phase (1 to 14 days)							
FBW	376.23 ^b	420.42 ^a	406.59 ^{ab}	380.87 ^b	418.36 ^a	7.53	0.002
ADG	23.98 ^b	27.14 ^a	26.15 ^{ab}	24.31 ^b	26.99 ^a	0.53	0.001
DFI	34.06	36.98	34.81	33.51	34.75	0.91	0.138
FCR	1.42	1.37	1.33	1.38	1.29	0.04	0.262
Grower phase (15 to 42 days)							
FBW	2557.78 ^b	2738.18 ^a	2696.78 ^a	2660.26 ^{ab}	2769.41 ^a	30.57	0.002
ADG	77.91 ^b	82.78 ^a	81.79 ^{ab}	81.41 ^{ab}	83.97 ^a	1.06	0.014
DFI	141.17	144.06	139.23	143.30	137.20	1.85	0.102
FCR	1.81 ^a	1.74 ^{ab}	1.70 ^{bc}	1.76 ^{ab}	1.64 ^c	0.02	<0.001
Finisher phase (43 to 56 days)							
FBW	3376.60 ^c	3627.95 ^a	3517.35 ^b	3511.10 ^b	3641.05 ^a	22.97	<0.001
ADG	58.49	63.56	58.61	60.78	62.26	2.85	0.663
DFI	149.71	144.13	138.28	144.64	131.05	5.43	0.196
FCR	2.56 ^a	2.28 ^{bc}	2.36 ^{ab}	2.38 ^{ab}	2.11 ^c	0.04	<0.001
Entire experimental period (1 to 56 days)							
FBW	3376.60 ^c	3627.95 ^a	3517.35 ^b	3511.10 ^b	3641.05 ^a	22.97	<0.001
ADG	59.58 ^c	64.06 ^a	62.09 ^b	61.98 ^b	64.30 ^a	0.41	<0.001
DFI	116.52 ^{ab}	117.31 ^a	112.88 ^{ab}	116.18 ^{ab}	110.05 ^b	1.59	0.028
FCR	1.96 ^a	1.83 ^b	1.82 ^b	1.88 ^b	1.71 ^c	0.01	<0.001
SR	83.93 ^b	96.43 ^a	96.43 ^a	94.64 ^{ab}	98.22 ^a	2.64	0.012

a, b, c, Means in the same row with different superscripts are significantly different; SEM= standard error of the mean; T1= without OA as a basal diet; T2= 60 mg oxytetracycline; T3= 16 g citric acid; T4= 8 g malic acid; and T5= combination of 8 g citric acid and 4 g malic acid per 1 kg of basal feed; FBW= final body weight; ADG= average daily gain; DFI= Daily feed intake; FCR= feed conversion ratio; SR= survival rate.

5.3.2. Carcass characteristics

The effects of dietary organic acid on the carcass characteristics of broiler chickens are presented in Table 3. The majority of carcass characteristics were significantly different ($P < 0.01$) among the treatments; however, the dressing percentage, wing, abdominal fat, and percentage of giblets were not significantly different ($P > 0.05$). The carcass yield significantly differed ($P < 0.05$) among the treatment groups. Broiler chickens fed in the mixture of citric and malic acid group achieved the highest yield, followed by those in the antibiotic group, whereas those in the untreated control group presented the lowest yield.

However, no significant difference was observed between the citric and malic treatment groups. No significant differences ($P>0.05$) in carcass cut parts (thigh, drumstick, or back or thorax) were detected between the organic acid-treated groups (T3, T4, and T5) and the antibiotic group. However, the T1 group presented significantly lower breast, back, and thorax yields than the other groups did. The combination of citric and malic acid and antibiotic treatment groups presented the highest breast weights, followed by the individual dietary supplemented citric and malic groups, whereas the untreated group presented the lowest breast weight. Giblet weight significantly varied ($P< 0.05$) among the treatments, with the greatest weight value in T5 and the lowest in T1.

Table 5:3 Effects of dietary supplementation with citric and malic acids and their mixture on the carcass performance of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM	<i>P</i> -value
SW (g)	3340.48 ^b	3596.86 ^a	3467.43 ^{ab}	3458.52 ^{ab}	3609.71 ^a	48.85	0.004
DCW (g)	2137.77 ^d	2430.85 ^{ab}	2316.14 ^{bc}	2306.74 ^c	2470.75 ^a	28.14	<0.001
DCP (%)	64.04	67.61	67.02	66.71	68.47	1.24	0.161
Thigh (g)	369.17 ^b	405.94 ^a	397.57 ^{ab}	412.68 ^a	428.38 ^a	7.65	<0.001
Drumstick (g)	273.89 ^c	326.79 ^{ab}	316.34 ^{ab}	303.81 ^{bc}	348.87 ^a	7.96	<0.001
Breast (g)	844.49 ^c	979.11 ^a	926.05 ^{ab}	915.41 ^b	983.69 ^a	14.89	<0.001
Back (g)	311.64 ^b	352.73 ^a	330.62 ^{ab}	331.79 ^{ab}	348.42 ^a	7.90	0.009
Wing (g)	245.73	251.92	248.45	239.30	260.76	8.45	0.490
Neck (g)	92.85 ^b	114.37 ^a	97.12 ^b	103.76 ^{ab}	101.62 ^b	2.97	<0.001
GW (g)	121.41 ^b	130.57 ^{ab}	127.78 ^{ab}	129.63 ^{ab}	130.11 ^a	2.31	0.042
GP (%)	3.64	3.63	3.70	3.75	3.64	0.10	0.892
AF (g)	58.18	61.97	56.83	62.22	64.66	2.447	0.180
AFP (%)	1.74	1.72	1.64	1.80	1.78	0.09	0.546

^{a, b, c}, Means in the same row with different superscripts are significantly different. SEM= standard error of the mean, T1= without OA as a basal diet, T2= 60 mg oxytetracycline per 1 kg of basal feed, T3= 16 g citric acid per 1 kg of basal feed, T4= 8 g malic acid per 1 kg of basal feed, and T5= mixture of 8 g citric acid and 4 g malic acid per 1 kg of basal feed; SW=slaughter weight; DCW= dressed carcass weight; DCP= dressed carcass percentage; GW= giblets weight; GP= giblets percentage, AFW= abdominal fat weight; AFP= abdominal fat percentage.

5.3.3. Evaluation of internal organs and gastrointestinal tracts

The effects of supplementation with dietary citric and malic acids, alone or in combination, on the relative organ weights and GIT measurements of the broiler chickens are presented in Table 5.4. Significant differences ($P<0.05$) in liver, kidney, and ceca weight proportions, as well as ceca length, were observed among the dietary treatments. The citric

acid group had the highest liver proportion, and the untreated control group (T1) had the lowest. However, antibiotic, malic, and the mixture of citric and malic (T5) were intermediate and not significantly different from each other. The highest kidney weight proportion was observed in T5 (mixed with citric and malic acids), which was significantly greater than that in the untreated group. However, antibiotics, citric, and malic groups presented intermediate values and were not significantly different from each other. The ceca weight proportion was varied significantly ($P<0.05$) varied among the treatment groups. Citric was significantly greater than that of both control groups, while antibiotic was lower ceca weight than those of the malic and mixed acid groups. The antibiotic group had longer ceca than all treatment groups, while citric, malic, and their mixture had intermediate lengths. No significant differences ($P>0.05$) were recorded in the relative weights of the pancreas, gizzard, small intestine, and large intestine or in gizzard width and thickness across all treatment groups.

Table 5:4 Effects of dietary supplementation with citric and malic acids and their combination on internal organ and gut parameters in broiler chickens

Parameters		T1	T2	T3	T4	T5	SEM	P-value
Liver	Proportion (%)	1.82 ^c	1.87 ^{bc}	2.02 ^a	1.96 ^{ab}	1.92 ^{abc}	0.03	0.003
Kidney	Proportion (%)	0.25 ^b	0.27 ^{ab}	0.30 ^{ab}	0.30 ^{ab}	0.32 ^a	0.01	0.022
Pancreas	Proportion (%)	0.20	0.23	0.23	0.21	0.28	0.02	0.165
Gizzard	Proportion (%)	1.32	1.35	1.25	1.26	1.21	0.06	0.455
	Width (mm)	48.37	47.41	48.26	48.41	48.29	0.99	0.562
	Thickness (mm)	15.36	15.94	16.01	15.88	16.86	0.39	0.151
SI	Proportion (%)	1.91	2.06	2.10	2.13	2.10	0.06	0.1490
	Length (cm)	214.90	232.39	234.09	231.61	233.41	5.79	0.133
LI	Proportion (%)	0.123	0.110	0.118	0.118	0.127	0.01	0.3421
	Length (cm)	13.24	14.29	13.68	13.30	14.78	0.63	0.338
Ceca	Proportion (%)	0.25 ^{bc}	0.22 ^c	0.35 ^a	0.31 ^{ab}	0.33 ^{ab}	0.01	<.001
	Length (cm)	20.20 ^b	23.01 ^a	21.50 ^{ab}	20.55 ^{ab}	20.08 ^{ab}	0.64	0.036

^{a, b, c}. Means in the same row with different superscripts are significantly different. SEM= standard error of the mean, T1= without OA as a basal diet, T2= 60 mg oxytetracycline per 1 kg of basal feed, T3= 16 g citric acid per 1 kg of basal feed, T4= 8 g malic acid per 1 kg of basal feed, and T5= mixture of 8 g citric acid and 4 g malic acid per 1 kg of basal feed; SI= small intestine, and LI= large intestine.

5.3.4. Economic efficiency analysis

The effects of dietary OA supplementation on the economic efficiency of broiler chickens are presented in Table 5.5. There were significant differences ($P<0.001$) in the majority of the economic evaluation parameters among the treatment groups. The groups treated with dietary OAs presented lower ($P<0.05$) feed cost values than did the control groups. Broiler birds fed the mixed diet presented significantly greater ($P<0.05$) total return and net profit values than those in T1 and T2. Compared with those of the untreated control group, higher values of total return and net profit were recorded from the citric acid- and malic acid-treated diets (T3 and T4), but lower values were recorded from the antibiotic group. Among all the treatment groups, the highest ($P<0.001$) collective economic efficiency measures (benefit ability ratio, profitability percentage, and net profit margin) were observed for the mixed OA-supplemented diets.

Broiler birds in the citric and malic groups presented lower ($P>0.05$) economic efficiency than those in the antibiotic group but greater ($P<0.001$) efficiency than those in the untreated group. Compared with those in untreated and antibiotic, the economic value of the dietary citric and malic acid mixture was greater ($P<0.001$). When broiler chickens were fed diets supplemented with citric or malic acid alone, their economic efficiency was greater than that of the untreated control but lower than that of the antibiotic control group. For citric and malic, economic efficiency increased by 9.20% and 5.6%, respectively, in comparison with that in the untreated treatment group.

Table 5:5 Effects of dietary supplementation with citric acid, malic acid, or their mixture on the economic efficiency of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM	P-value
Chick cost (USD/bird)	1.32	1.32	1.32	1.32	1.32	0.000	1.00
Mgt. cost (USD/bird)	1.51 ^b	1.55 ^a	1.55 ^a	1.55 ^a	1.55 ^a	0.004	<0.001
Feed cost (USD/bird)	3.86 ^b	3.89 ^a	3.74 ^d	3.85 ^c	3.65 ^e	0.021	<0.001
Additive cost (USD/bird)	0.00 ^d	0.02 ^c	0.33 ^a	0.32 ^b	0.32 ^b	0.035	<0.001
TVC (USD/bird)	6.68 ^c	6.79 ^d	6.95 ^b	7.05 ^a	6.84 ^c	0.029	<0.001
TFC (USD/bird)	0.48	0.48	0.48	0.48	0.48	0.000	1.00
TC (USD/bird)	7.17 ^e	7.27 ^d	7.43 ^b	7.53 ^a	7.33 ^c	0.029	<0.001
TR (USD/bird)	13.46 ^e	15.26 ^b	14.56 ^c	14.51 ^d	15.49 ^a	0.163	<0.001
NP (USD/bird)	6.29 ^e	7.99 ^b	7.12 ^c	6.98 ^d	8.17 ^a	0.159	<0.001
BCR	1.88 ^e	2.10 ^b	1.96 ^c	1.93 ^d	2.12 ^a	0.022	<0.001
EE or P.P (%)	87.80 ^e	109.80 ^b	95.80 ^c	92.80 ^d	111.50 ^a	2.166	<0.001
NPM (%)	46.76 ^e	52.34 ^b	48.94 ^c	48.12 ^d	52.72 ^a	0.541	<0.001
Add cost/TC (%)	0.00 ^e	0.34 ^d	4.49 ^a	4.31 ^c	4.32 ^b	0.473	<0.001
Add cost/TVC (%)	0.00 ^e	0.36 ^d	4.80 ^a	4.60 ^c	4.62 ^b	0.506	<0.001
REE of OAs from T1 (%)	100.00 ^d	--	109.15 ^b	105.64 ^c	126.98 ^a	2.467	<0.001
REE OAs from T2 (%)	--	100.00 ^b	87.28 ^c	84.48 ^d	101.54 ^a	1.972	<0.001

a, b, c, d, e Means in the same row with different superscripts are significantly different, SEM= standard error of the mean, T1= without OA as a basal diet, T2= 60 mg oxytetracycline per 1 kg of basal feed, T3= 16 g citric acid per 1 kg of basal feed, T4= 8 g malic acid per 1 kg of basal feed, and T5= mixture of 8 g citric and 4 g malic acids per 1 kg of basal feed; TFC= Total fixed cost; TC= Total cost; TVC= Total variable cost; TR=Total return; NP= Net profit; BCR= Benefit-cost ratio; P.P.= Profitability percentage; NPM=Net profit margin, REE= Relative economic efficiency; USD= United States dollar.

5.4. Discussion

5.4.1. Growth performance of broiler chickens

The improved average daily gain in broilers fed the mixed organic acid diet throughout the starter, grower, and overall trial periods may be attributed to a synergistic effect of the mixed acids, which increased nutrient utilization for energy production and protein synthesis. The results of the present study are in line with Kil DongYong *et al.*, (2011), who reported that dietary OAs can improve carbohydrate and protein utilization in pigs. This might be due to the ability of OAs to maintain an optimum pH in the stomach (Tomar *et al.*, 2020), allowing suitable conditions for proteolytic enzymes (pepsin, trypsin, and chymotrypsin), leading to better breakdown of complex protein molecules into smaller peptides and amino acids. This process is essential for increasing the efficiency of protein

digestion and absorption, increasing the availability of nutrients to chickens. Moreover, by improving protein digestion, proteolytic enzymes reduce the burden on the digestive system, leading to a healthier gut environment, which enhances nutrient absorption and the immune system and reduces the risk of infections (Steyl, 2019; Nguyen & Kim, 2020). By lowering the gut pH, OAs improve nutrient digestion and absorption in chickens through increased enzyme activity and feed retention time (Yaseen *et al.*, 2024; Kholif *et al.*, 2025), and this increased nutrient use allows chickens to meet their energy demand. In support of this study Kim *et al.* (2015), broiler chickens fed diets containing various levels of dietary OAs presented greater retention of dry matter (DM) and protein. Dietary OA supplementation in broiler chicks increases the activity of digestive enzymes, which are crucial for the digestion and absorption of dietary nutrients (Yang *et al.*, 2019; Mahfuz, *et al.*, 2021; Yaseen *et al.*, 2024).

The findings of the present study on the improvement in broiler chickens' body weight performance caused by dietary organic acid supplementation are supported by different scholars. A study by Bhandaria *et al.* (2021) reported that broiler chickens in body weight gained dietary organic acid supplementation (2402 g) compared with the control group (2276 g) at the end of a 42-day experiment. The findings of Kamal and Ragaa (2014) also indicated that in 42-day-old broilers, body weight gain and the FCR were increased in a diet supplemented with OAs. Hence, greater body weight gain was achieved through a direct antimicrobial effect, reducing the digesta pH level in the GIT while acting as a barrier to pathogens and buffering reactivity in conjunction with enhanced nutrient digestibility (D. K. Dittoe *et al.*, 2018; Kholif *et al.*, 2025). Similar to the current findings, scholars (Hassan *et al.*, 2010; Ghazalah *et al.*, 2011; Polycarpo *et al.*, 2017) reported that organic acid supplementation improved broiler chickens' body weight gain and FCR, possibly through its beneficial effects on the serum protein concentration and mucosa of the GIT. Furthermore, OAs enhance the absorption of minerals throughout the formation and development of bones (Hafeez *et al.*, 2014). As a result, a healthy bone structure offers a surface area for the growth and attachment of muscles. According to Islam *et al.* (2012), the addition of up to 0.75% citric acid to broiler diets further improved the availability of minerals such as Ca, P, and Mg for the bone development of broiler chickens. Specifically, Nezhad *et al.*, (2011)

reported that compared with the addition of phytase, citric acid supplementation increased the availability of P. Increased bone mineral content, density, and breaking strength may be indicators of the effects of dietary citric acid on mineral metabolism (Islam *et al.*, 2012).

During the overall growth period, reduced feed intake was noted in the groups fed mixed OAs, possibly because the combined effect of OAs may play an important role in energy production, resulting in increased digestion and absorption of nutrients (Dittoe *et al.*, 2018). Another possible attribute could be that OAs modify the gastrointestinal microbial population, enhancing nutrient digestibility and absorption by promoting digestive enzyme secretion and increasing the feed retention time (Rodjan *et al.*, 2018; Kholif *et al.*, 2025). Therefore, broilers in the mixed OA treatment group consumed less feed because they simply satisfied the energy requirements of their bodies as a result of improved nutrient utilization. More specifically, Kim *et al.* (2022) reported that citric and malic acids, which are intermediates of the Krebs cycle of organisms during energy production, can enhance the digestion and absorption of dietary nutrients by chelating various cations and increasing the activities of some digestive enzymes. The same results reported by Adil *et al.* (2011) revealed that, compared with the control diet, birds fed diets supplemented with OAs presented significantly reduced cumulative feed consumption.

The similar trend of improved growth and FCR for mixed organic acid-fed broilers during the grower, finisher, and entire phases of the current study could be attributed to nutrient utilization efficiency and gut health (Thirumeigmanam *et al.*, 2006). Owing to their physiochemical properties, mixed OAs have a better synergistic effect than single OAs do (Kil *et al.*, 2011). Compared with a control group, (Waghmare *et al.*, 2025) reported that broiler chickens supplemented with a blend of OAs presented significant improvements in average body weight, average daily gain, and FCR. A similar finding was reported by Rodríguez-Lecompte *et al.*, (2012), who reported that, compared with the control diet, dietary supplementation with different levels of citric acid improved the daily weight gain and FCR of broilers. Although this study lacked nutrient digestibility, a previous study showed that dietary supplementation with 0.25% or 0.75% citric acid to broilers increased mineral (Ca, P, and Mg) digestibility.

The improved survival rates in the organic acid-supplemented broiler groups compared with those receiving non supplemented might be due to the potential effects of OAs in the feed and GIT through the inhibition of photogenic microbial growth and the promotion of beneficial bacteria (Waghmare *et al.*, 2025). This contributes to a healthier gut environment and plays a crucial role in broiler survival rates. Moreover, OAs can lower the pH in the gut, creating an environment less hospitable for harmful bacteria (Kholif *et al.*, 2025) and reducing the incidence of bacterial infections, which are a major cause of mortality in broiler chickens (Brzoska *et al.*, 2013). Additionally, OAs have anti-inflammatory properties that improve general immune function and lessen the intensity of intestinal inflammation (Dai *et al.*, 2021), increasing the survival rate of broiler chickens compared with those in untreated groups. This finding aligns with that of Brzoska *et al.*, (2013), who reported that broiler chickens fed different levels of dietary OAs had lower mortality rates than broilers fed a control diet.

5.4.2. Carcass Evaluation

In this study, the higher dressed carcass weight of broiler chickens fed a mixture of citric and malic acids might be attributed to improved growth and better feed utilization. This implies that broiler chickens fed organic acid-treated diets have improved protein digestion and absorption (Sureshkumar *et al.*, 2021); consequently, higher protein deposition in the thigh, drumstick, and breasts leads to higher dressed carcass weights than in the control groups. The findings of the current study align with previous findings. Dressed carcass weight was significantly greater in groups fed a diet supplemented with a 0.2% organic acid mixture than in those fed a control diet (Hassan *et al.*, 2010). Similarly, Fascina *et al.*, (2012) reported that the carcass characteristics of broiler chickens improved because of different levels of dietary organic acid supplementation in the broiler diets. Another study by Yang *et al.*, (2019) demonstrated that supplementing a basal diet with citric acid (0.5%), and AGPs (avilamycin, 0.001%), or their combination resulted in higher carcass weights, with the combination group outperforming control chicks.

5.4.3. Organ and gastrointestinal measurements

The differences in the relative liver and kidney weights between the treatment groups may have resulted from the various effects of supplementation with citric and malic acids and their mixture on the growth and function of internal organs. The significantly greater liver proportions in the citric acid and malic acid groups and their mixture-supplemented groups could indicate enhanced liver metabolism, potentially related to improved nutrient processing or detoxification processes, thereby improving liver health and performance compared with those of the untreated control groups. The results of the present study, however, are not in line with those of previous scholars (Marín-Flamand *et al.*, 2014; Mohamed *et al.*, 2014), who reported that no effect on liver weight observed in broiler chickens supplemented with OAs affected the liver weight of broiler chickens. This difference might be attributed to the type, dose level, and synergetic effect of citric and malic acids and the genetics of the bird. The citric and malic acid mixture group presented the largest relative kidney proportion, which might potentially influence renal function or electrolyte balance. The increased kidney weight proportion in a mixture of citric and malic acids could be due to the interaction of acids in the mixture, which could stimulate metabolic processes in the gut; potentially, increased kidney filtration and excretion of metabolites leading to an increase in the workload could result in greater kidney weight.

The increased ceca weight resulting from the addition of citric acid, malic acid, or their mixture might have a potential stimulatory effect on the gut microbiota or improve nutrient absorption, leading to increased cecal content. Moreover, the intermediate performance of citric and malic acids in a mixture and separately in terms of both ceca weight and length shows a possible synergistic effect depending on the acid combination and concentration. Furthermore, the impact of citric acid, malic acid, and their mixture could support gut health by modifying pH, affecting microbial ecology, and enhancing gastrointestinal functioning (Saleem *et al.*, 2020). Similarly, Rodríguez-Lecompte *et al.*, (2012) reported that a mixture of 1.0% sorbic acid and 0.2% citric acid significantly increased the villus width, height, and area of the duodenum, jejunum, and ileum of broiler chicks at slaughter. According to Ocak *et al.*, (2006), broiler birds fed diets with malic acid concentrations of 0.4, 0.8, and 1.2 g/kg presented greater gastrointestinal weights than those

fed the control diet. Organic acids promote gut epithelial cell proliferation, increasing intestinal tissue weight and altering mucosal morphology (Waghmare *et al.*, 2025). Moreover, OAs lower the gastrointestinal pH, increasing pepsin activity and stimulating pancreatic enzyme secretion (Khan & Iqbal, 2016). This can increase the production of pancreatic juice rich in trypsinogen, chymotrypsinogen A, chymotrypsinogen B, procarboxypeptidase A, and procarboxypeptidase B, thereby improving protein digestion (Adil *et al.*, 2011).

5.4.4. Economic efficiency

Compared with untreated chickens, broiler chickens fed diets supplemented with OAs presented a lower feed cost, likely due to the beneficial effects of OAs on digestion and nutrient absorption (Ndelekwute *et al.*, 2019). As a result, birds receiving OAs can meet their needs for nutrients with reduced feed consumption. However, the experimental groups incurred higher TVC and TC, attributed to the higher prices of citric and malic acids than both control diets did. The significantly greater TR of broilers fed a mixed OA-supplemented diet with higher dressed carcass weights sold at higher prices led to greater TR. Compared with those in all the other treatment groups, broiler chickens fed diets supplemented with mixed OAs presented improved economic efficiency measures, including the BCR, P.P, and NPM. This improvement is likely due to the synergistic effects of mixed OAs, which enhance growth performance and carcass yield, leading to greater returns from carcass sales.

The study revealed that the use of a mixture of citric and malic acids increased the economic efficiency by 27.20% compared with that of the untreated control group and by 1.50% compared with that of the antibiotic group. Broiler chickens fed diets with citric and malic acids alone also presented greater economic efficiency than those fed the untreated control diet did, although they did not do better than the antibiotic-treated group did. This result may be attributed to better growth performance relative to that of the untreated group, as well as the higher market cost of OAs than that of antibiotics. The results of the present study agree with Mohamed *et al.*, (2018), citric acid influences the feed cost and would therefore be low, whereas gains can be made from increased growth and reduced mortality.

Additionally, Islam *et al.*, (2012) reported that while adding 0.5% citric acid increased the cost of the diet, it improved growth and feed efficiency, which led to higher production profits than the negative control. The supplementation of dietary OAs was economically better and increased the profit per chick in the group-fed diet supplemented with 2% fumaric acid followed by 2% lactic acid in the diet (Adil *et al.*, 2011). Broiler diets supplemented with OAs mixture up to 0.2% slightly improved carcass parameters; however, growth performance and meat quality were not affected (Mohamed *et al.*, 2018). According to Chowdhury *et al.*, (2009), feed costs increased gradually in all treatment groups except for the control when citric acid and avilamycin were added to the diet. However, chickens fed citric acid had the lowest production cost per kilogram of live weight of broilers, followed by chicks fed avilamycin, the control, and the combination.

5.5. Conclusion and Recommendations

According to the results of the present study, broiler chicken performance can be improved by supplementation with a mixture of citric and malic acids. The inclusion of OAs in the broiler chickens' diet resulted in greater improvements in the starter, grower, and finisher stages in terms of biological and economic parameters. Compared with individual organic acid supplementation, a mixture of citric and malic acids outperforms other methods, such as weight gain, FCR, survival rate, dressed carcass yield, and economic efficiency, but is comparable to the antibiotic-treated group. Similarly, the survival rate of the chickens improved with a mixture of citric and malic acids. Therefore, a mixture of citric and malic acids can be recommended to replace antibiotics in broiler chicken production without compromising the performance and well-being of the chickens.

CHAPTER 6: EFFECT OF DIETARY CITRIC AND MALIC ACIDS SUPPLEMENTATION ON LAYING PERFORMANCE, EGG QUALITY TRAITS, AND ECONOMIC EFFICIENCY OF COMMERCIAL LAYER HENS

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Effect of Dietary Organic Acids Supplementation on Laying Performance, Egg Quality Traits and Economic Efficiency of Commercial Layer Hens

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Abstract

Organic acids have been demonstrated as a substitute for antibiotic growth promoters in poultry production; however, research is still being conducted due to the contradictory results in the biological and economic performances of poultry birds. This study was conducted to evaluate the effect of organic acids on laying performance, egg quality traits, and economic efficiency of commercial layer hens. In a complete randomized design, 260, 25-week-old layer hens weighing an initial body weight of 1609 ± 18.5 g were randomly assigned to 5 treatment groups consisting of 4 replicates. Treatments were basal diets without any additive (T1); with 30mg oxytetracycline (T2), with 16gm citric acid (T3), 8gm malic acid (T4); and a combination of 8g citric + 4gm malic acid (T5) per kg of basal diets. The results revealed that a significantly higher difference ($p < 0.001$) was observed in hen day

egg production, egg quality traits, and FCR. However, there were no significant differences ($P>0.05$) in body weight gain and feed intake among the treatment groups. Layer hens fed organic acid diets improved hen-day egg production by 85.51% to 86.54% as compared to T1 (77.75%) and T2 (80.07%). Overall, mixed acid (T5) reflected the highest performance in terms of FCR, albumin weight, egg mass, total return, and net profit. T5 improved the profitability by 13.26% and 11.82% and relative economic efficiency by 16.83 % and 17.40% than T1 and T2, respectively. In conclusion, supplementation with all dietary organic acids can replace antibiotics effectively, but mixed organic acid does the best in terms of both the biological and economic performance of layer hens.

Keywords: Economic efficiency; Egg quality; Hen-day egg production; Layer hen; Organic acid

6.1. Introduction

Antibiotics have been extensively used to boost the production performance of commercial egg layer chickens (Markowiak & Ślizewska, 2018), by reducing pathogenic bacteria and modulating gastrointestinal microbiota (Khan & Iqbal, 2016; Gadde *et al.*, 2017). However, due to the development of antibiotic resistance, the use of antibiotics as performance promoters for poultry production was banned by the European Union in 2006 (Agbai *et al.*, 2020). The prohibition of antimicrobials has significantly impacted poultry productivity and raised concerns over meeting growing global demand. There is an urgent need for new feed additive alternatives to improve performance in poultry, safeguard health, and foster sustainable production without the use of antibiotics. Consequently, scholars are continuously searching for other alternatives (Diarra & Malouin, 2014), to improve feed efficiency and animal health (Shalaei *et al.*, 2014), and reduce the cost of production (Adil *et al.*, 2011). Among other alternatives, organic acid feed additives have gained great attention because of their antimicrobial activity against pathogenic bacteria and the fact that these compounds can induce a pH reduction in the GIT, which can improve nutrient utilization in poultry diets (Kim *et al.*, 2015; AM *et al.*, 2017). Organic acids are generally regarded as safe and have been approved by most member states of the EU to be used as feed additives

in animal production (Archana *et al.*, 2016). According to Rahman *et al.*, (2008), organic acid supplementation of laying hens' diets has a positive effect on feed conversion efficiency, persistency of egg laying, egg quality parameters, and economic viability.

Maintaining egg quality is essential to ensure its quality with safety to achieve higher egg production and economic efficiency (Świątkiewicz *et al.*, 2010). Organic acid supplementations improve egg quality traits which are the most important influencing factors of economic profitability in the egg production poultry industry (Shalaei *et al.*, 2014; Fouladi *et al.*, 2018). Dietary organic acids can inhibit the growth of harmful microorganisms in the feed (Fouladi *et al.*, 2018) and consequently maintain the microbial balance in the GIT (Rahman *et al.*, 2008). Inclusion of these acids in chicken diets help to maintain an optimum pH in the stomach (Saki *et al.*, 2011), which allows correct activation and function of proteolytic enzymes for enhanced protein digestion in the stomach, and stimulates feed consumption (Hassan *et al.*, 2010). Moreover, they increase the digestibility of peptides and mineral micronutrients like Ca, P, Mg, Zn, and Mo and also serve as substrates in the intermediary metabolism (Fouladi *et al.*, 2018).

Although the role of different organic acids in improving feed utilization, growth, egg quality, and economic efficiency has been well documented, their dose, combination, and inclusion levels in the diet have been questioned by many researchers (Anjum & Chaudhry, 2010). Moreover, there is scanty information on dietary supplementations of citric acid, malic acid and their combined effects on laying performance, egg quality traits, and economic feasibility of commercial laying chickens. Additionally, there is a lack of knowledge on the effect of dietary organic acids supplementations in poultry nutrition as a whole, and in commercial egg-laying nutrition as a specific in Ethiopia. Therefore, the current study aims to evaluate the effect of dietary organic acid supplementation on laying performance, egg equality traits, and economic efficiency of commercial egg-laying hens.

6.2. Materials and Methods

6.2.1. Description of the study Site

The study was carried out for 120 days at the poultry farm of the Bahir Dar University animal farm at Zenzelma Campus located in Northwest Ethiopia at 11° 37' north latitude and 37° 28' east longitudes and an elevation of 1912 meters above sea level. The annual average temperature was 29 °C, and the annual rainfall of the area was 1428 - 1521mm. During the study period, daily reading data of the inside experimental house temperature and relative humidity were taken in Celsius degree and percent, respectively with the help of a Digital Thermo-Hygrometer. The average temperature was 23.76 °C with 30.53 °C maximum and 16.69 °C minimum and 59.93% of relative humidity was recorded. The average mean THI during the experimental period was 70.10% (Figure 6.1).

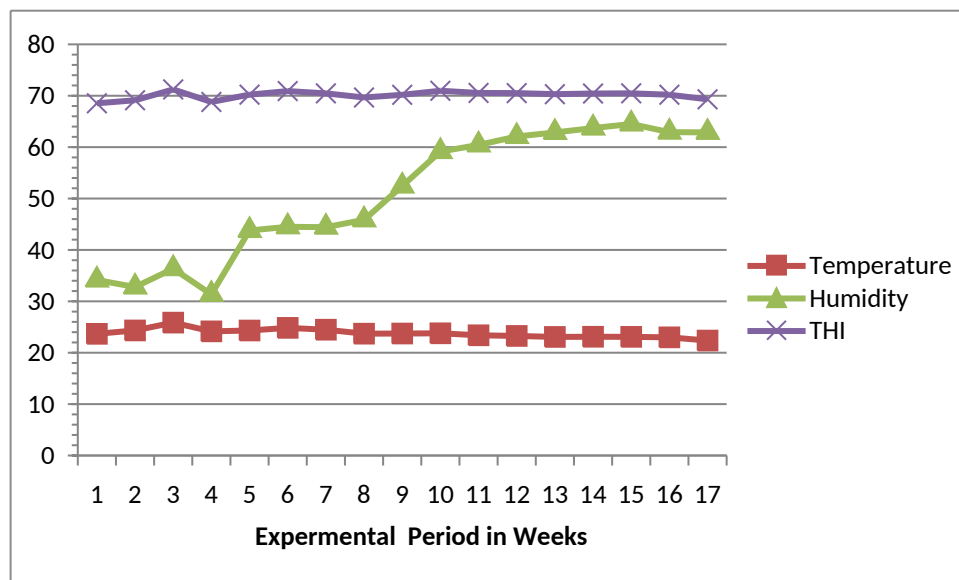


Figure 6:1 Average temperature, relative humidity, and temperature-humidity index of the experimental house during the study period (120 d).

6.2.2. Experimental layer hens and their management

For this feeding trial, a total of two hundred sixty, 25 weeks of old Bovans Brown layer hens were used. The study was conducted in a poultry farmhouse with partially open sides covered with a wire mesh. The experimental house was cleaned using tap water and disinfected by formalin (10%). Surrounding areas and all equipment were cleaned thoroughly and disinfected before the hens' arrival. Based on the floor space requirement per layer birds, the house was partitioned into 20 separate pens of equal size (2 x 2 m²) using wire mesh. Each pen was provided with feeding and watering troughs and egg-laying nests. A suitable wood shaving litter material was spread evenly over the floor of each pen to a depth of 10 cm. For the control of light, a 100-watt electric bulb was used. Electric light was provided in the experimental house for 18 hours. The layer hens were screened for visible physical defects, individually weighed using an electronic precision balance, and randomly distributed into 20 pens, consisting of 13 birds per pen. They were acclimated to experimental diets for 7 days and fed for 120 days. Throughout the feeding trial period, mash-type feed was provided based on the breeder guideline of Bovans Brown layer hens. Strict bio-security measure was taken and a footbath was used at the gate of the experimental house.

6.2.3. Experimental design and treatments

The experiment was conducted in a completely randomized design (CRD) consisting of five treatments with four replications per treatment. A total of 260-layer chickens with an average body weight of 1609.28 ± 18.45 g were randomly assigned to the treatments, consisting of 52 chickens per treatment (13 layers of chickens per replicate). The experimental treatments were a basal diet without supplementation (T1), 30 g/kg oxytetracycline (T2), 16 g/kg citric acid (T3), 8 g/kg malic acid (T4), and a mixture of 8 g/kg citric acid and 4 g/kg malic acid (T5). Oxytetracycline and organic acid levels in this study were selected based on EFSA guidelines and previously reported effective dosages for poultry performance (Koutsoumanis *et al.*, 2021; Ali & Hassan, 2010; Bhatt & Sharma, 2012; Qiu *et al.*, 2022). The experimental diet was a mash-type commercial layer feed

purchased from a commercial animal feed factory. The chemical composition (% dry matter basis) of the commercial layer feed is shown in Table 6.1. Organic acids and oxytetracycline were purchased from local suppliers. Then, both the OAs and OTC were added on top of the basal feed and mixed thoroughly.

Table 6:1 Chemical composition of commercial layer hen diet (% dry matter basis)

Parameters	Proportions
Metabolizable energy (kcal/kg)	2800
Crude protein (%)	16.5
Crude fat (%)	6
Crude fiber (%)	6
Ash (%)	12.5
Calcium (%)	3.2
Moisture (%)	11

6.2.4. Data collection and measurements

6.2.4.1. Production performance

Feed intake: Feed offered and refused per pen were taken throughout the experimental period. Refused feed per pen was collected and measured the next morning before giving the daily feed. Feed intake was calculated as a difference of feed offered and refused. Then mean daily feed intake was calculated by dividing daily feed intake by the number of hens multiplied by the experimental period.

Body weight gain: The experimental layer hens were weighed individually on the first day of the commencement of the experiment (initial weight) and then the final weight was taken at the end of the trial. The average body weight gain for each replicate was calculated by subtracting the initial weight from the final weight and dividing by the number of layers.

Egg production: Eggs in a pen were collected three times a day (9:00, 11: 00 and 15: 00), and the sum was taken as daily egg production. The rate of lay for each replicate was expressed as the average percentage of hen-day egg production. According to Hunton

(1995), the percentage of hen-day egg production (HDEP) was calculated as the number of eggs collected per day divided by the number of hens present that day multiplied by 100.

Egg weight and egg mass: The collected eggs were individually weighed immediately after collection for each replication and then the average egg weight was calculated by dividing the total sum of egg weight by the number of eggs. After the average egg weight was determined, egg mass daily was calculated by following the formula.

$$\text{Egg mass (g/hen/day)} = \frac{\text{Number of eggs per day} \times \text{Average egg weight per replicate}}{\text{Number of layer hens per replicate}}$$

Feed conversion ratio (gm. feed / gm. egg): The FCR was determined by dividing the average daily feed intake by the average daily egg mass per hen.

Survivability of layer hens: Throughout the study period, the death of hens was recorded as it occurred. At the end of the study, the mortality percentage was calculated by dividing the sum of dead hens by the number of originally stocked hens multiplying by 100. Then after, the survival rate of layer hens was calculated by subtracting the percentage of mortality from 100.

6.2.4.2. Egg quality evaluation parameters

During the experimental period, egg quality parameters were assessed every two weeks. Each time, 12 fresh eggs per treatment (3 eggs per replicate) were randomly selected for external and internal egg quality evaluation.

External egg quality: Egg weight, egg length, egg width, shape index, shell weight, shell thickness, and shell ratio were determined. The individual eggs were weighed with a digital sensitive balance to the nearest 0.01gm accuracy. The egg length and breadth were measured with an electronic digital caliper and expressed in millimeters (mm) and the egg shape index was calculated as the ratio of width to length multiplied by 100 (Smith, 2001). Eggshell weight and thickness were measured after the eggshell was broken on flat, smooth, and transparent glass placed on the leveled table. The weight of eggshells with membranes was measured in grams. After the shell membranes were removed eggshell thickness was

determined by measuring the two ends (narrow and wide) and middle part of the eggshell with an electronic digital caliper, then the average was taken as the eggshell thickness.

Internal egg quality: Internal quality traits such as albumen and yolk heights were measured using a tripod micrometer (TSS, ENGLAND). Yolk weight was determined using a sensitive balance and albumen weight was calculated as egg weight minus the sum of yolk and shell weight. Albumen and yolk ratios were calculated by taking their weights as the percentage of total egg weight. The Yolk diameter was measured using a ruler. The yolk-to-albumen ratio was calculated as the weight of the yolk divided by the weight of albumen multiplied by 100. The Haugh Unit value was calculated according to Haugh (1937), using the formula: $HU = 100 \log (H + 7.57 - 1.7W^{0.37})$; Where: HU = Haugh Unit; H = Observed height of the albumen (mm); W = Weight of the eggs in grams.

6.2.4.3. Economic Analysis

A partial budget analysis was performed to evaluate the economic viability of organic acids. Cost calculations were based on feed cost per kg, cost of organic acids, total feed cost, and Oxytetracycline cost per hen. Evaluation of the economic parameter was estimated based on the egg production performance of single-layer birds. Estimation of total fixed cost (TFC) includes cost for experimental house preparations and equipment. Total variable cost (TVC) was estimated from costs of management, feed, and feed additives (oxytetracycline, citric and malic acids).

Total cost (TC) was estimated as the sum of TFC and TVC. Total return (TR) was calculated from the sale price of one egg in the market, net profit (NP) was calculated by subtracting TC from TR and the benefit-cost ratio (BCR) was estimated as the ratio of TR to TC. To examine the economic advantage of dietary OAs supplementation in both T1 and T2 groups, the percentage of economic efficiency (EE) was calculated as the ratio between NP divided by TC multiplied by 100. The net profit margin (NPM) was calculated by dividing the NP by the TR, and the obtained result was multiplied by 100. Similarly, added costs were calculated as the ratio of additives cost divided by TC and TVC multiplied by 100. To evaluate the relative economic efficiency (REE) of dietary organic acids, the percentage of

REE was calculated as the ratio between the economic efficiency percentages of organic acids groups to the economic efficiency percentage of T1 and T2 groups.

6.2.5. Statistical analysis

The collected data were subjected to one-way ANOVA using the general linear model (GLM) procedure of SAS version 9.2. Tukey's test was employed to identify the significant difference between the treatment means at $P < 0.05$. The model used for statistical analysis was $Y_{ij} = \mu + T_i + e_{ij}$, where, Y_{ij} is the response variable, μ is the overall mean, T_i is the treatment effect (of the i th treatment) and e_{ij} is the random error.

6.3. Results

6.3.1. Effect of dietary organic acid on production performance of layer hens

The effect of the dietary OAs' supplementation on the production performance of Bovans Brown layer hens is presented in Table 6.2. Feed intake and body weight change of layer hens did not differ ($P>0.05$) among treatments. Organic acid-supplemented groups had a higher percentage of hen-day egg production and egg mass was higher in all organic acid-treated groups compared to T1. T2 was not significantly different from T3 and T4 but was lower than T5. Egg weight was lower ($P<0.05$) in T1 as compared to organic acid-treated diets (T3, T4, and T5), while the values for T1 and T2 were similar. Layer hens fed organic acid-treated diets had better ($P<0.001$) feed conversion ratios than T1. A lower ($P<0.05$) survivable rate of hens was observed in T1 than in the rest treatment groups.

Table 6.2. Effect of dietary organic acids supplementation on production performance of Bovans Brown layer chickens

Parameters	T1	T2	T3	T4	T5	SEM	<i>P-value</i>
Feed intake (g/hen/day)	118	118	117	117	117	0.541	0.394
Initial body weight (g/hen)	1610	1610	1610	1608	1609	18.454	1.000
Final body weight (g/hen)	1782 ^b	1803 ^{ab}	1805 ^{ab}	1799 ^{ab}	1816 ^a	6.860	0.042
Body weight change (g/hen)	172	193	195	191	207	19.737	0.797
HDEP (%)	77.6 ^c	81.5 ^{bc}	85.5 ^{ab}	85.2 ^{ab}	88.8 ^a	1.644	0.002
Egg weight (g)	60.8 ^b	62.9 ^{ab}	63.4 ^a	63.4 ^a	64.6 ^a	0.615	0.008
EM (g/hen/day)	47.2 ^c	51.4 ^{bc}	54.2 ^{ab}	54 ^{ab}	57.4 ^a	1.269	0.001
FCR	2.51 ^a	2.32 ^{ab}	2.16 ^{bc}	2.18 ^{bc}	2.05 ^c	0.059	0.001
Survivability (%)	94.2 ^b	100 ^a	100 ^a	100 ^a	100 ^a	0.859	0.001

^{a, b, c} Means in the same row with different superscripts are significantly different; SEM = standard error of the mean; EM= egg mass; T1= without OA as a basal diet; T2= 30 g oxytetracycline; T3= 16 g citric acid; T4= 8 g malic acid; and T5= combination of 8 g citric acid and 4 g malic acid per 1 kg of basal feed.

6.3.2. Effect of dietary organic acids supplementation on egg quality traits

The effect of dietary OAs supplementation on external and internal egg quality traits is presented in Table 6.3. Significant ($P<0.001$) differences were observed among treatment groups in shell weight, shell thickness, and shell ratio; while egg length, width, and shape index did not differ among treatments. Layer hens fed organic acids-supplemented diets had

higher ($P<0.001$) values in shell weight than T1 and T2. Similarly, T1 had a lower value in shall thickness compared to organic acid-supplemented groups, while the value for T2 was similar with all treatment groups. T5 had a higher shell ratio than all treatment groups, while all the rest groups had similar values in shell ratio. A statistical difference ($P<0.05$) was observed among treatment groups in yolk color while, no differences were recorded among treatment groups in all other internal egg quality evaluation parameters. Layer hens fed organic acid-supplemented diets had higher ($P<0.001$) yolk color than T1 and T2.

Table 6:3 Effect of dietary organic acids supplementation on egg quality performance of Bovans Brown layer chickens

Parameters	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	<i>P-value</i>
<i>External Egg Quality</i>							
Egg length (mm)	42.80	44.19	43.38	43.35	43.87	0.405	0.196
Egg width (mm)	33.71	34.29	33.76	34.23	34.11	0.138	0.057
Shape index (%)	78.80	77.62	77.83	78.98	77.80	0.775	0.619
Shell weight (g)	5.65 ^c	5.86 ^{bc}	6.37 ^a	6.23 ^{ab}	6.39 ^a	0.086	<0.001
Shell thickness (mm)	0.315 ^c	0.336 ^b	0.347 ^{ab}	0.342 ^{ab}	0.354 ^a	0.003	<0.001
Shell ratio (%)	9.24 ^b	9.21 ^b	9.98 ^a	9.74 ^{ab}	9.92 ^a	0.147	0.001
<i>Internal Egg quality</i>							
Albumen weight (g)	37.13 ^b	41.03 ^{ab}	40.71 ^{ab}	40.46 ^{ab}	41.33 ^a	0.941	0.040
Albumen height (mm)	7.86	7.94	8.20	7.56	8.23	0.377	0.721
Albumen length (mm)	84.61	80.37	77.99	78.79	82.25	2.349	0.314
Albumen index (%)	9.32	9.91	10.63	9.62	10.06	0.680	0.717
Albumen ratio (%)	60.70	64.44	63.79	63.27	64.02	1.692	0.563
Haugh unit	88.24	88.00	89.05	85.76	89.37	2.176	0.790
Yolk weight (g)	16.64	16.66	17.12	17.12	16.92	0.281	0.606
Yolk height (mm)	11.93	12.04	12.00	12.19	11.91	0.180	0.826
Yolk length (mm)	31.21	31.81	31.68	31.42	31.43	0.208	0.319
Yolk color	3.97 ^b	4.31 ^b	5.58 ^a	5.61 ^a	5.82 ^a	0.285	0.001
Yolk index (%)	38.24	37.85	37.88	38.78	37.89	0.593	0.773
Yolk ration (%)	27.21	26.15	26.82	26.77	26.22	0.495	0.544
Yolk - albumin ratio (%)	44.83 ^a	40.60 ^b	42.08 ^{ab}	42.41 ^{ab}	40.97 ^b	0.674	0.004

^{a, b, c}, Means in the same row with different superscripts are significantly different; SEM= standard error of the mean; T1= without OA as a basal diet; T2= 30 g oxytetracycline; T3= 16 g citric acid; T4= 8 g malic acid; and T5= combination of 8 g citric acid and 4 g malic acid per 1 kg of basal feed.

6.3.3. Economic efficiency of layer hens supplemented dietary organic acid

The effect of dietary OAs supplementation on the economic efficiency of layer hens is presented in Table 6.4. Statistical differences ($P < 0.001$) were noticed across treatment groups in total variable cost, total cost, total return, and net profit. No ($P > 0.05$) differences in benefit-cost ratio, profitability percentage, net profit margin, relative economic efficiency, and added costs of dietary organic acid supplementations. Organic acids-supplemented groups had higher total variable and production costs than T1, while, T2 was lower than T3 and T5 but similar to T4. Layer hens fed OA-supplemented groups had higher ($P < 0.05$) values in total return than T1 and T2. Net profit was lower ($P < 0.05$) in T1 as compared to organic acid-supplemented groups (T3, T4, and T5), while T2 was similar to OA-groups.

Table 6:4 Effect of dietary organic acids supplementation on economic efficiency performance of Bovans Brown layer chickens

Parameters	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	<i>P-value</i>
TVC (ETB)	559.25 ^c	585.50 ^b	604.74 ^a	595.18 ^{ab}	600.68 ^a	2.462	<0.001
TFC(ETB)	59.48	59.48	59.48	59.48	59.48	0.000	1.000
TC (ETB)	618.73 ^c	644.97 ^b	664.22 ^a	654.66 ^{ab}	660.16 ^b	2.462	<0.001
TR (ETB)	1117.18 ^c	1173.83 ^{bc}	1231.41 ^{ab}	1226.78 ^{ab}	1279.22 ^a	23.671	0.027
NP (ETB)	498.44 ^b	528.86 ^{ab}	567.18 ^{ab}	572.12 ^{ab}	619.06 ^a	23.827	0.004
BCR	1.81	1.82	1.85	1.87	1.93	0.037	0.154
PAP (%)	80.56	82.00	85.38	87.39	93.82	3.729	0.153
NPM (%)	44.59	44.87	46.01	46.63	48.35	1.097	0.163
AC/TC (%)	0.00 ^e	3.71 ^d	7.45 ^a	5.86 ^c	6.65 ^b	0.017	<.001
AC/TVC (%)	0.00 ^e	4.08 ^d	8.18 ^a	6.44 ^c	7.31 ^b	0.019	<.001
REE _{from T1} (%)	100.00	102.36	106.21	108.67	116.83	5.628	0.299
REE _{from T2} (%)	100.22	100.00	105.98	108.80	117.40	9.656	0.700

^{a, b, c}, Means in the same row with different superscripts are significantly different; SEM= standard error of mean; T1= without OA as a basal diet; T2= 30 g oxytetracycline per 1 kg of basal feed, T3= 16 g citric acid per 1 kg of basal feed; T4= 8 g malic acid per 1 kg of basal feed; and T5= mixture of 8 g citric and 4 g malic acids per 1 kg of basal feed; TFC= Total fixed cost; TC= Total cost; TVC= Total variable cost; TR=Total return; NP= Net profit; BCR= Benefit ability ratio; PP= Profit ability percentage; NPM=Net profit Margin, AC= Add cost; REE= Relative economic efficiency; OAs= organic acids; ETB= Ethiopian Birr.

6.4. Discussion

6.4.1. Production performance

The enhanced final body weight of layers given a diet supplemented with organic acids compared to T1 may be attributable to the organic acids' ability to lower feed and stomach pH to optimal levels to control harmful microorganisms, and enabling proper activation and function of proteolytic enzymes, which enhances nutrient utilization for energy production and protein synthesis (Dai *et al.*, 2020; Abbas *et al.*, 2022). It might also be attributed to the antibiotic roles of dietary organic acids. Diets containing organic acids had the greatest impact on reducing pathogenic microorganisms such as *E. Coli* and *Salmonella* population in the ileum (Ji *et al.*, 2023), reducing competition for nutrients between layers and microorganisms (Fouladi *et al.*, 2018), the digestibility of feeds and the absorption of nutrients in the GIT increased (Hassan *et al.*, 2010), consequently the nutrient needed for maintenance and production could be secured. Overall, organic acids can exert a good antibacterial effect by destroying the cell barrier, while, their combination may have a synergistic anti-biofilm effect for better results (Ji *et al.*, 2023).

The improved hen-day egg production observed in hens fed OAs supplemented diets is likely due to the lowered gastrointestinal pH, which enhances pepsin activity, improves protein digestion, and inhibits the proliferation of harmful bacteria such as *E. coli* and *Salmonella* (Ji *et al.*, 2023). These effects collectively contribute to better nutrient utilization, health, and overall productivity (Fouladi *et al.*, 2018). The results of the current study agree with those of Soltan (2008), who reported that dietary OAs supplementation at 780 ppm significantly increased egg production by approximately 5.77% compared to the untreated group. Organic acid supplementation at 520 ppm in the laying hen diet improved the persistence of lay feed conversion and eggshell quality during 67-74 weeks of age. Similarly, supplementation with organic acids significantly ($P < 0.05$) increased hen-day egg production by approximately 2.26, 8.0, and 9.84% in diets containing 260, 520, and 780 ppm organic acids, respectively, compared to the untreated group (Rahman *et al.*, 2008). Dhiab (2020) reported that 4% citric and 4% lactic acid and their 8% mixture enhanced egg production and feed conversion.

In this study, dietary OAs supplementation significantly improved egg weight, which may be attributed to the positive effect of OAs on the conversion of digested protein, fat, carbohydrate, and micronutrients into eggs. Organic acids also enhance the uptake of digested proteins and important minerals (Abbas *et al.*, 2022). In addition, OAs play a role in ATP production in cells. These factors potentially lead to improved energy metabolism, promote protein and fat digestion and deposition, and improve metabolic protein transportation in eggs. Consequently, OAs could potentially reduce the use of antibiotic growth promoters in poultry production because of their AMR in human and animal health. The current result disagrees with that of a previous study by Rahman *et al.*, (2008) who reported no statistical difference in egg weight between the OA supplementation groups and the untreated group. Similarly, Rahman *et al.* (2012) reported no significant difference was observed among different levels of OAs and control diets on layer egg weight. These differences might be attributed to many factors, including the level of organic acids, basal diet differences, layer breeds, and the environment where the experiment was conducted. The effects of environment particularly temperature have affected the egg weight of the layer (Talukder *et al.*, 2010).

Similarly, the highest egg mass was recorded in Bovans Brown layer hens fed a mixed organic acid diet. The possible factor for higher egg mass might be the positive effect of mixed organic acids on the conversion of digested protein, fat, carbohydrate, and micronutrients into eggs. They also enhance the uptake of digested proteins and other important minerals (Abbas *et al.*, 2022). These results are in line with those of Soltan (2008), who observed no effect of OAs on feed intake but improvements in egg mass and feed efficiency.

The OAs mixture group (T5) showed an advanced FCR compared to other treatment groups. This might be due to the anti-microbial activity of organic acids altering the ileal mucosa by increasing the height and width of the villus; and tunica mucosal width (Kaya *et al.*, 2014), promotes nutrient digestion and uptakes, and thus can lead to increased feed efficiency. The collective role of citric and malic acids affects layers by increasing the efficiency of nutrient utilization positively, contributing to improved egg weight, and making birds in T5 best in FCR. The better FCR indicated that mixed OAs can be

successfully used as a feed additive for laying hens. The result was similar to the finding of previous studies, (Fouladi *et al.*, 2018; Baghban-Kanani *et al.*, 2019), they observed significantly higher FCR in laying hens fed organic acids compared to those fed the basal diet.

6.4.2. External egg quality

The current study showed a significant improvement in eggshell weight, shell thickness, and shell ratio observed for layer hens fed an organic acids-treated diet. This improvement may be a result of increased mineral and protein absorption (Abbas *et al.*, 2022) and phytate phosphorus utilization (Kaya *et al.*, 2014). This increment in the absorption of minerals is reflected in the increased calcium deposition of the shell and results in improved shell quality (Kaya *et al.*, 2014). In our study, layers fed the mixed organic acid diets showed significantly enhanced shell quality traits; this might be a synergistic effect of combined organic acids rather than single ones to support mineral utilization. According to earlier studies show that the low pH resulting from the use of organic acids (Shalaei *et al.*, 2014), leads to an increase in the gut microbial phytase activity as well as phytic acid dissolution (Fouladi *et al.*, 2018). Following this process, the digestion and absorption of calcium and phosphorus were improved (Rahman *et al.*, 2008). The same result was reported by Fouladi *et al.*, (2018) who observed that the use of organic acids supplements in the experimental diet resulted in a significant increase in eggshell weight from 0.95 (%) for the diet without organic acids supplement to 1.15, 1.05 and 1.04 (%) respectively and eggshell thickness significantly increased from 0.21 mm for diet without organic acids supplements to 0.26, 0.25 and 0.24 mm, respectively. The results of the Świątkiewicz *et al.*, (2010) indicate that selected feed additives that lower the pH of the diet and intestinal content could have a positive effect on the eggshell quality. In contrast with the current finding, Kaya *et al.*, (2014) reported that different levels of dietary supplementing OAs mixture had non-significant differences in the shell thickness.

6.4.3. Internal egg quality

Higher albumen weight was noted for layer hens fed in dietary organic acids treated diet than T1. This might be due to dietary organic acids within the diet led to a significant increase in plasma albumin compared to negative control (Al-Harthi & Attia, 2015). In the current study, significantly higher yolk color was observed in all dietary organic acid-supplemented diets (T3, T4, and T5) compared to T1 and T2. This shows dietary organic acid supplementation increased yolk color. This may be attributed to the increased absorption of dietary carotenoids due to organic acid supplementation, and then transferred to egg yolk and making it more colorful. The result agreed with the finding of Al-Harthi & Attia, (2015), they observed that citric acid at 0.2% in the diet significantly increased egg yolk color compared to negative control. In contrast Kaya *et al.*, (2014) reported that supplementing the diets of laying hens with different levels of a mixture of dietary organic acid obtained non-significant differences in the yolk color and significant differences in yolk index. According to Rahman *et al.*, (2008), dietary organic acids had no effect ($P>0.05$) on shape index, yolk index, HU, and yolk color score. The differences in the yolk color, albumin index, and shell thickness with the above cited one may be due to variations in the amount and level of the dietary organic acid, layers breeds, and management conditions.

6.4.4. Economic efficiency

Organic acids treated groups recorded higher costs of variable and total costs; it may be largely due to the higher price of dietary OAs in the diet. However, higher values of total return and net profit were obtained in layers fed organic acid-treated diets. This might be due to the promoting effect of dietary organic acid supplementation on hen-day egg production resulting in higher returns from egg sales. A better profitability percentage was noticed in layer hens fed organic acid-treated groups than in T1 and T2 groups. While mixed organic acid groups had more advanced than single organic acid groups. Moreover, the profitability percentage of T5 was improved by 13.26% and 11.82% than T1 and T2, respectively. The relative economic efficiency was increased by 6.21% (T3), 8.67% (T4) and 16.83% (T5) from T1; and by 5.98 % (T3), 8.80 % (T3) and 17.40% (T3) from T2

group. The current study is in agreement with Rahman *et al.*, (2008), that the addition of OAs with a basal diet increases the economic efficiency of layer productivity.

6.5. Conclusion and Recommendations

From the current finding, all dietary OAs supplementation in egg layer hens improved hen day egg production, egg weight, egg mass, feed conversion ratio, shell weight, shell thickness, yolk color, total return, net profit, and survival rate. However, significantly superior values in laying persistency, laying performance, feed conversion efficiency, shell quality, total return, and net profit were recorded in layers-fed mixed organic acid-treated diets. Also, in the same trend better profitability percentage and relative economic efficiency were recorded from the mixture of OAs. Conclusively, mixed OAs at 8g citric and 4g malic acids per 1 kg of egg layer hens' ration can be recommended to replace antibiotic feed additives.

CHAPTER 7: SUMMARY, CONCLUSION AND RECOMMENDATION

7.1. Summary

This dissertation involved a multifaceted evaluation with logically interconnected objectives, starting from the availability of OAs and their utilization practices, and the role of organic acid supplements in commercial broiler and layer chicken production. The first study, the survey assessment, emphasized the assessment of the availability of OAs and their utilization practices in commercial chicken productions. This study revealed that OA feed additives were not currently used in commercial chicken farming in the surveyed areas, despite their local availability in Ethiopia for industrial purposes. All farmers reported having no prior information about OAs feed additives, which is highlighting a significant gap in awareness. However, OAs are being imported in the country for different industry uses such as.....The presence of OAs, including butyric, propionic, fumaric, sorbic, lactic, malic, tartaric, and citric acids, represents a valuable opportunity to use in commercial chicken production if awareness created among chicken farmers.

The second study, evaluated the effects of dietary citric and malic acid supplementation as alternatives to conventional antibiotics on the performance and economic efficiency of Cobb500 broiler chickens. The findings showed significant improvements ($P<0.05$) in final body weight (FBW) and average daily gain (ADG) across the starter, grower, finisher, and overall periods in broiler chickens fed OAs compared with the non-treated control. A mixture of citric and malic acids consistently achieved performance comparable to the antibiotic-treated group, particularly in FBW and FCR. Citric acid alone also improved growth parameters, while malic acid alone and the non-treated control showed lower FBW and ADG. The OA mixture group consistently produced the best FCR, outperforming single-acid treatments and comparable antibiotic supplementation across growth phases of broiler chickens. In 56 days, overall experimental periods, the inclusion of OAs demonstrably improved growth performance and FCR in broiler chickens. The OA mixture group had higher FBW than the untreated control group while being statistically similar to the antibiotic-treated control group. Broilers fed diets supplemented with OAs performed better (62.09 g, 61.98 g, and 64.30 g in T3, T4, and T5,

respectively) in ADG compared with the T1 group (59.58 g), while they were comparable to the antibiotic-treated group (64.06 g). A mixture of citric and malic acids shows the better FCR across all treatment groups; however, single OAs and antibiotic-treated groups had shown intermediate FCR values with no significant differences between them. Furthermore, the untreated control group had a significantly lower survival rate compared with all treatments. The highest carcass yield (2470.75 g) was recorded in T5, followed by T2 (2430.85 g), and T3 and T4 presented an intermediate value, while T1 was the lowest. The study found significant differences in liver, kidney, and ceca weight proportions among dietary treatments, with single and mixed OAs having the highest liver proportion. Broiler chickens fed diets supplemented with citric and malic acids demonstrated improved economic efficiency; however, their mixture exhibited the most significant improvements across key economic efficiency metrics. Profitability increased by 23.7 and 1.7 percent, and REE by 26.98 and 1.54 percent in the OAs mixture, compared to the untreated and antibiotic treated groups, respectively. The finding of this research highlights the mixture of dietary citric and malic acid is a more effective antibiotic replacement strategy than individual acid supplementation; it improves the growth performance, carcass characteristics, and economic efficiency of Cobb500 broiler chickens.

The third study, evaluated the effect of dietary citric and malic acids supplementation on laying performance, egg quality traits, and economic efficiency of commercial layer hens revealed that significantly higher difference ($P < 0.001$) was observed in hen day egg production, egg quality traits, and FCR between treatment groups. However, there was no significant difference recorded among treatment groups in body weight gain and feed intake. Layer hens fed on OA supplemented diets improved the hen-day egg production by 85.51% to 86.54% as compared to non-treated (77.75%) and antibiotic-treated (80.07%) groups. Overall, the combination of citric and malic acids, compared with single OA as well as both control groups. Moreover, citric and malic acids mixed acid reflected the highest performance in terms of FCR, albumin weight, egg mass, total return, and net profit. Mixture of citric and malic acids improved the profitability by 13.26% and 11.82% and REE by 16.83 % and 17.40% than non-treated and antibiotic-treated groups, respectively.

7.2. Conclusions and Recommendations

This dissertation research indicates that organic acids are not currently employed as feed additives in commercial chicken farming within the study regions, despite their availability for industrial purposes in Ethiopia. The reason for this absence of use is the lack of information available to chicken farmers, which necessitates awareness creation to address the knowledge gaps among farmers, thereby encouraging the adoption of organic acid feed additives. Feeding trials have provided compelling evidence of the advantages of citric and malic acids for both broiler and layer chickens. When these acids were included in the diet, particularly in combination, there were notable enhancements in growth performance, FCR, carcass characteristics, survival rate, and economic efficiency in Cobb500 broilers. Similarly, layer hens experienced improved egg production, egg quality, FCR, survival rate, and profitability. These results indicate that incorporating citric and malic acids into the diet can effectively replace antibiotics, with the combination of 8 g/kg citric acid and 4 g/kg malic acid delivering the most significant improvements in both production and economic outcomes for commercial broiler and layer chicken production.

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APPENDIX

Appendix 1. Questionnaires used for the Study

BAHIR DAR UNIVERSITY
College of Agriculture and Environmental Sciences,
Department of Animal Sciences
PhD Program in Animal Production

I. Interview Questioners for Commercial Chicken Producers

A. Characteristics of respondent

1. Name_____ City_____ Sub-city_____
2. Sex: A) Male; B) Female
3. Age:_____
4. Marital status: A) Single, B) Married, C) Divorced; D) Widowed
5. Family Size:

Age Group (year)	Number	Remark
<15		
15- 30		
31- 45		
46- 60		
> 60		

6. Education level:
A) Layman; D) Grades 9-12; F) Graduate in degree
B) Read and write; E) Graduate in and above;
C) Grades 1-8; Diploma;
7. If you are graduate in BSc and above other than animal sciences profession; what is your field of study?_____
8. Occupation: A) Small scale chicken production B) Large scale chicken production
9. For how long you have been working in chicken farm?_____

10. What is your responsibility in this chicken farm (multiple responses possible)

A) Farm owner;

C) Employee (please state your

B) Manager

responsibility)

11. How many workers involved in this chicken farm? _____

S/N	Education Level	Number of Workers	Remark
1	Layman		
2	Read and write		
3	Grades 1-8;		
4	Grades 9-12;		
6	Diploma graduate		
7	Degree & above		

B. Management of commercial farms

1. What type of chicken breed you are keeping in your farm?

S/N	Breed class	Number	Remark
1	Crossbred		
2	Exotic (Pure breed)		

2. To which production system is your chicken farm grouped and year of establishment?

Production System	Population			Year Established
	Layer	Broiler	Dual	
Semi-intensive				
Intensive				

3. How many and which classes of chickens are found in your farm currently?

Class	Age	Population	Breed
Layer	Starters (0 - 8 Weeks)		
	Growers(9 - 20 Weeks)		
	Layers (> 20 Weeks)		
	Total		
Broiler	Starters (0-3 Weeks)		
	Growers(4 - 5 Weeks)		
	Finisher(6 – 8 Weeks)		
	Total		
Dual	Starter(0-8 Weeks)		
	Grower(9-20 Weeks)		
	Total		

4. If your chickens are layer, do you follow all-in-all-out production system? A) Yes, B)

No

5. If your chickens are layer, what is the type of housing?
- A) Deep litter system with concrete floor C) Cage system
B) Deep litter system without concrete floor D) Other (specify)
6. If your chickens are broiler, what is the type of housing you use for your layer chicken?
- A) Deep litter system B) Other (specify)
7. What is your chicken house orientation?
- A) North-South direction C) Other (please specify)
B) East- West direction
8. What is the approximate area allocated for your chicken? (Number of chicken per m²).

Chicken Type	Chicken Age	Number of Chicken per m ²
Layer	Starters (0 - 8 Weeks)	
	Growers(9 - 20 Weeks)	
	Layers (> 20 Weeks)	
Broiler	Starters (0-3 Weeks)	
	Growers(4 - 5 Weeks)	
	Finisher(6 – 8 Weeks)	
Dual	Starter(0-8 Weeks)	
	Grower(9-20 Weeks)	

9. What is the source of money to finance your chicken farming?
- A) Chicken sales; C) Crop sales; E) Other (specify)
B) Egg sales; D) Livestock sales;
10. Where is your feed source for your chickens?
- A) Feed industries; D) Home-made feed;
B) Wholesalers and retailers; E) Other source(Please specify)
C) Farmer unions;
11. Do you know the nutrient content of your chicken feed? A) yes; B) No
12. Do you know the amount of feed offered per day? A) yes; B) No
13. If your answer for question number 12 is yes, how many grams of feed provide per chicken?

Chicken Type	Physiological stage of Chicken	Feed provided for one chicken per day	Remark
Layer	Starters (0 - 8 Weeks)		
	Growers(9 - 20 Weeks)		
	Layers (> 20 Weeks)		
Broiler	Starters (0-3 Weeks)		
	Growers(4 - 5 Weeks)		
	Finisher(6 – 8 Weeks)		

Dual	Starter(0-8 Weeks)		
	Grower(9-20 Weeks)		

14. How many times you provided feed to your chicken per day?
15. Do you have light source for your chicken? A) Yes, B) No
16. Do you have water source for your chicken? A) Yes, B) No
17. If your answers yes for question number 12, please specify your water source _____
18. Do you have the plan to expand your chicken farm? A) yes; B) No
19. If your answer to question number 14 is yes, at what scale level expand?
20. Do you have training related to feed quality and feeding strategy of chickens?
21. Do you have training related to biosecurity of chicken farm? A) Yes; B) No
22. Do you use antibiotics for your chickens? A) Yes; B) No
23. For what purpose antibiotics provided for chickens?
 - A) For treatment of sick chicken,
 - B) For disease prevention,
 - C) For growth promotion,
 - D) For maximized production
24. If yes, who recommend to the use antibiotics in your farm for intended use?
 - A) Veterinary professionals
 - B) Extension workers (DAs)
 - C) Friends that have chicken farm
 - D) Self-prescription
25. Is there any local source used to your chicken instead of antibiotics? A) Yes; B) No
26. If yes, what are the alternatives?
27. Do you have information about the negative effects of antibiotic? A) Yes, B) No
28. If yes, how do you keep your chicken healthy in sustainable manner?

C. Availability and utilization practice of organic acids

1. What do you provide from local sources when your chickens are feeling sick or you want to be healthy? A) yes; B) No

If yes, please mention the name, source, dose and methods of provide.

Name of the Source	Botanical Composition	Dose	Method of provide		
			By adding to feed	By adding to water	Directly

2. Do you have information about the role of feed additives in chicken production?
3. If your answer for number 2 yes, do you provide it to your chickens A) Yes B) No
4. If your answer for number 3 yes, for what purpose you provide feed additives to your chickens?
 - A) For growth promotion
 - B) For maximized production
 - C) For disease prevention
 - D) For treatments of sick chickens
5. Who recommend using feed additives to your chickens?
 - A) Veterinary professionals
 - B) Extension workers (DAs)
 - C) Friends that have chicken farm
 - D) Indigenous knowledge
 - E) Other (please specify)
6. If your answer for question number 1 is yes, from where do you get those feed additives to your chickens?
 - A) From veterinary drug shops
 - B) From feed suppliers/retailers
 - C) Government veterinary clinic
 - D) Privet veterinary clinic
 - E) Human pharmacy
 - F) Open market
 - G) From indigenous knowledge
7. Do you use organic acid feed additives for your chicken? A) Yes; B) No
8. If your answer for question number 7 is yes, which of the following organic acids provide to your chicken? Please mention the dose and methods of provide.

Organic Acids	Dose and Methods of Providing		
	By adding to feed (gm./kg)	By adding to water (amount / litter)	Other (please Specify)
Formic acid			
Acetic acid			
Propionic acid			
Butyric acids			
Lactic acid			
Malic acids			
Citric acid			
Fumaric acids			
Tartaric acids			
Other (please specify)			

9. To which production system and chicken type you provide organic acids feed additives?

Production System	Chicken Type		
	Layer	Broiler	Dual

Semi-intensive commercial			
Intensive commercial			

10. To which class of chicken age you provide organic acids feed additives?

Class	Age	Yes	No
Layer	Starters (0 - 8 Weeks)		
	Growers(9 - 20 Weeks)		
	Layers (> 20 Weeks)		
Broiler	Starters (0-3 Weeks)		
	Growers(4 - 5 Weeks)		
	Finisher(6 – 8 Weeks)		
Dual	Starter(0-8 Weeks)		
	Grower(9-20 Weeks)		

11. How long do you provide organic acids to your chickens?

A) Always,

B) Sometimes

12. Why you give organic acids feed additives to your chickens?

13. How is organic acids feed additive dosage determined before providing to your chickens?

14. If your answer for question number 6 is do not provided organic acid feed additives to your chicken, reason out why not giving it?

A) Lack of awareness about organic acid feed additives in chicken nutrition

C) Expensive price

D) Because of antibiotic availability

E) Other (please specify)

B) Unavailability

II. Checklist for Key-Informants Discussion

1. Study site:_____

2. Name of Key Informants

3. What is your view about chicken production?

4. Do you have information about feed additives in chicken production?

5. If yes, what are its functions for chicken?

6. Do you think the feed additives are available in the study area? If yes, please list them according to their availability?
7. Do you have knowledge about functions of organic acids? If yes, please mention its function and type of organic acids.
8. Do you have information about the availability of organic acids in Ethiopia?
9. Do you have information about the use of organic acids in chicken production? If you have please mention it.
10. Do you have knowledge on the use of organic acids other than chicken production? If yes, please mention it for what purpose to be used?

III. Checklist used for Suppliers

1. Name of respondent _____
2. Trade name _____
3. Do you supply poultry feed additives to your costumers?
A) yes; B) No
4. If your answer for question number 1 is yes, which type of feed additives supply to your costumers?
5. If you supply organic acids to your costumers, which type of organic acids supply to your costumers?
6. Who are your costumers of organic acids? Please rank them based on use of the customer?
7. From where you access this organic acids?
8. How is the availability of organic acids in your organization?
A) Always available;
B) Not always available;
C) Depend on the demand or order of your customers;
D) Other (Specify)
9. What do you think about future of organic acids in Ethiopia?

Appendix 2: ANOVA Tables

A) ANOVA tables for the effect of OAs supplementation on broiler growth performance, nutrient utilization at 56 days

1. Analysis of variance for the effect of OAs on broilers initial body weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	0.00222000	0.00055500	0.00	1.0000
Error	15	10.28740000	0.68582667		
Corrected Total	19	10.28962000			

R-Square	Coeff Var	Root MSE	IBW Mean
0.000216	2.043948	0.828147	40.51700

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	0.00222000	0.00055500	0.00	1.0000
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	0.00222000	0.00055500	0.00	1.0000

2. Analysis of variance for the effect of OAs on broilers final body weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	183437.6680	45859.4170	21.72	<.0001
Error	15	31670.5300	2111.3687		
Corrected Total	19	215108.1980			

R-Square	Coeff Var	Root MSE	FBW Mean
0.852769	1.299918	45.94963	3534.810

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	183437.6680	45859.4170	21.72	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	183437.6680	45859.4170	21.72	<.0001

3. Analysis of variance for the effect of OAs on broilers body weight gain

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	183440.1590	45860.0398	21.37	<.0001
Error	15	32194.5634	2146.3042		
Corrected Total	19	215634.7224			

R-Square	Coeff Var	Root MSE	FBWG Mean
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0.850699	1.325825	46.32822	3494.293
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Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	183440.1590	45860.0398	21.37	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	183440.1590	45860.0398	21.37	<.0001

4. Analysis of variance for the effect of OAs on broilers average daily gain

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	58.46003000	14.61500750	21.35	<.0001
Error	15	10.26835000	0.68455667		
Corrected Total	19	68.72838000			

R-Square	Coeff Var	Root MSE	ADG Mean
0.850595	1.325950	0.827379	62.39900

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	58.46003000	14.61500750	21.35	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	58.46003000	14.61500750	21.35	<.0001

5. Analysis of variance for the effect of OAs on broilers cumulative feed intake

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	466615.2007	116653.8002	3.68	0.0281
Error	15	476094.1956	31739.6130		
Corrected Total	19	942709.3963			

R-Square	Coeff Var	Root MSE	CFI Mean
0.494972	2.776287	178.1561	6417.066

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	466615.2007	116653.8002	3.68	0.0281
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	466615.2007	116653.8002	3.68	0.0281

6. Analysis of Variance for the effect of OAs on broilers average feed intake

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	148.8008500	37.2002125	3.67	0.0281
Error	15	151.8381500	10.1225433		
Corrected Total	19	300.6390000			

R-Square	Coeff Var	Root MSE	AFI Mean
0.494949	2.776503	3.181594	114.5900

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	148.8008500	37.2002125	3.67	0.0281
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	148.8008500	37.2002125	3.67	0.0281

7. Analysis of variance for the effect of OAs on broilers FCR

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	0.13007000	0.03251750	25.34	<.0001
Error	15	0.01925000	0.00128333		
Corrected Total	19	0.14932000			

R-Square	Coeff Var	Root MSE	FCR Mean
0.871082	1.949056	0.035824	1.838000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	0.13007000	0.03251750	25.34	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	0.13007000	0.03251750	25.34	<.0001

B) ANOVA tables for the effect of OAs supplementation on carcass characteristics of broiler chickens at 56 days

1. Analysis of variance for the effect of OAs on broilers on slaughter weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	296993.8578	74248.4645	5.18	0.0035
Error	25	358068.3000	14322.7320		
Corrected Total	29	655062.1578			

R-Square	Coeff Var	Root MSE	SW Mean
0.453383	3.424646	119.6776	3494.598

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	296993.8578	74248.4645	5.18	0.0035
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	296993.8578	74248.4645	5.18	0.0035

2. Analysis of variance for the effect of OAs on broilers dressed carcass weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	405814.8590	101453.7147	21.34	<.0001
Error	25	118854.2492	4754.1700		
Corrected Total	29	524669.1081			

R-Square	Coeff Var	Root MSE	DC Mean
0.773468	2.956142	68.95049	2332.449

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	405814.8590	101453.7147	21.34	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	405814.8590	101453.7147	21.34	<.0001

3. Analysis of variance for the effect of OAs on broilers thigh weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	11218.60409	2804.65102	7.98	0.0003
Error	25	8782.86990	351.31480		
Corrected Total	29	20001.47399			

R-Square	Coeff Var	Root MSE	Th. Mean
0.560889	4.656196	18.74339	402.5473

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	11218.60409	2804.65102	7.98	0.0003
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	11218.60409	2804.65102	7.98	0.0003

4. Analysis of variance for the effect of OAs on broilers drumstick weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	18588.80642	4647.20161	12.20	<.0001
Error	25	9520.13997	380.80560		
Corrected Total	29	28108.94639			

R-Square	Coeff Var	Root MSE	Dr Mean
0.661313	6.215901	19.51424	313.9407

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	18588.80642	4647.20161	12.20	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	18588.80642	4647.20161	12.20	<.0001

5. Analysis of variance for the effect of OAs on broilers breast weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	77007.2890	19251.8222	14.47	<.0001
Error	25	33257.5389	1330.3016		
Corrected Total	29	110264.8279			

R-Square	Coeff Var	Root MSE	Br Mean
0.698385	3.922919	36.47330	929.7490

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	77007.28895	19251.82224	14.47	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	77007.28895	19251.82224	14.47	<.0001

6. Analysis of variance for the effect of OAs on broilers back and thorax weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	6417.87717	1604.46929	4.28	0.0090
Error	25	9374.40005	374.97600		
Corrected Total	29	15792.27722			

R-Square	Coeff Var	Root MSE	Ba Mean
0.406393	5.779726	19.36430	335.0383

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	6417.877167	1604.469292	4.28	0.0090
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	6417.877167	1604.469292	4.28	0.0090

7. Analysis of variance for the effect of OAs on broilers wing weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	1510.14105	377.53526	0.88	0.4898
Error	25	10720.99905	428.83996		
Corrected Total	29	12231.14010			

R-Square	Coeff Var	Root MSE	Wi Mean
0.123467	8.308961	20.70845	249.2303

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	1510.141047	377.535262	0.88	0.4898
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	1510.141047	377.535262	0.88	0.4898

8. Analysis of variance for the effect of OAs on broilers neck weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	1582.225233	395.556308	7.46	0.0004
Error	25	1326.194033	53.047761		
Corrected Total	29	2908.419267			

R-Square	Coeff Var	Root MSE	Ne Mean
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0.544016	7.144547	7.283389	101.9433
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Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	1582.225233	395.556308	7.46	0.0004
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	1582.225233	395.556308	7.46	0.0004

9. Analysis of variance for the effect of OAs on broilers abdominal fat weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	1515.807280	378.951820	5.69	0.0021
Error	25	1665.749067	66.629963		
Corrected Total	29	3181.556347			

R-Square	Coeff Var	Root MSE	AF Mean
0.476436	14.69824	8.162718	55.53533

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	1515.807280	378.951820	5.69	0.0021
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	1515.807280	378.951820	5.69	0.0021

10. Analysis of variance for the effect of OAs on broilers giblets weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	373.885280	93.471320	2.90	0.0424
Error	25	806.389600	32.255584		
Corrected Total	29	1180.274880			

R-Square	Coeff Var	Root MSE	GW Mean
0.316778	4.433637	5.679400	128.0980

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	373.8852800	93.4713200	2.90	0.0424
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	373.8852800	93.4713200	2.90	0.0424

11. Analysis of Variance for the effect of OAs on broilers non-edible weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	35965.0166	8991.2542	2.12	0.1080
Error	25	105959.7560	4238.3902		
Corrected Total	29	141924.7727			

R-Square	Coeff Var	Root MSE	NEO Mean
0.253409	9.876080	65.10292	659.1980

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	35965.01665	8991.25416	2.12	0.1080
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	35965.01665	8991.25416	2.12	0.1080

C) Appendix 2.3. ANOVA tables for the effect of OAs supplementation on egg laying performance of layer hens in 120 days

1. Analysis of Variance for the effect of OAs on layer hens commutative feed intake

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	74227.1533	18556.7883	1.10	0.3916
Error	15	252661.1842	16844.0789		
Corrected Total	19	326888.3375			

R-Square	Coeff Var	Root MSE	CFI Mean
0.227072	0.918691	129.7847	14127.13

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	74227.15328	18556.78832	1.10	0.3916
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	74227.15328	18556.78832	1.10	0.3916

2. Analysis of Variance for the effect of OAs on layer hens average daily feed intake

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	5.12653000	1.28163250	1.10	0.3941
Error	15	17.54032500	1.16935500		
Corrected Total	19	22.66685500			

R-Square	Coeff Var	Root MSE	ADFI Mean
0.226169	0.918542	1.081367	117.7265

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	5.12653000	1.28163250	1.10	0.3941
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	5.12653000	1.28163250	1.10	0.3941

3. Analysis of Variance for the effect of OAs on layer hens FCR

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	0.49557000	0.12389250	8.81	0.0007
Error	15	0.21095000	0.01406333		
Corrected Total	19	0.70652000			

R-Square	Coeff Var	Root MSE	FCR Mean
0.701424	5.289426	0.118589	2.242000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	0.49557000	0.12389250	8.81	0.0007
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	0.49557000	0.12389250	8.81	0.0007

4. Analysis of Variance for the effect of OAs on layer hens initial body weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	15.79453	3.94863	0.00	1.0000
Error	15	20433.20312	1362.21354		
Corrected Total	19	20448.99766			

R-Square	Coeff Var	Root MSE	IBW Mean
0.000772	2.293454	36.90818	1609.284

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	15.79453000	3.94863250	0.00	1.0000
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	15.79453000	3.94863250	0.00	1.0000

5. Analysis of Variance for the effect of OAs on layer hens final body weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	2433.456250	608.364063	3.23	0.0423
Error	15	2823.953125	188.263542		
Corrected Total	19	5257.409375			

R-Square	Coeff Var	Root MSE	FBW Mean
0.462862	0.761866	13.72092	1800.963

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	2433.456250	608.364063	3.23	0.0423
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	2433.456250	608.364063	3.23	0.0423

6. Analysis of Variance for the effect of OAs on layer hens body weight change

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	2574.28553	643.57138	0.41	0.7966
Error	15	23375.28125	1558.35208		
Corrected Total	19	25949.56678			

R-Square	Coeff Var	Root MSE	BWC Mean
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0.099203	20.59483	39.47597	191.6790
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Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	2574.285530	643.571383	0.41	0.7966
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	2574.285530	643.571383	0.41	0.7966

7. Analysis of Variance for the effect of OAs on hen-day-egg-production

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	296.4594000	74.1148500	6.86	0.0024
Error	15	162.1707750	10.8113850		
Corrected Total	19	458.6301750			

R-Square	Coeff Var	Root MSE	HDEP Mean
0.646402	3.927105	3.288067	83.72750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	296.4594000	74.1148500	6.86	0.0024
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	296.4594000	74.1148500	6.86	0.0024

8. Analysis of Variance for the effect of OAs on average egg weight

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	31.83043000	7.95760750	5.26	0.0075
Error	15	22.68355000	1.51223667		
Corrected Total	19	54.51398000			

R-Square	Coeff Var	Root MSE	AEW Mean
0.583895	1.951302	1.229730	63.02100

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	31.83043000	7.95760750	5.26	0.0075
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	31.83043000	7.95760750	5.26	0.0075

9. Analysis of Variance for the effect of OAs on egg mass

Sum of					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	234.2946200	58.5736550	9.09	0.0006
Error	15	96.6099000	6.4406600		
Corrected Total	19	330.9045200			

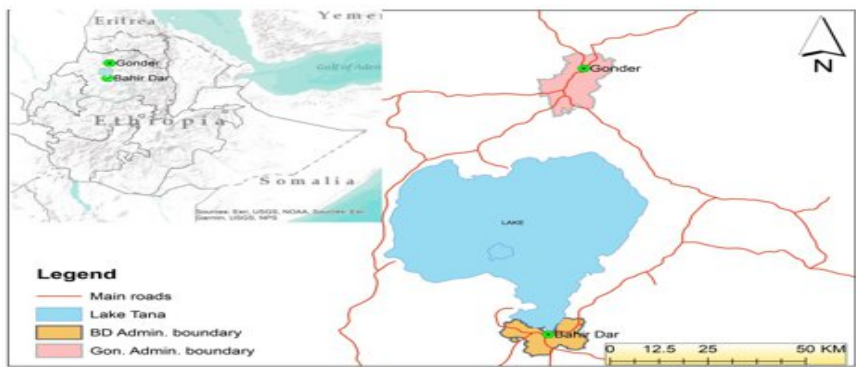
R-Square	Coeff Var	Root MSE	EM Mean
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0.708043	4.804524	2.537846	52.82200
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Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	4	234.2946200	58.5736550	9.09	0.0006
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	4	234.2946200	58.5736550	9.09	0.0006

Appendix 3: Figures

Appendix Figure 3.1. Map of the study areas for survey study



Appendix Figure 3.3. Photos taken while conducting the survey evaluation of commercial chicken farms



Appendix Figure 3.2. Experimental houses for animals evaluation



Appendix Figure 3.4. Photos shown experimental house and equipment preparations for layer hen evaluation



Appendix Figure 3.5. Photos shown experimental house and broiler chickens management



Appendix Figure 3.6. Photos shown experimental feed preparations



Appendix Figure 3.6. Photos shown body weight, egg production and quality evaluations of layer hens



Appendix Figure 3.6. Photos taken during measurements of body weight, carcass yield and GIT of broiler chickens

