



**GROWTH AND HEALTH PROMOTING EFFECTS OF *Nigella sativa*
L., *Trigonella foenum-graecum* L. AND *Curcuma longa* L. IN BROILER
CHICKEN**

PhD Dissertation

By

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Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Animal Production Studies

PhD Program in Animal Production

April 2018

Bishoftu, Ethiopia

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**A Dissertation Submitted to the College of Veterinary Medicine and Agriculture,
Addis Ababa University in Fulfillment of the Requirement of the Degree of Doctor
of Philosophy in Animal Production**

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L. and *Curcuma longa* L. IN Broiler Chicken

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STATEMENT OF THE AUTHOR

First, I declare that this dissertation is my benefit work and that all sources of material used for this dissertation have been duly acknowledged. This dissertation has been submitted in partial fulfillment of the requirements for a PhD degree at Addis Ababa University, College of Veterinary Medicine and is deposited in the University/College library to be made available to borrowers under the rules of the Library. I solemnly declare that this dissertation is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

I the Author of this desertation was born on February 27, 1986 in Addis Ababa, Ethiopia. I attended my elementary school education at Karamara and Netsanet Chora Elementary Schools and high school Education at Dejazmach Wondirad Secondary and Preparatory School between 1992 and 2005 G.C. After completion of my high school education, I joined Jimma University, College of Agriculture and Veterinary Medicine in 2005 then graduated with a BSc degree in Animal Science in 2008.

Soon after graduation, I rejoined the School of Graduate Studies of the same University to pursue my second degree majoring in Animal Production and graduated in 2010.

Upon completion of my MSc study, I was employed by Hawassa Poultry Breeding and Multiplication Centre as animal feed and production expert. After serving for 5 months I joined the Southern Agricultural Research Institute, Jinka Agricultural Research Centre and worked as a junior livestock researcher for a year. I then was employed by Wolaita Sodo University in December 2012 for teaching, research and community services from which I was given the opportunity to pursue my PhD study in 2014 in the School of Graduate Studies of Addis Ababa University, College of Veterinary Medicine and Agriculture in the Department of Animal Production Studies.

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DEDICATION

*I dedicate this piece of work to the Almighty God. For from him, through him,
and to him are all things to him be glory forever!! Amen.*

ABBREVIATIONS

ADG	Average Daily Gain
AGP	Antibiotics Growth Promoters
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AOAC	Association of Official Analytical Chemists
AST	Aspartate aminotransferase
BW	Body Weight
CF	Crude Fiber
CP	Crude Protein
EDTA	Ethylene Diaminetetra-Acetic Acid
EE	Ether extract
ELISA	Enzyme- Linked Immunosorbent Assay
FCR	Feed Conversion Ratio
GIT	Gastro Intestinal Tract
GLM	General Linear Model
Hb	Hemoglobin
HDL	High-Density Lipoprotein
HI	Haemagglutination Inhibition
HMG-CoA	Hydroxy-Methyl-Glutaryl Coenzyme A
IBD	Infectious Bursal Disease
LDL	Low-Density Lipoprotein
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
ME	Metabolizable Energy
NCD	Newcastle Disease (NCD)

ABBREVIATIONS (*Continued*)

NFE	Nitrogen Free Extract
NVI	National Veterinary Institute
PCV	Packed Cell Volume
PFA	Phytogenic or phytobiotic Feed Additives
PGG	Pentagalloyl Glucose
RBC	Red Blood Cell
RNA	Ribonucleic acid
SAS	Statistical Analysis Software
SBM	Soybean Meal
SGOT	Serum Glutamic Oxaloacetic transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SRBC	Sheep Red Blood Cell
WBC	White blood Cell
WHO	World Health Organization

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GROWTH AND HEALTH PROMOTING EFFECTS OF *Nigella sativa* L., *Trigonella foenum-graecum* L. AND *Curcuma longa* L. IN BROILER CHICKEN

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ABSTRACT

Two experiments were conducted to examine the growth and health promoting efficiency of black cumin and fenugreek seeds and turmeric rhizome. The aim of phase I experiment was to determine the optimum dosage levels of seeds of black cumin and fenugreek and rhizome of turmeric as phytobiotic feed additives. The phase II experiment was aimed at the synergistic effects of the different blends of phytobiotic feed additives against commercial antibiotics (Oxytetracycline). For the first experiment (phase I), six treatments containing black cumin, fenugreek and turmeric at the levels of 1 and 2% each of the total diets and a control diet were used. In phase II, six experimental diets containing negative control basal diet (T0), positive control basal diet + oxytetracycline at 0.035% (T1), basal diet + a blend of black cumin seed and fenugreek (T2), basal diet + a blend of black cumin seed and turmeric powder (T3), basal diet + a blend of fenugreek seed and turmeric powder (T4) and basal diet + a blend of black cumin seed + fenugreek seed + turmeric powder (T5) at equal proportion were used. A total of 315 and 270 (Cobb 500) day-old broiler chicks were randomly allocated to 7 and 6 treatment combinations for experiment I and II, respectively with 3 replications per treatment. Chemical analysis of the feeds used, feed consumption, weight gain, carcass characteristics, hematological parameters, serum biochemistry profile, immune response, ceacum microbial load and economic viability were measured. The results of Phase I indicated that; turmeric and black cumin at both levels ensured high feed conversion ratio (FCR) ($P \leq 0.05$) than the control birds. However, there were no significant differences ($P \geq 0.05$) in BW, BW gain and ADG among treatments. There were significant differences ($P \leq 0.05$) in commercial carcass and edible carcass yield among the treatments. Furthermore, turmeric at 2%, black cumin and fenugreek seeds at 1% had significant ($P \leq 0.05$) positive effects on Hb and RBC of broilers. Birds fed turmeric and

*fenugreek at both levels in the diets had reduced serum glucose level ($P \leq 0.05$). While, birds fed turmeric at 2%, black cumin at 1% and fenugreek at both levels showed lower serum cholesterol levels ($P \leq 0.05$) in broilers. The immunomodulatory response against Newcastle disease (NCD) antibody titer was improved and more pronounced ($P \leq 0.05$) in birds fed turmeric powder and fenugreek seed at 2%. Antibody response increased against infectious bursa disease (IBD) in birds fed turmeric powder at 2% and black cumin at 1% than the control birds during the 21st day of age. Higher net return was obtained from birds fed turmeric at both levels and black cumin at 1%. The result of Phase II revealed that there were no significant differences ($P \geq 0.05$) in BW gain and ADG between T5 and antibiotics fed birds without negative impact on carcass components and do not have toxin to impair the development of the internal organs ($P \geq 0.05$). Lowest feed consumption (T2, T3, T4 and T5) and improved FCR obtained from birds fed different blends of phytobiotic additives treatments (T3 and T5) than the controls T0 and T1 ($P \leq 0.05$). The PCV, RBC, MCV and MCHC values were significantly different ($P \leq 0.05$) between treatments. Birds fed in T5, T4 and T3 test diets had showed enhanced ($P \leq 0.05$) hypoglycemic and hypocholesterolemic effects than the birds in T1 and T0 diets. The mixture of phytobiotic additives (T2, T4 and T5) were superior than the antibiotics in lowering total coliform and *E. coli* bacteria of the cecum ($P \leq 0.05$). Generally, turmeric at 1-2% and black cumin at 1% can be used for profitable broiler production without negative effects on biological performances of birds. Blends of the three medicinal plants can substitute commercial antibiotics and impart positive effects on growth and health performances of broilers.*

Keywords: Black cumin; Biochemical; Broiler; Carcass characteristics; Fenugreek; Growth performance; Immunomodulatory; Phytobiotics; Turmeric

1. INTRODUCTION

The industrialization of poultry production and the need for the improvement of nutritional efficiency have accelerated the use of antibiotics as feed additives in animal feeding for many decades. The objective outlined by scientists is to increase production while maintaining animals in good health. The use of antibiotics in poultry feeds as a growth promoter is beneficial in improvement of production parameters and diseases prevention (Dono, 2012).

The routine uses of sub-therapeutic levels of antibiotics often referred to as antibiotic growth promoters (AGP) in broiler feeds have been a common practice for more than 50 years to prevent potential diseases as well as to robust gut health, increase meat yield and improve feed efficiency (Wang *et al.*, 2015; Dono, 2012; Gaskins *et al.*, 2002). However, the aggravated utilization of antibiotics in bulk as growth promoters has led to the increased resistance of pathogens to the antibiotics and the accumulation of antibiotic residues in animal products and in the environment. In other hand, use of antibiotics in poultry nutrition increases the risks of antibiotic resistance in human society (Toaha *et al.*, 2016; Nisha, 2008). This situation required most countries in the world to ban or limit the use of antibiotics in poultry feeds and launched the "antibiotic-free" labeled feeds (Toaha *et al.*, 2016; Cogliani, 2011; Lee *et al.*, 2004).

However, the removal of antibiotics as growth promoters has led to reduced growth performance and feed efficiency as well as increased the incidences of certain animal diseases (Wierup, 2000). This negatively affects the poultry sector and feed industries and the drives to search for other alternatives. As a result, natural alternatives with similar beneficial effects as antibiotics growth promoters have been intensified (Khan, 2011). Huge interest and some useful researches on various Natural Growth Promoters (NGPs) such as phytobiotics (essential oils, powders, extracts and phytochemicals), organic acids, clay minerals, egg yolk antibodies, exogenous enzymes, recombinant enzymes, nucleotides, polyunsaturated fatty acids and miscellaneous

compounds have increased the impetus for revisiting and to look for new, useful feed additives that can enhance health and productivity of birds (Hashemi and Davoodi, 2010).

Medicinal plants are also known as phytobiotic /or phyto gene feed additives (PFA) they are often very complex mixtures composed of bioactive components including several groups of plant products depending on the origin and purpose of production. The positive effects of medicinal plants or phytobiotic additives can be expected primarily through beneficial effects on the growth and health of individuals as well as by improving the immune system and stimulate beneficiary antimicrobial activities (Windisch *et al.*, 2008).

Several authors (Prajapat, 2016; Al-Kassie *et al.*, 2011a; Jang *et al.*, 2007; Platel and Srinivasan, 2004; Guo *et al.*, 2004a) have indicated that the use of medicinal plants, containing compounds, such as wide varieties of herbs, spices and/or essential oils (e.g., thyme, turmeric, fenugreek, black cumin, oregano, rosemary, marjoram, yarrow, garlic, ginger, green tea, hot pepper, coriander, cinnamon) would stimulate the digestive enzymes production and activity; and induce a higher secretion of bile acids

An increased use of medicinal plants in animal nutrition is the result of their positive properties including bactericidal, fungicidal, antiviral, antioxidant capacity, growth-promoting efficacy, immune stimulating effects, stimulation of the secretion of digestive enzymes and absorption of nutrients (Cross *et al.*, 2007; Ertas *et al.*, 2005). Although detailed mechanisms of their actions in broiler chickens remains unclear (Puvaca *et al.*, 2013). However, some study indicated that the positive impacts of medicinal plants is the result of synergy between complexes of active molecules in the plants.

Jang *et al.* (2007) and Lee *et al.* (2003), essential oils used as feed additives for broilers to enhance the activities of trypsin and amylase. It has been suggested that those dietary spices can induce changes in cell membrane fluidity and permeability properties, resulting in an increased absorption of micronutrients from the small intestine (Prakash and Srinivasan, 2010). Furthermore, active plant compounds are the potential effectors on microbial communities and therefore could be considered as alternatives in controlling the

intestinal microbial population (Burt, 2004; Lee *et al.*, 2004). Due to their aromatic properties, phytogetic additives may affect the sensory characteristics of the food, such as taste and smell (Puvaca *et al.*, 2013; Cross *et al.*, 2007; Ertas *et al.*, 2005).

The main reasons for the application of medicinal plants as feed additives are on reducing the risk of intestinal imbalance, occurrence of diarrhea (especially in young animals), improve feed consumption, improve growth performance, increase productivity, reducing mortality rates, improving the quality of the final product, the absence of withdrawal period and harmful residues in animal products (Mathe and Mathe, 2008) and production of organic animal products. Black cumin, fenugreek seeds, and turmeric rhizome are known to have an antioxidant, antimicrobial, immunomodulatory, hypoglycemia, hypocholesterolemia, hepatoprotective, hepatostimulatory properties (Gowda *et al.*, 2009; Kavirasan, 2007). In addition, fenugreek seed extracts have a unique feature of anti-hyperthyroidism and properties of anti-sterility and anti-androgenic effects (Tahiliani and Kar, 2003; Kamal *et al.*, 1993).

The synergistic effect of phytoiotics compounds have been reported in studies with essential oils, seeds and leaves (Amerah *et al.*, 2011; Tiihonen *et al.*, 2010; Mehala and Moorthy 2008a; Mitsch *et al.*, 2004). The combination of herbal powders and essential oils are more effective than a single herb administration (Khaligh *et al.*, 2011) because the interaction of two or more agents to produce a blended event is invariably larger than the sum of their individual effects (Van Vuuren and Viljoen, 2011).

Even though Ethiopia abounds in herbal wealth and innumerable medicinal plants/herbs possession, their use in the poultry still awaits exploitation by the scientific research methods. However, rural poultry farmers use some medicinal plants only when their birds get sick rather than using them as prevention and production promoters. On the other hand, researches conducted comparing the synergistic effects of the selected medicinal plants in poultry feeding is inadequate. Although studies have been conducted so far comparing different inclusion levels of the selected medicinal plants, the results were inconclusive and contradictory.

So far, no research finding has been reported on the utilization of locally available variety of black cumin, fenugreek seeds and turmeric rhizome as broiler feed additive in Ethiopia. Thus, it is worthwhile to evaluate the growth and health promoting effects of some of the phytobiotic feed additives in broiler chicken production. Therefore, the present study was conducted to examine the effects of different levels of black cumin seed (*Nigella sativa* L.), fenugreek seed (*Trigonella foenum* L.) and turmeric rhizome (*Curcuma longa* L.) on growth and health performance of broiler chicken with the following general and specific objectives:

General Objective

- To evaluate the growth and health promoting effects of black cumin, and fenugreek seeds and turmeric rhizome as a natural or phytobiotic feed additives in the diets of broiler chickens

Specific Objectives

- To evaluate the effects of different inclusion levels of black cumin, and fenugreek seeds and turmeric rhizome on the growth and carcass characteristics of broiler chickens,
- To assess the effects of different inclusion levels of black cumin, and fenugreek seeds and turmeric rhizome on the hematological, biochemical and immunomodulation of broiler chickens,
- To analyze the synergetic effects of the different blends of black cumin and fenugreek seeds and turmeric rhizome against commercial antibiotics (Oxycytetracyclin) on the growth, carcass characteristics, hematological and serum biochemical profiles, immunomodulation and ceacal microbial loads of broiler chickens,
- To determine the partial budgets of the inclusion of black cumin and fenugreek seeds and turmeric rhizome alone or in different combinations in broiler diets.

2. LITERATURE REVIEW

2.1. Medicinal Plants

Since ancient times, medicinal plants have been used because of their preservatives and medicinal properties along with the characteristics of imparting aromas as well as flavors to the food. For centuries in traditional or folkloric medicine as well as veterinary medicine aromatic plants (also called as herbs and spices), their essential oils as well as extracts have been used for treating human and animals (Midrarullah *et al.*, 2014). The World Health Organization estimates that about 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Over 50% of all modern clinical drugs are of natural product origin (Kirbag *et al.*, 2009).

Plants contain a wide range of bioactive compounds, such as phenolics, flavonoids, terpenoids, carotenoids, saponins, alkaloids, flavours and fragrances. Plant extracts with these bioactive molecules are widely used in the food, pharmaceutical and cosmetic industries (Windisch *et al.*, 2008; Cowan, 1999). Medicinal plants had possessed antimicrobial (Guo *et al.*, 2004a), antioxidant (Hashemi *et al.*, 2009) and immune enhancement (Guo *et al.*, 2004b).

Nowadays, natural products obtained from plants are gaining interest by the consumers as natural additives (Toghyani *et al.*, 2010). Herb and plant extracts used in animal feeds, called phytobiotic/ or phytogenic feed additives (PFA) are defined as compounds of plant origin incorporated into animal feeds to enhance the livestock productivity through the improvement of digestibility, nutrient absorption and elimination of pathogens residents in the animal gut (Athanasiadou *et al.*, 2007).

The large variety of plant compounds used as phytobiotic or PFA are assembled according to their origins and treatments, such as herbs and spices (fenugreek, garlic, anise, cinnamon, turmeric, black cumin, coriander, oregano, chili, pepper, rosemary and thyme) and essential oils (Kamel, 2000).

The content of active substances in these products can vary greatly depending on what part of the plant is used (seeds, leaves, roots or rhizome, bark, flowers, or buds), the harvest season and geographical origins. The technique of treatment (cold, steam distillation, extraction or maceration with non-aqueous solvent) also changes the active substances and related compounds in the final product (Alloui *et al.*, 2014; Windisch *et al.*, 2008).

Use of herbal plant considered safe without having side effects for enhancing the performance of birds. Herbs could have expected to serve as feed additives due to their suitability and preference, reduced risk of toxicity, minimum health hazards, environment friendliness and lower cost of production (Tanweer *et al.*, 2012).

2.2. Mode of Action of Medicinal Plants

Most of the herbal preparations contain various bioactive molecules namely flavonoids, polyphenols, lignin, and alkaloids which shows many pharmacological activities, such as antibacterial, antiviral, anti-inflammatory, antifungal, antioxidant, analgesic (painkiller) and anticarcinogenic properties (Gowda *et al.*, 2009; Kavirasan, 2007; Amin *et al.*, 2005).

The antimicrobial mode of action is supposed to be due to the hydrophobicity of the phenolic compounds which can enter the bacterial cell membrane leading to disintegration of cell membrane, denaturing and coagulating proteins, leakage of ions and eventually bacterial cell death (Bakkali *et al.*, 2007; Burt, 2004). The antimicrobial activities of phytochemicals can vary, for instance due to tannins act by deprivation of iron; binding with hydrogen or *via* interactions non-specifically with proteins of the virus or bacteria such as enzymes (Scalbert, 1991). The antiviral activity of some medical plant is due to its potent interaction with viral protein (Gangopadhyay *et al.*, 2011) and inhibition of the viral RNA synthesis (Song *etf al.*, 2005). Many phyto-chemicals such as pentagalloyl glucose (PGG) and oligonol a low molecular weight polyphenol derived from lychee fruit extracts showed strong anti-influenza activity by inhibiting virus entry into host cells, inhibits reactive oxygen species-dependent, blocking the extracellular

signal regulated kinase phosphorylation results inactivation of the virus (Liu *et al.*, 2011; Gangehei *et al.*, 2010).

The possible mechanisms of action of medicinal plants or herbs in the animals for growth promotion include changes in the intestinal microbiota, increased digestibility and nutrient absorption, improvement of the immune response, morphological and histological modifications of the gastrointestinal tract and antioxidant activity (Prajapat, 2016; Hashemi and Davoodi, 2010). There are evidences that plant contents have digestion-stimulating properties that have intrinsic bioactivities on animal physiology and metabolism. The reason for improvement in nutrient absorption are due to stimulation in secretions of saliva, bile and enhanced enzyme activities (Prajapat, 2016; Al-Kassie *et al.*, 2011a; Platel and Srinivasan, 2004). The improved nutrient digestibility consequently enhances the performance and health status of broilers. Furthermore, the medicinal plant phytochemical compounds relieve the host animals from immune defense stress during critical situations to increase the intestinal availability of essential nutrients for absorption, thereby helping animals to grow better within the framework of their genetic potential (Windisch *et al.*, 2008; Hashemi and Davoodi, 2010).

The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their active constituents (Ivanova *et al.*, 2005). Herbs have been demonstrated to work through the anti-oxidative mechanisms serving as free radical scavengers promoted by polyphenols, flavonoids, ascorbic acid and terpenoids which possess the ability to protect the cell organelles from damage caused by free radicals induced oxidative stress either by inhibiting the initiation or propagation of oxidative chain reactions (Ghaffar and El-Elaimy, 2012). Studies have shown that the plants active principle agents in the herbs have a strong capability for scavenging superoxide radicals, hydrogen peroxide and nitric oxide from activated macrophages, reducing iron complex and inhibiting lipid peroxidation (Gowda *et al.*, 2009; Khan *et al.*, 2012a). In addition, herbs and spices or PFA have more beneficial effect on immune system by stimulatory roles toward non-specific (T-cell-mediated) immune responses. Also, stimulating the non-specific immunomodulation, essentially granulocytes, macrophages

and natural killer cells as well as increase phagocytosis (Rajput *et al.*, 2013; Hashemi and Davoodi, 2012; Jagetia and Aggarwal 2007).

2.3. Medicinal Plants/Phytobiotics of Interest

2.3.1. Black cumin seeds (*Nigella sativa* L.)

2.3.1.1. Origin, distribution and description

Black cumin known by the Latin name *Nigella sativa* Linnaeus of the variety hispidula that belongs to the botanical family Ranunculaceae. It is native to the Mediterranean region and cultivated in North Africa, Asia, and Southeastern Europe. The largest producers of black cumin seed are Egypt, India, Pakistan, Iran, Iraq and Turkey (Ali and Blunden, 2003). Black cumin is an annual herbaceous plant growing in countries bordering the Mediterranean Sea and Asia, mostly as garden flower (Cheikh-Rouhou *et al.*, 2008).

There are four species of *Nigella*: (*Nigella arvensis* L., *Nigella deserti*, *Nigella assyriace* and *Nigella sativa* L.). The first three species are very rare. The *Nigella sativa* L. grows to a maximum height of about 40–70 cm and has finely divided foliage and pale blue and white flowers. From the fruit capsules, many small caraway-type black seeds are produced (length: 2.5 to 3.5 mm and width: 1.5 to 2 mm) (Amin and Hosseinzadeh, 2015).

2.3.1.2. Chemical composition of black cumin seeds

Black cumin seeds contain various composition including proteins, carbohydrates, oil, vitamins and minerals. According to Abaza (2001), the percentages of crude protein (CP), ether extract (EE), crude fiber (CF), ash and nitrogen free extract (NFE) were 24.46, 28.38, 11.44, 5.69 and 23.49%, respectively. Atta (2003) found that the seed has nutrient contents in the range of 96.2-93% DM, 22.0-40.35% EE, 20.85-31.2% CP, 3.7-4.7% ash and 24.9-40.0% carbohydrate. The mineral composition was 0.36% Ca, 0.65% P, 0.28%

Na, 0.25% Mg, 0.79% K, 34.19 mg/100g Fe, 5.63 mg/100g Zn, and 0.87 mg/100g Cu. These minerals are important for the normal function and growth of the body. The levels of vitamins were 10.19 µg/g for α-tocopherol, 2.28 µg/g for d-tocopherol, 0.18 µg/g for retinol, 1.38 µg/g for vitamin D2 1.85 µg/g for vitamin K1 and 2.15 µg/g for vitamin K2 (Iman *et al.*, 2006). However, the composition varies with the geographic distribution, time of harvest, and cultivation methods (Kamal *et al.*, 2010).

Fatty acid compositions of black cumin were myristic (12.97–13.23%), palmitic (9.47–13.34%), oleic (15.17–24.15%), and linoleic (54.32–70.81%) (Cheikh-Rouhou *et al.*, 2008). The fatty acids like linoleic, oleic and palmitic acids of the Ethiopian black cumin account for more than 93% of the total fatty acids, which were almost similar to 92.5% as reported by Cheikh-Rouhou *et al.* (2008). In fully ripened seeds, saturated fatty acids represent 6.5% of fatty acids, while 93.5% account for unsaturated fatty acids (Vatansev *et al.*, 2013).

The Ethiopian black cumin seeds has 18 amino acids of which the individual amino acids constituted; Arginine 18.3 mg/g, aspartic acid 15.8 mg/g, threonine 8.4 mg/g, serine 6.9 mg/g, glutamic acid 43.2 mg/g, alanine 9.0 mg/g, cyctine 3.6 mg/g, valine 12.7 mg/g, methionine 2.1 mg/g, isolucine 9.0 mg/g, leucine 13.0 mg/g, tyrosine 3.0 mg/g, phenylalanine 7.6 mg/g, histidine 5.5 mg/g, lysine 9.3 mg/g, ammoina 11.4 mg/g and proline 10.7 mg/g (Mariod *et al.*, 2012).

2.3.1.3. *Pharmaceutical and therapeutic uses of black cumin seed*

Black cumin is used for medicinal purposes, as herb and oil, which is traditionally used for medicinal purposes for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver functions, circulatory and immune system support and for a general wellbeing (Jahan, 2012). The Middle East, Northern Africa, Far East and Asia uses black cumin for centuries in folkloric (herbal) medicine all over the world in the treatment and prevention of many diseases and conditions that include asthma, diarrhea and dyslipidemia. Seeds/oil has anti-inflammatory, analgesic,

antipyretic (prevent fever) and antimicrobial activities. Especially the oil decreases the blood pressure and increases respiration (Ali and Blunden, 2003).

The black cumin seeds have been reported to have many biological properties and uses in pharmaceutical industries, and medicinal purposes. Many medicinal properties have been noticed including antidiabetic (Meral *et al.*, 2001), anti-parasitic, antibacterial, antifungal, and anthelmintic, antidiarrheal, anti-tumor agent and stimulatory effect on the immune system (Vatansev *et al.*, 2013). Study showed that the black cumin seeds had potent effect towards protection from decreases in hemoglobin (Hb) and leucocyte or WBC counts, and its inhibitor against aflatoxin formation by 85-90% (Nair *et al.*, 1991). The bioactive component or the active constituents of black cumin is the volatile oils, such as thymoquinoline, dithymoquinoline, nigellone, thymol, nigellidine, nigellimine and thymohydroquinone (Swamy and Tan, 2000), which also have antitumor properties, treating asthma, respiratory congestion and coughs (Zahoor *et al.*, 2004). According to El-Dakhakhny *et al.* (2000) black cumin oil shows a favorable effect on the serum lipid pattern where the administration of the oil caused a significant decrease in serum total cholesterol, LDL, triglycerides and a significant elevation of serum HDL levels.

2.3.1.4. Effects of black cumin seeds as phytobiotics additives on growth performance of poultry

Black cumin seed and its extracts in poultry diets have shown an improvement in live body weight (BW), BW gain and feed conversion ratio (FCR). Many Researchers Siddiqui and Sayed (2015); Ashayerizadeh *et al.* (2009); Abu-Dieyeh and Abu-Darwish (2008); Abaza *et al.* (2008) reported that inclusion of black cumin seeds and oils in broiler diets improved the BW and FCR. Similarly, Khan *et al.* (2012b) found out that 2.5 and 5.0% of black cumin seed fed to birds had significantly improved FCR than the antibiotics and control diet fed to birds. El-Ghamry *et al.* (2002a) indicated that chicks fed a diet supplemented with 0.4% black cumin seeds showed a significantly increased BW, BW gain and improved FCR at 6 weeks old compared with chicks not fed with black cumin seeds.

According to Durrani *et al.* (2007) black cumin at 40 g per kg feed resulted in significantly higher BW gain, with lowest feed intake and better feed efficiency. Similarly, Al-Beitawi and El-Ghousein (2008) reported that supplementation of broiler diet with 1.5% black cumin seeds increased BW gain and enhanced FCR. El-Soud (2000) found that BW gain, feed intake and FCR were highly improved for quails receiving a diet supplemented with 2% black cumin seeds.

2.3.1.5. Effects of black cumin seeds as phytobiotics additives on carcass characteristics of poultry

Most Researchers reported that black cumin seeds inclusion in poultry diets, have shown an improvement in carcass weight and quality and do not possess negative outcome in carcass, for instance Durrani *et al.* (2007); El-Bagir *et al.* (2006) reported that addition of black cumin seeds to broiler diets resulted in an increased dressing percentage, weight of thigh and breast muscle. Osman (2002) mentioned that the addition of 1 g/kg feed of black cumin oil to a broiler diet significantly increased a dressing percentage. On the other hand, El-Khalek and El-Naggar (2016) also reported a no change in carcass, liver and gizzard weights after feeding of black cumin oil inclusion at 1 ml/kg for broilers.

Abbas and Ahmed (2010) reported also that birds fed on diet supplemented with 1% black cumin did not show a significant effect on liver, gizzard, heart and abdominal fat percentage. Similarly, Abdel-Hady *et al.* (2009); Durrani *et al.* (2007) reported that black cumin seed had no significant effect on carcass fat percentage. While, Shewita and Taha (2011); Tollba and Hassan (2003) found that supplementation of 2% black cumin seeds accompanied by decreased fat percentage. Black cumin seeds' essential oil at the level of 60 mg/kg diet had no effect on carcass weight of Japanese quails, but it caused in a lower percentage of abdominal fat (Denli *et al.*, 2004). Earlier study by Abdo (1998) reported that 0.3% black cumin seed in broiler diet resulted in better growth and meat quality of broilers. In contrary reported by El-Ghamry *et al.* (2002a) black cumin at 0.2% inclusion rate resulted with significant reduction in dressing

percentage. Also, Abbas and Ahmed, (2010) reported that ground black cumin seeds in concentrations of 0.25 to 0.75%, and Nasir and Grashorn, (2010) reported black cumin seed at 1% or 2% of the diet of broiler had lower performance and carcass quality.

2.3.1.6. Effects of black cumin seeds as phytobiotics additives on blood constituents of poultry

Hematological analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional and environmental management in human and veterinary medicine. Black cumin seeds inclusion in broiler diet yielded a significant increase in hematological parameters such as, red blood cells (RBCs), hemoglobin (Hb) and packed cell volume (PCV) (Siddiqui and Sayed, 2015; Alimohamadi *et al.*, 2014; Shewita and Taha, 2011). The desirable influence of black cumin seed on hematology might be to the presence of highly active components, particularly thymoquinone and thymohydroquinone, which have strong antioxidant activities (Arslan *et al.*, 2005). On other hand, Al-Nazawi and El-Bahir (2012) reported that black cumin seed had no significant effect on PCV and Hb concentration of broiler chicken.

Serum protein depends on the availability of dietary protein; the proteins of the black cumin seeds diets were more available to the birds by increasing serum protein in birds fed diets with high levels of black cumin (Khan *et al.*, 2012b; Hassan *et al.*, 2007; Tollba and Hassan, 2003). According to Al-Betawi and El-Ghousein (2008) reported that crushed black cumin seed at 1.5 or 2.5% inclusion in diets of broiler increase serum total proteins.

Black cumin seeds seems to have hypoglycemic effects in birds when supplemented in different inclusion levels. According to Hossain (2014), reported that black cumin seeds at 1% inclusion in diets of broilers reduce the blood glucose than the antibiotics and control. Similarly, black cumin seed oil at 0.1 ml/kg reduced the serum glucose of broiler

than with antibiotics (avilamycin) and control diet fed to birds (Saleh, 2014). El-Bagir *et al.* (2010) reported that addition of black cumin seeds to broiler diets resulted in decrease in blood glucose.

Black cumin seeds have reported to possess a favorable effect on serum lipid profile by decreasing its total cholesterol, low-density-lipoprotein (LDL), triglycerides and by elevating the high-density-lipoprotein (HDL) level (El-Dakhakhny *et al.*, 2000). The plasma triglyceride, cholesterol and LDL concentration levels were predominantly lower in the chickens fed diets supplemented with 20 g/kg crushed black seed (Ghasemi *et al.*, 2014). Abdel-Majeed (1999) reported that feeding 0.7% black cumin seeds to broilers resulted in a significant reduction of serum total lipids concentration. Al-Beitawi *et al.* (2009) reported that replacing the antibiotics (bacitracin) with crushed black cumin seeds in broiler diets significantly reduced the serum cholesterol. Similarly, feeding of powdered black cumin to laying hens has shown to lower serum cholesterol and triacylglycerol concentrations, which indeed was associated with a decrease in egg yolk cholesterol (Akhtar *et al.*, 2003). Black cumin seed contains an appreciable amount of sterols, especially β -sitosterol, which has the ability to inhibit the absorption of dietary cholesterol from the intestine (Khan *et al.*, 2012b). Reduction in serum cholesterol attributed to the lowering effect of thymoquinone and monosaturated fatty acids on the synthesis of cholesterol by hepatocytes or the fractional reabsorption from the small intestine (Brunton, 1999).

Alteration in serum enzymes activities under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm. Generally, alanine aminotransferase (ALT) or serum glutamic oxaloacetic transaminase (SGOT), aspartate aminotransferase (ASP) or serum Glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) considered as liver enzymes that increased with liver damage (hepatocellular degeneration). Black cumin seed is more effective in reducing the elevated levels of SGOT, SGPT and ALP enzymes (Khan *et al.*, 2012b). The non-hepatotoxic nature of black cumin proved in the study performed by Hassan *et al.*

(2007), who found unaltered and normal activities of serum SGOT, SGPT and ALP as well as retained architecture of liver after black cumin treatment in Japanese quails.

2.3.1.7. Effects of black cumin seeds as phytochemicals additives on immune response of poultry

Abou-Egla *et al.* (2001) noted that mortality tended to be lower in birds fed diets containing 4.59% black cumin seeds. Similarly, no mortality noted in chicks fed diets with a black cumin seeds at 0.4% level (El-Ghamry *et al.*, 2002a). According to Shewita and Taha's (2011), a significant level-dependent improvement in antibody titer against Newcastle Disease (NCD) observed in black cumin supplemented birds. In addition, there was a significantly increased lymphoid organ ratio (bursa and thymus) with increasing black cumin seed levels. Similarly, Al-Beitawi *et al.* (2009) showed that antibody titers against NCD and Infectious Bursal Disease (IBD) increased significantly with crushed black cumin seeds (Khan *et al.*, 2013). Using black cumin seeds at the level of 40 g/kg enhanced antibody production against NCD and IBD in broiler chickens (Durrani *et al.*, 2007).

According to Ghasemi *et al.* (2014), black cumin seeds at 20 g/kg improved the primary and secondary antibody titers against sheep red blood cell (SRBC) in male broilers. Moreover, increased in anti-SRBC antibody response has been reported in laying hens also (23 weeks of age) that fed diets supplemented with 15 g/kg black cumin seeds (Yalçın *et al.*, 2012). The SRBC antigen reaction is dependent on the cellular aspects of the immune response through stimulation of the T-cell receptor (Leshchinsky and Klasing, 2001).

Umar *et al.* (2016) detected that antibody titer against avian influenza virus (H9N2) in turkeys was significantly higher and developed faster in birds fed thymoquinone, which is one of the major active agent in black cumin seeds. Osman and Barody (1999) illustrated that black cumin seeds supplementation had highly significant effect on the immune response of broilers. Abdo (1998) reported that 0.3% black cumin seeds in

broiler diet gave the best growth, meat quality and immunity of broilers. The improvement in the immune response of black cumin seeds might be because of the sufficient quantities of pharmacologically active constituents, such as thymol, nigellidine, nigellimine, thymoquinone, dithymoquinone and thymohydroquinone (Swamy and Tan, 2000), that are able to induce pharmacological effects against antigenic (antigen) challenge. Similarly, Islam *et al.* (2004) and Swamy and Tan (2000) evidenced that some constituents of black cumin exert stimulatory roles toward T-cell-mediated immune responses.

2.3.1.8. Antimicrobial effects of black cumin seeds

The intestinal microflora plays an important role in the health status of host animals. In general, intestinal bacteria divided into species that exert either harmful (pathogenic) and beneficial effects on the host health. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria to inhibit the colonization of invading pathogens (Guo *et al.*, 2004b). Black cumin seed has been reported to have a good antimicrobial property against most of gram-positive bacteria and some of gram-negative e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella boydii*, *Vibrio cholera* and *Escherichia coli*. The active compound thymoquinone, representing 18.4-24% of the volatile oil of black cumin seeds, possess antibacterial, antioxidant, antihistaminic (antialergic) and antiinflammatory activities (Arslan *et al.*, 2005). Salman *et al.* (2005) reported that black cumin seeds oil have good antimicrobial properties against sensitive as well as multi-drug resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The black cumin seed shown to be effective against total coliform count in the intestine of broilers (Erener *et al.*, 2010). Khan *et al.* (2012b) reported that a reduction in cecal total coliform and *E. coli* bacteria population in chicks fed a diet containing black cumin or antibiotic when compared with the control diet. El-Khalek and El-Naggar (2016) reported that total coliform bacteria count reduced due to supplementation blends of

black cumin, oregano, ginger and thyme. Black cumin has various components that affect bacterial activities and inhibit aflatoxin production by fungi (Maraqa *et al.*, 2007).

Bourgou *et al.* (2010) explained that the antibacterial activity of black cumin seeds is due to the thymoquinone substances, such as terpenes. Bakkali *et al.* (2007) observed that the antibacterial effects of black cumin might be linked with the efficacy of its essential oil, which can disturb mitochondrial membranes and cellular function of bacteria and eukaryotic cells and result in cell death.

2.3.2. Fenugreek seeds (*Trigonella foenum-graecum* L.)

2.3.2.1. Origin, distribution and description

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant dicotyledonous, self-pollinated plant from the member of the legume family of leguminosae. Although grown as a spice in most parts of the world, the species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past (Petropoulos, 2002).

Fenugreek said to be originated from Southeastern Europe and Western Asia, however the exact origin has been widely debated. Fenugreek is believed to indigenous to the Mediterranean region of the "Old World", while an alternate Asian origin for the crop has also been advocated since its presence and use are ancient in India, Southern China, the Middle East (Basu *et al.*, 2014). Currently, fenugreek crop spread and grow in many parts of the world, especially around the Mediterranean and on the West Coast of the Black Sea. Its presence also noted in the Middle East, Egypt, Ethiopia, India, Northern Africa, and the United States (Basu *et al.*, 2014).

Fenugreek is an erect annual plant with trifoliate leaves, divided into toothed leaflets. It produces solitary or paired yellow-white flowers tinged with violet. The seeds are hard, yellowish brown and angular. Some are oblong, some rhombic, other virtually cubic, with a side of about 3 mm. A deep furrow all but splits them in to two (Basu *et al.*, 2014).

Fenugreek seeds are known for its pleasantly bitter slightly sweet seeds. Available in whole and ground form used to flavor many foods including curry powders, spice blends and teas. The fenugreek seed contains a central hard, yellow embryo surrounded by a corneous and comparatively large layer of white, semi-transparent endosperm (Betty, 2008), which contains the mucilage (galactomannan gum), lipids, protein, and protease inhibitors. They are available whole and dried, or as a dull yellow powder, ground from the roasted seeds (Mullaicharam *et al.*, 2013).

2.3.2.2. Chemical composition of fenugreek seeds

The nutritional composition of fenugreek seeds is contain contained 28.4% CP, 9.3% CF and 7.1% EE and have many other chemical compounds. Ethiopian fenugreek seed varieties have 94-93 DM, 3 ash, 28-30 CP, 7-8 EE, 6-7% CF and 44-46 of total carbohydrates (Birhane, 2012). According to the Kochhar *et al.* (2006) reported fenugreek seeds contain 88.2% DM, 25.8 CP, 6.53 EE, 3.26 ash, 6.28 CF and 58.13% of total carbohydrates on DM basis. However, El-Nasri and El-Tinay (2007) found that 28.4 CP, 9.3 CF, and 7.1% EE. Chemical composition and antioxidant activities of husk (seed coat) and endosperm of fenugreek seeds have revealed that endosperm has the highest content of saponin (4.63%) and protein (43.8%) In contrast, the husk (seed coat) contains higher amount of polyphenols (103.8 mg of Gallic acid equivalent) and total dietary fiber (77.1%). Fenugreek seeds are considered as an appetizer and helps in digestion; improve growth performance and health (El-Wafa *et al.*, 2003). Fenugreek seeds is especially rich in choline, niacin and nicotinic acid, which are reported in fenugreek seed whereas germinating seeds contain pyridoxine, cyanocobalamine, calcium pantothenate, biotin and vitamin C (Sharma, 1986).

Mature seeds contain amino acid, fatty acid, vitamins, and saponins. Fenugreek contains large quantities of folic acid 84 mg/100 g (Toppo *et al.*, 2009). According to Birhane (2012) fenugreek contain Na (102-154), K (9750-10967), Ca (502.9-625.6), Mg (1090-1443), P (4879-6750), Fe (69-99) and Zn (38-52 mg/kg). The fatty acid profile dominated

by unsaturated acids, namely, oleic, linoleic and linoleic acids accounting for 16.3, 50 and 24.4%, respectively of the total fatty acids (El-Nasri and El-Tinay, 2007)

The main chemical constituents of fenugreek seed are fibers, flavonoids, polysaccharides, steroid saponins and some identified alkaloids viz, trigonelline and choline (Toppo *et al.*, 2009). Some of the anti-nutritional factors constitute in fenugreek seed such as; tannin 269, saponin 6320 and phytate 111 mg/100 g. Phytates potentially form complexes with minerals and dietary proteins and decreases their bioavailability (Reddy, 2002). The fenugreek varieties studied by Birhane (2012), which are grown in Ethiopia, are found to have lower contents of phytates and tannins as compared to the fenugreek varieties grown in India and other countries.

2.3.2.3. *Pharmaceutical and therapeutic uses of fenugreek seeds*

Fenugreek seed have several pharmacological effects, including antioxidant property (Kavirasan, 2007), gastro protective (Suja, 2002), anti-inflammatory, antipyretic (preventing or alleviating fever) (Ahmadiani *et al.*, 2001), anthelmintic, antibacterial and antimicrobial properties (Safaa, 2007), hypoglycemia (Mamoun *et al.*, 2014), hypocholesterolemia (Srinivasan, 2006), anti-hyperthyroidism (Tahiliani and Kar, 2003), anti-sterility and anti-androgenic effects (Kamal *et al.*, 1993), anti-cancer (Amin *et al.*, 2005) and stimulate the appetite by the action of neurin, biotin and trim-ethylamine on the nervous system (Al-Habori and Roman, 2002; Mullaicharam *et al.*, 2013; Zeweil *et al.*, 2015).

Fenugreek seeds contain alkaloids, including trigonelline, gentianine and carpaine compounds, also contain an especial amino acids called 4-hydroxyisoleucine and a fiber fenugreekine, a component that have hypoglycemic activity (Mullaicharam *et al.*, 2013). However, the astringent smell, like maple syrup, of sweat and urine limits their acceptability for consumption (Birhane, 2012). Compound responsible for the strong maple-syrup odour is due to sotolon, which was reported to be found predominantly (95%) in fenugreek (Meghwal and Goswami, 2012).

2.3.2.4. Effects of fenugreek seeds as phytobiotics additives on growth performance of poultry

Fenugreek seed has been used as a growth promoter particularly in diets of broiler chickens. Inclusion of fenugreek seeds in the diet significantly improves the BW of broiler chickens (Abaza, 2001; Guo *et al.*, 2004a; Guo *et al.*, 2004b; Yatoo *et al.*, 2012; Qureshi *et al.*, 2015). According to Mamoun *et al.* (2014) broiler chicks fed diets containing 1% fenugreek seeds flour recorded heaviest BW and best feed conversion efficiency compared to the control diet. The mechanism of the effect was unclear, although the stimulation of the digestive system of broilers by fenugreek suggested as possible causes of action. Also, there are reports indicating that broilers fed fenugreek contained diets increased in their feed intake (Michael and Kumawat, 2003; Alloui *et al.*, 2012; Mamoun *et al.*, 2014). The increase in feed consumption of fenugreek attributed to the carbohydrates and their main components, galactomannan, which stimulate the appetite and digestive process in animals (Michael and Kumawat, 2003; Alloui *et al.*, 2012).

According to Elbushra (2012) BW gain, FCR and protein efficiency ratio improved for chicks fed diets supplemented with 0.5% or 1.5% fenugreek seeds. Also, incorporation of 1% germinated fenugreek seeds resulted in increased egg production with no adverse effects on egg quality (Hassan *et al.*, 2004). The supplementation of layers feed with 0.5% fenugreek seeds improved the egg production by 2.23% as compared to that of the control group and significantly decreased the feed intake as well (Abaza, 2007). Further, it improves the feed efficiency with reduction in feed cost when used as phytobiotic feed additive in broiler chicken diets (Azoua, 2001).

2.3.2.5. Effects of fenugreek seeds as phytobiotic additives on carcass characteristics of poultry

Fenugreek seed supplementation to diets improved the carcass characteristics of broiler chicks (Alloui *et al.*, 2012; Elagib *et al.*, 2013). Fenugreek powder in broiler diet had a

significant effect on the dressing percentage and with no significant effect on other carcass characteristics and subjective meat quality (Mamoun *et al.*, 2014). A study on effects of the seed extract (aqueous) on the visceral organs of broiler chicks concluded that aqueous extract of fenugreek seed had ample good effects on the weight of visceral organs (Farman *et al.*, 2009).

Mamoun *et al.* (2014) reported that the supplementation of fenugreek seeds at 1% level in the diet of broiler chicken caused significant improvement in the intestinal length. A significant effect on the digestive parts and increase in length and weight of intestines has recognized due to dietary inclusion of fenugreek seeds 5-40 g/kg in diet of broiler chicken (Duru *et al.*, 2013). Moreover, Bhaisare *et al.* (2014) found that dietary inclusion of fenugreek seeds at 0.5% level in the diet of Nandanam turkey poults for eight weeks' duration improved in dressed weight and attributed it to the antimicrobial properties of fenugreek. The positive effect on intestinal morphology could prolong the contact between the digesta and mucosal epithelium, which may be more effective for nutrient absorption (Boguslawska-Tryk *et al.*, 2012). However, Researchers indicated that fenugreek had no significant effect on slaughters parameters (dressing percentage and carcass length) (Farman *et al.*, 2009; Guo *et al.*, 2004; Abaza, 2001). Supplementation of fenugreek seeds had no significant effect on intestinal length when calculated in terms of per 100 g of BW (Weerasingha and Atapattu, 2013).

2.3.2.6. Effects of fenugreek seeds as phytobiotics on the blood constituents of poultry

The inclusion of fenugreek seeds at 10 g/kg of diet in broiler breeder chicken significantly improved the PCV, RBC and Hb concentration and attributed this improvement in erythropoiesis to the enhancement of antioxidant activities in RBCs, which decreases the production of free radicals that destroy Hb and cause hemolysis of RBCs (Abdul-Rahman, 2012). According to Bhaisare and Thyagarajan (2014), the Hb content were significantly higher when fenugreek seeds used in turkey poults, indicating that certain bioactive principles in fenugreek seeds have positive effect on haemopoietic process in the body.

Many researchers report that incorporation of dietary fenugreek seeds in broilers diets reduced serum glucose level of broiler chicken (Qureshi *et al.*, 2015; Mamoun *et al.*, 2014; Safaei *et al.*, 2013; Abdul-Rahman, 2012; Eman, 2011). The hypoglycemia effects of fenugreek seed mode of action had not been fully elucidated; however, Schryver, (2002) ascertained that the reduction in the serum glucose levels may be related to the presence of special amino acid (4-hydroxyisoleucine), which found abundantly in fenugreek seed that stimulate directly β -cell stimulation in the pancreases to increases insulin secretions, thus improves glucose uptake by the body rather than stay in the blood.

Incorporation of dietary fenugreek seeds in broilers at 1% level significantly decreased the blood cholesterol levels (Mamoun *et al.*, 2014; Safaei *et al.*, 2013; Abdul-Rahman, 2012). There was a reduction in blood cholesterol levels by inclusion of fenugreek seeds at 40 g/kg in diet of broiler chickens (Duru *et al.*, 2013). Supplementation of fenugreek powder on the broiler diets significantly decreased serum cholesterol, total proteins, albumin and calcium (Alloui *et al.*, 2012; Elagib *et al.*, 2013; Mamoun *et al.*, 2014). Abbas (2010) ascertained that the effect of fenugreek seeds on broiler performance at the rate of 3 g/kg of diet and showed significant reduction in serum cholesterol level. Similarly, Abdel-Rasoul and Yousif (2003) showed that fenugreek seed powder as capsules (750 mg/kg BW) decreased the blood cholesterol in broiler chickens. Similar results were observed by El-Ghamry *et al.* (2002b) with Muscovi duckling who demonstrated that total cholesterol value in plasma of fenugreek seeds (1.5%) treatments were significantly lower than those of the control groups. Ground fenugreek seeds given to laying hens at 6 g/hen/day resulted in reduction in serum cholesterol levels (Abdouli *et al.*, 2014). Addition of fenugreek to the diets of turkey poults revealed a decreasing trend in serum triglyceride level as compared to control turkey (Bhaisare and Thyagarajan, 2014).

The fenugreek seed supplementation in bird hypocholesterolemic effects mode of action includes competition with cholesterol at binding sites or interference with the cholesterol biosynthesis in the liver. Saponins and resins in fenugreek seed which inhibit bile acid and cholesterol absorption from intestine (Mukhtar *et al.*, 2013), hence have a potential

role in prevention of arteriosclerosis (Mullaicharam *et al.*, 2013). Further, fenugreek seeds have hepatostimulatory and hepatoprotective effect in broiler chickens as imputed by lower SGOT, SGPT and ALP enzyme secretion levels were recorded indicating that it has no toxic effect in liver and kidney as well as in other organs (Qureshi *et al.*, 2015; Ali and Ismail, 2012; Toppo *et al.*, 2009).

2.3.2.7. Antimicrobial effects of fenugreek seeds

The antibacterial and antifungal role of fenugreek seed has recently been shown. The extracts contain effective antimicrobial agents. Study by Ahmad (2016) showed that fenugreek oil has a positive effect on microbial health by lowering the total bacterial count, Salmonella and *E. coli* of the laying hens and thus can be used instead of commercial antibiotics. However, not much literature reported regarding the *in vivo* effect of fenugreek in poultry. Qureshi *et al.* (2015) investigated the *in vitro* antibacterial activity of fenugreek and reported the 2.1 mm of zone of inhibition for the concentration of 0.05 mg/ml of extract against *E. coli*. Similarly, *in vitro* antibacterial activity of methanolic extract of fenugreek against *E. coli* has been reported by Dash *et al.* (2011) and ascribed to the flavonoids, saponins and phenols present in it (Schryver, 2002).

In a study by Haouala *et al.* (2008) found that all parts of the fenugreek plant showed antifungal potential and the magnitude of effect varies with plant parts and species of fungus such as *Fusarium graminearum*, *Botrytis cinerea*, *Alternaria sp.*, *Rhizoctonia solani* and *Pythium aphanidermatum* (Haouala *et al.*, 2008). It could suggest that fenugreek is an important source of biologically active compounds useful for developing better and novel antifungal drugs.

2.3.3. Turmeric rhizome (*Curcuma longa* L.)

2.3.3.1. Origin, distribution and description

Turmeric or *Curcuma longa* L, is a rhizomatous herbaceous perennial herb and member of the Zingiberaceae (ginger) family along with the other members like ginger, cardamom and galangal. It belongs to the genus (*Curcuma*) that consists of hundreds of species of plants that possess rhizomes and underground root like stems (Gowda *et al.*, 2009; Prasad and Aggarwal, 2011). Turmeric is a plant, with a large rhizome and large leaves and yellow flowers and is a close relative to the ginger plant. The plant grows to a height of 3 to 5 feet and has oblong pointed leaves, which bears funnel-shaped yellow flowers. Dried *Curcuma longa* is the source of turmeric, the ingredient that gives curry powder its characteristic yellow color, which is used widely for spice and food colorant, native to tropical South Asia (Southeast India). However, it is cultivated extensively in Asia, mostly in India and China and other countries with tropical and sub-tropical climates (Abdel-Wahab and El-Bahr, 2012; Prasad and Aggarwal, 2011).

2.3.3.2. Chemical composition of turmeric rhizome

The nutrient compositions of turmeric rhizome includes 6.72 of CP, 5.04 of EE, 3.96 of CF and 7.72% of ash (Basavaraj *et al.*, 2010). Nutritional analysis showed that 100 g of turmeric rhizome contains 69.9 g total carbohydrates, 21 g dietary fiber, 3 g sugars, 8 g protein, 10 g total fat, 0.2 g Ca, 0.26 g P, 10 mg Na, 2500 mg K, 47.5 mg Fe, 0.9 mg thiamine, 0.19 mg riboflavin, 4.8 mg niacin, 50 mg ascorbic acid (Balakrishnan, 2007).

More than 100 components have been isolated from turmeric among the main components of the root is a volatile oil, containing turmerone and there are other coloring agents called curcuminoids in turmeric rhizome. Turmeric rhizome also contains up to 5% essential oils curcumin, a polyphenol (Prasad and Aggarwal, 2011). Turmeric is also a good source of ω -3 fatty acid and α -linolenic acid 2.5% (Goud *et al.*, 1993).

2.3.3.3. *Pharmaceutical and therapeutic uses of turmeric rhizome*

In folk medicine, turmeric has used in therapeutic preparations over the centuries in different parts of the world. In Ayurveda (Hindu alternative medicine) practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones and relieving arthritis (Prasad and Aggarwal, 2011).

In modern pharmaceutical and therapeutic turmeric has found to convey antioxidant (Park *et al.*, 2000; Osawa *et al.*, 1995), antiviral (Liu *et al.*, 2013a), antibacterial (Liu *et al.*, 2013b), antifungal (Gowda *et al.*, 2009), anticancer (Garg *et al.*, 2008), hepatoprotective (Miyakoshi *et al.*, 2004), cardioprotective (Mohanty *et al.*, 2006), hypoglycemic and hypocholesterolemic properties (Qasem *et al.*, 2016; Abou-Elkhair *et al.*, 2014).

The active phytochemical substances of turmeric were curcumin, tetrahydrocurcuminoids, dimethoxycurcumin and bismethoxycurcumin (Al-Sultan, 2003). According to researchers Chattopadhyay *et al.* (2004); Wuthi-Udomler *et al.* (2000); Osawa *et al.* (1995) the medicinal properties of rhizome of turmeric are related to the active ingredients are called curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, turmeronols and tetrahydrocurcuminoids.

The antioxidant property of curcumin reduces the activity of reactive oxygen species and elevates the antioxidant enzymes such as, superoxide dismutase, catalase and glutathione peroxidase levels in the blood (Joe and Lokesh, 1994). Turmeric is known to have hepatoprotective characteristics. Reduced the liver enzymes (AST, ALP and ALP), which are mostly secreted during liver damages or toxicities (Hosseini-Vashan *et al.*, 2012; Basavaraj *et al.*, 2011; Reddy, 2010).

2.3.3.4. *Effects of turmeric rhizome as phyto-biotic additives on the growth performance of poultry*

There was an improvement in BW and FCR of broilers fed turmeric at 0.50% inclusion level (Al-Jaleel, 2012). Al-Sultan (2003) showed also that the higher BW gain and FCR was achieved on broilers receiving 0.5% turmeric in diets followed by birds receiving diets with 0.25%, 1% than the control group birds. Similarly, Durrani *et al.* (2006) reported that the basal diets supplemented with 0, 0.25, 0.50 and 1% levels of turmeric revealed a significant improvement in BW gain and FCR and did not cause a significant mortality.

Sahin *et al.* (2012) studied that the active constituents of turmeric called curcumin at 200 and 400 mg/kg in the diets of quails under heat stress condition and resulted in improvement of the growth performance of quails and antioxidation in the liver. There was an increase in feed intake, final BW weight and BW gain in response to increasing the dietary curcumin supplementation. However, different reports by Emadi and Kermanshahi (2006) revealed that 0.25, 0.50 and 0.75% of turmeric powder in chick diets had no effects on BW gain, feed intake and FCR. In addition, supplementation of turmeric powder at level of 3.3, 6.6 and 10 g/kg diets had improved FCR, but no substantial change on feed intake and BW gain of broilers (Nouzarian *et al.*, 2011).

Inclusion of 0.5% turmeric powder which containing 1.48% total curcuminoid (74 mg/kg), to the aflatoxin B1 diet increased the feed intake (906 *versus* 858 g), but significantly improved weight gain (746 *versus* 662 g) in chicks, suggesting antioxidant protection by turmeric powder meal (Gowda *et al.*, 2009). An increased BW gain might be due to the oxidant of turmeric that stimulates protein synthesis by bird's enzymatic system (Osawa *et al.*, 1995) by reducing intestinal pH (Siew *et al.*, 2005). Therefore, turmeric could be effective as natural additive on poultry use for overall performance.

2.3.3.5. *Effects of turmeric rhizome as phyto-biotic additives on carcass characteristics of poultry*

Many researches were interested to use medicinal plants in order to replace antibiotic in animal feed to avoid residue in the final products. Scholars indicated a higher dressing percentage as well as breast weights of broilers fed a diet containing 5 g/kg of turmeric powder (Hussein, 2013; Fascina *et al.*, 2012; Durrani *et al.*, 2006; Osawa *et al.*, 1995). Correspondingly, Wang *et al.* (2015); Ukoha and Onunkwo (2016a) recorded the highest dressed and breast weights in birds fed a diet containing 3% turmeric powder. Durrani *et al.* (2006) found that basal diets supplemented with 0.25, 0.50 and 1.0% level of turmeric on broilers during five weeks showed that dressing percentage, thigh and breast weights were significantly increased, but liver, heart and gizzard weight had not changed. Moreover, a 3.3, 6.6 and 10 g/kg turmeric powder on carcass yield, heart, pancreas, spleen, and intestine did not significantly differ. There was a significant decrease in abdominal fat and liver weights due to increasing level of turmeric powder in the diets (Nouzarian *et al.*, 2011). However, Al-Jaleel (2012) reported that there was no change for edible carcass parts at 0.50% inclusion rate of turmeric in broiler diets.

Emadi and Kermanshahi (2006) reported that supplementation of 0.25, 0.50 and 0.75% level of turmeric in broiler diets had no effects on liver, spleen and pancreas weights, but heart and abdominal fat were significantly reduced. Also, Wang *et al.* (2015) reported that dietary supplementation with turmeric at 150-200 mg/kg and 100 to 300 mg/kg, respectively markedly reduced the abdominal fat ratio compared to that of the control group birds. Turmeric at the rate of 10 % in feed resulted in degenerative effect in the liver of broilers, but no change in the structure of liver and pancreas at 1% inclusion of turmeric (Namagirilakshmi *et al.*, 2010).

2.3.3.6. *Effects of turmeric rhizome as phytobiotic additives on blood constituents of poultry*

Inclusion of turmeric as a phytobiotic additive in poultry diets had positive effect in hematology and biochemical indices of the birds. According to Ukoha and Onunkwo (2016b); Sugiharto *et al.* (2011) the Hb, PCV and RBCs were increase due to supplementation of turmeric to diets of chicks. While, Kumari *et al.* (2007) were noticed an increased in Hb concentration in birds supplemented with turmeric containing diets than the control diet fed birds. However, inclusion of turmeric powder at 0.5 to 1.5% resulted in no detrimental effect on PCV, RBC, Hb, and WBC, but there was an increase in Albumin and globulin concentration levels (Al-Jaleel, 2012).

Turmeric supplementation can also influences some of protein metabolites indices of chickens. According to Kumari *et al.* (2007), significantly, higher levels of serum total protein and globulin were noticed of broilers fed a diet containing turmeric powder at 1 g/kg, but the serum albumin level was low. Al-Noori *et al.* (2011) found a significant increase in serum total protein concentration in broiler chickens that received a diet containing turmeric powder at the levels of 0.5% and 1%. However, Emadi *et al.* (2007) suggested that there was no significant effect on a total protein and albumin concentrations in broiler chickens treated with the levels of 0.25%, 0.5% and 0.75% of turmeric powder at 21st day of age. Similarly, Ahmadi (2010) reported that 0.3 g/kg of turmeric powder had no significant effect on serum total protein, albumin and globulin.

The hypoglycemic effect of turmeric in birds was witnessed by researchers Qasem *et al.* (2016); Abou-Elkhair *et al.* (2014); Ahmadi (2010), who indicated that turmeric supplementation has lowered the blood glucose level in chicken. In addition, Gowda *et al.* (2009) found that 444 mg curcumin per kg significantly reduced the serum glucose concentration. However, Kumari *et al.* (2007) reported that the serum glucose was not significantly affected by turmeric powder added to broiler feeds at levels of 0.25, 0.5, 0.75 and 1 g/kg. The reduction in serum glucose might attributed to the turmeric active agents that increase the activities of glucose transporters, which are a family of

transmembrane proteins that help to carry glucose across the plasma membrane (Kumari *et al.*, 2007).

Turmeric supplementations had positive effects in lowering the cholesterol level (hypercholesteremic) in the blood of chickens (Fallah and Mirzaei, 2016; Hussein, 2013; Hosseini-Vashan *et al.*, 2012). Blood total cholesterol and LDL-cholesterol decreased and HDL-cholesterol increased when heat-stressed birds fed turmeric (Hosseini-Vashan *et al.*, 2012). Turmeric rhizome powder at 0.5-1.5% and 0.5 g/kg containing diets exhibited significantly decreased levels of blood serum cholesterol and LDL-cholesterol and while plasma level of HDL-cholesterol was significantly increased compared with the control birds (Abo El-Maaty *et al.*, 2014; Arslan *et al.*, 2017). The hypocholesterolemic effect of turmeric justified as related to the altered activity of effective enzymes in cholesterol metabolism, HMG-CoA reductase (3-hydroxy3-methylglutaryl Coenzyme A) the rate limiting enzyme of cholesterol synthesis and mediated by the stimulation of hepatic cholesterol 7 α -hydroxylase enzymes which converts cholesterol to bile acids and facilitating the biliary cholesterol excretion (Kim and Kim 2010; Daneshyar *et al.*, 2011).

According to the study of Emadi and Kermanshahi (2007), supplementation of turmeric meal in the diets at the rate of 2.5-7.5 g/kg reduced the concentrations of ALP and ALT in the blood of broiler chickens. Similarly, Hosseini-Vashan *et al.* (2012) reported significant reductions in the activities of ALT, AST and ALP enzymes in broiler chickens fed 0.4% and 0.8% turmeric powder under heat stress. Similarly, Abo El-Maaty *et al.* (2014) demonstrated that turmeric at 0.5 g/kg in broiler has hepatoprotective ability as manifested by reduction in AST and ALT in blood plasma.

Turmeric's hepatoprotective effect is mainly a result of its antioxidant properties, as well as its ability to decrease the formation of proinflammatory cytokines. Furthermore, turmeric has been shown to protect liver against a variety of toxicants including aflatoxin B1 in mice, rats, broiler chicken and ducks (Gowda *et al.*, 2009; Yarru *et al.*, 2009; Park *et al.*, 2000).

Nevertheless, Ahmadi (2010) reported that 0.3 g/kg of turmeric powder had no significant effect on ALT and AST enzymes compared with the control group. Mehala and Moorthy (2008b); Kumari *et al.* (2007) reported also that the activities of some liver enzymes, ALT, AST and ALP concentrations did not change with the dietary inclusion of turmeric powder. In addition, Basavaraj *et al.* (2011) reported that absence of any alteration in ALP of broilers fed turmeric as additives. No significant change in ALT, AST and ALP perceived that there were no side and direct effects of supplementations of turmeric powder in different combinations on chicken liver performance.

2.3.3.7. Effects of turmeric rhizome as phyto-biotic additives on immune response of poultry

Turmeric supplementation at the level of 0.5 to 1.5% significantly improved antibody titer against NCD and IBD in broilers (Arslan *et al.*, 2017). Similarly, Zaki *et al.* (2016) indicated that HI titres against NCD were higher in turmeric supplemented than the control birds, while, Fallah and Mirzaei (2016); Reddy (2010) indicated that birds receiving turmeric powders had higher antibody titer against NCD than the control groups.

According to Qasem *et al.* (2015), findings, turmeric powder inclusion at the rates from 1 to 2% significantly improve the antibody against NCD, while antibody against IBD was significantly higher when turmeric powder supplemented at the rates of 1.4 and 1.6%. Nayaka *et al.* (2012) also observed a significantly higher antibody response in birds supplemented with turmeric and vitamin-E against NCD when added to diets either alone or in combination.

The antibody of broilers was increases in turmeric fed broilers challenged with EtMIC 2, an apical complex protein that places an important role in host cell invasion of *Eimeria* species parasites (Lee *et al.*, 2010). Turmeric rhizome powder also proves to stimulate the expression of genes involved in the antioxidant and immune systems of

broiler chickens, Yarru *et al.* (2009) showed that 5.0 g/kg turmeric meal supplementation had beneficial effects on the stimulation of genes involved in antioxidant function (CYP450) and immune function (IL-6 and IL-2). Turmeric reasonably boosts and modulate the activation of T-cells, B-cells, macrophages, neutrophils and natural killer (NK) cells (Rajput *et al.*, 2013).

2.3.3.8. Antimicrobial effects of turmeric rhizome

Turmeric could control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the chicken's gut resulting in balanced gut microbial ecosystem that leads to better feed utilization reflected by final BW and BW gain (Al-Mashhadani, 2015).

Curcumin is one of active components of the turmeric and has antibacterial, antifungal and antiprotozoal activities (Basavaraj *et al.*, 2010). Namagirilakshmi *et al.* (2010) showed that the use of turmeric as feed additive in broiler chickens significantly reduced small intestinal total microbial counts. Khosravifar *et al.* (2014) reported that the colony forming units of coliform bacteria in digesta of ileum of birds supplemented with turmeric powder from 0.5 to 1.5 were significantly lowered than the control in Japanese quails. Similarly, Samarasinghe *et al.* (2003) reported that turmeric powder reduced coliform bacteria in duodenum of broiler chicks. The anticoccidial effects of dietary inclusion of 1% curcumin was observed in chickens after infection of *Eimeria maxima* and *E. tenella* species. Improved BW gain, reduced lesion scores and oocyst counts were shown only against *E. maxima* (Allen *et al.*, 1998). Later Abbas *et al.* (2010) reported that dietary supplementation with 3% turmeric powder was effective against a mild infection of *E. tenella*. In addition, Lee *et al.* (2010) reported that fecal oocyst shedding from birds experimentally infected with *E. acervulina* was significantly decrease when broiler chickens were fed with a diet containing turmeric. Therefore, turmeric is effective in antimicrobial agents against some multiple drug-resistant bacteria and may have important implications for preventing or treating infections caused by these microorganisms in poultry.

2.4. Synergetic Effects of Phytobiotics Additives

Synergy defined as the interaction of two or more agents to produce a combined effects greater than the sum of their individual effects (Van Vuuren and Viljoen, 2011). In a medicinal research field, however, the understanding of synergy is complicated. Spinella (2002) has classified the concept of synergy broadly into two main categories based on the mode of actions: pharmacodynamic and pharmacokinetic synergy (Spinella, 2002). The first type of synergy describes two or more agents that work on the same receptors or biological targets that result in enhanced therapeutic outcomes through their positive interactions. The second type of synergy results from interactions between two or more agents during their pharmacokinetic processes (absorption, distribution, metabolism and elimination) leading to changes of the agents quantitatively in the body and hence their therapeutic effects (Van Vuuren and Viljoen, 2011; Spinella, 2002).

The use of medicinal plants or phytobiotics for enhancement of growth probably as the result of synergistic effects among molecules those are active as well as complex. Better BW gains and higher feed intake have been noticed in 0.25% herbals combination of amla, turmeric and tulsi as feed additives on broilers (Reddy *et al.*, 2012). The inclusion of blend of thymol and cinnamaldehyde in feeds was shown to improve the BW gain in broilers (Tiihonen *et al.*, 2010; Amerah *et al.*, 2011).

Mehala and Moorthy (2008a) indicated that inclusion of turmeric and *Alo vera* mixtures resulted in a higher BW gain. According to Khaligh *et al.* (2011), different medicinal plant blends such as alfalfa leaves, cornflower, senna leaves and absinthe significantly increased the final BW and BW gain of birds. Also, feed intake in birds fed a mixture of leaves, cornflowers, senna leaves and absinthe was higher than those fed blends of alfalfa leaves meal, cinnamon, burdock root, and licorice root (Khaligh *et al.*, 2011).

On the other hand, a synergistic effect of phytobiotic compounds have been reported in studies with essential oils. Addition of a blend of essential oil from basil, caraway, laurel, lemon, oregano, sage, tea and thyme (100 g/ton) in broiler diets has been demonstrated to

improve the BW gain with positive effects on FCR (Khattak *et al.*, 2014). The reason for the increase in voluntary feed intake resulting in improved weight gain was due to the flavor and palatability of feed by the aromatic essential oils (Kroismay *et al.*, 2006). Similarly, the synergetic effect of combination between each 2.5 kg/ton chamomile flower heads and black cumin in broiler diets was superior in BW and BW gain than either marjoram leaves, coriander seeds, thyme flowers and harmala seed treatments or control birds (Abaza, 2001).

A combination of herbal powders might tend to be more effective than a single herb administration (Khaligh *et al.*, 2011). One commercial blend of phytonutrients (containing carvacrol, cinnamaldehyde and capsicum) oleoresin approved by EU as the first botanical feed additive to improve performance in broilers. Several research trials performed with this commercial blend demonstrated with consistent improvement in growth and feed efficiency (Pirgozliev *et al.*, 2015; Bravo *et al.*, 2014; Karadas *et al.*, 2014). According to Kirubakaran *et al.* (2016), fenugreek and garlic combinations resulted in higher BW and BW gain than the control broiler birds. The same study revealed that feed intake was significantly higher in broilers fed garlic and black paper combinations when compared to control at 6 weeks of age (Kirubakaran *et al.*, 2016). The improvement in BW gain might be due to the presence of antimicrobial, antioxidant properties and anti-stress factors in herbal preparations (Mehala and Moorthy, 2008a; Reddy *et al.*, 2012). Feed efficiency was better in broilers fed with herbals either alone or in combination (Reddy *et al.*, 2012).

Al-Kassie *et al.* (2011b) reported that the inclusion of a mixture of hot red pepper and black pepper at levels of 0.75 and 1% in the diets significantly improved the dressing percentage of broilers. On the other hand, dietary supplementation of herbals alone or in combination at 0.25 and 0.50% did not significantly affect percent weights of liver, heart, gizzard, spleen and bursa (Reddy *et al.*, 2012; Gupta and Charan, 2007). An improved dressing percentage and giblet weight was observed when broilers fed diets with amla and turmeric and their combination (Singh *et al.*, 2007).

Dietary supplementation of 100 mg/kg essential oil blends with 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin as active constituents improved the concentration of anti-oxidants in the liver of broiler chickens (Karadas *et al.*, 2014). Supplementation of herb blends consisting of 0.05% garlic powder, 0.3% cinnamon powder and 0.03% of each of the following dried herbs: yarrow, rosemary, thyme, basil and oregano improved egg laying capacity of hen by 1.79% as compared with a control groups (Gerzilov *et al.*, 2015). However, easy and practical application, availability and less cost known as advantages of the whole herbs application as compared to the extracted or essential oil forms.

Soliman *et al.* (1999) studied the synergistic effect of feeding black cumin and garlic on broiler performance and immunity. Their results showed that using 0.3% black cumin in broiler diets improved the development of immunity. The immunity to NCD antibody titre was above protective levels of 28 to 211 in birds fed amla and turmeric mixtures (Reddy *et al.*, 2012). Similarly, supplementing broilers with amla, tulsi and turmeric powder in combination resulted in a high antibody titre to NCD vaccination (Reddy, 2010). Combination of alfalfa leaves meal, cinnamon (*Cinnamomum zeylanicum*), burdock root, licorice root, thyme (*Thymus vulgaris*), caraway (*Carum carvi* L.), bishop's weeds (*Carum copticum*) blends of diets fed broilers also resulted in higher antibody titers against NCD vaccine as compared to the control birds (Khaligh *et al.*, 2011).

Al-Beitawi and El-Ghousein (2016) reported that *Nigella sativa*, *Pimpinella anisum* and *Thymus vulgaris* mixture were significantly improve the antibodies of broiler birds against IBD during 42 days of age. Antibody titer against avian influenza virus (H9N2) in turkeys was significantly higher and developed faster in birds fed thymoquinone and curcumin, alone and in combination (Umar *et al.*, 2016). Supplementation of amla and turmeric powder at 5 g/kg of feed in broiler diets resulted in nil mortality in broilers (Singh *et al.*, 2007) and less mortality by inclusion of amla, tulsi and turmeric 0.25 and 0.5% of the diets (Reddy, 2010). One hundred percent livability where observed when *Aloe vera* and turmeric combination mix in broiler diets (Mehala, and Moorthy,

2008a). Therefore, phytobiotic mixtures had positive influence in chicken growth and health performance due to their active constituents of synergetic effects.

2.5. Commercial Antibiotics as Growth Promoters

Antibiotics are chemical substances derived initially from certain fungi, bacteria and other organisms that can inhibit the growth and destroy harmful microorganisms (Davey, 2000). Numerous studies have reported that growth enhancement properties of antibiotics are closely related to interactions with the microbes in the gut. Antibiotic growth promoters (AGP) can help to control the diseases by selectively modifying and improving the gut microflora, reducing bacterial fermentation and preventing infectious diseases and results in health status improvement (Dono, 2012). All these changes lead to an increase in nutrient availability for the animals, allowing enhanced feed efficiency and being able to achieve better growth performance (Hernández *et al.*, 2006). Antibiotics supplementation in the diets promotes growth and plays a key role in poultry production (Wang *et al.*, 2015). Moreover, the use of antibiotic in poultry diets gives significant economic advantages as it facilitates better production efficiency, thus allowing consumers to purchase high quality poultry products at lower price (Dono, 2012).

Nevertheless, the aggravated utilization of antibiotics in the presence of low antibiotic levels give rise to resistant pathogens to survive and multiply, thereby producing antibiotic resistance pathogens in the animals and the accumulation of antibiotic residues in animal products (Abaza, 2001; Lee *et al.*, 2004; Cogliani, 2011; Toaha *et al.*, 2016). Despite these debates on the role of antibiotics (AGP) use in conferring antimicrobial resistance to human pathogens, the EU issued a ban on the approval for antibiotics as growth promoters since January 2006 on precautionary grounds (Hashemi and Davoodi, 2011; Wang *et al.*, 2015). Presently, there is an increasing interest to find alternative substances and strategies to improve the health status of farm animals for human consumption. Among those, alternatives, phytobiotic or herbal products have received increased attention since they are more widely acceptable as natural additives and candidate to replace AGP.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiments were conducted in the Poultry Farm of College of Veterinary Medicine and Agriculture of Addis Ababa University, Bishoftu between July 2015 - February 2016. The area is situated at 47 km East of Addis Ababa at an altitude of 1900 m above sea level, latitude of 8°44'N and longitude of 38°57'E. The average (25 years) annual rainfall is 851mm with an average minimum and maximum temperature of 8.9°C and 26°C, respectively and the average relative humidity is 58.6% (DZARC, 2003).

3.2. Preparation of Medicinal Plants

Three medicinal plants, namely *Nigella sativa* L. (black cumin) seeds, *Trigonella foenum-graecum* L. (fenugreek) seeds and *Curcuma longa* L. (turmeric) rhizome purchased from the vicinity local markets for incorporate in the diets of broiler chickens as phytobiotic feed additives. The black cumin, fenugreek seeds and turmeric rhizome were washed with tap water and sun dried under shade. Then, the dried turmeric rhizome, black cumin and fenugreek seeds were coarsely grounded by manual mill and stored in polythelene bags until required for the formulation of experimental rations.

3.3. Experimental Diets

Common feed ingredients used to formulate the experimental diets of the two consecutive studies were corn grain, wheat bran, noug seedcake (*Guizotia abyssinica*), soybean meal (SBM), vitamin premix, salt, limestone, methionine and lysine. Ground form of black cumin seeds, fenugreek seeds and turmeric rhizome were included as phytobiotic feed additives in the formulation of the treatment diets. All the ingredients except SBM, wheat bran, vitamin premix methonine and lysine were hammer-milled in sieve size of 5 mm and stored until required.

Table 1. Chemical composition of feed ingredients and studied feed additives

Ingredient	DM (%)	Nutrients (%DM)						ME (Kcal/kg DM)
		CP	CF	EE	Ash	Ca	P	
Maize	90.33	6.89	4.62	2.4	9.11	1.66	0.30	3300.08
SBM	94.17	37.37	5.95	12.55	6.34	2.12	0.65	3847.28
NSC	92.93	36.79	16.03	8.16	7.50	1.44	0.65	2667.04
Wheat Bran	86.6	11.0	25.0	4.80	7.00	0.11	1.15	1709.02
Black cumin	94.37	25.77	14.73	36.88	12.29	1.94	0.75	4149.29
Fenugreek	92.77	46.71	8.30	7.03	7.98	1.62	0.25	3271.64
Turmeric	87.63	8.70	3.42	7.04	7.04	1.33	0.36	3743.39

* Nutrient per DM percentage, CF = Crude fiber; DM = Dry Matter; CP = Crude protein; EE = Ether Extract; Ca = Calcium; P = Phosphorous; SBM = Soybean meal, NSC = Noug seedcake; ME = (kcal/kg DM)

Based on the results of chemical analysis of ingredients the phase I experimentation, seven treatment diets containing black cumin, fenugreek and turmeric each at levels of 1 and 2% of the total ration and control diets were formulated: The treatment diets included:

- T₀: Control basal diet
- T₁: Diet containing black cumin seeds at 1%
- T₂: Diet containing black cumin at 2%
- T₃: Diet containing fenugreek seeds at 1%
- T₄: Diet containing fenugreek seeds at 2%
- T₅: Diet containing turmeric rhizome at 1%
- T₆: Diet containing turmeric rhizome 2%

For phase II experiment, six treatment broiler rations containing blends of different phytoantic additives and control diet were formulated at lower levels (1%) set for each of the medicinal plants as shown below.

- T₀: Basal diet (Negative control)
- T₁: Basal diet + oxytetracycline 0.035% (Positive control)
- T₂: Basal diet + a mixture of black cumin + fenugreek
- T₃: Basal diet + a mixture of black cumin + turmeric
- T₄: Basal diet + a mixture of fenugreek + turmeric
- T₅: Basal diet + a mixture of black cumin + fenugreek + turmeric

The diets were formulated to be nearly isocaloric and isonitrogenous as shown in Table 2 and 3 with metabolizable energy (ME) content of 3000 kcal/kg DM and CP content of 22% during the starter phase 1-28 days of age and ME content of 3200 kcal/kg DM and CP content of 20% during the finisher phase of 29- 49 days of age (Leeson and Summers, 2005). All birds offered the respective weighed diets and had free access to clean water during the entire experimental periods.

Table 2. Proportion of ingredients and their respective gross composition of phase I experimental diets

Ingredient %	Starter phase (1-28 d)							Finisher phase (29-49 d)						
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Maize	56.97	56.00	56.00	57.50	58.31	57.75	57.00	60.00	60.00	59.52	61.5	61.50	60.00	60.00
SBM	28.00	28.00	28.00	28.48	28.30	29.61	29.67	20.00	20.00	20.00	20.00	19.00	20.45	20.00
Wheat bran	5.2	5.22	5.00	4.00	3.00	3.00	3.00	12.37	11.20	11.00	10.00	10.00	10.00	9.00
NSC	6.2	6.20	5.55	5.55	5.00	5.25	5.00	4.00	4.27	4.00	4.00	4.00	5.00	5.50
Limestone	2.33	2.28	2.18	2.20	2.10	2.18	2.12	2.40	2.30	2.2	2.22	2.18	2.25	2.18
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin-mineral premix	0.5	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.30	0.5	0.17	0.17	0.17	0.12	0.12	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine HCl	0.15	0.3	0.25	0.25	0.27	0.24	0.24	0.20	0.20	0.25	0.25	0.29	0.27	0.29
Black cumin	0.00	1.00	2.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	0.00	0.00	0.00	0.00
Fenugreek	0.00	0.00	0.00	1.00	2.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	0.00	0.00
Turmeric	0.00	0.00	0.00	0.00	0.00	1.00	2.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Composition (% DM basis)														
DM	92.25	92.35	92.0	91.69	91.14	92.05	91.84	93.021	93.04	93.06	93.71	93.36	93.46	92.01
CP	21.75	21.95	21.93	21.60	21.90	21.80	21.92	19.60	19.50	19.75	20.00	20.05	19.61	19.63
ME (kcal/kg DM)	2970	2995	2999	2898	2890	3005	2992	3198	3196	3199	3111	3117	3117	3123
EE	4.80	6.83	5.71	4.76	4.27	4.67	4.56	4.92	5.42	5.63	4.05	4.36	4.35	4.42
CF	6.54	6.67	6.57	6.73	6.18	6.53	6.64	7.56	7.71	7.52	8.78	8.74	7.6	7.54
Ca	1.06	1.04	1.00	1.10	1.05	1.35	1.40	1.04	1.13	1.12	1.02	1.16	1.07	1.09
P	0.46	0.49	0.5	0.48	0.46	0.44	0.48	0.57	0.59	0.56	0.55	0.57	0.53	0.58

SBM= Soybean meal, NSC= Noug seed cake, T₀ = Control basal diet, T₁= 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, vitamin-mineral Premix contains, Vitamin A 1000 000 IU, Vitamin D 3200 000 IU. Vitamin E 1000 mg, Vitamin K₃ 225 mg, Vitamin B₁ 125 mg, Vitamin B₂ 500 mg, Vitamin B₃ 1375 mg, Vitamin B₆ 125mg, Vitamin B₁₂ 2 mg, Vitamin PP (niacin) 4, 000 mg, Folic Acid, 100 mg, choline chloride 37,500 mg, Calcium 29.7 %, Iron 0.4 %, Copper 0.05 %. Manganese 0.6 %, Zinc 0.7%, Iodine 0.01 % Selenium 0.004 %.

Table 3. Proportion of ingredients and their respective gross composition of phase II experimental diets

Ingredient %	Starter phase (1-28 d)						Finisher phase (29-49 d)					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Maize	37.00	37.00	35.00	32.00	31.50	32.00	46.85	46.85	46.65	45.50	46.00	46.00
SBM	30.00	30.00	30.00	30.00	30.00	30.00	31.50	32.00	30.00	29.50	29.00	28.00
Wheat bran	16.00	16.00	16.00	17.00	17.00	15.00	7.00	7.00	7.00	7.650	7.65	7.65
NSC	14.00	14.00	14.00	16.00	17.00	17.30	11.00	11.00	11.00	12.00	12.00	12.00
Limestone	1.75	1.75	1.75	1.75	1.25	1.40	2.50	2.50	2.20	2.20	2.20	2.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methonine	0.10	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.05
Lysine HCl	0.15	0.15	0.15	0.15	0.15	0.15	0.10	0.10	0.10	0.10	0.10	0.10
Oxytetracycline 30%	0.00	0.035	0.00	0.00	0.00	0.00	0.00	0.035	0.00	0.00	0.00	0.00
Black cumin	0.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	1.00
Fenugreek	0.00	0.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	1.00
Turmeric	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00
Total	100	100	100	100	100	100	100	100	100	100	100	100
Composition (% DM basis)												
DM	90.86	90.21	90.31	90.09	90.09	90.05	91.01	91.47	91.38	91.81	91.35	91.92
CP	20.73	21.03	21.32	21.57	22.12	22.30	19.85	20.0	20.0	19.79	19.85	19.7
ME (kcal/kg DM)	3032	3025	3042	3017	3019	3051	3177	3201	3187	3174	3162	3165
EE	3.80	3.83	3.71	3.76	3.81	3.78	3.72	3.74	3.69	3.72	3.73	3.71
CF	6.56	6.71	6.52	6.88	7.04	6.92	5.54	5.57	5.44	5.61	5.59	5.53
Ca	0.89	0.89	0.89	0.89	0.71	0.77	1.17	1.06	1.05	1.05	1.05	1.05
P	0.61	0.61	0.61	0.62	0.63	0.61	0.53	0.53	0.52	0.52	0.52	0.5

SBM = Soybean meal, NSC= Noug seed cake, T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, Vitamin-mineral Premix contains, Vitamin A 1000 000 IU, Vitamin D 3200 000 IU. Vitamin E 1000 mg, Vitamin K₃ 225 mg, Vitamin B₁ 125 mg, Vitamin B₂ 500 mg, Vitamin B₃ 1375 mg, Vitamin B₆ 125mg, Vitamin B₁₂ 2 mg, Vitamin PP (niacin) 4, 000 mg, Folic Acid, 100 mg, choline chloride 37,500 mg, Calcium 29.7 %, Iron 0.4 %, Copper 0.05 %. Manganese 0.6 %, Zinc 0.7%, Iodine 0.01 % Selenium 0.004 %.

3.4. Experimental Treatments and Design

A total of 585 one-day old broiler chicks (Cobb 500) were purchased from Alema farm-Koudijs Feed P.L.C. For the first experiment (Phase I), 315 one-day old unsexed broiler chicks with average initial BW of 40.60 ± 0.64 g (Mean $\pm$ SD) were randomly divided into seven dietary treatments and three replications per a treatment having 15 chicks per a replicate. For the second experiment (Phase II), a total of 270 one-day old unsexed broiler chicks with average initial BW of 39.04 ± 0.45 g were randomly allocated into six dietary treatments and three replications per a treatment having 15 chicks per a replicate. The experimental design was complete randomized designs (CRD) for both consecutive experiments.

3.5. Management of Birds

The birds were raised till to 49-days in a tin-shed poultry house with partially opened wall and covered by wire-mesh of concrete floor. The room was partitioned to have 21 and 18 pens for the two consecutive experiments, respectively. The chicks under different experimental treatments were kept in a separate pen using wire-mesh and wood partition of equal size of 1.8 m^2 (0.25 m^2 per bird). Before the commencement of the actual experiment, the room, watering and feeding troughs and utensils were thoroughly washed and sprayed, disinfected with disinfectant chemicals (HI7) and the room was fumigated by mixing potassium permanganate (KMnO_4) powder with formalin 10% solution.

The chicks were brooded using 200-watt bulbs suspended with gradual height adjustment as sources of heat and light for each pen to ensure adequate and uniform distribution of heat and light. The lightening program was with a 23 h constant light schedule. A thickness of about 7 cm 'Teff' (*Eragrostis tef*) straw was used as a deep litter material. The brooding temperature was maintained at 32°C during the first week. It was then gradually lowered by 2°C until it reached the room temperature of 24°C towards the end of the experiment. Ambient temperature and relative humidity were measured using wall mount thermometer and hygrometer.

The birds were vaccinated against Newcastle disease (NCD) (Hitchner-B1 strain) at 7-days of age through intraocular and Lasota strain a booster dose at 21-days of age and Infectious Bursal Disease (Gumboro) at the age of 14 and 28 days and administered through drinking water obtained from the spring.

Strict bio-security measures were followed throughout the experimental periods. Water troughs were cleaned daily. The entrance of persons was restricted. Work cloth and feet wear were changed beforehand any activity. All birds were kept under close supervision throughout the experimental period for abnormal signs and other suspicious activities. A sick bird was promptly isolated to a separate pen and excluded from the experiment. Dead birds were disposed and buried in burial pit far from the poultry house after post mortem examination.

3.6. Data Collection

3.6.1. Chemical analysis

Samples of medicinal plants (phytobiotic feed additives) and major feed ingredients were ground by using a Wiley mill (Thomas® Wiley Cutting Mill) to pass through a 1 mm screen for proximate composition at National Veterinary Institute (NVI) animal nutrition laboratory prior to experimental feed formulation. Feed samples were analyzed for DM, ether extract (EE), CF and ash according to the procedure of AOAC (1998). The nitrogen contents determined by Kjeldhal procedure and the CP content was determined as $N \times 6.25$. Calcium and total phosphorus contents were determined by atomic absorption and Vanado-Molybdate method, respectively (AOAC, 2000). The metabolisable energy (ME) content of the experimental diets were calculated by the formula according to Wiseman (1987) as $ME \text{ (kcal/ kg DM)} = 3951 + 54.4EE - 88.7CF - 40.80Ash$.

3.6.2. Body weight change

Birds were weighed in group at the beginning and on weekly basis until the end of the experiment. A bench balance (Xianghai, model ACS-C with 0.001 gram sensitivity) was used to take body weight measurement. Average BW gain was calculated as the difference between the two successive weights divide by number of birds. Average daily gain (ADG) was calculated as the mean of final BW change to the number of experimental days.

3.6.3. Feed consumption and conversion ratio

The amount of feed offered and refused per pen were recorded daily. Feed consumption was determined as the difference between the feed offered and refused. Feed conversion ratio (feed consumed to produce a unit of gain) was computed as the ratio of average feed consumption to average BW gain.

3.6.4. Performance index

Performance index (PI) was calculated during the studied growth periods according to the equation of North (1981). Performance Index (PI) = (BW gain per kg) / FCR) x 100.

3.6.5. Mortality

Mortality recorded as encountered throughout the experimental period and mortality percentage was calculated as number of dead birds divide by number of total birds multiplied by 100.

3.6.6. Carcass processing and measurement

At the end of each experiment, two birds from each replicate were randomly selected for carcass analysis. The selected birds were starved for twelve hours and weighted before slaughtering. The slaughter BW was taken before slaughtering.

The birds were humanly slaughtered by severing the jugular vein, exsanguination followed by dipping into hot water and de-feathering by hand plucking. Carcasses were manually eviscerated (removing of heart, crop, pancreas, kidney, lungs, proventriculus, small intestine, large intestine, caeca and urogenital tracts) and suspended from the evisceration line and allowed to drain for 15 minutes prior to weighing.

The breast, wings, thighs with drumsticks and back were used to evaluate the carcass yield/weight on a commercial basis. The dressing percentage was calculated as the proportion of carcass weight to slaughter weight multiplied by 100. Gizzard and liver are edible in most places and included in the edible carcass component. They were added to the carcass weight and another version of dressing percentage was calculated. The eviscerated carcass such as breast, wings, thighs with drumsticks and back were separated and weighed and then their individual weights were divided by the slaughter weight and multiplied by 100 to determine percentage weights of each component. The giblets (heart, gizzard and liver) were weighed and the percentage was calculated as the proportion of slaughter weight. The fat around the proventriculus, gizzard, abdominal wall and the cloacae were collected and weighed; and fat percentage was calculated as the proportion of the slaughter weight (Etalem, 2013).

3.6.7. Hematological and serum biochemistry profile determination

Samples of blood were collected from two birds of randomly selected per each pen was carried out at the end of the experimental periods. About 2-3 ml blood samples were collected from the wing vein using a 5 ml sterile disposable syringe of 22-gauge needle. Half of the blood was transferred into heparinized tube containing EDTA, for the hematological assay. The second half of the blood was transferred into non-heparinized tubes, later on the coagulated blood samples were centrifuged for 15 minutes at 4000 rpm, and the clear serum was separated and stored in a deep freezer at -20°C for pending biochemical analysis. All the hematology and biochemical profile determination were undertook at pathology and biochemistry laboratory of Addis Ababa University, college of veterinary medicine and agriculture.

3.6.7.1. Hematological profiles determination

Samples of non-coagulated blood was tested shortly after collection of blood for estimating the Packed Cell Volume (PCV), which was determined by microhaematocrit method (Schalm *et al.*, 1975). Haemoglobin (Hb) concentration was measured by Sahli's method (Sahli, 1905). The Red Blood Cell (RBC) and White Blood Cell (WBC) counts were counted using Neubauer haemocytometer under light microscope (Schalm *et al.*, 1975).

Additionally, RBC indices include MCV, MCH and MCHC, which were calculated from Hb, PCV and RBC as prescribed by Jain (1986). Mean corpuscular volume (MCV) is the expression of the average volume of individual erythrocytes calculated: $MCV = (PCV \times 10) / RBC$ and expressed as femtoliters (fL). Mean corpuscular hemoglobin (MCH) is the expression of the average hemoglobin content of a single erythrocyte and was calculated as $MCH = (Hb \times 10) / RBC$ and expressed as picograms (pg). The mean corpuscular hemoglobin concentration (MCHC) is the expression of the volume hemoglobin within the erythrocytes and was calculated: $MCHC\% = (Hb \times 100) / PCV$.

3.6.7.2. Serum biochemical profile determination

Serum total protein and albumin were determined according to Doumas (1971) and Witt and Trendelenburg (1982). Globulin concentration was calculated as the difference between total protein and albumin. Albumin to globulin ratio g/100 ml was also calculated. Total cholesterol was determined according to the procedure outline by Watson (1960). Serum glucose determined using glucose oxidase method prescribed by Trinder (1969). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) follows the proposed optimized formulation of the International Federation of Clinical Chemistry (IFCC) (Bergmeyer *et al.*, 1986), while measurement of Alkaline phosphatase (ALP) was as per the German Society for Clinical Chemistry (GSCC, 1972). Samples were analyzed using the instrument HumaStar 80 automated chemistry analyzer (HUMAN Gesellschaft fuer Biochemical und Diagnostic GmbH, Germany).

The instrument was calibrated using calibrator (Autocal) and quality control samples normal (Humatrol N) and pathological (Humatrol P) were run for validation before running samples for tests.

3.6.8. Immunological response measurement

To assess the humoral/or non-specific antibody response against Newcastle Disease (NCD) and Infectious Bursal Disease (IBD) virus 1 ml/chick blood samples were drawn into Eppendorf tubes from wing vein of birds (two birds per replicate), on day 21 and 42 of the experiment and transported to NVI serology laboratory. Blood samples allowed to clot for 2 hours prior to collecting serum. Then the serum was separated by centrifugation at 2000 rpm for 10 min and stored at -20°C for use.

For NCD antibody titer assayed by haemagglutination inhibition (HI) test were used according to the procedure described by Xu (1990). Briefly, a 2-fold serial dilution of serum ranged from 1:2 to 1:256 (2^2 - 2^8). After inactivation at 56°C for 30 minute, was made in a 96-well, V-shaped bottom microtiter plate containing 50 µl of NCD virus antigen (4 hemagglutination units) was added to all the wells except for the last row (the controls). The antigen serum mixture was incubated for 10 min at 37°C. Fifty micro-liters of a 1% rooster erythrocyte suspension then added to each well and the wells re-incubated for 30 minutes. A positive serum, a negative serum, erythrocytes, and antigens were also included as controls. The highest dilution of serum causing complete inhibition of erythrocyte agglutination considered at the end point. The mean titer expressed converted to log 2 values of the highest dilution that displayed anti-NCD HI (Xu, 1990). While, enzyme link immunosorbent assay (ELISA) applied for IBD as described by Thayer and Beard (1998) using commercial kits provided by Affini Tech.Ltd., AR. The mean antibody titer expressed converted to log 10.

3.6.9. Cecum bacterial load determination

From each euthanized bird for carcass evaluation, the ileum, starting from the Meckel's diverticulum to 4 cm above the ileo-caecal junction was quickly dissected and the caecal contents of each bird pooled on universal bottle placed on icebox and transport to NVI microbiology laboratory for enumeration of microbial populations. Microbial load determined from one gram of the cecal content was diluted 1:9 (w/v) with distilled water. Samples serially diluted from 10^{-1} to 10^{-7} . Each dilution poured Eosin Methylene Blue (EMB) agar for *E. coli*, which were incubated at 37 °C from 8 to 24 h (Collee *et al.*, 1989) whereas, on violet red bile agar VRBS (Merck) was used for total coliform bacteria at 37°C for 24 h (Cheesbrough, 1985). Then plates counted between 24 and 48 h after inoculation (Collee *et al.*, 1989; Cheesbrough, 1985). The counted data were transformed to $\log^{10}$ cfu/ml.

3.6.10. Partial budget analysis

Partial budget analysis was conducted to determine the economic efficiency or profitability of the inclusion of the phytobiotic additives at the given level in broiler diets. The analysis took into consideration the cost of feed (which is variable cost) consumed by the chicks, purchasing price of chicks and selling prices of the carcass of broilers, while other costs (labor cost, vaccination cost, house rent, light cost) were assumed to be similar for all the treatments.

Cost of feed consumed per bird was obtained from multiplying feed consumed per bird by feed cost per kilogram of feed. The total variable costs (TVC) include cost of day-old broiler chick and cost of feed consumed per bird. The total income or total return (TR) obtained from the average carcass weight of broiler multiplied by the selling price of carcass at the supermarket (80 birr/kg). Net income or net return (NR) were calculated as the amount of money left when total variable costs (TVC) was subtracted from the total returns (TR) as follows (Knott *et al.*, 2003): $\text{Net return (NR)} = \text{TR} - \text{TVC}$.

The marginal rate of return (MRR) measures increases in net return (NR) associated with each additional unit of expenditure (TVC) and it was calculated as follows (Knott *et al.*, 2003): Marginal rate of return (MRR) = NR / TVC.

3.7. Statistical Analysis

The collected data were subjected to the analysis of variance test for each parameter using the general linear models (GLM) procedures of SAS statistical package version 9.3 (SAS, 2010). Duncan's multiple range test was used to detect the differences among the treatment means at ($P \leq 0.05$) (Duncan, 1955).

The model used was:

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

Where: Y_{ij} = the j^{th} the observation with treatment i

μ = overall mean

α_i = the i^{th} treatment effect (growth performance, carcass, hematology, biochemical, antibody titer & caecal microbial load)

ε_{ij} = The random error of variation normally and independently distributed ($0, \delta^2$)

4. RESULTS

4.1. Phase I Experiment

4.1.1. *Growth performance of broilers*

As shown in Table 4, the difference in feed consumption between the treatment diets was highly significant ($P \leq 0.001$) during the starter, finisher period and overall. Birds consumed significantly ($P \leq 0.05$) more on diets with fenugreek (T3 and T4) and control during the finisher phase and overall. Broilers consumed low amount ($P \leq 0.05$) on diets with black cumin (T1 and T2) and turmeric (T5 and T6) compared to the control during the finisher phase and overall.

The effect of supplementing black cumin, fenugreek and turmeric at 1 and 2% levels on the final BW, BW gain and ADG of broiler chicks during the starter and finisher phases are shown in Table 4. The initial BW of chicks at day-old showed nearly similar values with no significant differences ($P \geq 0.05$) among the treatment groups. Also, the final BW, BW gain and ADG during the starter and finisher phase were not significantly different ($P \geq 0.05$) among the treatment groups.

As shown in Table 5, it was clear that a highly significant difference ($P \leq 0.001$) was shown among treatments in FCR during the starter, finisher and entire experimental phases. Phytobiotic feed additive treatment diets T1, T2, T5 and T6 ensured improvement of the FCR ($P \leq 0.05$) than the control diet group during starter phases. Inclusion of black cumin and turmeric at both levels in the diets resulted in exceedingly ($P \leq 0.05$) improved FCR as compare to the control and fenugreek fed birds during the finisher phases and overall. There is no significant difference ($P \geq 0.05$) between birds fed black cumin and turmeric at both levels. However, fenugreek at 1 and 2% levels were inefficient in FCR as compared to the birds fed the control diet ($P \leq 0.05$) during the finisher and overall.

Table 4. Effects of dietary inclusion of black cumin, fenugreek and turmeric on growth performance of broilers

Parameter (g/bird)	Treatment							P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
Feed intake								
Starter	1928.9 ^a ±22.0	1449.9 ^b ±14.2	1490.9 ^b ±39.0	1900.0 ^a ±9.8	1794.6 ^a ±14.6	1410.2 ^b ±21.4	1434.3 ^b ±4.7	0.001
Finisher	3187.6 ^a ±20.3	2161.4 ^b ±44.3	2391.5 ^b ±89.5	3595.4 ^a ±32.9	3422.2 ^a ±15.6	2516.2 ^b ±46.0	2531.4 ^b ±71.6	0.001
Overall	5116.5 ^a ±18.2	3611.3 ^b ±30.8	3882.1 ^b ±12.8	5495.4 ^a ±42.2	5216.8 ^a ±30.2	3926.4 ^b ±67.9	3965.7 ^b ±71.9	0.001
Initial BW	40.66±0.4	40.67±0.7	40.45±0.2	40.22±0.2	41.0±0.3	40.89±0.6	40.2±0.2	0.882
Final BW								
Starter	881.1±11.4	847.8±21.2	862.9±29.4	811.3±22.0	791.3±31.2	858.0±40.8	909.6±29.7	0.123
Finisher	2088.9±31.5	2072.3±25.9	1956.6±66.8	1911.21±20.9	1856.3±0.9	2070.17±28.0	2097.10±37.1	0.315
BW gain								
Starter	840.5±11.0	807.1±20.6	822.44±29.2	771.1±22.0	750.4±31.2	817.1±40.4	869.3±29.5	0.116
Finisher	1207.8±22.5	1224.56±26.0	1093.7±37.5	1099.9±41.4	1064.9±32.1	1212.17±19.4	1187.55±22.4	0.623
Overall	2048.2±31.5	2031.7±26.0	1916.2±66.6	1871.0±21.1	1815.4±0.9	2029.3±20.8	2056.9±37.0	0.315
ADG(g/bird/day)								
Starter	30.0±0.4	28.8±0.7	29.4±1.0	27.5±0.8	26.8±1.1	29.2±1.4	31.0±1.1	0.116
Finisher	57.5±1.1	58.3±1.2	52.1±1.8	52.4±1.9	50.7±1.5	57.7±9.0	56.6±1.1	0.623
Overall	41.8±0.6	41.6±0.5	39.1±1.4	38.2±0.4	37.0±0.2	41.4±4.2	42.0±0.8	0.315

T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, BW= Body weight, ADG = Average daily gain SE = Standard error of the mean, P=Probability.

Table 5. Effects of dietary inclusion of black cumin, fenugreek and turmeric on FCR, performance index and mortality of broilers during the starter and finisher phases

Parameter	Treatment							P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
FCR								
Starter	2.3 ^a ±0.0	1.8 ^b ±0.1 ^b	1.8 ^b ±0.0	2.5 ^a ±0.1	2.4 ^a ±0.3	1.7 ^b ±0.1	1.7 ^b ±0.1	0.001
Finisher	2.6 ^b ±0.2	1.8 ^c ±0.0	2.2 ^c ±0.0	3.3 ^a ±0.1	3.2 ^a ±0.1	2.2 ^c ±0.3	2.1 ^c ±0.0	0.001
Overall	2.5 ^b ±0.1	1.8 ^c ±0.0	2.0 ^c ±0.0	2.9 ^a ±0.2	2.9 ^a ±0.2	1.9 ^c ±0.2	1.9 ^c ±0.0	0.001
Performance index								
Starter	36.6 ^{bc} ±0.5	45.0 ^{ab} ±2.5	45.4 ^{ab} ±2.1	31.3 ^c ±1.7	32.4 ^c ±5.6	47.5 ^a ±4.1	52.8 ^a ±3.7	0.002
Finisher	46.4 ^{ab} ±4.7	69.4 ^a ±2.7	50.0 ^{ab} ±1.6	33.7 ^b ±2.4	33.2 ^b ±0.7	60.8 ^a ±18.6	55.7 ^{ab} ±1.2	0.031
Overall	82.4 ^{bc} ±5.5	114.3 ^a ±2.1	94.6 ^{ab} ±3.5	63.7 ^c ±1.2	63.6 ^c ±3.8	106.5 ^{ab} ±20.7	106.7 ^{ab} ±2.2	0.003
Mortality%								
Starter	2.2±2.2	0.0±0.0	0.0±0.0	4.4±2.2	4.4±2.2	0.0±0.0	2.2±2.2	0.579
Finisher	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-
Overall	2.2±2.2	0.0±0.0	0.0±0.0	4.4±2.2	4.4±2.2	0.0±0.0	2.2±2.2	0.579

*^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$); T₀ = Control basal diet, T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, FCR = Feed conversion ratio, SE = Standard error of the mean, P = Probability.

4.1.2. Performance index (PI) and mortality

Results presented in Table 5, indicated that there were significant differences ($P \leq 0.05$) among treatments in the performance index (PI) during the different experimental periods. During the starter phase diet with turmeric at 1 and 2% (T5 and T6) significant upturned PI values ($P \leq 0.05$) as compared to the control and fenugreek at both levels (T3 and T4) fed birds. However, birds fed with fenugreek at both levels and control diet recorded the lowest PI values, respectively and was not significantly different ($P \geq 0.05$).

Performance index of broilers during the finisher phase, diet with black cumin (T1) and turmeric at 1% (T5) levels showed a substantial increase of performance index as compared with the control group, however, it was not statistically significant different ($P \geq 0.05$) compare to the control group. Among all tested dietary levels of phytobiotic additives, lower level of black cumin (T1) had the best values of PI when compared to that of 2% inclusion rate. Similarly, turmeric and fenugreek perform better at lower inclusion rate in the broiler diets. Moreover, throughout the experimental period, inclusion of black cumin at 1% (T1) in broiler diets resulted in an increased PI value as compared to birds fed the control and fenugreek diets ($P \leq 0.05$). However, there was no significant difference ($P \geq 0.05$) between birds fed with fenugreek at both levels and the control birds. Furthermore, no mortality recorded during the finisher phase, although there were no significant differences ($P \geq 0.05$) in mortality among the treatments during the starter phase and throughout the experimental periods.

4.1.3. Carcass characteristics of broilers

Carcass characteristics of broilers fed diets with different phytobiotic additives at different levels are presented in Table 6 and 7. The slaughter weight were not significantly different ($P \geq 0.05$) among treatments. However, there were a significant difference ($P \leq 0.05$) in commercial carcass yield, edible carcass yield and their respective dressing percentages.

Table 6. Effects of dietary inclusion of black cumin, fenugreek and turmeric on carcass components of broiler chickens

Parameter	Treatment							P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
Slaughter weight (g)	2076.7±38.8	2003.3±46.9	1995.0±37.1	1878.3±42.9	1901.8±18.8	2020.0±11.2	2084.17±37.4	0.565
Carcass yield (g)	1371.1 ^{ab} ±18.6	1302.8 ^{ab} ±43.2	1273.12 ^{ab} ±10.7	1184.6 ^b ±30.1	1161.8 ^b ±80.3	1313.8 ^{ab} ±67.9	1423.8 ^a ±36.4	0.034
*Dressing %	66.07 ^{ab} ±0.7	64.97 ^{bc} ±0.9	63.88 ^{bc} ±0.8	63.08 ^{cd} ±0.9	60.89 ^d ±0.8	65.15 ^{bc} ±1.1	68.29 ^a ±0.9	0.000
Edible carcass weight (g)	1524.1 ^{ab} ±17.1	1460.5 ^{abc} ±44.7	1437.1 ^{abc} ±14.2	1330.42 ^{bc} ±30.2	1303.62 ^c ±60.9	1476.33 ^{abc} ±75.8	1576.67 ^a ±40.2	0.035
**Dressing %	73.5 ^{ab} ±0.7	72.9 ^b ±0.7	72.1 ^b ±0.8	70.8 ^{bc} ±0.5	68.4 ^c ±0.8	73.2 ^{ab} ±1.4	75.6 ^a ±0.8	0.000

*^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$); T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, * dressing % calculated only commercial carcass yield, ** dressing % calculated by including gizzard & liver, SE = Standard error of the mean, P = probability.

Inclusion of turmeric rhizome at 2% (T6) resulted in the uppermost commercial carcass yield, edible carcass weight and their respective dressing percentage, however, there was no statistically significant difference ($P \geq 0.05$) in comparison with the control (T0) birds. Nevertheless, fenugreek at both levels of 1 and 2% (T3 and T4) fed birds had lower values in commercial carcass weights, edible carcass weights and their respective dressing percentages than the birds fed the control diet ($P \leq 0.05$).

As shown in Table 7, the commercial carcass components including breast, back weights and their percentage were significantly different ($P \leq 0.05$) among treatments. However, there were no significant differences ($P \geq 0.05$) among treatments in back percentage, thigh with drum weight, wings weight and its percentage. Broiler supplemented with turmeric at 2% diets was exhibited higher ($P \leq 0.05$) breast meat weight and percentage than the T1, T2, T3 and T4 group. Nevertheless, there were no statistical differences ($P \geq 0.05$) in breast weight and percentage between T6 and control (T0) group birds. Fenugreek at both levels of 1 and 2% (T3 and T4) fed birds had lower breast meat weight than the birds fed the control diet ($P \leq 0.05$). The lower back weight was obtained from T4 fed birds ($P \leq 0.05$) as compared to the control. Whereas the uppermost weight was obtained from T1, however it was not statistically different ($P \geq 0.05$) as compared to the control and turmeric at both levels of 1 and 2% (T5 and T6) diet fed birds.

The relative weight of internal organs such as liver, heart, GIT weights and its percentages and gilet weight were not significantly different ($P \geq 0.05$) among treatments. However, there were significant difference ($P \leq 0.05$) among treatments in gilet percentage, gizzard weight and their percentage and abdominal fat weight and its percentages. Fenugreek inclusion at 2% had a significant effect ($P \leq 0.05$) on gizzard weight and gilet percentage as compared to the control and other phytobiotic additive treatment group birds. In addition, the abdominal fat weight was significantly reduced ($P \leq 0.05$) due to dietary inclusion of turmeric at 1 to 2 % compared to that of the control and the other phytobiotic additive treatment birds. Similarly, the abdominal fat percentage was significantly lower ($P \leq 0.05$) in T6 than the control and other phytobiotic additive treatment groups.

Table 7. The absolute and relative weight of carcasses and internal organs of broiler chickens

Parameter	Treatment							P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
Breast (g)	638.9 ^{ab} ±21.1	579.7 ^{bc} ±33.8	548.8 ^{bc} ±6.6	532.4 ^c ±15.5	533.83 ^c ±57.3	602.18 ^{abc} ±42.4	685.88 ^a	0.014
Breast %	30.8 ^{ab} ±1.0	28.9 ^{bc} ±1.2	27.5 ^c ±0.5	28.4 ^{bc} ±0.6	27.9 ^c ±0.8	29.7 ^{bc} ±0.7	32.9 ^a ±0.6	0.000
Thigh+Drum (g)	430.2±9.6	415.3±14.8	423.1±8.0	387.3±12.5	370.9±40.1	425.2±25.9	442.3±10.8	0.198
Thigh+ Drum%	20.7±0.4	20.8±0.9	21.2±0.5	20.6±0.6	19.4±0.6	21.1±0.5	21.2±0.4	0.262
Wing (g)	79.5±1.0	78.7±1.9	78.4±1.6	77.5±2.6	73.56.2	81.1±3.3	78.7±3.3	0.793
Wing%	3.8±0.04	3.9±0.1	3.9±0.1	4.1±0.1	3.9±0.1	4.0±0.1	3.8±0.1	0.065
Back (g)	222.5 ^{ab} ±7.5	229.2 ^a ±20.8	222.8 ^{ab} ±8.4	187.4 ^{bc} ±7.5	183.6 ^c ±19.9	205.4 ^{abc} ±5.1	216.9 ^{ab} ±5.1	0.039
Back %	10.7±0.3	11.4±0.9	11.2±0.4	10.0±10.22	9.7±0.4	10.4±0.9	10.4±0.1	0.309
Gizzard (g)	32.0 ^b ±2.3	33.4 ^b ±1.4	31.4 ^b ±1.1	37.1 ^{ab} ±1.5	39.9 ^a ±1.9	35.0 ^{ab} ±1.0	36.0 ^{ab} ±3.1	0.038
Gizzard%	1.54 ^c ±0.1	1.7 ^{bc} ±0.1	1.57 ^c ±0.04	2.0 ^{ab} ±0.1	2.1 ^a ±0.1	1.8 ^{bc} ±0.6	1.7 ^{bc} ±0.2	0.001
Liver (g)	47.9±2.6	46.3±1.7	43.4±1.9	42.5±3.0	49.3±3.1	42.5±2.3	45.7±2.9	0.374
Liver %	2.3±0.1	2.3±0.1	2.2±0.1	2.3±0.2	2.7±0.3	2.1±0.0	2.2±0.1	0.163
Heart (g)	11.5±0.8	10.1±0.6	11.5±0.9	11.3±0.8	10.9±1.0	10.9±0.5	11.3±0.5	0.844
Heart %	0.6±0.04	0.5±0.03	0.6±0.1	0.6±0.03	0.59±0.1	0.6±0.04	0.5±0.02	0.673
Giblet (g)	91.5±5.1	89.7±2.1	86.3±1.3	90.9±3.2	100.1±4.4	88.4±2.9	92.9±2.4	0.129
Giblet %	4.4 ^b ±0.2	4.5 ^b ±0.1	4.3 ^b ±0.1	4.9 ^{ab} ±0.2	5.4 ^a ±0.4	4.4 ^b ±0.1	4.5 ^b ±0.2	0.005
GIT (g)	87.5±7.8	90.0±7.0	95.7±7.8	95.0±7.2	101.7±8.5	84.4±4.3	98.0±9.3	0.682
GIT %	4.2±0.4	4.5±0.4	4.8±0.4	5.1±0.4	5.5±0.5	4.2±0.2	4.9±0.4	0.192
Abdominal fat (g)	30.5 ^a ±4.2	31.4 ^a ±5.1	31.6 ^a ±2.6	30.9 ^a ±5.7	23.0 ^{ab} ±4.0	16.0 ^b ±3.1	14.5 ^b ±7.5	0.039
Abdominal fat %	1.5 ^{ab} ±0.2	1.6 ^{ab} ±0.3	1.6 ^{ab} ±0.1	1.6 ^a ±0.3	1.1 ^{abc} ±0.2	0.8 ^{bc} ±0.1	0.4 ^c ±0.3	0.006

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$); T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, GIT = Gastrointestinal tract, SE = Standard error of the mean, P = probability.

4.1.4. Hematological profiles

The hematological indices result of broiler birds fed a basal diet incorporated with graded levels of black cumin, fenugreek or turmeric are summarized in Table 8. Incorporation of any of these three phytobiotic feed additives to broiler diets influenced the hemoglobin (Hb), packed cell volume (PCV) and red blood cell (RBC). However, there was no significant difference ($P \geq 0.05$) among treatments regarding the white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values

The Hb concentration showed a significant increment ($P \leq 0.05$) for treatment T1 and T6 as compared to the control (T0). On the other hand, there were no significant differences ($P \geq 0.05$) between treatments T2, T3, T4 and T5. Hemoglobin concentration of birds was not following a trend at the dietary levels of 1 and 2%.

The PCV percentage was greater for T1 as compared to the control (T0) and the rest of phytobiotic feed additives treatment groups, however, PCV values were not significantly different ($P \geq 0.05$) among T0, T1, T3, T4, T5 and T6. Nevertheless, T2 and T3 birds had less PCV percentages than the birds fed the control diet ($P \leq 0.05$).

The RBC count was higher for T6 and T3 as compared to the control T0 ($P \leq 0.05$). The lower RBC count was recorded from T0 group birds, but, T1, T2, T4 and T5 group birds lacked significant differences ($P \geq 0.05$) compared to the control. As indicated in Table 8 the RBC count of birds was not following a trend at the dietary levels of 1 and 2% ($P \geq 0.05$).

Table 8. Hematological profile of broiler chickens fed diets with different levels of black cumin, fenugreek and turmeric

Parameter	Treatment							P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
Hb (g/dl)	10.1 ^c ±0.2	11.3 ^a ±0.3	10.8 ^{abc} ±0.3	10.3 ^{bc} ±0.3	10.5 ^{bc} ±0.2	10.4 ^{bc} ±0.3	11.0 ^{ab} ±0.3	0.023
PCV (%)	42.0 ^{abc} ±0.9	47.8 ^a ±1.2	39.5 ^{bc} ±2.0	39.0 ^c ±1.6	45.0 ^{abc} ±1.9	45.5 ^{ab} ±3.1	45.7 ^{ab} ±2.1	0.021
RBC (10 ⁶ cell/mm ³)	3.8 ^c ±0.2	4.3 ^{abc} ±0.2	4.3 ^{abc} ±0.25	4.5 ^{ab} ±0.1	4.0 ^{bc} ±0.1	4.1 ^{abc} ±0.2	4.6 ^a ±0.1	0.035
WBC (10 ³ cell/mm ³)	5.6±0.9	6.03±0.6	5.90±0.4	5.43±0.3	6.07±0.2	5.93±0.6	6.23±0.5	0.090
MCV (fL)	111.4±4.7	113.9±6.1	95.2±9.7	86.7±3.2	112.7±6.7	113.4±10.5	100.0±3.5	0.058
MCH (Pg)	26.8±1.1	26.9±1.1	25.6±1.6	23.1±1.1	26.2±0.9	25.7±1.3	24.2±0.8	0.259
MCHC (%)	24.1±0.5	23.8±0.8	27.81±2.3	26.9±1.9	23.6±1.3	23.7±2.4	24.4±1.4	0.394

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$). T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, Hb = hemoglobin, PCV = Packed cell volume, RBC = Red blood cell, WBC = White blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, One deciliter (dl) = 10⁻¹⁰ Liter, One femtoliter (fL) = 10⁻¹⁵ L, One pictogram (Pg) = 10⁻¹² grams, SE = Standard error of the mean, P = probability.

4.1.5. Biochemical indices of serum

The results of serum biochemical parameters are illustrated in Table 9. The blood chemistry constitutes showed that there were highly significant differences ($P \leq 0.001$) among the treatments on serum total proteins, while serum glucose, total cholesterol and aspartate aminotransferase (AST) enzyme activities ($P \leq 0.05$). However, there were no significant differences ($P \geq 0.05$) in some protein metabolites (albumen, globulin, A/G ratio) liver injury indicators: alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzyme activities among the treatments.

The serum total protein was significantly increased ($P \leq 0.05$) in birds fed the three phytobiotics additives at both levels as compared to the birds fed on the control diets. Particularly, treatment T6 had higher serum protein than the rest of treatments. Nevertheless, there were no significant level dependent ($P \geq 0.05$) pattern in black cumin T1, T2, and fenugreek T3 and T4 supplemented birds on total serum protein concentration.

The serum glucose was significantly reduced ($P \leq 0.05$) in birds which were fed treatment T3, T4, T5 and T6, respectively than the control (T0) diet fed birds. However, there were no significant differences ($P \geq 0.05$) between black cumin at both levels (T1 and T2) and control T0 birds in serum glucose.

As shown in Table 9, there was a significant difference ($P \leq 0.05$) in serum total cholesterol of broilers. Serum cholesterol level was significantly ($P \leq 0.05$) reduced in treatment T1, T3, T4 and T6 as compared to the control T0 birds. However, there was no significant difference ($P \geq 0.05$) that observed among T2, T5 and T0 birds in concentration of total cholesterol. In addition, there was no significant level dependent ($P \geq 0.05$) pattern in serum cholesterol level between treatments.

The effect of different phytobiotic additives on liver enzyme hepatotoxicity (AST, ALT and ALP) are illustrated in Table 9. Serum aspartate aminotransferase (AST) activity was significantly different ($P \leq 0.05$) among treatments. The low serum AST concentration was obtained from the birds on the diet T4, T6, T3 and T5, respectively compared to the control T0 birds. In addition, there were significant ($P \leq 0.05$) level dependent differences between fenugreek treatment T3 and T4 birds, as the level of inclusion increases the AST enzyme decreases. However, there were no statistically significant differences ($P \geq 0.05$) between treatment T1 and T2 as compared to the control birds on the AST enzyme activities levels. However, supplementation of diet with black cumin, fenugreek and turmeric at 1 and 2% levels did not change significantly ($P \geq 0.05$) the concentration of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in comparison with the control group birds.

Table 9. Serum biochemical parameters of broilers fed on diets with different levels of black cumin, fenugreek and turmeric

Parameter	Treatment							P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
Total protein (g/dl)	4.0 ^c ±0.2	4.7 ^{ab} ±0.1	4.7 ^{ab} ±0.1	4.5 ^b ±0.7	4.6 ^b ±0.1	4.6 ^{ab} ±0.8	4.9 ^a ±0.3	0.001
Albumen (g/dl)	3.5±0.2	3.4±0.4	3.6±0.1	3.2±0.3	3.2±0.2	3.4±0.2	3.3±0.7	0.87
Globulin (g/dl)	0.5±0.3	1.3±0.04	1.0±0.1	1.3±0.2	1.3±0.2	1.2±0.1	1.6±0.1	0.08
A/G ratio	0.15±0.1	0.53±0.2	0.28±0.5	0.48±0.2	0.43±0.1	0.37±0.1	0.52±0.1	0.31
Glucose (mg/dl)	264.2 ^a ±12.9	242.3 ^{abc} ±12.9	261.3 ^{ab} ±9.5	212.1 ^c ±14.8	228.6 ^{bc} ±9.3	228.3 ^{bc} ±9.6	230.0 ^{bc} ±8.2	0.01
Cholesterol (mg/dl)	158.7 ^a ±6.5	130.5 ^b ±9.9	140.2 ^{ab} ±8.6	131.9 ^b ±9.7	137.4 ^b ±2.7	141.4 ^{ab} ±5.8	122.7 ^b ±4.3	0.02
AST (IU/L)	339.4 ^a ±20.0	308.1 ^{ab} ±19.0	305.0 ^{ab} ±19.3	253.1 ^{bc} ±32.8	232.8 ^c ±11.6	271.2 ^{bc} ±10.6	248.1 ^{bc} ±19.4	0.01
ALT (IU/L)	12.6±1.9	11.6±2.5	10.5±1.4	9.3±1.4	10.9±1.3	12.6±1.0	10.5±1.6	0.78
ALP (IU/L)	531.2±58.0	650.5±57.1	625.0±55.7	588.1±46.0	516.1±64.7	567.8±25.7	509.3±52.9	0.39

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$); T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, A/G = Albumen to Globulin ratio, Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, One deciliter (dl) = 10⁻¹⁰ Liter, SE = Standard error of the mean, P = Probability.

4.1.6. Immunomodulatory effects of phytobiotic feed additives on broilers

Table 10 presents the mean hemagglutination inhibition (HI) titre values of antibody against the most infectious respiratory disease, which is Newcastle Disease (NCD) at 21st and 42nd days of age. Results demonstrated that there were significant differences ($P \leq 0.05$) in antibody titer against NCD at 21st and 42nd days of age among treatments.

Table 10. Antibody titer for NCD immune response (at 21st and 42nd days) of broilers fed diets with graded levels of black cumin, fenugreek seeds and turmeric rhizome

Treatment	NCD titer	
	21 st day	42 nd day
T ₀	5.33 ^b ±0.49	5.0 ^b ±0.45
T ₁	5.50 ^b ±0.22	5.0 ^b ±0.45
T ₂	5.83 ^b ±0.17	6.17 ^{ab} ±0.47
T ₃	6.17 ^{ab} ±0.31	5.50 ^{ab} ±0.22
T ₄	6.0 ^{ab} ±0.37	6.67 ^a ±0.33
T ₅	5.83 ^b ±0.31	5.83 ^{ab} ±0.40
T ₆	6.83 ^a ±0.17	5.50 ^{ab} ±0.22
P-value	0.041	0.035

*^{a,b} Means±SE in a column with different superscripts differ at ($P \leq 0.05$), T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric. NCD = Newcastle Disease, SE = Standard error of the mean, P = Probability.

The mean value of antibody titer against NCD was higher ($P \leq 0.05$) in birds fed diets T₆, T₃ and T₄ at 21st sampling day. An increase in antibody was more pronounced in birds diets turmeric at 2% (T₆) as compared to the control and the rest treatments ($P \leq 0.05$). However, NCD antibody titer of birds supplemented with black cumin and fenugreek at both levels and turmeric at 1% (T₅) were not significantly different ($P \geq 0.05$) compared to the control groups. On the other hand, at 42nd day of age higher antibody response was recorded from T₄, T₂, T₅, T₆ and T₃ treatment birds. The NCD antibody titer of birds fed fenugreek at 2% (T₄) was significantly higher ($P \leq 0.05$) than the control birds. However, there were no significant difference ($P \geq 0.05$) was detected in NCD antibody titers of T₁, T₂, T₃, T₅ and T₆ as compare to the control T₀ birds at 42nd day of age.

There were no consistent level dependent differences ($P \geq 0.05$) between treatment in response to NCD at 21st and 42nd days of age.

Table 11. Antibody titer for IBD immune response (at 21st and 42nd days) of broilers fed diets with graded levels of black cumin, fenugreek seeds and turmeric rhizome.

Treatment	IBD titer	
	21 st day	42 nd day
T ₀	303.90 ^b ±56.86	118.43±73.08
T ₁	787.61 ^a ±94.72	174.99±11.01
T ₂	549.89 ^{ab} ±27.91	126.20±53.13
T ₃	319.71 ^b ±93.05	160.34±10.14
T ₄	572.11 ^{ab} ±13.71	143.23±75.46
T ₅	577.92 ^{ab} ±15.71	147.31±10.24
T ₆	824.65 ^a ±77.18	246.91±15.44
P-value	0.034	0.982

* ^{a-b} Means±SE in a column with different superscripts differ ($P \leq 0.05$), T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric. IBD = Infectious bursal disease, SE = Standard error of the mean, P = Probability.

Table 11 shows that the mean antibody titre against Infectious Bursal Disease (IBD) at 21st and 42nd days of age. Results demonstrated that there were significant differences ($P \leq 0.05$) in antibody titer against IBD at 21st day of age. Feed incorporated with different phytobiotic feed additives had the highest antibody titer against IBD at 21st day of age. The upper most antibody titer was obtained from T₆ and T₁ fed birds ($P \leq 0.05$) respectively, as compared to the control birds. The IBD antibody titer increased in all phytobiotic additive treated groups, nevertheless there were no statistical differences ($P \geq 0.05$) as compare to the control birds. However, at 42nd day of age, antibody titer for IBD was not statistically different ($P \geq 0.05$) among treatments.

4.1.7. Partial budget analysis

The profitability of broilers supplemented with different levels of phytobiotic additives from partial budget analyses is given Table 12. The purchasing price of chicks and selling prices of the broilers carcass were assumed similar for all the treatments. The highest price of feed per kg was recorded in T₁ and T₂ diets.

However, the lower price per kg diets was incurred in the control T0 ration followed by T5, T3, T6 and T4 diets, respectively. The lowest price feed consumed per bird was obtained from turmeric at both levels T5 and T6 followed by black cumin at both levels of T1 and T2 diets. However, the fenugreek at both levels of T3 and T4 and the control T0 diets had the higher prices feed consumed per bird.

The highest net return from the sale of carcass was obtained at T6 followed by T5, T1 and T0 diets. The least net returns was earned from birds consumed fenugreek at both levels T3 and T4 and black cumin at 2% diets (T2), respectively. The highest marginal rate of return was obtained at T6 (1.31 birr) followed by T5 (1.17 birr) and T1 (1.13 birr) diet groups. However, the marginal rate of return was less than the control (0.97 birr) for T4, T3 and T2 groups.

Table 12. Economic efficiency of inclusion of graded levels of phytobiotic feed additives in broiler chickens

Description	Treatment						
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Cost (ETB)							
A day-old chick cost (ETB)	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Total feed consumed/bird (kg)	5.12	3.61	3.88	5.49	5.21	3.92	3.97
Per unit feed cost (ETB)	7.00	7.99	8.60	7.33	7.60	7.25	7.37
Total feed cost (birr/bird)	35.80	28.88	33.40	40.31	39.69	28.46	29.22
Total cost (ETB)	55.80	48.89	53.40	60.31	59.69	48.46	49.22
Revenue							
Average carcass weight (kg)	1.37	1.30	1.27	1.18	1.16	1.31	1.42
Price/kg of carcass (ETB)	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Total return (ETB)	109.69	104.23	101.85	94.77	92.94	105.11	113.90
Net return/bird (ETB)	53.89	55.34	48.45	34.45	33.26	56.65	64.68
Marginal rate of return	0.97	1.13	0.91	0.57	0.56	1.17	1.31

T₀ = Control basal diet, T₁ = 1% black cumin, T₂ = 2% black cumin, T₃ = 1% fenugreek, T₄ = 2% fenugreek, T₅ = 1% turmeric, T₆ = 2% turmeric, ETB = Ethiopian birr, 1 ETB = 0.05 USD (as current exchange rate).

4.2. Phase II Experiment

The trial were designed to evaluate the synergetic effect of different blend of black cumin, fenugreek and turmeric on feed consumption, BW gain, FCR, carcass characteristics, hematological parameters, serum biochemistry profile, immune response and cecum microbial load of broiler chickens and the economic efficiency.

4.2.1. Growth performance

The combined effects of phytobiotic feed additives of (black cumin, fenugreek and turmeric) on the growth performance of broiler chicken are summarized in Tables 13. There were no marked differences ($P \geq 0.05$) on feed consumption among treatment birds during the starter phase. However, there were highly significant differences among treatments on feed consumption during finisher ($P \leq 0.001$) and entire periods ($P \leq 0.05$). All blends of phytobiotic additives had lower ($P \leq 0.05$) feed consumption than the negative control (T0) and antibiotics (T1) diets fed birds during the finisher phase and overall as well. Nearly similar body weight of broiler chicks was allocated in six dietary treatments at the beginning of the experiment ($P \geq 0.05$). The final BW, BW gain and ADG during the starter phase were not significantly different ($P \geq 0.05$) among treatments. During finisher phases, T2 birds showed significantly ($P \leq 0.05$) lower BW than the negative control (T0) and positive control (T1) birds; while values for T3, T4 and T5 were not significantly different ($P \geq 0.05$) as compare to the controls (T0 and T1).

The BW gain and ADG showed highly significant differences at the finisher phase ($P \leq 0.001$) and throughout experiment period ($P \leq 0.05$) among the treatments. However, there were no significant differences ($P \geq 0.05$) in BW gain and ADG among treatments during starter phase. The BW gain for the birds in T5 was highest ($P \leq 0.05$) as compared to T0, T1, T2 and T3 at the finisher phases. For entire period, a higher BW gain were achieved from birds fed blends of the three phytobiotics additives diets at T5 and was comparable ($P \geq 0.05$) with the result found at T1.

Table 13. Effects of dietary inclusion of black cumin, fenugreek and turmeric mixtures on the growth performance of broilers

Parameter (g/bird)		Treatment						P-value
		T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	
Feed intake								
	Starter	1727.3±19.9	1861.1±72.8	1887.7±77.3	1876.8±16.1	1888.7±41.6	1857.4±17.6	0.868
	Finisher	3614.5 ^a ±47.6	3576.5 ^a ±12.8	2942.8 ^b ±16.4	2799.0 ^b ±67.5	2948.1 ^b ±93.4	2910.1 ^b ±60.8	0.000
	Overall	5341.8 ^a ±67.5	5437.6 ^a ±85.7	4830.4 ^b ±93.7	4675.8 ^b ±83.6	4836.7 ^b ±61.7	4767.5 ^b ±78.5	0.016
Initial BW		39.1±0.2	39.1±0.2	39.1±0.2	39.3±0.0	38.9±0.4	38.7±0.3	0.638
Final BW								
	Starter	870.6±73.0	890.0±15.0	845.1±42.7	885.7±2.9	809.3±23.6	853.1±10.8	0.654
	Finisher	1988.5 ^{ab} ±44.1	2065.5 ^a ±36.4	1685.9 ^b ±63.2	1999.2 ^{ab} ±33.4	2006.0 ^{ab} ±67.2	2080.8 ^a ±43.7	0.029
BW gain								
	Starter	831.5±12.9	850.9±14.9	806.0±22.7	846.3±2.9	770.4±23.1	814.4±10.9	0.655
	Finisher	1117.9 ^b ±22.6	1175.5 ^b ±22.1	840.8 ^c ±22.9	1113.6 ^b ±31.6	1196.6 ^{ab} ±31.0	1227.7 ^a ±38.7	0.001
	Overall	1949.4 ^b ±35.5	2026.4 ^a ±36.3	1646.8 ^c ±44.6	1959.9 ^b ±33.4	1967.1 ^{ab} ±54.2	2042.1 ^a ±43.4	0.028
ADG (g/bird/day)								
	Starter	29.7±2.6	30.4±0.5	28.8±1.5	30.2±1.1	27.5±0.8	29.1±0.4	0.655
	Finisher	53.2 ^a ±3.4	55.9 ^a ±1.1	40.0 ^b ±1.1	53.0 ^a ±1.5	57.0±2.4	58.5 ^a ±1.8	0.001
	Overall	39.8 ^b ±2.9	41.4 ^a ±0.7	33.6 ^c ±1.3	40.0 ^{ab} ±0.7	40.1 ^{ab} ±1.3	41.7 ^a ±0.9	0.028

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$), T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, BW = Body weight, ADG = average daily gain, SE = Standard error of the mean, P = Probability.

However, the BW gain of birds fed T2 showed significantly lower ($P \leq 0.05$) BW gain than the T0 and the rest treatments diet fed birds. Birds fed on diet supplemented with blends of black cumin and fenugreek diets (T2) was gained lower ADG ($P \leq 0.05$) than the controls group (T0 and T1). However, there were no marked differences ($P \geq 0.05$) on ADG between the controls (T0 and T1) and blends of phytobiotics additives T3, T4 and T5 birds during the finisher phase. For entire period, birds fed T5 attained higher ADG than the negative control (T0).

The results of the effects of blends of different phytobiotic feed additives on FCR, performance index and mortality of the broiler chickens are summarized in Table 14. The FCR at the starter phase was not significantly ($P \geq 0.05$) different among the treatments. However, there were highly significant differences ($P \leq 0.001$) among treatments on FCR during finisher and entire periods ($P \leq 0.05$). All the blends of phytobiotic additives except T2 ensured improved ($P \leq 0.05$) FCR as compared with the control T0 and antibiotics T1 group birds during finisher phase, while during overall it was found that blends of phytobiotic additives T5 and T3 had improved FCR ($P \leq 0.05$) than antibiotics and negative control birds.

4.2.2. Performance index (PI) and mortality

Results presented in Table 14 indicated that there were no-significant differences among the treatments in performance index (PI) during the starter phase ($P \geq 0.05$). During the finisher phase, the PI result indicated that there was substantial performance difference ($P \leq 0.05$) in birds fed blends of phytobiotic feed additives in T5 as compared with the T0 and T1 ($P \leq 0.05$). While, blends of phytobiotics additives in T4 and T3 resulted in comparable ($P \geq 0.05$) with the antibiotics (T1) diet fed birds. The lower PI was recorded from blends of T2 and was significantly lower than the controls group (T0 and T1) birds. Throughout the experimental period, the performance index of T2 had performed less ($P \leq 0.05$) than the negative control (T0) diet fed birds. While, T5, T4 and T3, resulted in comparable ($P \geq 0.05$) with T1 supplemented birds. There were no significant differences ($P \geq 0.05$), in mortality among the treatments during starter, finisher periods and overall.

Table 14. Effects of dietary inclusion of black cumin, fenugreek and turmeric mixtures on performance index and mortality of broilers.

Parameter	Treatment						P-value	
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅		
FCR								
Starter	2.1±0.1	2.2±0.1	2.4±0.0	2.2±0.0	2.5±0.0	2.3±0.0	0.062	
Finisher	3.3 ^{ab} ±0.2	3.0 ^b ±0.2	3.5 ^a ±0.1	2.5 ^c ±0.1	2.5 ^c ±0.2	2.4 ^c ±0.1	0.001	
Overall	2.8 ^{ab} ±0.1	2.7 ^{abc} ±0.1	2.9 ^a ±0.0	2.4 ^{cd} ±0.1	2.5 ^{bcd} ±0.1	2.3 ^d ±0.1	0.005	
Performance index								
Starter	40.1±2.8	39.1±2.7	34.4±2.3	38.2±5.4	31.5±1.4	35.9±1.5	0.088	
Finisher	34.8 ^c ±4.2	38.8 ^{bc} ±2.7	24.0 ^d ±1.0	44.6 ^{abc} ±3.6	49.1 ^{ab} ±5.8	51.9 ^a ±2.7	0.002	
Overall	71.3 ^b ±7.7	75.9 ^a ±5.3	56.2 ^c ±2.9	82.4 ^a ±4.3	80.3 ^a ±6.1	87.7 ^a ±3.8	0.017	
Mortality %								
Starter	8.9±2.2	4.4±2.2	6.7±2.2	4.4±2.2	6.7±0.0	4.4±2.2	0.458	
Finisher	4.4±2.2	4.4±2.2	2.2±2.2	0.0±0.0	0.0±0.0	2.2±2.2	0.541	
Overall	11.1±2.2	8.9±2.2	8.9±2.2	4.4±2.2	6.7±0.0	6.7±0.0	0.488	

*^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$), T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, FCR = Feed conversation ratio, SE = Standard error of the mean, P = Probability.

4.2.3. Carcass characteristics

The carcass characteristics, including the slaughter weight, plucked weight, commercial carcass yields, edible carcass yields and their respective dressing percentages, breast weight, thigh with drum weight, wings and back weight and their percentages did not show significant differences ($P \geq 0.05$) among the treatments as shown in Tables 15 and 16. In addition, the internal organs relative weight of gizzard, heart, giblet and GITs and their percentages did not show significant difference ($P \geq 0.05$) among the treatments. However, the relative weight of abdominal fat and liver percentages were significantly different ($P \leq 0.05$) among treatments as shown in Table 16.

The relative weights of giblet component (gizzard, liver and heart) were not changed ($P \geq 0.05$) by the treatments except liver percentage ($P \leq 0.05$). Phytobiotic additive blends in T2 changed liver percentage ($P \leq 0.05$) in birds as compared to the control group (T0 and T1). In addition, blends of phytobiotics additives (T5 and T2) significantly ($P \leq 0.05$) reduced the abdominal fat compared to the antibiotics (T1) and negative control (T0) birds. However, their abdominal fat percentage did not show significant differences ($P \geq 0.05$) among the treatments.

Table 15. Effects of different blends of black cumin, fenugreek and turmeric additives on carcass characteristics of broilers

Parameter	Treatment						P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	
Slaughter weight	1928.3±69.7	2071.7±56.4	1675.0±42.2	2003.3±54.7	1996.7±76.6	1920.0±63	0.080
Carcass yield (g)	1216.8±56.5	1301.0±57.1	1016.7±24.2	1215.5±68.4	1225.8±49.1	1241.8±58.0	0.062
*Dressing %	63.0±1.0	62.4±1.6	60.7±0.8	60.7±1.5	61.4±0.6	65.4±3.3	0.395
Edible carcass weight (g)	1293.1±57.0	1376.8±50.3	1090.6±24.0	1285.2±72.3	1294.5±54.5	1317.2±61.4	0.830
**Dressing %	67.0±0.9	66.1±1.6	65.2±0.7	64.2±1.7	64.8±0.5	69.4±3.6	0.387

*T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, * dressing % calculated only on commercial carcass yield, ** dressing % calculated by including gizzard & liver, SEM = standard error of the mean, P = Probability.*

Table 16. Effects of different blends of black cumin, fenugreek and turmeric feed additives on the relative weights of carcass yields and internal organ weights of broilers.

Parameter	Treatment						P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	
Breast (g)	555.0±35.8	598.2±47.2	476.3±7.4	538.7±30.8	539.3±29.5	550.0±32.4	0.239
Breast%	28.7±1.0	28.7±0.9	28.51±0.7	26.9±1.0	27.0±1.1	28.8±1.2	0.580
Thigh + Drum (g)	410.5±18.2	432.5±27.4	332.8±13.6	416.3±24.3	417.3±24.9	422.2±26.5	0.054
Thigh + Drum %	21.3±0.3	20.9±0.7	19.8±0.5	20.8±0.5	20.9±0.8	22.3±1.5	0.500
Wings (g)	73.7±2.0	82.2±4.9	66.3±2.7	74.0±4.1	75.0±2.3	75.3±4.0	0.097
Wings%	3.8±0.1	3.97±0.1	4.0±0.1	3.7±0.1	3.8±0.1	4.0±0.2	0.573
Back (g)	177.7±5.0	188.2±27.2	140.7±5.9	186.5±13.2	194.2±16.4	194.3±7.6	0.123
Back %	9.2±0.3	8.9±0.9	8.4±0.4	9.3±0.3	9.7±0.7	10.4±0.9	0.363
Gizzard (g)	36.7±2.0	32.9±1.9	31.5±1.7	35.1±2.6	36.2±1.4	37.5±2.7	0.339
Gizzard %	1.9±0.1	1.6±0.1	1.9±0.1	1.8±0.1	1.7±0.03	2.0±0.2	0.234
Liver (g)	39.6±1.2	42.9±3.2	42.9±2.3	41.5±2.6	44.6±1.9	37.9±4.2	0.547
Liver %	2.1 ^b ±0.1	2.1 ^b ±0.1	2.6 ^a ±0.1	2.1 ^b ±0.1	2.2 ^{ab} ±0.1	2.0 ^b ±0.2	0.047
Heart (g)	8.5±0.8	9.6±0.9	8.3±0.9	10.2±1.7	10.2±0.6	9.1±0.5	0.528
Heart%	0.4±0.03	0.5±0.02	0.5±0.1	0.4±0.1	0.5±0.03	0.5±0.02	0.862
Giblet (g)	84.8±2.4	85.4±4.6	82.7±4.6	76.5±9.3	78.9±9.1	84.4±4.8	0.891
Giblet %	4.4±0.1	4.1±0.1	4.9±0.3	3.8±0.5	3.9±0.4	4.5±0.3	0.144
GIT (g)	72.2±7.2	83.±5.4	72.6±4.8	76.3±2.4	87.5±6.1	83.8±9.2	0.437
GIT %	3.7±0.3	4.0±0.2	4.3±0.2	4.1±0.3	4.4±0.3	4.5±0.7	0.718
Abdominal fat (g)	27.4 ^{ab} ±3.8	34.3 ^a ±4.4	19.3 ^c ±4.8	31.2 ^{ab} ±5.1	26.9 ^{ab} ±3.7	16.2 ^c ±3.2	0.039
Abdominal fat %	1.4±0.2	1.7±0.2	1.1±0.3	1.6±0.2	1.4±0.2	0.9±0.2	0.130

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$), T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, GIT= Gastrointestinal tract, SE = Standard error of the mean, P = Probability.

4.2.4. Hematological profiles

Table 17 showed the effect of blends of black cumin, fenugreek and turmeric as feed additives on the hematological profile of broilers. The concentration of hemoglobin (Hb) level did not show significant ($P \geq 0.05$) differences among the treatments. The value of packed cell volume (PCV) showed highly significant differences ($P \leq 0.001$) among treatments. Packed cell volume was increased significantly ($P \leq 0.05$) in blends of phytobiotic additive T2 as compared to the negative control (T0) and antibiotics (T1) diets fed birds. While, the PCV value in birds feed diets with blends of phytobiotic additive (T5, T3, T4) had comparable ($P \geq 0.05$) with the antibiotics (T1) groups. The PCV values were significantly also, increased ($P \leq 0.05$) as compared to the negative control (T0) diet fed birds.

The RBC counts were significantly ($P \leq 0.05$) different among the treatments. Birds feed diets with antibiotics had significantly ($P \leq 0.05$) increased RBC counts. While blends of phytobiotic additive (T2 and T4) was resulted in comparable ($P \geq 0.05$) RBC count with the antibiotic T1 fed birds. However, there were no significant ($P \geq 0.05$) changes in RBC count between the negative control and birds fed blends of phytobiotic feed additives (T3 and T5). White blood cell (WBC) counts did not show a significant ($P \geq 0.05$) difference among treatments.

The MCV were significantly ($P \leq 0.05$) different among the treatments and were significantly increased ($P \leq 0.05$) in treatments T2, T3 and T5 than the antibiotics and negative control (T0 and T1). However, there were no significant ($P \geq 0.05$) changes in MCV between T4 and the controls (T0 and T1). The MCHC were significantly ($P \leq 0.05$) different among the treatments. A higher MCHC value was recorded from control groups (T0 and T1) fed birds; while the lowest value was obtained from the treatments T2 and T3. However, there were no significant ($P \geq 0.05$) changes in MCHC between T5, T4 and the antibiotics (T1) fed birds. The MCH were not significantly ($P \geq 0.05$) different among the treatments.

Table 17. Hematological indices of broilers fed blends of black cumin, fenugreek and turmeric

Parameter	Treatment						P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	
Hb (g/dl)	10.4±0.2	11.7±0.4	10.8±0.6	10.6±0.4	10.7±0.2	11.5±0.5	0.218
PCV (%)	21.7 ^c ±0.8	26.5 ^b ±1.4	32.7 ^a ±1.3	28.7 ^b ±0.9	28.0 ^b ±1.5	29.7 ^{ab} ±0.8	0.000
RBC (10 ⁶ cell/mm ³)	2.6 ^b ±0.6	3.1 ^a ±0.2	2.9 ^{ab} ±0.1	2.6 ^b ±0.1	2.8 ^{ab} ±0.1	2.7 ^b ±0.1	0.025
WBC (10 ³ cell/mm ³)	5.4±0.8	5.5±0.4	5.7±0.4	5.5±0.5	5.47±0.6	5.70±0.4	0.666
MCV (fL)	85.2 ^b ±4.6	88.0 ^b ±8.5	115.1 ^a ±6.1	113.8 ^a ±8.6	102.0 ^{ab} ±7.0	111.3 ^a ±4.3	0.008
MCH (Pg)	40.8±48.3	38.6±3.0	38.1±2.4	41.9±3.2	38.8±1.5	43.1±2.2	0.606
MCHC (%)	48.3 ^a ±1.9	44.3 ^{ab} ±1.5	33.6 ^c ±2.7	36.9 ^c ±1.4	38.8 ^{bc} ±2.7	38.9 ^{bc} ±2.0	0.000

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$), T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, Hb = Hemoglobin, PCV = Packed cell volume, RBC = Red blood cell, WBC = White blood cell, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, One deciliter (dl) = 10⁻¹⁰ Liter, One femtoliter (fL) = 10⁻¹⁵ Liter, One pictogram (Pg) = 10⁻¹² grams, SE = standard error of the mean, P = Probability.

4.2.5. Serum biochemical indices

The results of biochemical profile due to inclusion of blends of black cumin, fenugreek and turmeric as phytobiotic feed additives in broilers are illustrated in Table 18. The serum protein, globulin, glucose, total cholesterol and ALP levels were significantly ($P \leq 0.05$) different among the treatments. However, there were no significant ($P \geq 0.05$) differences in serum albumen, A/G ratio, AST and ALT levels among the treatments.

The serum total protein was higher ($P \leq 0.05$) on birds fed phytobiotics additive blends of T3 compared to the negative and positive controls (T0 and T1) group birds. However, there were no significant ($P \geq 0.05$) difference in serum total protein between T3, T5 and T2. The serum globulin was also higher ($P \leq 0.05$) in T3 as compared to the controls (T0 and T1) and rest of the treatments. However, there were no significant ($P \geq 0.05$) differences revealed in albumen and A/G ratio on birds fed the different treatment diets.

The serum glucose concentration was significantly ($P \leq 0.05$) reduced in birds fed with blends of phytobiotic additives of T4, T5 and T3 than the negative control T0. In addition, serum glucose of birds fed with blends of phytobiotic additive T5 and T3 was resulted in comparable ($P \geq 0.05$) with the antibiotics T1 fed birds. However, there was no significant ($P \geq 0.05$) difference observed among T0, T1 and T2 birds in serum glucose.

The serum total cholesterols shown in Table 18 were significantly ($P \leq 0.05$) different among the treatments. The phytobiotic additives in T4 and T5 fed bird had lower ($P \leq 0.05$) serum total cholesterol as compared to the control T0 and T1. However, there were no significant ($P \geq 0.05$) changes in serum cholesterol on birds fed blends of phytobiotic additives (T2 and T3) as compared to the negative and positive controls (T0 and T1).

The effect of blends of different phytobiotic additives on liver enzyme hepatotoxicity (AST, ALT and ALP) is presented in Table 18. There were significant ($P \leq 0.05$) differences among the treatments on liver enzyme ALP concentration. Results of both the

blends of phytobiotic additives (T5 and T4) and antibiotics fed birds (T1) were significantly ($P \leq 0.05$) reduced in ALP activities as compared to the negative control (T0). In addition, serum ALP activities of birds fed with blends of phytobiotic additive (T5 and T4) were resulted in comparable ($P \geq 0.05$) with the antibiotics (T1) fed birds. However, there were no significant ($P \geq 0.05$) differences revealed on ALT and AST concentrations among the treatments.

Table 18. Serum biochemical parameters of broilers fed blends of black cumin, fenugreek and turmeric

Parameter	Treatment						SEM	P-value
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅		
Total protein (g/dl)	2.5 ^b ±0.2	2.7 ^b ±0.2	3.3 ^{ab} ±0.2	4.5 ^a ±0.8	2.8 ^b ±0.2	3.5 ^{ab} ±0.3	0.19	0.02
Albumen (g/dl)	1.5±0.2	1.50±0.2	2.0±0.3	1.7±0.2	1.5±0.2	1.8±0.3	0.09	0.59
Globulin (g/dl)	1.0 ^b ±0.0	1.2 ^b ±0.2	1.3 ^b ±0.2	2.8 ^a ±0.7	1.3 ^b ±0.2	1.7 ^b ±0.3	0.17	0.02
A/G ratio	0.8±0.1	0.9±0.2	0.8±0.3	1.8±0.4	1.1±0.3	1.1±0.3	0.12	0.16
Glucose (mg/dl)	203.7 ^a ±20.6	180.0 ^{ab} ±13.6	185.0 ^{ab} ±12.8	161.7 ^{bc} ±16.8	135.3 ^c ±6.8	153.2 ^{bc} ±7.9	6.49	0.02
Cholesterol (mg/dl)	153.7 ^a ±13.9	151.7 ^a ±10.4	158.5 ^a ±12.2	136.2 ^{ab} ±14.0	114.3 ^b ±10.2	110.2 ^b ±12.4	12.37	0.03
AST (IU/L)	131.6±17.3	155.5±11.7	153.0±20.6	153.2±36.6	134.5±29.8	132.7±12.8	8.97	0.94
ALT (IU/L)	13.9±3.6	11.9±2.1	14.7±2.8	13.8±2.1	7.9±2.0	10.6±3.9	1.15	0.56
ALP (IU/L)	1451.8 ^a ±14.5	1014.1 ^{bc} ±28.6	1362.7 ^{ab} ±14.7	1078.3 ^{abc} ±17.6	1040.5 ^{bc} ±10.3	876.1 ^c ±18.4	57.88	0.02

* ^{a-c} Means±SE within a row with different superscripts differ significantly ($P \leq 0.05$), T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, A/G= Albumen to globulin ratio, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, One deciliter (dl) = 10⁻¹⁰ Liter, SE = standard error of the mean, P = Probability,

4.2.6. Immunomodulatory effects of phytobiotic feed additives

Tables (19 and 20) present the effects of blends of phytobiotic feed additives as immune-stimulants against NCD and IBD. Results demonstrated that there were no significant ($P \geq 0.05$) differences in antibody titer against NCD at 21st and 42nd days of age among the treatments as shown in Table 19. Numerically higher NCD antibody titer was recorded at T5 and lower at treatment T3 at 21st day of age. Similarly, T5 resulted in higher NCD antibody titer; however, treatment T4 resulted in lower antibody titer against NCD at 42nd day of age.

Table 19. Antibody titer for NCD immune response at 21st and 42nd days of age in broilers fed different blends of black cumin, fenugreek and turmeric

Treatment	NCD titer	
	21 st day	42 nd day
T ₀	6.67±0.42	5.50±0.62
T ₁	6.33±0.21	5.33±0.21
T ₂	6.50±0.2	5.33±0.56
T ₃	6.17±0.40	5.50±0.22
T ₄	6.67±0.56	4.83±0.30
T ₅	6.83±0.31	6.00±0.26
P-value	0.82	0.49

* T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, NCD = Newcastle disease, SEM = standard error of the mean, P = Probability.

There were no significant differences ($P \geq 0.05$) in antibody titer against IBD at 21st and 42nd days of age among treatments as shown in Table 20. The results demonstrated that numerically, treatment T1, T4 and T5 had the highest antibody titer against IBD at 21 and 42nd day of age.

Table 20. Antibody titer for IBD immune response at 21st and 42nd days of age in broilers fed different blends of black cumin, fenugreek and turmeric

Treatment	IBD titer	
	21 st day	42 nd day
T ₀	518.72±66.72	227.73±86.51
T ₁	564.57±84.78	247.57±77.92
T ₂	545.45±86.56	230.11±62.30
T ₃	559.52±64.12	232.51±67.03
T ₄	562.84±65.89	246.14±91.06
T ₅	561.74±46.84	234.19±84.54
P-value	0.97	0.99

* T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, IBD = Infectious bursal disease, SEM = standard error of the mean, P = Probability

4.2.7. Caecum microbial counts

The results for caecal microbial load count for total coliform bacteria and *Escherichia coli* are shown in Table 21. There were significant ($P \leq 0.05$) differences in the population of total coliform and *E. coli* bacteria among the treatments. There was a reduction ($P \leq 0.05$) in total coliform bacteria in chicks fed different blends of phytobiotic additives (T₄, T₅ and T₂) as compared with the antibiotics (T₁) and the control (T₀). However, there were no significant ($P \geq 0.05$) differences between black cumin and turmeric mixture (T₃), antibiotics (T₁) and control diet fed birds (T₀) in the total coliform bacteria count. Reduction in *E. coli* bacteria population in chicks of treatment T₅, T₄ and T₂ were equivalent ($P \geq 0.05$) with the antibiotics (T₁) fed birds. However, there was a pronounced significant ($P \leq 0.05$) increase in *E. coli* bacteria population in broilers that received the negative control diet (T₀) than birds fed supplemented with blends of phytobiotic feed additives (T₅, T₄ and T₂), but comparable with the antibiotics group diets.

Table 21. Caecal microbial load counts for total coliform and *E. coli* bacteria

Treatment	Total Coliform count (cfu/ml)	<i>E. coli</i> (cfu/ml)
T ₀	7.31 ^a ±0.52	7.05 ^a ±0.13
T ₁	6.62 ^a ±0.57	6.15 ^{ab} ±0.56
T ₂	5.62 ^b ±0.16	5.46 ^b ±0.09
T ₃	6.50 ^a ±0.60	6.40 ^{ab} ±0.55
T ₄	5.42 ^b ±0.06	5.42 ^b ±0.7
T ₅	5.53 ^b ±0.12	5.26 ^b ±0.14
SEM	0.22	0.20
P-value	0.037	0.027

* ^{a,b} Means±SE in a column with different superscripts differ ($P \leq 0.05$), T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, cfu = coliform forming unit, SE = Standard error of the mean, P = Probability.

4.2.8. Partial budget analysis

According to the partial budget analysis, the profitability of broilers supplemented with different blends of phytobiotic additives is illustrated in Table 22. The purchasing price of chicks and selling prices of the broilers carcasses were assumed to be similar for all the treatments. The highest price of feed per kg was recorded in diets of T₅ followed by T₂ and T₃. However, the lower price per kg diets was incurred for the control T₀ followed by T₁ and T₄ diets. The lowest price of total feed consumed per bird was obtained for T₄ followed by T₃, T₂ and T₅ diets. However, diets both the positive and negative control groups (T₁ and T₀) diets had the higher price of feed consumed per bird. The highest net return was obtained for T₁ followed by T₄, T₅ and T₃ diets. The least net returns was earned from birds consumed black cumin and fenugreek seeds mixture (T₂) diet. The highest marginal rate of return was obtained from T₁ followed by T₄ and T₃ diet groups. However, the marginal rate of return of T₅ was lower and comparable with the negative control groups.

Table 22. Economic efficiency of inclusion of different mixtures of phytobiotic additives in broiler chickens

Description	Treatment					
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Cost (ETB)						
A Day old chick cost (ETB)	20.00	20.00	20.00	20.00	20.00	20.00
Total feed consumed/bird (kg)	5.34	5.44	4.83	4.67	4.84	4.77
Per unit feed cost (ETB)	7.58	7.69	8.60	8.45	8.00	8.71
Total feed cost (birr/bird)	40.49	41.82	41.55	39.50	38.69	41.55
Total cost (ETB)	60.49	61.82	61.55	59.50	58.69	61.55
Revenue						
Average carcass weight (kg)	1.22	1.30	1.02	1.22	1.23	1.24
Price/kg of carcass	80.00	80.00	80.00	80.00	80.00	80.00
Total return (ETB)	97.35	104.08	81.33	97.24	98.07	99.35
Net return/bird (ETB)	36.85	42.26	19.78	37.74	39.38	37.80
Marginal rate of return	0.61	0.69	0.32	0.64	0.67	0.62

T₀ = Control basal diet, T₁ = Basal diet + antibiotics, T₂ = Basal diet + black cumin + fenugreek, T₃ = Basal diet + black cumin + turmeric, T₄ = Basal diet + fenugreek + turmeric, T₅ = Basal diet + black cumin + fenugreek + turmeric, ETB = Ethiopian birr, ETB = Ethiopian birr, 1 ETB = 0.05 USD (as current exchange rate).

5. DISCUSSIONS

5.1. Phase I

5.1.1. *Effects of phytobiotic feed additives on growth performance*

The low feed consumption observed in broiler fed diets supplemented with black cumin at 1% in this study was in agreement with report of Durrani *et al.* (2007); Abbas and Ahmed (2010), who mentioned addition of 1 and 4% ground black cumin seeds to the broiler diets had significantly decreased the feed consumption. Similarly, the feed consumption observed on broilers fed turmeric diets was comparable with the results found by Mondal *et al.* (2015), who observed non-significant differences in feed intake of broilers fed turmeric at 0.5, 1 and 1.5% of the basal diet.

The decrease in feed consumption in black cumin and turmeric diets group birds might be due to the increase in digestion retention time in the gizzard. In contrast, other researchers also reported that turmeric powder fed birds had a higher feed intake (Mondal *et al.*, 2015; Al-Jaleel, 2012). However, feed consumption of broilers fed fenugreek included diets coincided with the report of many researchers who observed increased feed consumption of broilers during 42 days of age by inclusion of fenugreek seeds in the diets of broilers (Mamoun *et al.*, 2014; Alloui *et al.*, 2012; Michael and Kumawat, 2003).

The increase in feed consumption of fenugreek seed might be related to different perspectives that fenugreek as phytobiotic feed additives improve palatability and could be attributed to the carbohydrates and their main components, galactomannan, which stimulate the appetite and digestive process in animals (Alloui *et al.*, 2012; Michael and Kumawat, 2003). Fenugreek also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system (Zeweil *et al.*, 2015; Al-Habori and Roman, 2002).

The non-significant effects of treatment diets on the final BW and BW gain found in the present study was in agreement with the report of Qasem *et al.* (2015); Mondal *et al.* (2015); Nouzarian *et al.* (2011), who reported that broilers fed turmeric powder at 0.025, 0.050, 0.075, 1.0, 10.0 and 20.0% had no effect on final BW.

The results of the current study were in-line with the reports of Abbas and Ahmed (2010), who found black cumin supplementation at 1.5% had no changes on final BW and BW gains. Similarly, Al-Mufarrej (2014) also reported black cumin inclusion levels of 0.7%, 1.4%, 2.1% or 2.8%, had no effect on final BW and BW gain in broiler chickens. Contrary to this findings, Ashayerizadeh *et al.* (2009) reported that black cumin at 1 kg/ton supplementation exhibited a higher BW gain compared to the control diet fed broilers. The results of the present study on final BW and BW gain are in agreement with the findings of Abbas (2010), who reported non-significant effects on BW and BW gain of consuming fenugreek seed. Contrary to the current study, Mahejabin *et al.* (2015) reported a higher final BW in broilers supplemented with fenugreek seed.

It has been proposed that immunomodulation effects of the phytobiotic additives may have antagonistic effects on BW gain, because more nutrients were repartitioned to synthesize antibodies. Similarly, the lower BW gain in fenugreek supplemented birds maybe mediated by anti-nutritional factors in fenugreek seeds such as tannin, sapononin and phytate might have contributed to the effects. Tannin causes to bind the dietary protein and digestive enzymes forming complexes that are not readily digestible (Soetan and Oyewole, 2009), while, phytates potentially form complexes with minerals and dietary proteins and decreases their bioavailability (Reddy, 2002).

The improved FCR obtained in broilers fed black cumin seed in this study was in agreement with the findings of Guler *et al.* (2006), who evaluated supplementation of black cumin seeds at 1% to broilers from 1 to 42 days of age improve FCR. An increased nutrient digestibility is likely to be responsible for the improvement of FCR as they increased the digesta retention time in the gizzard (Gonzalez-Alvarado *et al.*, 2007).

The main active constituent called thymoquinone and other components (carvone, anethole, carvacrol and 4-terpineol) of black cumin may stimulate the digestive enzymes secretion in the intestinal mucosa and pancreas. This improves the digestion of dietary nutrients and feed efficiency.

The improved FCR of the current study was also exhibited at both levels of turmeric treated groups. This result was in-line with report of other researchers who reported that broilers that received diet with 0.4 and 0.5% of turmeric powder utilized their diets more efficiently than the control broilers (Al-Mashhadani, 2015; Al-Jaleel, 2012; Durrani *et al.*, 2006; Al-Sultan *et al.*, 2003; Wuthi-Udomler *et al.*, 2000). The turmeric powder can promote protein deposition, improve the transportation of metabolic products and improve FCR in birds resulting in promoting growth (Fallah and Mirzaei, 2016). It also considered that turmeric powder has stimuli digestion enzyme secretion that create microbial eubiosis in the gastrointestinal (Langhout, 2000; Hertrampf, 2001).

On the other hand, supplementing broilers with fenugreek seed at 1 and 2% levels resulted in the lowest FCR that might be due to fenugreekine, a steroidal sapogenin peptide ester which slows carbohydrate absorption and inhibits glucose transport. This is in line with report of scholars who reported fenugreek seeds have significantly decreased the FCR in broiler chicks (Abed and Kadhim, 2014; Alloui *et al.*, 2012; Motlaq and Sadik, 2011).

5.1.2. Effects of phytobiotic feed additives on performance index (PI) and mortality

The effect of the phytobiotic additive treatments on the performance over the experimental periods and measured parameters are obviously variable. Black cumin at 1% level inclusion exhibited high performance index than those received the control diet birds. This may be attributable to higher BW gain and lower feed consumption. Similar results were found by Abaza *et al.* (2008), who cleared that the addition of black cumin seed oil at 0.1% into broiler chick diet significantly improved the PI value

than the negative control and antibiotics group birds. Also, Osman (2002); El-Gendi *et al.* (2000) indicated that feeding chicks on diet containing 0.5 kg/ton of Bio-tonic (included of black cumin seed oil) as a feed additive improved the performance index value. However, birds fed fenugreek at both levels showed low performance index than the control group. It may be attributed to inefficient nutrient utilization of the consumed feed.

The mortality was negligible, for black cumin and turmeric group with no difference among all groups as shown in Table 5. A similar observation was made by Abu-Dieyeh and Abu-Darwish (2008); Durrani *et al.* (2006), who reported that supplementing diets with turmeric and black cumin at 1.25, 2.5 and 5% did not cause any mortality of broilers and this, might be the antibacterial activities of black cumin and turmeric might have suppressed the pathogenic bacteria.

5.1.3. Effects of phytobiotic feed additives on carcass characteristics

The highest commercial carcass weight and dressing percentage were found in birds fed turmeric powder at 2% diet compared to the other phytobiotic additive treatments however, it was not statistically significant compared to the control group as shown in Table 6. The current results are in agreement with the previous finding Hady *et al.* (2016); Wang *et al.* (2015), demonstrated that no change in dressing percentage when broilers fed with turmeric powder at 0.75% and 100-300 mg/kg containing diets as compared to the control. In disagreement with the current study, Mondal *et al.* (2015); Al-Jaleel (2012); Durrani *et al.* (2006) the dressing percentages were significantly increased when 0.5, 1.0 and 1.5% turmeric powder was included in the diets as compared to the control group.

The carcass weights and dressing percentage were not affected in black cumin fed birds as compared to the control birds. This is in agreement with the findings of El-Khalek and El-Naggar (2016), who used black cumin oil at 1 ml/kg and demonstrated no significant changes in carcass weight and dressing percentage. In the current study

lower carcass weight and dressing percentage was obtained in fenugreek diets fed birds. This is in-line with Weerasingha and Atapattu (2013), who fed broilers with diets containing 3, 4 and 5% of fenugreek and demonstrated lower carcass weight than the control birds. On other hand, the current results disagree with Alloui *et al.* (2012) and Abbas (2010), who reported no significant changes on carcass yield and dressing percentage when broilers supplemented with fenugreek at 3 g/kg of the diet. In addition, Mamoun *et al.* (2014) added that boilers fed on diets supplemented with 1% and 2% fenugreek recorded significantly higher dressing percentages compared to the control.

The broilers fed diets containing turmeric at 2% as phytobiotic additives had numerically higher breast yield than the control diet groups. This is in agreement with earlier studies of Ukoha and Onunkwo (2016a); Wang *et al.* (2015); Hussein (2013); Fascina *et al.* (2012), who indicated a higher dressing percentage as well as breast weights of broilers fed a diet containing 3% and 5 g/kg of turmeric powder. The increased breast weight maybe related to the presence of essential compounds flavonoids in the turmeric powder, which prevent oxidation of lipids that allow high amounts of lean cut to accumulate in the organs and proportions of the organs (Shawle *et al.*, 2016) this also, reduce the risk of hyperlipidemia in consumers and increase acceptability.

Supplementation of fenugreek at both levels was resulted a lower breast weight. This is in disagree with Khan *et al.* (2011), who studied the effect of aqueous seed extract of fenugreek in drinking water at 30 ml/L and has reported a beneficial impact on the weight of breast, thigh and drumstick of broiler chicks. However, in the current study, gizzard weight and percentage were higher in birds supplemented with fenugreek at 2% of the diet. Additionally, the giblets percentage (liver, heart and gizzard) increased in the birds fed a diet containing fenugreek at 2% of the diet. The increased weight of the gizzard and giblet reflects the increasing digestive or metabolic capacity of birds (Mushtaq *et al.*, 2014).

The results of the current study indicated that turmeric incorporation on broiler diets has the potential to reduce fat deposition in the abdominal area of broilers, which is regarded as waste in the poultry industry. In accordance with the current results, Wang *et al.* (2015); Rajput *et al.* (2013); Emadi and Kermanshahi (2006), who reported that dietary supplementation with turmeric at 0.75 g/kg and 100-300 mg/kg markedly reduced the abdominal fat ratio compared to that of the control group birds. The decrease in abdominal fat might be due to turmeric active agent called curcumin that has the potential to inhibit hepatic fatty acid synthase (FAS) activity and increased β -oxidation of fatty acids leading to an effective decrease in fat storage (Sugiharto *et al.*, 2011; Smith, 1994). Whereas, the results of the current study in contradiction with Mehala and Moorthy (2008b), where turmeric supplementation of broilers did not significantly reduce the abdominal fat.

5.1.4. Effects of phytobiotic feed additives on hematological indices

The phytobiotic feed additives at both levels demonstrated positive effects on broiler bird hematology indices as shown in Table 8 and they were within the normal reference range of healthy chicken given by Campbell (2012) included PCV: 23.0-55.0%, Hb: 7.0-18.6 g/dl and RBC: $1.3-4.5 \times 10^6/\text{mm}^3$. Hematological indices are reflecting the physiological responsiveness of the animal to its external and internal environments, including feeds and feeding. The result of the current study are in accordance with many researchers, who reported that feeding diet with black cumin seeds yielded a significant increase in Hb in broiler chicken (Alimohamadi *et al.*, 2014; Shewita and Taha, 2011). Also, turmeric inclusion at 2% level increased Hb and RBC level, which is in agreement with Ukoha and Onunkwo (2016b); Sugiharto *et al.* (2011), who reported that Hb, RBC were increased due to supplementation of turmeric in broiler diets. The desirable influence of black cumin seed at 1% on Hb might be to the presence of highly active components particularly thymoquinone and thymohydroquinone which have strong antioxidant activities (Arslan *et al.*, 2005). In addition, the high concentration of Hb in turmeric at 2% and black cumin might be due to hepato-stimulatory effects resulting in the synthesis of more Hb in the bone marrow,

which is under the control of erythropoietic factors released by hepatic cells. Contrary to the current study, Al-Nazawi and El-Bahir, (2012) reported that black cumin and turmeric had no significant effects on Hb concentration. In addition, similar to the current study neither turmeric (Al-Jaleel, 2012) nor black cumin seeds (El-Bagir *et al.*, 2010) changed the PCV percentage of broiler chickens.

It can be therefore said that the higher RBC in the birds fed diet supplemented with turmeric at 2% and fenugreek at 1% might be attributed to firstly, the lowered lipid peroxide in erythrocyte membrane, resulting in a decreased susceptibility of erythrocyte to haemolysis (Alimohamadi *et al.*, 2014; Toghyani *et al.*, 2010). Secondly, the observed increase in Hb and RBC concentrations in turmeric 2% fed birds are probably because of reduced severity of the infection and possible increase in the synthesis of the protein as shown in Table 9, which might be due to the improved nutrient utilization and assimilation into the blood stream for use by the birds and enhanced blood formation due to availability of essential nutrients. Third, the increased RBC count in turmeric at 2% and fenugreek at 1% diet fed birds could be explained due to the rich sources of iron and zinc (Jani *et al.*, 2009) which are essential components or precursors in the formation of RBC and this enhances absorption of iron and protein that contributes to increased erythropoiesis in bone marrow.

The inclusion of the phytobiotic feed additives at both levels in the diets of the broilers did not have negative effect on red blood cell indices (MCV, MCH and MCHC). These RBC indices were used to measure the size and Hb content of erythrocytes and values are useful in diagnosing various forms of anaemia. MCV and MCH are decreased in iron deficiency anemia and increased hemolytic anemia (Tietze, 2012). However, the current study shows that there was no significant cause of anaemia in all treatment groups.

The results of this study are in agreement with Ukoha and Onunkwo (2016); Al-Nazawi and El-Bahir (2012), who reported black cumin seed and turmeric rhizome supplementation have no negative effect in broiler chicken as results, there were no significant differences in MCH and MCHC values.

5.1.5. Effects of phytobiotic feed additives on serum biochemical indices

The inclusion of the phytobiotic feed additives at both levels in the diets of the broilers did not had negative impact on the biochemical profile as shown in Table 9. The serum total protein values obtained in this study were within the normal range (4.55-6.46 g/dl) that reported by Udoiyong *et al.* (2010) indicating nutritional adequacy of the diets.

The present study showed significant increases in total protein due to inclusion of turmeric, black cumin and fenugreek at 1 and 2% in broiler diets as compared to the control group. As serum protein depends on the availability of dietary protein, the proteins of the turmeric diets were more available to the birds. In agreement with the current study, Al-Noori *et al.* (2011) also reported a substantial increase in serum total protein concentration in broiler chickens that received a diet containing turmeric powder at levels of 0.5% and 1%. Vamsidhar (2015) indicated that supplementation of dietary fenugreek at 0.5-2% in the diet of quails significantly increased serum total protein. A higher value indicates that there is enzyme hydrolysis of dietary proteins explained that the blood pool serves as a major source of amino acids needed for the synthesis of proteins (Scott, 1970). Contrary, to the current study, Toghyani *et al.* (2010); El-Ghamry *et al.* (2002a) reported that serum total protein was not changed of broiler chicks fed on 4 g/kg black cumin seeds. Similarly, Qureshi *et al.* (2015); Mamoun *et al.* (2014) reported that serum protein was not changed due to inclusion of fenugreek in diets of broilers.

The present study revealed that there were no significant differences in serum albumen, globulin and albumen to globulin ratio (A/G ratio) by feeding different levels of turmeric, black cumin and fenugreek and values of all groups were considered within the normal reference range of healthy chicken given by Abdi-Hachesoo *et al.* (2011). Similar results had been reported by Abou-Elkhair *et al.* (2014) and Sethy *et al.* (2016), who reported the absence of any effect on albumin levels in broiler chickens treated with turmeric powder. Similar to the current result, Abbas (2010) reported that fenugreek did not affect the serum albumin, globulin and A/G ratio of broilers.

The inclusion of the phytobiotic feed additives at both levels in the diets of the broilers lower the serum glucose and total cholesterol and they were within the normal reference range given by Reece *et al.* (2015) which are glucose: 130–270 mg/dl and Cholesterol: 125–200 mg/dl. The present finding on the serum glucose effect of fenugreek seeds at 1% was in harmony with the report of other researchers (Qureshi *et al.*, 2015; Safaei *et al.*, 2013; Abdul-Rahman, 2012).

The reduction serum glucose in fenugreek fed birds might be attributing to the presence of galactomannanas that reduce glycemia and due to pectins, which delay stomach emptying and glucose absorption from intestine (Ali *et al.*, 1995). Fenugreek regulates blood sugar through the enzymes sucrase and alpha-amylase, by inhibiting these two enzymes, which slow the breakdown of carbohydrates into sugar further reducing blood sugar levels in the body (Raju *et al.*, 2001). In addition, the direct β -cell stimulation by amino acid (4-hydroxyisoleucine) present abundantly in fenugreek seeds, which stimulate the pancreases to release insulin secretion, thus improves glucose uptake or tolerance. Contrary to the current finding, Weerasingha and Atapattu (2013) and Mamoun *et al.* (2014) indicated that serum glucose not changed in broiler chicks fed on fenugreek seed.

The lowering serum glucose of birds fed turmeric at both levels was observed in the current study comes in accordance with Qasem *et al.* (2016); Abou-Elkhair *et al.* (2014); Ahmadi (2010), who reported that turmeric supplementation was lowered serum glucose. Similarly, Gowda *et al.* (2009) reported that the active ingredient of turmeric called curcumin at 444 mg/kg significantly reduced serum glucose concentration. The reduction in serum glucose might be due to better utilization or uptake of glucose by muscle and adipose tissue by the birds and it is possible that turmeric powder enhances the activity of glucose transporters proteins that help to carry glucose across the plasma membrane. On the contrary, some researchers reported that supplementation of 0.5-3% turmeric rhizome powder had no significant effect on glucose concentration in broilers (Fallah and Mirzaei, 2016; Ukoha and Onunkwo, 2016b; Abou-Elkhair *et al.*, 2014).

The current result on reduction in serum total cholesterol turmeric powder at 2% in diet of broiler was in accordance with Arslan *et al.* (2017); Fallah and Mirzaei (2016); Abo El-Maaty *et al.* (2014), who reported that broiler chickens feed turmeric powder at 5-15 g/kg containing diets exhibited significantly lowered serum cholesterol levels of broiler chicken. The reduction in serum cholesterol of birds fed turmeric it may related to the altered activity of effective enzymes in cholesterol metabolism, HMG-CoA reductase (3-hydroxy3-methylglutaryl Coenzyme A) which is the rate limiting enzyme of cholesterol synthesis. hypocholesterolemic properties of turmeric may also, mediated by the stimulation of hepatic cholesterol 7 α -hydroxylase enzymes which converts cholesterol to bile acids and facilitating the biliary cholesterol excretion through feces (Daneshyar *et al.*, 2011; Kim and Kim, 2010). In addition, higher activity of hepatic cholesterol-7 α -hydroxylase has been evidenced in rats fed turmeric active compound called curcumin (Srinivasan and Sambaiah, 1991). However, some researchers reported turmeric powder was not altering the cholesterol in broiler (Sethy *et al.*, 2016; Reddy *et al.*, 2012; Basavaraj *et al.*, 2011). The difference in results may be due to variety and method of processing differences, low inclusion level and harvesting stages.

The reduction of serum cholesterol of birds fed black cumin seeds at 1% was in agreement with some scholars findings that feeding broiler chicks with 1.5-3% black cumin seed reduces serum cholesterol compared with control diet (Al-Beitawi *et al.*, 2009; Al-Beitawi and El-Ghousein, 2008). The reduction in serum cholesterol may be due to the lowering effect of thymoquinone on the synthesis of cholesterol by hepatocytes or to fractional reabsorption from the small intestine (Brunton, 1999). In addition, black cumin seed also contain an appreciable amount of sterols, especially β -sitosterol, which may inhibit the absorption of dietary cholesterol (Khan *et al.*, 2012b).

Fenugreek also reduced serum cholesterol levels, the results of which are in agreement with Qureshi *et al.* (2015); Mamoun *et al.* (2014); Safaei *et al.* (2013); Abdul-Rahman (2012); who reported that incorporation of fenugreek seeds in broilers at 1% level significantly decreased the blood cholesterol. The reduction in the serum cholesterol level maybe attribute to the presence of different alkaloids, flavonoids and saponins,

the steroidal saponins in fenugreek seeds (diosgenin, yamogenin, tigogenin and neotigogenin), resins, hemicelluloses, mucilage, tannins and pectin in fenugreek seeds. This might inhibit the bile acid and cholesterol absorption from the intestine. The ability of fenugreek some saponins to form mixed micelles with bile acids and the mixed micelles are not available for reabsorption and consequently carried over into the large bowel and results would be loss by fecal excretion of cholesterol and bile acids with a consequent reduction in the serum cholesterol, liver and other tissues (Kutlu *et al.*, 2001). High levels of serum cholesterol may result in its deposition on the wall of the blood vessels, may eventually harden and block important vessels result in destruction of heart tissue or myocardial infarction. Therefore, it has a potential role in prevention of arteriosclerosis (Mullaicharam *et al.*, 2013). On the other hand, Weerasingha and Atapattu (2013) found out that serum cholesterol levels was not affected by the dietary fenugreek supplementation on broiler chicken.

The enzyme activities of ALT and ALP were not significantly different among treatments. However, the enzyme activity of AST in serum of chicks fed a diet-containing fenugreek and turmeric significantly decreased as compared to control diet fed birds and the values were in normal reference range specified by Meluzzi *et al.* (1992) for the healthier broiler chicken (568–8,831 IU/l). This indicate that there was no a detrimental or toxic effect on liver due to feeding phytobiotic additives at both levels. The current study results in agreement with Hosseini-Vashan *et al.* (2012); Abdel-Azeem *et al.* (2000), who reported that turmeric at 0.4 or 0.8% supplementation decreased serum AST level in broiler chicken. Turmeric's hepatoprotective effect is mainly a result of active compounds, which have antioxidant properties (Maheshwari *et al.*, 2006).

The present study was in concordance with the findings of Qureshi *et al.* (2015); Bhaisare and Thyagarajan (2014); Ali and Ismail (2012); Meghwal and Goswam (2012); Toppo *et al.* (2009), who reported that fenugreek has no negative effect on AST, ALT and ALP levels in broiler chickens. However, a contradictory report by El-Husseiny *et al.* (2002) in broiler chicken revealed that addition of fenugreek seeds on

bird diets significantly increased AST and ALT values as compared to control, which indicated that supplementation of fenugreek seed at higher dose, may result in liver damage.

5.1.6. Immunomodulatory effects of phytobiotic feed additives

As shown in Table 10 and 11, the effects of phytobiotic additives on antibody titer against NCD and IBD at 21st and 42nd day of broilers age. Inclusion of turmeric at 2% on broiler diets was superior in stimulating immune activity by increasing the antibody titer against NCD and IBD at 21st day of age, which coincided with the finding of Arslan (2017) confirming that turmeric inclusion at the level from 0.5 to 1.5% significantly improved antibody titer against NCD and IBD in broiler. Similarly, Qasem *et al.* (2015) indicated that turmeric inclusion at 1 to 2% significantly improved antibody titer against NCD, while the antibody against IBD was significantly higher when turmeric powder was added in the feed at the rates of 1.4 and 1.6%. Zaki *et al.* (2016); Fallah and Mirzaei (2016) reported that antibody titres against NCD were higher in turmeric supplemented birds than the control diet fed birds. Turmeric has profound immune modulating activities. This may attributed due to the sterol molecules which is found in turmeric that seems to target specific T-helper lymphocytes, helping normalize their functioning, resulting in improved T-lymphocytes activities (Rajput *et al.*, 2013; Jagetia and Aggarwal, 2007). However, there was no significant change in antibody titer to NCD at 42nd day of age between turmeric at both levels and control diet fed birds. This is in agreement other finding of Nouzarian *et al.* (2011); Mehala and Moorthy (2008b), who reported that supplementing diet with turmeric powder to broilers at 0.33, 0.66 and 1.0% didn't bring any significantly change in the antibody titer against NCD.

Supplementing fenugreek at 2% in broiler diets boost the antibody against NCD. These in-line with the results obtained by Abed and Kadhim (2014); Abid *et al.* (2011), who reported that supplementing diet with fenugreek seed to broilers at 0.5-2% significantly enhanced the antibody titer against NCD at 42nd day of age. From the obtained results

there was no significant change in antibody titer to NCD at day 21st and 42nd between birds fed diet containing black cumin seed at both levels and the control group. These results agreed with that of Shewita and Taha (2011); Toghyani *et al.* (2010), who found that there was non-significant impact of feeding black cumin seed on antibody titers against NCD. However, black cumin seed at 1% supplementation had immunostimulant effect during 21st day of age against IBD. This is in line with Al-Mufarrej (2014), who reported that black cumin seeds at the levels of 1% or 1.4% enhanced immune responsiveness expressed by higher antibody titer against IBD in broiler chickens. There are few studies, which have revealed the mechanism of action on the immune-stimulatory compounds of medicinal plants, but the exact molecular mechanisms of some medicinal plants have not fully understood. The possible explanations for immunomodulation mechanisms of phytobiotic feed additives (turmeric, fenugreek and black cumin) might be attributed to first, inclusion of turmeric powder, fenugreek and black cumin seeds into the broiler diets lead to increase serum protein as shown in Table 9, which may accordingly increase the level of circulating antibodies in the blood. Second, turmeric, fenugreek, and black cumin active principles possessing antibacterial and antioxidant activities, which might induce positive effects on the immunity. However, broiler antibody titer against NCD and IBD appears to decrease at 42nd day of age, which might be attributed to the decreased in maternal antibody withdrawn from their system as their age increases.

5.1.7. Economic efficiency of phytobiotic feed additives

In the present study, the mean feed cost per bird revealed a substantial positive effect of turmeric at 1%, black cumin at 1% and turmeric at 2% incorporation in the diets reduced the production cost of broiler chicks as shown in Table 12. The current results are in accordance with Durrani *et al.* (2006), who observed lower feed cost per broiler supplemented with turmeric powder at 0.50% in ration compared to control rations. Kumar *et al.* (2005) also reported that feed cost per bird was less for the group supplemented with turmeric at 1 g/kg as compared to control. Similarly, Mehala and Moorthy (2008a) indicated that inclusion of turmeric had lowered feed cost than the

control group. Whereas, Tripathi (2015) indicated that lowest feed cost per kg BW gain of broiler birds was observed in feed supplemented with turmeric powder at 0.5% followed by control group and ginger. The current study in accordance with Durrani *et al.* (2007), who obtained that black cumin at 40 g/kg feed resulted in significant reduction total feed cost in broiler.

The highest net return (64.68 birr) was obtained from broilers fed ration containing 2% turmeric followed by turmeric and black cumin at 1% rations. The reason might be due to higher growth rate and heavier carcass weight, as a result of better feed conversion efficiency of the chicks that were supplemented with higher level of turmeric than the control. These findings is in agreement with Al-Sultan (2003), who concluded that the use of turmeric as feed additive at 0.50% levels enhanced the overall performance of broiler chickens and cost effectiveness. According to the finding of Hossain *et al.* (2014), higher profit found in the 1% black cumin supplemented feed for broiler production than control diets. Durrani *et al.* (2007) black cumin at 40 g/kg feed resulted in higher net return in broiler. On the contrary, the least net returns was found from birds consumed fenugreek at both level and black cumin at 2% diets. Fenugreek at both levels and black cumin at higher level is not considerable profitability or economically efficient in broiler production. The difference in the net return among treatments was mainly due to the high cost of black cumin and fenugreek and low body weight gain (carcass yield) of broilers at a higher level of inclusion.

The marginal rates of return imply that increases in net income associated with each additional unit of expenditure. In the current study, additional 1.31 birr and 1.17 birr profits for turmeric addition at 2% and 1%, respectively. Also, there is an increase in 1.13 birr for black cumin at 1% in broiler diets. Therefore, the results of this study indicated that diets containing turmeric at both level and black cumin at 1% are potentially profitable than the higher level of black cumin inclusion in the diet.

5.2. Phase II

5.2.1. Effects of blends of phytobiotic feed additives on growth performance

The inclusion of different phytobiotic additives mixtures (black cumin, fenugreek and turmeric) brings improvement in BW gain, ADG and FCR of broiler chicken as compared to the negative control group as shown in Table 13 and 14. The current findings are in harmony with many scholars (Al-Kassie *et al.*, 2011a; Fascina *et al.*, 2012; Al-Beitawi and El-Ghousein, 2015; El-Khalek and El-Naggar, 2016) indicated that BW gain and ADG significantly improved as a result of black cumin mixture with other medicinal plants in broiler diets. Similarly, Abdel-Rahman *et al.* (2014), indicated that turmeric and fenugreek herbal mixture diet significantly increases the BW gain of birds. Similarly, Dono (2012) combination of turmeric and garlic at the rate of 5 and 10 g/kg had synergetic effects revealed by increasing the BW gain. According to Ali and Fateh (2014) also reported that fenugreek seeds and mint leaf blends improved the BW gain of broiler birds. However, from the current study black cumin and fenugreek mixture (T2), birds performed less in BW gain and ADG than the negative control birds. On the contrary, Yatoo *et al.* (2012) reported that combination of black cumin and fenugreek at 0.5% each improved the BW and BW gain and FCR of broiler birds.

In addition, the current result indicated that black cumin, fenugreek seeds and turmeric powder mixtures (T5) were comparable or perform similar to the antibiotics treatment birds in BW gain and ADG. This is in agreement with Samarasinghe *et al.* (2003), who reported equivalent performance of birds fed turmeric at 1% with the synthetic antibiotics treated birds in ADG. The inclusion of mixture of medicinal plants that brought improvement of BW gain and ADG growth probably due to the results of synergistic effects among the phytochemical molecules of black cumin (nigella, thymoquinone and thymohydroquinone), turmeric (curcuminoids and curcumin) and fenugreek (fenugreekine and trigonelline) that are active as well as complex that exist in plants, which had antioxidant and antibacterial properties seems to stimulate protein synthesis and digestion of proteins as a results, in increasing weight gains.

In the current study, the different phytobiotic mixtures had lower feed consumption rate than the negative control and antibiotics fed birds. This is in agreement with El-Bahr and Al-Azraqi (2014), who revealed that turmeric and black cumin mixtures fed broilers had low feed consumption rate but increase in BW gain. However, Abdel-Wahab and El-Bahr (2012) noted that low synergistic effect of black cumin and turmeric mixture was observed in the relative promotion of growth performance whereas feed utilization efficiency remained unchanged in fish. The low feed intake and BW gain in black cumin and fenugreek mixture (T2) fed birds might be due to the high proportion of soluble fiber in the fenugreek containing mixtures, which forms a gelatinous structure, which maybe result in slowing down the digestion and absorption of feed from the intestine and create a sense of fullness in the abdomen and thus suppressing appetite and resulting in more weight loss (Geetha *et al.*, 2011).

The current FCR improvement due to the three phytobiotic mixtures and blends of turmeric and fenugreek are in agreement with Prajapat (2016); Qureshi *et al.* (2015), who reported that FCR was achieved in blends of fenugreek seeds, dandelion and enzyme mixture increase the growth performance of the birds. Similarly, Al-Beitawi and El-Ghousein (2015) reported that FCR significantly improved at 7 weeks of age because of black cumin, thyme and anise addition as a mixture to the basal ration of broiler chicken. According to Dono (2012), combination of turmeric and garlic at the rate of 5 and 10 g per kg had synergetic effects revealed by increasing FCR. The improvement in FCR due to the synergetic effects of phenolic acids present in medicinal plants mixture, which act as antimicrobial and cause sterilization of the gastrointestinal tracts. As a result, it improved feed utilization of the available feed and results in increasing weight gains (Windisch *et al.*, 2008).

5.2.2. Effects of blends of phytobiotic feed additives on performance index (PI) and mortality

As shown in Table 14, blends of phytobiotics additives treatment performance index (PI) better than the negative control group except black cumin and fenugreek mixtures (T2). However, treatments T5, T4 and T3 are comparable with the antibiotics treated group in PI. These results are in line with the findings of Prajapat (2016), who indicated that mixture of fenugreek and tulsi at 0.5% inclusion in broiler diets resulted in highest performance index as compared to the control group. The improved in PI attributed due to the synergetic effect of different bioactive ingredients in phytobiotic additives and the antioxidants activity properties of medicinal plants mixtures that might improve the feed efficient utilization as a results increase body weight gain of the birds.

The mortality percentage was not significantly different among treatments. A similar observation was made by El-Bahr and Al-Azraqi (2014), revealed that turmeric and black cumin mixtures fed broilers had less mortality rate than the control group. Low mortality of broilers might be the synergistic antibacterial activity of black cumin and turmeric mixtures suppressed the *E. coli* bacteria, which suppress growth and cause mortality.

5.2.3. Effects of blends of phytobiotic feed additives on carcass characteristics

Carcass characteristics of broilers relative to final body weight did not significantly differ among treatments as shown in Table 15. These are in agreement with Abou-Elkhair *et al.* (2014); Mahejabin *et al.* (2015), who found no difference on carcass traits in broilers due to supplementing mixtures of phytobiotic feed additives. Correspond to the current findings, Al-Beitawi and El-Ghousein (2008); Karukarach *et al.* (2016) reported that carcass weight, wing, thigh and drumstick was not affected due to supplementing blended of herbal extracts to broiler chickens.

Similar to the current results, Al-Kassie *et al.* (2011a); Abou-Elkhair *et al.* (2014); Mahejabin *et al.* (2015) reported that the dressing percentage and edible giblets were not influenced by dietary supplements of mixture of (black paper, turmeric and coriander seed) and (neem, turmeric and papaya leaf extract), respectively. Furthermore, correspond to the present findings Hady *et al.* (2016) reported a dried coriander, turmeric and thyme mixtures had no significant difference in carcass dressing percentage. The current results are in agreement with El-Khalek and El-Naggar (2016), who demonstrated that blends of black cumin, ginger, thyme and oregano oil had no effects on liver and gizzard weight.

The combinations of three or two phytobiotics additives had no synergetic effect on giblet and its percentage. These results were in line with Reddy *et al.* (2012), who reported that mixtures of amla, tulsi and turmeric at 0.5% had no synergetic effect on giblet weights and its percentage of broilers. However, there was significant increase in liver percentage of broiler fed diet bearing black cumin and fenugreek mixture as compared to the rest of treatments. According to Bone (1979) indicated that the weights of the internal organs arise due to the increased metabolic rate to reduce toxic elements or anti-nutritional factors to non-toxic metabolites.

Blends of the three mixtures of phytobiotic additives and black cumin and fenugreek mixtures had positive effects in reducing abdominal fat. It is likely that the active component synergetic effects may decrease the activity of the enzymes acting as rate limiting enzymes in lipogenesis such as Acetyl-CoA carboxylase in fatty acids synthesis and increased β -oxidation of fatty acids (Sugiharto *et al.*, 2011; Smith, 1994) leading to an effective decrease in body fat storage. On contrary, Mehala and Moorthy (2008b) reported that no reduction in abdominal fat pad between treatment groups by inclusion of *Aloe vera* and turmeric powder mixtures in broiler diets. Hady *et al.* (2016) also, reported blends of coriander, turmeric, thyme and Sadeghi *et al.* (2012) mixtures of turmeric, thyme and cinnamon had no significant effect in reducing the abdominal fat respectively.

5.2.4. Effects of blends of phytobiotic feed additives on hematology indices

The synergetic effect of blends of phytobiotic feed additives increased the PCV percentage and RBC count as shown in Table 17. The values of hematological PCV, Hb, RBC indices fall within the normal physiological range of healthy broiler chickens (Campbell, 2012; Wakenell, 2010; Islam *et al.*, 2004).

The synergetic effect of blends of phytobiotic feed additives increase PCV value in T2 and T5 which consequently had values between the recommended range of healthy chicken (23 – 55%) as reported by Campbell (2012). The current result supported by Mahejabin *et al.* (2015), who showed that neem, turmeric and papaya leaf extract mixture increase the PCV of broilers. Similarly, Yatoo *et al.* (2012) reported that supplementing combination of black cumin and fenugreek seeds, increase PCV percentage of broiler birds. In disagreement with the current results, Al-Kassie *et al.* (2011a) reported that the broiler PCV percentage was not changed due to supplementation of turmeric and cumin mixture at 0.5%.

The RBC count in treatment T2 and T4 were comparable with birds in the commercial antibiotics. Nevertheless, El-Khalek and El-Naggar (2016); Al-Beitawi and El-Ghousein (2015) reported that addition of black cumin and other herbal mixtures improved RBC than the antibiotics group. The synergetic effect of blends of phytobiotic feed additives on PCV and RBC might be due to the high content of vitamins (tocopherols, B1, B2, B6, niacin, folic acid) and minerals (calcium, iron, sodium and potassium) presence in black cumin, turmeric and fenugreek (Jani *et al.*, 2009; Nergiz and Otles, 1993), which are essential components in the formation of RBC. Despite the fact, Hb, PCV and RBC values are within the normal range, broilers consumed diets containing phytobiotic additives mixture as compared to the control diet suggest that they improved nutrient utilization and assimilation into the blood stream for use by the birds and enhanced blood formation due to availability of essential nutrients. Contrary, Al-Kassie *et al.* (2011a) reported that RBC and Hb seem to decline due to supplementation of turmeric and cumin at 0.5% in the diet of broiler chickens.

The value of MCV and MCHC significantly vary in different treatments. The negative control and antibiotics group birds had a lower MCV value than the blends of phytobiotic additive treatments. While, the value of MCHC appears to decrease in different blends phytobiotic additive treatments as compared to the control birds. However, the range of the MCV and MCHC values did not deviate from normal known standards (80–127 fL) and (21–50%), respectively as reported by Wakenell (2010). It assumed that phytobiotic feed additives mixture had synergetic interaction with blood elements and pose no negative health challenge for growth of broiler chickens. Generally, normal hematological values portray the nutritional and health status of the animal. Thus, the values observed in the present study indicate the adequacy of nutrients for the birds.

5.2.5. Effects of blends of phytobiotic feed additives on biochemical indices

Blood biochemical indices directly express the status of metabolism, nutrition and health of the animals. Black cumin and turmeric mixture as phytobiotic additives resulted in increased total serum protein and globulin levels than the antibiotics (oxytetracycline) included diet fed birds as shown in Table 18. The serum total protein values obtained in this study are within the normal range cited by Thrall (2007) that is 2.5 to 4.5 g / dL. The results were in agreement with El-Khalek and El-Naggar (2016), who reported that addition of black cumin seeds, ginger, thyme and oregano oil mixtures improved plasma total protein and globulin. Similarly, Yattoo *et al.* (2012) reported that the total protein increased in black cumin and fenugreek mixture supplemented birds. In imitable study, El-Bahr and Saad (2008) concluded that dietary supplementation of black cumin seeds and turmeric mixture improved the serum protein of fish. As serum protein depends on the availability of dietary protein, the proteins of the black cumin and turmeric mixture diets may be more available to the birds to support the nutritional requirements. The increase in total protein might be the synergetic effect of the active compounds in black cumin (thymoquinone and thymohydroquinone) and turmeric (curcuminoids and curcumin) which have antioxidant properties maybe reduce protein oxidation. In addition, black cumin and turmeric mixture might have abundant proteolytic enzymes, which promote protein digestion, deposition and improve the transportation of metabolic protein

in birds. Contrary to the current findings, Toghyani *et al.* (2010); El-Bahr and Al-Azraqi (2014) indicated that plasma total protein was not changed in broiler chicks fed black cumin seeds and turmeric rhizome powder.

Globulin values vary among treatments. Globulin carries essential metals through the bloodstream to the various parts of the body and helps the body to fight infections. Elevated globulin levels are often pronounced in birds with increased production of antibodies (Agboola *et al.*, 2013). In this study, the globulin of birds fed blends of phytobiotics feed additives diets except black cumin and turmeric mixtures was comparable positively with the negative control and antibiotics diet fed birds and values are within the normal range for chicken that is 0.5 to 1.8 g/ dL as cited by Thrall (2007). In addition, serum albumin and A/G ratio was not changed among treatments, which is an indication that the phytobiotics feed additives diet did not precipitate any severe effects on the health status of the birds.

In the present study, serum glucose level was reduced in blends of fenugreek seed and turmeric powder than the negative and positive (oxytetracycline) controls constituted diet fed birds. However, the glucose level was within the normal ranges 130–270 mg/dl as reported by Reece *et al.* (2015). On the other hand, black cumin and fenugreek seeds mixtures and blends of black cumin, fenugreek seeds and turmeric powder were equivalent to the antibiotics (oxytetracycline) in lowering serum glucose. This is in agreement with Sameer *et al.* (2003), who reported that low doses (0.2 mg/ml) of vanadate in combination with fenugreek powder found to be comparable and effective in correcting altered carbohydrate metabolism in rats to the synthetic vanadate (0.6 mg/ml) drug. Comparable to the present findings, Abou-Elkhair *et al.* (2014) reported that mixtures of (0.5% of black pepper and turmeric) and (0.5% black pepper, turmeric and 2% coriander seeds) on the diet significantly lowered the blood glucose in broiler birds. Similar to the present study, El-Bahr and Al-Azraqi (2014) reported that the glucose level of diabetic rats (253.3%) has been reduced to 28.6% when rats have been treated with black cumin and turmeric mixture.

The hypoglycemic effect of the phytobiotic mixtures of fenugreek and turmeric containing ration might be due to the beneficial synergetic effects of flavonoids in phytobiotic additives mixtures, which some flavonoids possess insulin-like properties and thereby can reduce blood glucose uptake by muscle and adipose tissue (Benhaddou-Andaloussi *et al.*, 2010) or inhibition of intestinal glucose absorption (Meddah *et al.*, 2009). In addition, fenugreek contains 4-hydroxyisoleucine, which stimulate the pancreases to release insulin secretion, thus improves glucose tolerance (Schryver, 2002), as resulted low glucose accumulation in the blood.

The value of serum cholesterol obtained in the present study is similar to earlier report by Campbell (2012), who indicated that the serum cholesterol level in normal birds ranges from 100–250 mg/dl. Nevertheless, lower values of serum cholesterol in birds consumed diet containing blends of phytobiotics (black cumin, fenugreek and turmeric) and (fenugreek and turmeric) as compared to the negative and antibiotics containing diet fed birds and could be an indication of its hypocholestrolemic properties. This finding were in agreement with El-Khalek and El-Naggar (2016), who reported depression in the serum cholesterol level due to the supplementation of black cumin with fenugreek mixtures to the birds. Al-Kassie *et al.* (2011a) also reported a low cholesterol level in the blends of turmeric and cumin at 0.75 and 1%. Black cumin and turmeric mixture restored the cholesterol to normal level in diabetic rats and fish (El-Bahr and Al-Azraqi, 2014; El-Bahr and Saad, 2008). The hypocholesterolemic effect of (black cumin, fenugreek and turmeric mixtures) and (fenugreek and turmeric mixtures) might be due to the synergetic effect of the phytobiotics active compounds enhancing hepatic bile acid synthesis and increase degradation of cholesterol to fecal bile acids absorption from intestine, thereby, decreasing cholesterol level in blood.

Results of liver function enzymes showed that no significant change in level of AST and ALT compared to the negative control and antibiotics supplemented birds. These results were in agreement with El-Khalek and El-Naggar (2016), who reported that black cumin and other different medicinal plants mixtures do not have toxic effect in liver as indicated in AST and ALT activities.

The alkaline phosphate (ALP) activities were significantly lowered in birds supplemented with blends of phytobiotic additives (black cumin, fenugreek and turmeric) and (fenugreek and turmeric), which were also comparable to the antibiotics drugs. Reductions of these enzymes are important as their accumulation in the liver is related to toxicity and hepatocellular damage. On the other hand, according to Kogut and Powell (1993), also noted that increase plasma ALP activity appears to be a sensitive indicator of intestinal diseases, such as coccidia infections in the duodenum, jejunum, and cecum. Nevertheless, ALP level was within the standard range forwarded by Meluzzi *et al.* (1992) for the healthy chicken (568–8,831 IU), thus indicating the synergetic effects of phytobiotic feed additives mixtures may act as hepatoprotective agents.

5.2.6. Immunomodulatory effects of blends of phytobiotic feed additives

The blends of different phytobiotic additives on antibody titer against NCD and IBD at 21st and 42nd day of broilers age are illustrated in Tables 19 and 20. The current study did not result in significant changes among the treatments against NCD and IBD antibody titer neither at 21st nor 42nd day of age. This result is in accordance with Al-beitawi and El-Ghousein (2016) reported that black cumin, anisum and thyme mixtures did not improve the antibodies of broiler birds against NCD and IBD. Contrary to the current study, Reddy *et al.* (2012) indicated that feeding broilers with diets supplemented with turmeric, tulsi and amla powder mixture resulted in high antibody titre against NCD. Birds receiving turmeric and thyme mixture powders birds receiving had the highest antibody against NCD and Influenza during the 18th and 28th days compared to the control birds (Fallah and Mirzaei 2016).

There are many factors (e.g., stress, maternal immunity and environment) that can alter antibody titers of birds. It is apparent that the time of blood collection after inoculation of vaccine and the level of phytobiotic additives are important to get a response on antibody production against NCD and IBD virus.

The conflicting results from previous findings considering the effect of different mixture of turmeric, fenugreek and black cumin on the immune response of broilers might be the mechanisms of action of the phytobiotic additives to exert the desirable effects mainly depend upon the structure and the level used in addition to the metabolism (Barreto *et al.*, 2008). It is difficult to precisely determine the required active ingredients for exerting the positive effect due to the unknown dose required to elicit a response (Khan *et al.*, 2012a). Moreover, the chemical composition of the medicinal plants varies with different parts of the plants as well as environmental conditions, climate, soil type and harvesting time have a great extent account for the inconsistent reports.

5.2.7. Antibacterial effects of blends of phytobiotic feed additives

As the finding of this study, the synergistic effect of the three-phytobiotic mixtures suppressing the pathogenic bacteria (coliform forming bacteria and *Escherichia coli*) as shown in Table 21. Lower population of pathogenic bacteria might increase availability of nutrients, eliminate sub-clinical infections and reduce production of growth-depressing toxins or metabolites by intestinal microflora. The phytobiotics mixtures except, black cumin and turmeric mixture group birds had lower coliform and *E. coli* bacteria count than the antibiotics (Oxycytetracycline) containing diet fed birds. This results in agreement with Guo *et al.* (2004b), who reported that that the total aerobic bacteria and *E. coli* counts in the caecum of the birds fed the antibiotic i.e., apramycin, were significantly higher than those of the herbal extracts received birds. Ahmad *et al.* (2016) also, reported that black cumin seeds, fenugreek and black tea oil control harmful bacteria like *E. coli* and *Salmonella* at similar level to antibiotics drug i.e. lincomycin and lowered total bacteria count in the intestine of the laying hens.

In addition, the phytobiotics additives mixtures (black cumin and fenugreek) and (fenugreek and turmeric) had lower coliform bacteria than the negative control. This concur with the results of El-Khalek and El-Naggar (2016), who reported that total coliform counts significantly reduce in broiler due to supplementation of blends of black cumin, oregano, ginger and thyme mixtures. The antibacterial effect of phytobiotic

mixtures (black cumin, fenugreek and turmeric) demonstrated in the current study may attributed due to either by specific adhesion of pathogens through the ‘lectin receptor’ mechanism (agglutination) by blocking the adhesion of pathogens onto the mucosal layer of the intestine (Pusztai *et al.*, 1990). In addition, due to the bioactive compound of terpenes, which are the main constituents of the medicinal plants and are responsible for the bulk of their antimicrobial activity of plant sources (Bourgou *et al.*, 2010). Terpenes compound changing the ions diffusion through disturbance in bacteria cell walls, mitochondrial membranes, cellular function and result in cell death (Burt, 2004; Bakkali *et al.*, 2007). Also, the antibacterial effect of phytobiotic blends (black cumin and fenugreek), (fenugreek and turmeric) and (black cumin, fenugreek and turmeric) may be attributed due to increase of digestive enzymes secretion under the influence of their aromatic characteristics which limits pathogen bacteria growth or their toxins that can reduce growth efficiency of the birds which create microbial eubiosis in the gastrointestinal.

5.2.8. Economic efficiency of blends of phytobiotics feed additives

Blends of different phytobiotics additive treatments did not decrease the per unit feed costs as compared to that of the negative and antibiotics controls as shown in Table 22. However, this is due to the addition of two and three different types of medicinal plants as mixture feed additives at the same time, which resulted in constant increase in per unit feed cost. The feed cost per bird was revealed a substantial positive effect of blends of fenugreek and turmeric supplemented diets and black cumin and turmeric mixtures inclusion in the diets, respectively reduce the production cost of broiler chicks.

The highest net return (42.26 birr) was earned from antibiotics followed by (39.38 birr) from fenugreek and turmeric mixtures, (37.80 birr) from the three phytobiotics mixtures and (37.74 birr) from black cumin and turmeric mixtures rations, respectively. The current results in agreement with Hossain *et al.* (2014), who revealed that the antibiotics group obtained the highest profit than the turmeric and black cumin group in

broiler birds. The current result is also in agreement with Reddy *et al.* (2010), who reported a reduction of 4% feed cost per unit live BW gain in mixture of turmeric, amla and tulsi at 0.25%. Least net returns (19.78 birr) were earned from birds fed black cumin and fenugreek mixture than the control diets. The low profit is due to the high feed cost and low body weight gain or carcass weight of broilers. Increase in the net profit obtained from the birds that fed diets containing fenugreek and turmeric mixture might be attributed to the better feed utilization, which results in more growth and better FCR ultimately leading to higher carcass yield and a higher profit.

The highest marginal rate of return was obtained from the antibiotics containing diets followed by fenugreek and turmeric mixture diets and black cumin and turmeric mixture diet groups, this is in agreement with Mahmood *et al.* (2014), who concluded that the use of various herbal mixture as growth promoters in the ration exhibited an increase in the profit margin of broilers as compared to the control. However, the marginal rate of return of the black cumin and fenugreek mixture was lower than the control group. Therefore, from the current study blends of turmeric rhizome and fenugreek seed mixture at 1% each as a phytobiotic feed additives in broiler production is profitable almost the same as antibiotics growth promoters (AGP).

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The ban on the use of antibiotics as feed additives due to drug resistance and the concern about toxic residues in food products and increased demand for organic animal products have driven the use of natural additives for production and maintenance of animal health.

Black cumin and turmeric at both levels had better FCR values or better efficiency to utilize nutrients. Turmeric can be included as feed additive at a level of 1 and 2% in the total ration to reduce the abdominal fat. Turmeric at 2%, black cumin and fenugreek inclusion at 1% in broiler diets had favorable effects on haematopoiesis of broiler birds as evidenced by increased Hb and RBC constitutes.

Turmeric and fenugreek at both levels on the diet had better hypoglycemic effect on broilers. Fenugreek at both level, black cumin at 1% and turmeric at 2% of the diet had high hypocholesterolemic effect on broilers. The liver enzymes remains lowered in phytobiotic additives at both levels, which indicated that there was no detrimental or toxic effects and the hepatoprotective ability of the phytobiotic feed additives.

The antibody against NCD improved in birds fed turmeric and fenugreek at 2%. In addition, IBD antibody improved in birds fed black cumin at 1% and turmeric at 2% levels. An improvement is possible through inclusion of turmeric 1-2% and black cumin at 1% of diets for profitable broiler production without any negative effects on biological performance of birds.

Mixture of the three-phytobiotic additives improved the BW gain, ADG and FCR without negatively affecting the carcass and internal organs. Moreover, different blends of phytobiotic additives had some favorable effects equivalent to commercial antibiotics (Oxycyteracycline) on haematopoiesis (PCV and RBC) of broiler birds but results were not consistent for each blend of phytobiotic feed additives.

The improvement in some biochemical parameters in broiler fed blends of (black cumin with turmeric), (fenugreek with turmeric) as well as mixtures of the three phytobiotic additives (black cumin, fenugreek and turmeric) revealed the ability to lower blood glucose and cholesterol levels in broiler chickens.

The mixture of phytobiotic additives except black cumin and turmeric mixture diet had antibacterial properties superior than the commercial antibiotics in lowering pathogenic, bacteria (*E. coli* and total coliform) of the cecum as result improved gut health and lead to better growth performance. It may be concluded that mixture of phytobiotic feed additives except black cumin and fenugreek blend in diets may be convenient for profitable production of broilers as the same as the commercial antibiotics.

Therefore, blends of (black cumin and turmeric), (fenugreek and turmeric) and the mixture of the three medicinal plant as phytobiotics additives in the diet of broiler can substitute commercial antibiotic growth promoters, as they are safe, effective, and economical efficient and above all, will help in the production of organic broilers and save the health of humankind from ill effects of residual of antibiotic in animal products.

Generally, inconsistencies have been observed between the results of the current and earlier studies. These inconsistencies might be attributed to the differences in plant variety, plant parts used, environment, climate, soil, harvesting time, methodology and laboratory analysis.

6.2. Recommendations

- Detailed studies are needed on the active constituents of phytocompound to determine the optimal dietary inclusion levels of the phytobiotic additives in order to achieve the optimum BW gain of broilers.
- Black cumin seeds and turmeric rhizome at both levels can be used in broiler diets for improved feed efficiency.

- Turmeric at 2% and black cumin and fenugreek at 1% inclusion levels can be used in broiler diets for improvement of hematopoiesis of broiler birds.
- The three phytobiotic additives can be used in individual or combined forms in order to utilize their beneficial biochemical and hematological links of broiler chickens.
- Phytobiotic feed additives individual or combined form should be used as natural growth and health promoters in order to substitute commercial antibiotics.
- More studies are needed to fully understand the phytobiotic mixture mode of action on the immune system.
- Individual inclusion of turmeric, fenugreek and black cumin can be used as immune-stimulant adjuvants as promising novel approach for new vaccine development.
- Further studies are required regarding the effects of phytobiotic additives on the meat quality of broilers.

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8. APPENDICES

APPENDIX TABLE

Appendix Table 1. Experimental feed ingredients and their respective price per kg unit

Ingredients	Unit	Price (ETB)
Maize	1 kg	4.70
Soybean meal	1 kg	11.00
Wheat bran	1 kg	4.80
Noug seedcake	1 kg	6.00
Limestone	1 kg	0.50
Salt	1 kg	5.500
Vitamin-mineral premix	1 kg	45.00
Methonine	1 kg	190.00
Lysine HCl	1 kg	120.00
Oxytetracycline 30%	1 Pack	35.00
Black cumin	1 kg	80.00
Fenugreek	1 kg	40.00
Turmeric	1 kg	15.00

*ETB = Ethiopian birr

APPENDIX FIGURES



Appendix Figure 1. Black cumin containing diets set for mixing



Appendix Figure 2. Fenugreek containing diets set for mixing



Appendix Figure 3. Turmeric containing diets set for mixing



Appendix Figure 4. Manual mixing of treatment diets



Appendix Figure 5. Arrangement of treatments and replications pen



Appendix Figure 6. Day old chicks upon their arrival in phae (I)



Appendix Figure 7. Intraocular administration of Hitchner-B1 vaccinee at 7th day of age



Appendix Figure 8. Measuring bird group weight



Appendix Figure 9a. Cobb-500 broiler at the end of the experiment



Appendix Figure 9b. Cobb-500 broiler at the end of the experiment



Appendix Figure 10. Slaughtering and defeathering bird for carcass evaluation



Appendix Figure 11. Biochemical and PCV determination



Appendix Figure 12. HI test for antibody determination against NCD



Appendix Figure 13. Cecum sample culturing for bacteria determination