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EVALUATION OF OYSTER MUSHROOM (*Pleurotus ostreatus*), GARLIC (*Allium sativum* L.), GINGER (*Zingiber officinale* R.) POWDERS AND THEIR MIXTURES IN DIETS OF BROILER CHICKENS AS NATURAL GROWTH PROMOTERS

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

July, 2018
Bishoftu, Ethiopia

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A Dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Production

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As members of the examination board of the final PhD open defense, we certify that we have read and evaluated the dissertation prepared by Zena Kidane entitled **'Evaluation of Oyster Mushroom (*Pleurotus ostreatus*), Garlic (*Allium sativum* L.), Ginger (*Zingiber officinale* R.) Powders and their Mixtures in Diets of Broiler Chickens as Natural Growth Promoters.',** and recommended that it be accepted as fulfilling of dissertation requirement for the Degree of **Doctor of Philosophy in Animal Production**.

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DEDICATION

This dissertation is dedicated to my wife, Kidist Abebe Negussie.

STATEMENT OF THE AUTHOR

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

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BIBLIOGRAPHICAL SKETCH

I, the author of this dissertation, was born in Addis Ababa in 1981. I attended my primary education from 1987 to 1995 at Jerusalem Elementary School and my secondary education from 1996 to 1999 at Entoto Academic, Technical and Vocational School in Addis Ababa. After completion of my high school education, I joined the then Alemaya University of Agriculture in 1999 and graduated with Bachelor of Science degree in Animal Sciences in 2002. Soon after graduation, I joined the Ministry of Agriculture and Rural Development (Agricultural, Technical and Vocational Education and Training/ATVET) and worked as senior instructor at Kombolcha ATVET College for six years in the Department of Animal Science. In September 2008, I joined the School of Graduate Studies of Haramaya University to pursue my MSc study majoring in Animal Production. After completion of my MSc study, I then joined Wolkite University and served as Lecturer for one year in the department of Animal Production and Technology. Then, I joined Addis Ababa University, College of Veterinary Medicine and Agriculture in 2014 for my PhD study in Animal Production. During the course of my study, I have received International Diploma in Animal Feed from Aeres Training Centre International, the Netherlands.

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LIST OF ABBREVIATIONS

ADG	Average Daily Gain
AGP	Antibiotic Growth Promoter
ALT	Alanine Amino Transferase
ALP	Alkaline Phosphatase
AOAC	Association of Official Analytical Chemist
AST	Aspartate Amino Transferase
CF	Crude Fiber
CFU	Colony Forming Units
CG	Corn Grain
CP	Crude Protein
CRD	Completely Randomized Design
DM	Dry Matter
ECE	Economic Efficiency
EE	Ether Extract
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed Conversion Ratio
FI	Feed Intake
GAP	Garlic Powder
GIP	Ginger Powder
GIT	Gastro Intestinal Tract
GOT	Glutamic Oxaloacetic transaminase
GPT	Glutamic Pyruvic Transaminase
Hb	Hemoglobin
LDL	Low Density Lipoprotein
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
ME	Metabolizable Energy

NR	Net Return
NSC	Noug Seed Cake
OMP	Oyster Mushroom Powder
PCV	Packed Cell Volume
RBC	Red Blood Cells
REE	Relative Economic Efficiency
SBM	Soybean Meal
TA	Total Ash
TI	Total Income
TWBC	Total White Blood Cells
WB	Wheat Bran

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ABSTRACT

The study targeted evaluation of oyster mushroom (Pleurotus ostreatus), garlic (Allium sativium L.), ginger (Zingiber officinale R.) powders and their mixtures in broiler diets as natural growth promoters. Two separate experiments were conducted where the second trial was designed based on the results of the first trial. The first trial was conducted using 315 unsexed day-old broiler chicks of Cobb 500 strain which were divided randomly into seven groups (T_1 till T_7). Each represented a treatment (45 birds/treatment) with three replicates in a completely randomized design (CRD). The treatments were T_1- Control, from T_2 till T_7 the diets contained 1% oyster mushroom powder (OMP), 2% OMP, 1% garlic powder (GAP), 2% GAP, 1% ginger powder (GIP) and 2% GIP, respectively. The second trial was conducted using 275 unsexed one day-old broiler chicks of Cobb 500 strain which were divided randomly into six groups. Each group represented a treatment (45 birds/treatment) with three replicates in a CRD. The first group (T_1) fed on basal diets without antibiotic (negative control diet) and the second group (T_2) fed on basal diets with antibiotic (0.30 g oxytetracycline/kg, positive control diet). The other groups from T_3 till T_6 were fed on basal diet containing different mixtures (0.5% OMP + 1% GAP), (0.5% OMP + 1% GIP), (1% GAP + 1% GIP) and (0.33% OMP + 0.66% GAP + 0.66% GIP), respectively. Daily feed intake (FI) and weekly body weight gain (BWG) were recorded and carcass evaluation was made. Blood hematological, serum biochemical data and fecal microbial counts were recorded. The results of the first trial showed that inclusion of 2% GIP improves FI ($P<0.05$), whereas, BWG ($P<0.05$) and FCR

(P>0.05) were impaired due to inclusion of 2% OMP. Most of the carcass parameters considered were not significantly affected (P>0.05) showing that the herbs didn't impair the development of the organs. Positive effects were observed for the values of RBCs (all except T_3), TWBC (T_4) and PCV (T_5), whereas, the biochemical parameters measured were not affected. Profitability was the lowest for all herbal treatments. The results of the second trial showed that T_4 and T_5 significantly reduced FI (P<0.05). Phytogenic mixtures had no significant effect on BWG and FCR. Percent proportions of dressed carcass, eviscerated carcass and breast cuts were significantly (P<0.05) impaired for T_6. Reduced abdominal fat % (P<0.05) was recorded for T_2 and T_3. Some of the blood parameters considered, (RBC, TWBC, PCV, MCV, MCH and MCHC) were not altered (P>0.05). T_3, T_5 and T_6 exhibited hypocholesterolemic effect (P<0.05). The herbal mixture of GAP and GIP was superior over the antibiotics in lowering the number of total colony forming units (CFU) and Escherichia coli bacteria in the digesta of ileo-cecum (P<0.05) and also the FCR. From the economic point of view, the herbal mixtures were not as profitable as the controls. Generally sole treatments (treatment either with garlic or ginger) were not as beneficial as the mixture of the two herbs. From the current findings, it was concluded that oyster mushroom, garlic and ginger powders each at 1% inclusion level as well as all the four combinations of medicinal plants could be considered as potential growth promoters and the mixtures may also replace the antibiotics in broiler diets.

Key words: Biochemical; Broiler; Carcass characteristics; Feed additives; Garlic; Ginger; Growth performance; Hematology; Microbial load; Oyster mushroom

1. INTRODUCTION

Broiler production plays a major role in food security for the rapidly increasing Ethiopian human population. Their short production cycle, high feed efficiency and high biomass per unit of agricultural land are particularly attractive for the Ethiopian production system (Smith, 2001).With improved stock; broiler birds can attain a weight of 2-3 kg within five to six weeks. However, this production capacity is subject to availability of good quality feed and disease control (Elijah *et al.*, 2012).

The industrialization of poultry production and the improvement of feed nutritional efficiency have promoted the introduction of a number of feed additives including antibiotics which became widely used in animal feed for many decades with the objectives of increasing production and maintaining animal health (Elijah *et al.*, 2012). Feed additives are ingredients added to diets for reasons other than to supply nutrient to the animal (FAC, 1998). The economic benefit of feed additives is generally to lower production cost as a result of an improvement in production efficiency. Feed additives are typically used in small quantities and are classified both organic and inorganic in poultry industry. The organic feed additives are products derived from plants which are used in feeding animals to improve their performance (Cuppelt and Hall III, 1998; Nakatani, 2000). The inorganic feed additives are agrochemicals such as antibiotics.

For the past several decades, sub-therapeutic dosages of antibiotics have extensively been used as tool to prevent poultry diseases and increase animal performances related to growth and feed efficiency. Therefore, about 80% of domestic animals have been fed with synthetic compounds for the purpose of either medication or growth promotion (Lee *et al*., 2001). However the large utilization of antibiotics in poultry feed has led to an increasing resistance of pathogens to the antibiotics and the accumulation of antibiotic residues in animal products and in the environment (Elijah *et al.*, 2012). Moreover, drugs used as feed additives plus the use of low protein diets increase fat content of broiler carcasses (Gerard *et al.*, 2011; Khan *et al.*, 2011 and Oleforuh-Okoleh *et al.*, 2014). Many countries tend to minimize or prohibit the use of antibiotic feed additives because of the above mentioned deleterious side effects on both animals and human (Oleforuh-Okoleh *et*

al., 2014). Removing these growth promoters from broilers' diets resulted in low growth performance and less resistance against diseases (Elijah *et al.*, 2012). As this may negatively affect the profitability of the poultry industry, alternative feed formulation and management strategies that exclude antimicrobial growth promoters must be developed and evaluated under intensive farming conditions (Collet and Dawson, 2001; Khan *et al.*, 2011). Possible alternatives to antibiotics may be represented by plant products (Guo *et al.*, 2004).

Recently, research has become more focused on the use of naturally occurring phytogenics in replacing the chemically based feed additives (Herawati and Marjuki, 2011). Phytogenic feed additives, also known as phytogenic products are plant derived products, used in animal feeding to improve the performance through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (Toghyani *et al*., 2012). Compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have been proven to be natural, less toxic, residue free and are thought to be ideal feed additives in food animal production (Hashemi *et al*., 2008). Some phytogenic feed additives have been successfully incorporated into the feeding standards of diets birds without any deleterious effect or toxic residues (Oyekunle and Owonikoko, 2002).

Prominent among these are oyster mushroom (*Pleurotus ostreatus*), garlic (*Allium sativum* L.) and ginger (*Zingiber officinale* R.). The potential for mushroom production in Ethiopia is rich and small-scale mushroom enterprises are already established and began to supply fresh edible mushrooms, particularly the oyster mushroom to the local markets (Tiret, 2009). Mushrooms in the form of extracts and dried powder as feed additives have been proved to have a good efficacy with a wide safety range and could be used as alternative for antibiotics (Abdalla *et al.*, 2009; Kavyani *et al.*, 2012). According to Chang and Miles (2004), the medicinal part of the oyster mushroom is in its fruiting body and polysaccharides in *Pleurotus* species belong to (1´3)-β-D-glucans. Oyster mushrooms are known to be rich sources of various bioactive substances, like anti-bacterial, anti-fungal, anti-viral, anti-parasitic, anti-oxidant, anti-inflammatory, anti-proliferative, anti-

cancer, anti-tumor, cytotoxic, hypo-cholesterolemic, anti-diabetic, anti-coagulant, hepato-protective compounds, among others (Lindequist *et al*., 2005; Ajith and Janardhanan, 2007).

Next to onion, garlic is the second most widely cultivated *Allium* species in Ethiopia and the production is estimated to be over 1,665.28 tones (CSA, 2018). Garlic has played an important dietary as well as medicinal roles for centuries. Even today, the medicinal values of garlic is wide-spread (Zeray and Mohammed, 2012). The production is carried out throughout the country both under irrigation and rain fed condition in different agro climatic conditions (CSA, 2006). Garlic has bioactive components, like sulfur containing compounds (*Alliin*, *Diallylsulfides* and *Allicin*) that act as antibacterial, antifungal, anti-parasite, antiviral, antioxidant, anti-cancerous and vasodilator characteristics (Kamal and Abo, 2012). In broilers, it was reported that garlic as a natural feed additive, improved growth and feed conversion ratio (FCR), and decreased the mortality rate (Tollba and Hassan, 2003; Onu, 2010; Puvača *et al*., 2014). Different authors also reported that garlic supplementation can improve carcass yield in broiler chickens (Lewis *et al*., 2003; Demir *et al*., 2008; Kamal and Abo, 2012). Garlic has also been found to lower serum, liver and tissue cholesterol (Stanaćev *et al*., 2012) and inhibit bacterial growth (Cavallito *et al*., 1994)

Ginger, a member of the family *Zingeberaceae*, is an important tropical herbaceous perennial plant, with the rhizome valued for its culinary and medicinal properties. Ginger production for the extraction of oleoresins and essential oils, as well as the direct use of rhizomes for culinary purposes is increasing, worldwide (FAO, 2008). In 2007, it was the second widely cultivated spices next to chilies in Ethiopia (Girma *et al*., 2008; MoARD, 2008). Ginger as natural growth promoters can be potential alternatives for common artificial growth promoters like antibiotics (Demir *et al*., 2005). The main important compounds in Ginger are *gingerol*, *gingerdiol* and *gingerdione* which have the ability to stimulate digestive enzymes, affect the microbial activity and have anti-oxidative activity (Dieumou *et al*., 2009). It is well studied that ginger had a significant effect on performance and many blood serum traits. According to Oleforuh-Okoleh *et al.* (2014),

the very minute amounts of ginger had a positive effects on feed intake (FI), body weight gain (BWG) and FCR of broiler chickens. It had also a very strong impact as anti-lipidemic effect on serum cholesterol and triglycerides (Arkan *et al*., 2012). Feeding ginger powder (GIP) or aqueous extract of ginger also improves carcass weight, dressing percentage and other carcass parameters in birds (Alcicek *et al.*, 2004; Tollba *et al.*, 2007; Ademola *et al.*, 2009; Javed *et al.*, 2009).

A lot of studies have indicated that mixtures of garlic and ginger had positive effects on performance of broilers in terms of feed intake FI, BWG and FCR; prevent high cholesterol level and resulted in the highest profitability ratio than their sole inclusion and also in comparison with control diets (Ademola *et al.*, 2007; Ademola *et al.*, 2009; Onu, 2010; Safa, 2014(a); Oleforuh-Okoleh *et al.*, 2015). It has been also reported that different mixture levels of garlic and GIP had positive effects on carcass parameters. According to scholars (Sarica *et al.*, 2005; Ademola *et al.*, 2009; Gholam *et al.*, 2013; Safa, 2014 (a)) mixture of GAP and GIP enhanced slaughter weight, meat quality, commercial cuts, carcass percentages and decreased the percentages of abdominal fat. Similar results were also reported for the mixtures of garlic, mushroom and propolis (Daneshmand *et al*., 2012). These can be very good reasons to propose that the combined use of these three natural materials could be also of particular benefit and be useful as a substitute for antibiotics.

Generally garlic, ginger and oyster mushroom have gained prominence due to their wide range of properties not only in improving performance, but also in many other ways (Elijah *et al*., 2012). Several studies have identified the separate use of these natural products. This study focused on both the separate and combined effects of garlic, ginger and oyster mushroom and to evaluate synergetic and complementary effects between these three in broiler chickens. Perhaps there are no reported studies about the combined effects of the above three natural substances on poultry performance and so far no research finding has been reported on the utilization of oyster mushroom, garlic and ginger as broiler feed additive in Ethiopia.

Specific objectives

- To evaluate the effects of different inclusion levels of oyster mushroom, garlic and ginger in boiler diets as feed additives on feed intake, growth performance and carcass traits in broiler chickens;
- To evaluate the effects of different inclusion levels of oyster mushroom, garlic and ginger in broiler diets as feed additives on hematobiochemical indices in broiler chickens;
- To evaluate the effects of different mixtures of oyster mushroom, garlic and ginger in boiler diets as feed additives on feed intake, growth performance and carcass traits in broiler chickens;
- To evaluate the effects of different mixtures of oyster mushroom, garlic and ginger in broiler diets as feed additives on hematobiochemical indices and cecal microbial counts in broiler chickens;
- To access the economic viability of inclusion of oyster mushroom, garlic, ginger and their mixtures as feed additives against the commercial antibiotics in broiler chickens diet.

2. LITERATURE REVIEW

2.1. The Use of Antibiotic as Feed Additives in Poultry Industry

It is important to make a demarcation between antibiotics used in the treatment and prevention of disease in farm animals (prescribed therapeutic and prophylactic use), which differs from their use as feed additives to enhance growth (Castanon, 2007). As feed additives, antibiotics are used at low concentrations of 2.5-50 ppm (depending on the compound used) (Hashemi and Davoodi, 2010). Generally feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin or to improve the animals' performance and health. The initial use of antibiotics in diets arose from the discovery in the late 1940's, in the United States that including the fermentation products of *Streptomyces aureofaciens* (a strain of bacteria) in the diets of simple-stomached animals, such as pigs and poultry resulted in growth responses (Frost, 1991). In the next 50 years, the use of antibiotics as feed additives in pig and poultry production became virtually universal (Hashemi and Davoodi, 2010).

The mechanism by which growth-promoting antibiotics improve growth performance is not known with certainty, but some mechanisms have been proposed by Jensen (1993): (1) nutrients are more efficiently absorbed because of a thinner small-intestinal epithelium (the reduction in intestinal weight as a result of feeding antibiotics is considered to be one of the mechanisms by which nutrient absorption is improved) (Gordon and Bruckner,1961a, b) ; (2) nutrients are spared because competing micro-organisms are reduced; (3) micro-organisms responsible for subclinical infections are reduced or eliminated; (4) production of growth-depressing toxins or metabolites by the gastrointestinal micro biota is reduced.

Frequently overlooked issues on the debate regarding the problems associated with the use of antibiotic feed additives are the substantial benefits derived from their use in food-producing animals. Scientifically documented benefits from the inclusion of low levels of antibiotics in animal feeds are indicated below (Cervantes, 2012).

Use of antibiosis as feed additives helps prevent subclinical diseases, such as necrotic enteritis in poultry. This is the main reason that antibiotic feed additives are used at sub-therapeutic levels in animal feeds, because they are used to prevent sub-clinical disease. Sub-clinical necrotic enteritis of poultry has been shown to have a significant adverse impact on flock performance and condemnations at the processing plant (Kaldhusdal and Hofshagen, 1992; Lovland and Khaldhusdal, 2001). Previous research has demonstrated that the effectiveness of an antibiotic feed additive to improve performance parameters such as growth rate and FCR is directly correlated with its ability to control *Clostridium perfringens*, the causative agent of clinical and sub-clinical necrotic enteritis of chickens and turkeys (Stutz and Lawton, 1984).

The other importance of antibiotic growth promoters is reduction of human pathogens, by improving flock uniformity, enhancing intestinal strength, minimizing gastrointestinal ruptures during evisceration and processing, and by reducing shedding of human pathogens such as *Salmonella* and *Campylobacter* species of bacteria. The use of antibiotic feed additives in animal feeds ultimately enhances the safety of the final products for the consumers (Cox *et. al*., 2003; Russell, 2003; Hurd, 2005). Chickens raised for the organic market without antibiotics have been shown to have a prevalence of *Campylobacter spp*. almost three times greater than that of conventionally-grown chickens (Heuer *et. al*., 2001).

Improved animal welfare is also the other benefit of antibiotic growth promoters (AGPs), because antibiotic feed additives have been scientifically shown to reduce immunologic stress even in “healthy chickens” kept under optimal sanitary, environmental and management conditions, their use contributes to enhance the welfare of food-producing animals (Roura *et. al*., 1992).

The use of AGP also improves production efficiency. This benefit is the result of better enteric health and prevention of nutrient degradation by the intestinal microflora. Typically, growth rate and FCR are improved which have led to class these additives as

"growth promoters", given what have been learned since this old term was coined many years ago, and the consequences from banning their use in the European Union (EU) (Casewell *et. al*., 2003; Phillips *et. al*., 2004; IFT Expert Report, 2006), where the prevalence of enteric diseases and the use of therapeutic antibiotics in food-producing animals have increased significantly since the bans, a more appropriate name would have been "health promoters" (Cervantes, 2006).

The improvements attained in growth rate and feed conversion is the other benefit of AGP. The same meat output can be maintained with a reduced number of animals and farms, and a reduced number of tons of feed resulting in more acres of the environment being preserved in its natural state results in preservation and less contamination of the environment. A recent scientific presentation estimated that a 0.04 improvement in FCR attributed to the use of antibiotic feed additives in a commercial turkey production operation would eliminate the need for an additional 5,525 tons of feed that without them would have had to have been produced and delivered, and as a consequence, an additional amount of excreta corresponding to this increase in feed tonnage would have been produced and disposed of into the environment without any additional gain in meat production (Tilley and Gonder, 2007).

According to Cervantes (2012), the use of antibiotic feed additives resulted in an improvement in production efficiency and the savings from the cost of production can be passed on to the consumers who can continue to enjoy an abundant supply of nutritious and safe meats at an affordable price

2.2. The Limitations of Antibiotic Feed Additives

There has been growing concern about public health risks resulting from antibiotic resistance, carcinogenic responses and other side effects of the residues in animal products. The extensive use of AGPs in poultry industry has resulted in a rapid appearance of resistant forms of microorganisms which are less sensitive to the antibiotics. A study demonstrated that 19 and 81% of the poultry meat and environmental

isolates analyzed were resistant to at least one of the tested molecules (enrofloxacin, ciprofloxacin, tetracycline and erythromycin) (Cesare *et al*., 2002). The population of antibiotic-resistant bacteria, which was established during the time when antibiotics were routinely used, has survived from generation to generation for over 60 years even in the absence of antibiotic exposure (Langlois *et al*., 1986). The most important potential route by which humans become infected with resistant bacteria is via the food chain, of which meat is the most significant source although other animal products, such as milk and eggs may be involved (Hinton, 1988). Nowadays, antibiotics have been banned and thus removed from diets of poultry in many countries. The effect of withdrawal of dietary antibiotics on growth and FCR of different farm animals is represented in the following Table.

Table 1. Effect of withdrawal of feeding antibiotics as performance promoters on growth performance and feed conversion efficiency in different species of farm animals

	Reduction in daily body weight gain (%)	Increase in feed per gain (%)
Veal calf production	7-8	4-5
Beef production	4	2
Weaned piglets	8	5
Growing pigs	5	3
Fattening pigs	2	1
Pig production	5	2
Growing chickens	3	2

Source: (Wenk, 2003a)

According to Wenk (2003a), the withdrawal of antibiotics had the greatest effect on reduction in daily body mass gain in veal calf production and weaned piglets, but seems to have less effect on fattening pigs and growing chickens. It also had the greatest effect on FI of veal calf production and weaned piglets. Improved biosecurity, vaccination, genetic selection and the use of natural alternative feed additives are some strategies that can be followed to reduce the use of antibiotics (Sun *et al*., 2005).

2.3. Phytogenics as Feed Additives

Phytogenic feed additives are products of plant origin used in animal feeding as non-nutrient substances to enhance their performance and health. Recently phytogenic feed additives gained importance due to the restriction of using antibiotics as growth promoters by European Union, but there is a lack of knowledge about their mode of actions (Windisch *et al*., 2008). Natural medicinal products originating from herbs and spices have also been used as feed additives for farm animals in ancient cultures for a long period of time. To differentiate from the plant products used for veterinary purposes (prophylaxis and therapy of diagnosed health problems), phytogenics were redefined by Windisch and Kroismayr (2006) as plant-derived products added to the feed in order to improve performance of agricultural livestock. Around the world, phytogenics have been investigated as natural sources of biologically important chemicals since efforts are being made to ban all types of in feed antibiotics in many countries. Antimicrobial, antifungal (Sivropoulou *et al*., 1996; Sivropoulou *et al*., 2007) and anti-parasitic properties of phytogenic substances (Guarrera, 1999) were also approved.

A successful alternative to AGPs should comply with certain characteristics. It should be able to mimic the mode of action or effect of the antimicrobial, and therefore have a significant beneficial impact on animal production and health which can be reflected in improved digestion, nutrient metabolism and absorption, as well as a decrease in incidence of diseases. It should also be generally regarded as safe to both the animal and human, be easy to apply and store and be cost-effective. Dry powder products, for instance, are easier to handle than liquid products. Low inclusion rates, heat stability and long shelf-life are all qualities that will make the products more attractive (Collett *et al*., 2001).

2.3.1. Classification of phytogenics

Phytogenics comprise a wide range of substances and thus have been further classified according to botanical origin, processing and composition. Phytogenic feed additives

include herbs, which are non-woody flowering plants known to have medicinal properties; spices, which are herbs with intensive smell or taste, commonly added to human food; essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits, and roots; and oleoresins, which are extracts derived by non-aqueous solvents from plant material (Jacela *et al*., 2010).

2.3.2. Plant secondary metabolites

The active compounds of phytogenics are secondary plant constituents. Their active principles are chemical compounds present in the entire plant or in specific parts of the plant that confers them therapeutic activity or beneficial effects (Martins *et al*., 2001). These substances have low molecular weights and are derived from the plant secondary metabolism, including glucosides, alkaloids (alcohols, aldehydes, ketones, ethers, esthers, and lactones), phenolic and poly-phenolic compounds (quinones, flavones, tannins, and coumarins), terpenoids (mono and sesqui-terpenoids, and steroids), saponins, mucilages, flavonoids, and essential oils (Martins *et al*., 2001). Antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytogenics exert positive effects on the growth performance and health of animals. Compounds (phytochemicals) in phytogenics are well known to have antimicrobial ability (Cowan, 1999). Polysaccharide components are considered to be the most important immunoactive components (Xue and Meng, 1996). A common feature of phytogenics is that they are a very complex mixture of bioactive components and they may exert multiple functions in the animal body (Wang *et al*., 1998; Windisch and Kroismayr, 2006). Growth enhancement through the use of phytogenics is probably the result of the synergistic effects among complex active molecules existing in phytogenics (Gauthier, 2002). However, the exact growth promoting mechanisms of phytogenics in broiler chickens are poorly understood.

2.3.3. The mechanisms of action of phytogenics

Wenk (2003b), reported that herbs, spices and their extracts can stimulate appetite and endogenous secretions such as enzymes or have antimicrobial, coccidiostatic or

anthelmintic activities in monogastric animals. The mechanisms of action of phytogenics can be further explained as follows.

2.3.3.1. Antimicrobial effect and competitive blocking of bacterial adhesion

Some bioactive substances from plants, like most antimicrobial agents, exert their effects by modulating the cellular membrane of microbes (Kamel, 2000). A strong increase in hydrophobicity of the microbial species in the presence of some plant extracts may influence the surface characteristics of microbial cells and thereby affect the virulence properties of the microbes (Kamel, 2001). This may be an important antimicrobial mechanism of some plant extracts. Various essential oil mixtures, which contain natural polyphenolic compounds or flavonoids as major active ingredients, have been identified as potential antimicrobial and antioxidant agents (Cruickshank, 2001; Friedman *et al*., 2004). Thus, supplementation of broiler diets with essential oil mixtures can create a healthier gut microflora, aiding optimum digestion and improving bird performance (Cruickshank, 2001).

Lectin-carbohydrate receptor interactions are the main mechanism in adhesion of pathogens to the brush border of the gut mucosal epithelium. Many prebiotic and phytogenic bioactive substances can have a direct effects on certain pathogenic bacteria either by specific adhesion of pathogens through the 'lectin-receptor' mechanism (agglutination) or by blocking the adhesion of pathogens onto the mucosal layer of the intestine (Pusztai *et al*., 1990).

2.3.3.2. Stimulation of digestive enzymes

Another possible mode of action of phytogenic bioactive compounds on growth performance of farm animals could be their effects on the activities of digestive enzymes. Xu *et al*. (2003) reported that dietary supplementation of fructo-oligosaccharides improved daily BWG of male broiler chickens by increasing the activities of amylase and protease. Furthermore, a study with broiler chickens indicated that feeding a diet

containing a commercial blend of essential oils (CRINA®) in combination with lactic acid induced a significant increase in activities of digestive enzymes of the pancreas and intestinal mucosa of birds, leading to a significant increase in growth (Jang *et al*., 2004). Although Lee *et al*. (2003) observed significantly higher amylase activity in the intestinal digesta of broiler chickens fed the same commercial essential oil mixture.

2.3.4. Effects of drying on phytochemical constituents of selected phytogenics

Processing or drying methods have variable effects on total phenolic content and antioxidant activity of plant samples, including little or no change, significant losses, or enhancement in antioxidant activity (Nicoli *et al*., 1999). Apati *et al*. (2010) suggested that the best temperature in the drying process for oyster mushroom fruiting bodies was around 40ºC. Drying mushrooms under the sun yields unhygienic and poor quality product (Gothandapani *et al*., 1997). According to Aishah and Rosli (2013), the β-glucan content of mushroom samples which undergo sun drying techniques has the highest β -glucan content (27.5%) followed by low heat air blow (25.8%) and the lowest when using gas laboratory oven method (24.1%). Syntsya *et al*. (2008) reported that the content of β-glucan was at 27.4 -39.2% in the pilei and 35.5- 50.0% in the stems of oyster mushroom in dry matter (DM). Aishah and Rosli (2013) concluded that sun drying is good in improving β-glucan content. Garlic powder is also thought to retain the same ingredients as raw garlic, however, the proportions and amounts of various constituents differ significantly (Harunobu *et al.,* 2001). Vankar *et al.* (2006) studied the stability of ginger components after heat treatment at 120°C and reported that the antioxidant activity of the powdered form were stable. Drying also enhanced the phytochemical constituents of rhizomes by stopping polyphenol oxidase activity compared to fresh samples that had higher polyphenol oxidase activity (Ali *et al*., 2016). Eze and Agbo (2011), reported that ginger is best preserved in its natural form under open-air sun drying conditions. However, Ebewele and Jimoh (1981), reported that sun drying of ginger results in loss of some volatile oils by evaporation and destruction of some heat sensitive properties.

Generally four factors may affect the effectiveness of phytogenic additives: plant parts and their physical properties, source, harvest time and compatibility with the other

ingredient(s) in the feed (Wang *et al*., 1998), which may also explain why 50% difference in BWG and 63% difference in FCR could happen when different kinds of phytogenics are used in chickens diets (Xing, 2004). Although phytogenics are a group of natural additives, research needs to be done into their mechanisms of actions, compatibility with diets, toxicity and safety assessments (based on the fact that some phytogenic might have harmful substance(s)) before they can be applied more extensively in poultry feed (Yang *et al*., 2009).

2.4. Oyster Mushroom

Mushroom is a special type of edible fungi forming flesh umbrella like fruiting bodies. They belong to the class of Basidiomycetes and order of Agaricales (Bahl, 1998). Mushrooms are not a true vegetable in the sense that it does not have any leaf therefore contains no chlorophyll, roots, or seeds and really does not need any light to grow. It is a fungus, which grows in the dark and propagates by releasing spores (Chang and Boswell, 1996). Mushrooms have now been recognized universally as food and are grown on commercial scale in many parts of the world, including Ethiopia (Okechukwur, 2011). Today about 7000 species of mushroom possess varying degrees of edibility, and more than 3000 species may be considered prime edible species, of which only 200 species have been experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, and about 10 species cultivated on an industrial scale. In addition, 2000 species have been suggested to possess medicinal properties (Chang and Miles, 2004).

Edible mushrooms, like *Pleurotus ostreatus* are known to be among the largest of fungi (Onuoha, 2007). *Pleurotus ostreatus* (oyster fungus) is an edible mushroom having excellent flavor and taste (Shah *et al*., 2004). China produces more than 85 percent of all oyster mushrooms grown worldwide (Chang, 1999).

Cultivation of oyster mushroom has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues. *Pleurotus* species are efficient lignin degraders, which can grow on

different agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandiak and Goyal, 1995). Growing oyster mushrooms convert a high percentage of the lingo-cellulosic substrate to fruiting bodies increasing profitability. Of them, *Pleurotus ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and economic way (Kues and Liu, 2000). It requires a short growth time in comparison to other edible mushrooms. All this makes *Pleurotus ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms (Kausar, 1998).

2.4.1. Oyster mushroom production in Ethiopia

The idea of establishing a mushroom farm with a view to produce and supply fresh mushrooms to the local market was first initiated by the African Mushroom Company in 1997. Soon after, the company, a pioneer in the activity, started to cultivate oyster mushroom and supply the market with the product. Although imported canned mushrooms were available in supermarkets and were used by hotels and restaurants, fresh mushrooms were new to the local market and the demand for fresh mushrooms started to grow. Subsequently, fresh button (*Agaricus bisporus*) and shiitake (*Lentinula edodes*) mushrooms were introduced to supermarkets, restaurants and international hotels (Tiret, 2009).

Small scale mushroom production represents an opportunity for farmers interested as an additional work, and is specially an option for farmers with no adequate farm lands (Marshall and Nair, 2009), especially in the current alarmingly increasing Ethiopian population.

Small-scale mushroom enterprises are already established and began to supply fresh edible mushrooms, particularly the oyster mushroom to local markets. Though efforts on growing the button (*Agaricus bisporus*) and shiitake (*Lentinula edodes*), is underway, the success story is based on the oyster mushroom (Dawit, 2014).

2.4.2. Nutrient composition of mushroom

Mushrooms represent one of the world's greatest untapped resources of nutritious food (Obodai *et al*., 2003). Mushrooms represent an excellent source of proteins, dietary fibers, minerals (P, K, Na, Ca and Fe) and B-vitamin (B_1, B_2, niacin, C, folic acid and pro-vitamin D ergosterol) and are low in fat (Moharram *et al*., 2008). Protein on DM basis in mushrooms can range 20-40% (Chang and Buswell, 1996) and is rich in essential amino acids, like lysine and leucine limited in cereal grains (Chang and Buswell, 1996). Oyster mushrooms are known to bear therapeutic ingredients, such as dietary fibers (chitins and chitosans) and phenolic compounds (Neyrinck *et al*., 2009).

Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered a health food (Chang and Mshigeni, 2001; Sadler, 2003). Furthermore, they contain significant amounts of carbohydrates and fibers (Chang and Buswell, 1996). In general, the gross composition of mushrooms is water (90%), protein (2-40%), fat (2-8%), carbohydrates (1-55%), fiber (3-32%) and ash (8-10%) (Firenzuoli *et al*., 2008).

2.4.3. Medicinal values of mushroom

Mushrooms have long been considered to have medicinal values. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic values as a source of food. Humankind has constantly searched for new substances that can improve biological functions and thereby make people fitter and healthier. Recently, Western society has placed a great emphasis on plants, herbs, and foods as sources of these health enhancers. These products have variously been called vitamins, dietary supplements, phytochemicals, nutraceuticals, and nutriceuticals. Dietary supplements are ingredients extracted from foods, herbs, plants, and fungal species that are not used as a regular food but which boost the immune system or otherwise help maintain health. Phytochemical (phytonutrient) is a more recent evolution of the term that emphasizes the plant source of such protective disease- preventing compounds (Chang and Miles, 2004).

Out of approximately 14,000 known species, 650 possess medicinal properties (Rai *et al*., 2005). According to Chang and Miles (2004), the medicinal part of the oyster mushroom is in its fruiting body. Oyster mushroom is used in traditional medicine to prevent or assist in more than 30 diseases or disorders. Antitumor activity was found in the polysaccharide fractions of the fruiting bodies of almost all *Pleurotus* species. These polysaccharides belong to (1´3)-β-D-glucans. Different glucans from *Pleurotus* have been found to enhance the activity of natural killer cells and lymphokine activated killer cells. In addition to modulating the immune system, *Pleurotus spp.* has hypoglycemic activity, antithrombotic effects, reduce inflammation and lower blood pressure and plasma lipid concentration. In addition, they have antioxidant activities. The investigation of antioxidant activity of different extract fractions (acidic, phenolic, alkaline and neutral) showed that the highest activities level was present in the phenolic fraction. It was also demonstrated that the extracts of fruiting bodies had higher antioxidant activity than mycelium and cultured liquid extracts. Differences in antioxidant activities between samples of extracts may be related to different fatty acid composition of their lipids. Thus, the content of unsaturated fatty acids in fruiting body extracts was higher than in mycelium and cultured liquid extracts (Chang and Miles, 2004). Table two shows biologically active compounds from oyster mushroom and their pharmacological effects.

Table 2. Biologically active compounds from oyster mushroom and their pharmacological effects

Active compound	Pharmacological effect	References
Pleuran (β-1, 3-glucan with galactose and mannose)	immuno-modulatory anti-tumor	El Enshasy and Rajni (2013)
Proteoglycan	hypoglycemic anti-oxidant	Tong *et al*. (2009)
Laccase	anti-viral	El Fakharany *et al*. (2010)
Pleurostrin (peptide)	anti-fungal	Chu *et al*. (2005)

In the feed industry there are many commercial products available based on β-glucans from mushrooms species. These species are cultivated on a commercial scale and have a rapid growth and are able to produce in a short time with high biomass. As a result they are able to provide a steady source of β-glucan to feed the needs of an enlarged market. These are used as feed supplements aiming at the stimulation of the animal's immune system and hence avoiding the overuse of antibiotics in the feed industry. Such immuno-stimulation was noticed with β-glucans from oyster mushroom fed to chickens (Muthusamy *et al*., 2013).

2.4.4. Using mushroom as a poultry feed additive

2.4.4.1. Effects on broiler performance

Guo *et al*. (2003) reported that mushroom extract as feed additive enhanced growth by improving the beneficial intestinal flora of the gut and reducing the harmful effects of certain bacterial enzymes, and with increase in the mushroom extract dose, birds tended to have higher BWG. Giannenas *et al*. (2010a) also showed improved performance of broiler chickens when adding 10 and 20 g/kg of an edible mushroom (*Agaricus bisporus*) to the diet.

2.4.4.2. Effects on serum biochemical constituents

Blood parameters had been shown to be major indices of physiological, pathological and nutritional states of an organism and changes in the constituents' compounds of blood when compared to normal values can be used to interpret the metabolic stage of an animal as well as quality of feed (Ranjit *et al.*, 2013). Besides, hematological values are important to clinico-pathological diagnosis such as traumatic injury, parasitism, organic disease, bacterial septicemia and nutritional deficiencies. Moreover, managing abnormalities in birds requires an understanding of how diseases change the biochemical function and electrolyte of the bodies. Because the clinical signs of illness in birds are

frequently subtle, clinical chemistry is necessary to evaluate cellular changes (Ritchie *et al*., 1994).

Abdalla *et al*. (2009) reported a significant increase in total protein and albumin in mushroom treated birds (11.75%, 17.5%, 23.5 g mushroom powder/kg) which could be due to the growth promoting effects of mushroom as prebiotic feed additive that beneficially affect the host by selective stimulation of growth or activity of one or a limited number of bacterial species in the colon. Thus benefiting host health by increasing absorption of nutrients.

According to Abdalla *et al*. (2009), the result of erythrogram showed a significant increase in red blood cell (RBCs) count, hemoglobin (Hb), packed cell volume (PCV) in mushroom treated group. Wasser *et al.* (2000) also reported that biologically active substances from higher basidiomycetes of mushroom stimulate hematogenesis. In the same aspect Fleischer *et al*. (2000) reported increased hemoglobin concentration in broiler chickens supplemented with *β*-D-glucan with the diet.

2.4.4.3. Effects on intestinal health

A more balanced biota population in the gastrointestinal tract (GIT) of poultry could lead to a greater efficiency in digestibility and utilization of feed, resulting in an enhanced growth and improved FCR (Ferket, 2004). Rehman *et al.* (2007a, b) reported that lactic acid producing bacteria may improve gastrointestinal function, feed digestibility and animal performance. Also, *Lactobacilli* may produce organic acids and other bactericidal substances (Neal *et al*., 2012). Giannenas *et al.* (2011) reported that, *lactobacilli spp*. population in the ileum of turkeys' poults supplemented with *Agaricus bisporus* mushroom were higher, and *Escherichia coli* counts were lower than control group. Similarly, in other trial the broilers diets supplemented with *Agaricus bisporus* mushroom at the level of 20 g/kg diet had higher *Lactobacilli spp*. population in the cecum and ileum compared with control group (Giannenas *et al*., 2010b). Furthermore, Giannenas *et al*. (2011) reported that fermentable polysaccharides content of mushrooms may improve

growth of *Lactobacilli* and *Bifido bacteria* populations and inhibited that of *Escherichia coli*. In other trial Stanley *et al.* (2000) reported that coliform bacteria decreased in turkeys' intestinal content when turkeys were supplemented with mannan oligosaccharides. Ashkan *et al*. (2014) concluded that supplementing broiler diet with 30 g mushroom/kg could induce favorable influences on intestinal health of broiler chickens. The *Lactobacilli spp*. population in birds supplemented with mushroom at the level of 30 g/kg was non-significantly higher than other groups at 45 days of age. Ashkan *et al*. (2014) also reported that *Escherichia coli* loads significantly decreased in broilers fed diets containing 5 g mushroom/kg. Table three summarizes the effects of inclusion of different levels of mushroom in broiler diets as observed by various workers.

Table 3. Summary of response of broilers to dietary inclusion of different levels of mushroom as phytogenics

Dietary dose of mushroom	Treatment effects	References
7.5 and 15 ppm *Pleurotus ostreatus*	reduced low density lipoprotein (LDL)	Ekunseitan *et al*. (2017)
1% and 2% dried mushroom (*Agaricus bisporus*)	showed improved performance	Giannenas *et al*. (2010a)
3% dried mushroom (*Agaricus bisporus*)	significant effect on immune responses	Kavyani *et al*. (2012)
0.5% dried mushroom (*Agaricus bisporus*)	significantly decreased *Escherichia coli* loads	Ashkan *et al*. (2014)
2% dried mushroom (*Agaricus bisporus*)	higher *Lactobacilli spp.* population in the cecum and ileum	Giannenas *et al*. (2010b)
3% dried mushroom (*Agaricus bisporus*)	induce favorable influences on intestinal health	Ashkan *et al*. (2014)
0.2% *Lentinus edodes* extract	increased *Bifidobacteria* and *Lactobacilli* and reduced *Escherichia coli loads* in cecal content	Guo *et al*. (2004)
3% *Agaricus bisporus*	reduced *Escherichia coli*	Kavyani *et al*. (2012)
1% and 1.5% *Pleurotus ostreatus*	improves carcass weight (g/bird)	Rani *et al*. (2016)
1 and 2% *Pleurotus ostreatus*	reduced breast meat fat content of Japanese quail	Vargas-Sánchez *et al*. (2018)

LDL = low density lipoprotein

2.5. Garlic (*Allium sativum* L.)

2.5.1. Garlic production in Ethiopia

Economic significance of garlic in Ethiopia is quite considerable. It is grown as spice and used for flavoring local dishes, and contributes to the national economy as export commodity (Fekadu and Dandena, 2006). In Ethiopia, garlic is one of the important bulb crops produced for home consumption and is a sources of income to many peasant farmers in many parts of the country (Metasebia and Shimelis, 1998). In 2015/16 total amount of garlic production in Ethiopia was 1,665.28 tone of bulbs and the total area covered was 18,807.94 ha (CSA, 2018).

2.5.2. Nutrient composition of garlic

Protein content of garlic was found to be considerably higher than that in other vegetables such as bean and pea (Gulfraz *et al*., 2014), but crude oil content was considerably lower. Among minerals, garlic is known to contain high level of potassium (21 g/kg), phosphorous (6 g/kg) followed by magnesium (1 g/kg), sodium (532.78 mg/kg), calcium (363.61 mg/kg) and iron (52.91 mg/kg). Vitamins like riboflavin, thiamin, nicotinic acid, vitamin C and vitamin E are other important chemical constituents (Alejandra *et al.*, 2010). Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B_1 and C, fiber and water. It also contains 17 amino acids to be found in garlic: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine (Josling, 2005).

2.5.3. Medicinal values of garlic

The majority of reported medicinal effects of this botanical appear to come from the sulfur containing compounds, high trace mineral content and enzymes. Most of the sulfur found in whole garlic cloves is of two types found in equal quantities: the S-alkyl-

cysteine sulfoxides and the γ-glutamyl-S-alkylcysteines. The most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 mg/g in fresh garlic or 30 mg/g dry (Lawson, 1998).

Typical garlic food preparation includes chopping, mincing, or crushing the garlic. When these traumas occur the odor-free cysteine sulfoxides are exposed to the allinase enzymes, and quickly convert to thiosulfanates, which give off garlic's characteristic aroma (Pedrazza *et al*., 2006). Up until recently, the therapeutic value of garlic has been attributed to the low molecular weight thiosulfinates. Although allicin is considered the major antioxidant and scavenging compound, studies are showing that other compounds may play stronger roles (Chung, 2006). In addition, newer research has characterized some polar compounds of phenolic and steroidal origin, which proffer various pharmacological properties. These latter compounds, in contrast to the thiosulfinates, are without odor, and are also heat stable (Lanzotti, 2006). Although not all of the active ingredients are known, ample research suggests that several bioavailable components likely contribute to the observed beneficial effects of garlic (Harunobu *et al.*, 2001). Table four summarizes the most important biologically active compounds available in garlic with some of their pharmacological effects.

Table 4. Biologically active compounds from garlic and their pharmacological effects

Active compound	Pharmacological effects	References
Allicin (diallyl-dithiosulfinate) (this compound does not occur in garlic until it is crushed or injured)	Antimicrobial and hypocholostromic effects	Londhe *et al*. (2011); Anthony *et al.* (2005); Josling (2001).
DDS (Diallyl disulphide)	Active against yeasts, anti-hyperlipidemic and hypoglycaemic actions	Avato *et al*. (2000) and Sela *et al*. (2004)
Ajoene	The most active antiviral compound present in garlic with antimicrobial properties	Weber *et al*. (1992); Azimi *et al*. (2011)
DTS (Diallyl trisulfide)	Active against yeasts and hypolipidemic effects	Avato *et al*. 2000); Lii *et al*. (2012)
SAC (S-allylcysteine) (the most abundant organosulfur compound found in aged garlic extract)	Antioxidant properties	Cruz *et al*. (2007)

2.5.4. Using garlic as a poultry feed additive

2.5.4.1. Effects on broiler performance

Pourali *et al.* (2010) suggested that allicin in garlic promotes the performance of the intestinal flora of broiler chickens thereby improving digestion and enhancing the utilization of energy, leading to improved growth. Ramakrishna *et al.* (2003) also indicated that garlic supplementation enhances the activity of pancreatic enzymes of rats and provides an environment for better absorption of nutrients.

Zekić *et al.* (2014) concluded that the addition of commercial GAP in amount of 2% in broiler chickens diet had significant influence on production performance, higher final body weight, as well as improved the nutritive quality of chickens' breast meat. Elagib *et al*. (2013a) clearly indicated that the best production performance parameters were attained by the birds those fed the diet supplemented with 3% level of GAP, the lower performance were attained by the birds fed 5% level and recommended that garlic can be used as feed additives at level of 3% to improve the overall performance of broiler chickens. Similarly Javandel *et al.* (2008) reported that feed consumption was significantly higher in birds fed diets with lower concentration of garlic 0.125 and 0.25% compared to higher level 0.5, 1 and 2%. Kumar *et al.* (2005), Afsharmanesh *et al.* (2008), Raeesi *et al*. (2010) and Mansoub and Nezhady (2011) also found positive effects of garlic supplementation on broiler performance. Ademola *et al.* (2009) reported that garlic supplementation improved the final live weights of broiler chickens. Fadlalla *et al.* (2010) and Raeesi *et al*. (2010) observed better FCR while incorporating garlic to broiler diets. El-Gamry *et al.* (2002), Tollba and Hassan (2003) indicated that, addition of GAP in broiler diets significantly improved FCR in broilers. Ademola *et al*. (2005), Demir *et al*. (2005) and Javendel *et al.* (2008) fed herbal plants (ginger and garlic) as growth promoters in broiler diets and observed a pronounced improvement in their BWG and FCR.

Contrary to the above findings, it was reported that feeding GAP at levels of 1.5, 3 and 4.5% had no effect on birds' performance (Konjufca *et al*., 1997). Using GAP in broilers' diet also had no significant effect on performance but it influenced meat quality and carcass yield positively (Horton *et al*., 1991).

2.5.4.2. Effects on carcass traits

According to Elagib *et al*. (2013a) birds fed diet containing 3% GAP attained the highest hot carcass weight, dressed weight, breast weight, fleshed breast weight and fleshed breast percentage, followed by the birds fed 0% level and the lowest yield was attained by the group fed 5% level. The group fed the diet containing 3% level attained the highest

dressed weight, and the lowest dressed weight was observed for 5% GAP (Elagib *et al*., 2013a). Raeesi *et al.* (2010) also reported a significant improvement on the carcass parts of broilers fed with garlic.

2.5.4.3. Effects on serum biochemical constituents

It was reported that feeding GAP at levels of 1.5, 3 and 4.5% caused a significant reduction in birds' serum and liver cholesterol (Kamal and Abo, 2012). The studies conducted by Prasad *et al.* (2009a) and Yeh and Liu (2001) showed that garlic was effective in lowering the total cholesterol via its effect on the plasma concentration of LDL cholesterol. Serum cholesterol was decreased by feeding garlic to layers (Chowdhury and Smith, 2002 and Lewis *et al*., 2003). According to Qureshi *et al*. (1983), broilers on diets containing the equivalent of 1, 2, 4, 6 and 8% garlic paste had lower serum cholesterol concentrations reduced by 18, 21, 24 and 25%, respectively. Zekić *et al*. (2014) concluded that the GAP is very powerful for cholesterol reduction in all chickens' tissues which makes that tissue with the lower cholesterol levels and safe for consumption by human with cardiovascular diseases.

Contrary to the above findings, Horton *et al.* (1991) reported that total serum cholesterol concentrations were not significantly affected by the supplementation of dietary GAP at different levels (0 and 1 g/kg) over a 35 days growth period. Some studies also suggested that commercial garlic oil, GAP and commercially available garlic extract may not be hypocholesterolemic (Berthold *et al.*, 1998; Crindle *et al.*, 1998). Chowdhury and Smith (2002) suggested that the relative stability of chemical ingredients in garlic and the duration of the study may affect responses, since Lawson *et al.* (1992) reported that alicin, the potentially active component in garlic, is unstable and poorly absorbed from the digestive tract.

According to Elagib *et al*. (2013a) birds fed on 0% level showed the highest hemoglobin percentage, whereas, there was no significant difference between the other groups fed on 3% and 5% levels of garlic powder in the diet. Similar results were reported by Ademola

et al. (2009); Prasad *et al.* (2009b) and Fadlalla *et al.* (2010), the latter due this decrease in Hb percentage to the presence of some hemolytic bioactive constituents and/or their metabolites in garlic.

Elagib *et al*. (2013a) reported no effect on most blood parameters due to garlic inclusion in broiler feed except for hemoglobin concentration. Hematological parameters in birds had been shown to be influenced by various factors including physiological (age, sex) (Alodan and Mashlay, 1999) environmental conditions (as season), diet contents and age (Seiser *et al.*, 2010). Table five summarizes the effects of inclusion of different levels of garlic in broiler diets as reported by different workers.

Table 5. Summary of response of broilers to dietary inclusion of different levels of garlic as phytogenics

Dietary dose of garlic	Treatment effects	References
1.4%	Improved FI and BWG	Oleforuh-Okoleh *et al*. (2014)
0.25%	Improved BWG	Onu (2010)
0.2%	Improved FCR	Onu (2010)
1 and 3%	Improved FCR	Raeesi *et al.* (2010)
250 ppm	Improved BWG and FCR	Kumar *et al*. (2010)
0.1%	Improved BWG and FCR	Mansoub (2011)
1.4%	Improved dressing percentage	Oleforuh-Okoleh *et al*. (2014)
1%	Improved dressed weight	Karangiya *et al*. (2016)
0.5 and 1%	Improved breast weight	Nikola *et al*. (2016)
0.1%; 1.4%; 1, 2 and 3%	Reduced abdominal fat percentage	Ashayerizadeh *et al*. (2009); Oleforuh-Okoleh *et al*. (2014); Huda *et al*. (2015).
0.2 and 0.4%; 0.1%; 2, 6 and 8%; 1%; 0.5, 1 g/kg; 0.5% and 1.0%; 1% and 2%.	Reduced serum total cholesterol	Jamal and Omar (2011); Rahimi *et al*. (2011); Khan *et al*. (2012); Hossain *et al*. (2014); Kharde *et al*. (2014); Puvača *et al*. (2014); Huda *et al*. (2015)
10, 20 and 40mg/kg/day essential oils	Reduced *Escherichia coli*, *Salmonella* and *Shigella* species of bacteria	Dieumou et al. (2009)
0.1% dose	Reduced *Escherichia coli* in the digesta of ileo-cecum	Rahimi *et al*. (2011)

2.6. Ginger (*Zingiber officinale* R.)

Ginger is an important plant with several medicinal, ethno medicinal and nutritional values (Kumar *et al*., 2011). Ginger is the underground rhizome of the ginger plant with a firm, striated texture. *Zingiber officinale* R., commonly known as ginger belongs to family *Zingiberaceae* (Weiss, 1997; Mc Gee, 2004).

2.6.1. Ginger production in Ethiopia

The major ginger growing area in Ethiopia includes wetter regions at altitude below 2000 m in Kefa, Illubabur, Gamo Gofa, Sidama, Wellega, Wolaita, and Kembata-Tambaro. Currently, it has become an important cash crop for farmers in southern and south-western parts of Ethiopia. The production of this spice has been expanding in most parts of the country, as it can be grown under varied climatic conditions. It thrives well in areas with altitudes from sea level to 1500 m, mean annual temperature of 20–32°C and with total rainfall greater than 1200 mm. Well-drained, fertile and friable soil with enough humus and neutral pH is the ideal soil type for the production of ginger (Hailemicheal *et al*., 2008; Asfaw and Demissew, 2009).

2.6.2. Nutrient composition of ginger

Ginger is widely used in a variety of foods because of its nutritional composition and flavoring compounds (Jyotsna *et al*., 2017). The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. The composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Govindarajan, 1982).

2.6.3. Medicinal values of ginger

In the fresh ginger rhizome, the gingerols were identified as the major active components (Hoffman, 2007). The sensory perception of ginger arises from two distinct groups of chemicals, namely volatile oils and non-volatile pungent compounds. The volatile oil components in ginger consists, mainly of sesquiterpene hydrocarbons, predominantly

zingeberene (35%), curcumene (18%) and farnesene (10%) (Govindarajan, 1982). Many of these volatile oil constituents contribute to the distinct aroma and taste of ginger. Non-volatile pungent compounds include gingerols, shogaols, paradols and zingerone that produce a 'hot' sensation in the mouth (Govindarajan, 1982).

Ginger extract also contains polyphenol compounds (6-gingerol and its derivatives) which possess high antioxidant activity. Although the digestion stimulating effect of this spice become known a long time ago, the stimulating effect on peptic juices, such as gastric juice, bile, pancreatic and intestinal juices was discovered later. Bile acids play a major role in the uptake of fats and each upset in the metabolism of fats would hinder food digestion as a whole, because the fatty particles cover the other food elements and make them inaccessible for the action of the digestive enzymes. Lipase is the other key factor which plays a vital role in fat digestion. When ginger was included in animal diets; it was found that there was a considerable increase in the pancreatic and intestine lipase (Platel and Srinivasan, 2000). Some of the medicinal values of ginger are explained below.

2.6.3.1. Antioxidant property of ginger

Ginger roots' extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity. Antioxidant activity is due to the presence of flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins (Stoilova *et al*., 2007). Antioxidant property of ginger is an extremely significant activity which can be used as a preventive agent against a number of diseases.

2.6.3.2. Antimicrobial property of ginger

Ginger has been traditionally exploited for having broad range of antimicrobial activities against both gram positive and gram negative bacteria and fungi. *In vitro* studies have shown that active constituents of ginger inhibit multiplication of colon bacteria. These bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted

with ginger (Gupta and Ravishankar, 2005). It inhibits the growth of *Escherichia coli*, *Proteus sp.*, *Staphylococci*, *Streptococci* and *Salmonella* (White, 2007).

2.6.3.3. Effects on serum biochemical constituents

It was reported that 6-gingerol and 6-shogaol are the major Gingerol and Shogaol present in the rhizome (Comell and McLachlan, 1972). Sharma and Shukla (1997), reported a significant blood glucose lowering effect of ginger juice in diabetic and non-diabetic animals. In addition, Ahmed and Sharma (1997), reported a significant hypoglycemic activity in rats after administration of ginger extract. Furthermore, Bhandari and Grover (1998), reported the blood glucose and blood urea were lowered after administration of ethanolic extract of ginger in diabetic rats. Ginger acts as a hypolipidemic agent in cholesterol-fed rabbits (Bhandari and Grover, 1998). Akhani *et al*. (2004) reported that ginger treatment significantly lowered both serum cholesterol and triglycerides. In addition, Fuhrman *et al*. (2000) reported that ginger decreased LDL-cholesterol, very low density lipoprotein (VLDL)-cholesterol and triglycerides levels in apolipoprotein-E deficient mice. Bhandari *et al*. (2005) found that, the ethanolic extract of ginger significantly reduced serum total cholesterol and triglycerides and increased the high density lipoprotein - cholesterol levels; also, the extract can protect tissues from lipid peroxidation and exhibit a significant lipid lowering activity in diabetic rats. The study of Gujaral *et al*. (1978) revealed that serum and liver cholesterol decreased when ginger was administered to hypercholesterolemic rats.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)/Glutamic Pyruvic Transaminase (GPT) are cytosolic enzymes which are highly concentrated in the liver and kidney and are only found in significant quantities in the serum when the cell membrane becomes leaky and even completely ruptured (Ngaha, 1981). Serum AST and ALT are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage (Naik and Panda, 2007). The presence of AST and ALT in the serum is an index of damage or injury to the organs. Ajayi, (2011) concluded that consumption of GIP has beneficial effect on hepatic functions of rats fed on

hypercholesterolemic diet. Biologically active compounds from ginger and their pharmacological effects is presented in the following Table (Table 6).

Table 6. Biologically active compounds from ginger and their pharmacological effects

Active compound	Pharmacological effects	References
Gingerol and gingerol related compound	Antioxidant, anti-tumor, anti-inflammatory activity, anti-microbial activity and hepato-protective activity	Masuda *et al*. (2004); Park *et al*. (2006); Plengsuriyakarn *et al*. (2012); Hu *et al*. (2012); Kim *et al*. (2011)
Paradol	Anti-oxidant, anti-cancerous and anti-microbial activity	Chung *et al*. (2001); Suresh *et al*. (2010); Galal *et al*. (1996);
Shogoal	Anti-oxidant and anti-inflammatory activity	Park *et al*. (2006); Choudhury *et al*. (2010)
Zingerone	Antioxidant, anti-inflammatory and anti-bacterial activity	Shin *et al*. (2005); Aeschbach *et al*. (1994)
Zerumbone	Anti-tumor and anti-microbial activity	Kirana *et al*. (2003); Abdul *et al*. (2008)
1-Dehydro-(10) gingerdione	Regulation of inflammatory genes	Lee *et al*. (2012)
Terpenoids	Induce apoptosis by activation of p53. ↓cancer	Liu *et al*. (2012)
Ginger flavonoids	Antioxidant activity	Rahman *et al*. (2011)

2.6.4. Using ginger as a poultry feed additive

2.6.4.1. Effects on broiler performance

The very minute amounts of ginger had a very strong anti-lipidemic effect on serum cholesterol and triglycerides plus it had also a positive effects on FI, BWG and FCR, improves feed digestion and stimulate enzymes secretion (Oleforuh-Okoleh *et al.*, 2014). Onimisi *et al.* (2005) and Ademola *et al.* (2009) reported that ginger supplementation to the broiler diets can increase body weight when supplemented up to 2% level. Scholars (Tollba, 2003; Herawati, 2006; Moorthy *et al*., 2009 and Herawati, 2010) also illustrated that birds fed with diets containing ginger up to 2% recorded better FCR than un-supplemented one. Al-Homidan (2005), found an improvement in weight gain of broilers when they were fed 2 and 6% ginger. Ademola *et al*. (2004) reported that inclusion of ginger at a level of 5.0 g/kg improved growth performance of broilers and the improvement rate slightly decreased at higher levels of 10 and 15 g/kg. Similarly Sadeghi *et al.* (2013) suggested that growth performance of broilers may positively respond to ginger inclusion at the level of 5.0 g/kg and over this level; it had negative effect on BWG. Ahmed *et al*. (2014) concluded that diets containing GIP had positive effects on the FBW, FI, BWG and FCR of broilers. Based on feed efficiency, best performance of broilers was observed in diet supplemented with 0.75 g GIP for 1 kg followed by 1.75 g for 1 kg diet as per NRC standard (control) (Ahmed *et al*., 2014). A study by the same authors also showed that the highest FI was obtained by the birds fed 1% GIP during second, third, fourth and fifth weeks compared with other levels of ginger (0%, 0.5% and 0.75%). According to Oleforuh-Okoleh (2014), birds fed on ginger in powder form showed a significant improvement on their daily BWG and daily FI than those fed the dietary treatment through infusion. Javed *et al.* (2009) reported that broiler chickens given aqueous extract of ginger showed an improved BWG. Zomrawi *et al*. (2013) also indicated that FI was significantly decreased for birds fed 1.5% and 2% GIP, respectively, whereas, BWG and FCR were not affected by the dietary inclusions of GIP. Based on a study by Zomrawi *et al*. (2013), the highest slaughter weight was recorded for

the birds that received 1% GIP with the lowest for the group fed 2% GIP. Birds fed 1% GIP also achieved higher dressing percentage.

Unlike the above mentioned significant performance improvements achieved through inclusion of GIP in broiler diets, few works indicate rather a non-significant effect or a rather reduced performance response to this herbal feed additive. Wafaa *et al*. (2012) reported the lack of a significant effect in FCR among treatments containing 0.5%, 1% and 1.5% GIP. El-Deek *et al*. (2002) observed that diet containing 1.0 g/kg of ginger did not affect the growth performance. In contrast, Al-Homidan (2005), observed reduced growth rate of starter broilers (1 to 4 week) when ginger was fed at the rates of 2% and 6% and similar findings were also reported by Ademola *et al*. (2009) who included 2% ginger in the diet of broilers.

2.6.4.2. Effects on carcass traits

Researchers (Alcicek *et al*., 2004; Tollba *et al*., 2007; Ademola *et al.*, 2009 and Javed *et al*., 2009) indicated that, carcass characteristic improved in broilers fed different levels of powder or aqueous extract of ginger from 1-42 days of age. Addition of ginger and its essential oils to broiler diets as growth promoters significantly reduced the abdominal fat of chickens (Rafiee *et al*., 2013; Valiollahi *et al*., 2013). In addition to the above, Ademola *et al*. (2009) also stated that ginger could be used as anti-lipidemic agents in broiler diets to lower abdominal fat pad. Also, Herawati and Marjuki (2011), reported a reduction in the fat content of broilers carcass fed ginger. According to Zomrawi *et al.* (2013), higher dressing percentage was achieved using 1% GIP in broiler diet.

2.6.4.3. Effects on serum biochemical constituents

It is obvious that ginger had a significant effects on performance and many blood serum traits (Arkan *et al.,* 2012). The hypolipidemic action of ginger supplementation can be used to lower risk factor of the cardiovascular diseases and cancer either in animals or human (Ademola *et al*., 2009). The supplementation of ginger reduced cholesterol levels

in blood serum because of its antioxidative action which is also a mechanism that could be used as anti-stress approach (Jang *et al*., 2007). The hypocholestrolemic action may be done by ginger acting as a potential inhibitor of cholesterol synthesis (Said *et al.*, 2010). Similarly, Arkan *et al.* (2012) showed that ginger have a positive effects on broilers' performance and lowering effects on blood serum cholesterol, triglycerides and glucose, which can refer to strong anti-oxidative action and potential anti-stress action.

According to Arkan *et al.* (2012) birds fed with 0.1% ginger and 0.2% ginger scored the lowest serum glucose, triglycerides and cholesterol compared with birds kept on control diets, while, ginger supplementation didn't affect the total blood protein. Al-Homidan (2005) and Ademola *et al.* (2009) reported a lower blood serum glucose and cholesterol while feeding broiler chickens with ration which contained up to 6% ginger. Wafaa *et al.* (2012) pointed that feeding broiler chickens with GIP at levels 0.5% and 1% lowered serum cholesterol levels. The same author also pointed that feeding broiler chickens with GIP at levels of 0.5% and 1% significantly lowered serum cholesterol levels. Zomrawi *et al*. (2013) showed that GIP at level of 2% significantly lowered serum cholesterol, glucose and protein concentration but it had adverse effects on performance and blood constituents and concluded that broiler chickens can tolerate up to 1.5% GIP in the diet without adverse effects on performance and blood constituents.

Ginger and garlic in broiler chickens diets have been recognized for their strong stimulating effects on the immune and digestive systems in birds (Horton *et al*., 1991; Gardzielewska *et al*., 2003). Response of broilers to different inclusion levels of ginger in the diet is summarized in Table seven. The variations between the results of many researchers could be attributed to differences in the source and composition of mushroom, garlic and ginger used, preparation process, feed inclusion levels, the overall diet composition, breed/strain of bird used in the study and environmental stress factors (Sadeghi *et al*., 2013; Oleforuh-Okoleh, 2014). Method of administration of the feed additives is also another factor affecting the performance of the birds. Although feeding of garlic and ginger in powder form and through water-based infusion significantly affected all the growth performance traits as studied by Oleforuh-Okoleh *et al*. (2014),

birds fed the test ingredients in powder form (1.4%) weighed significantly heavier and consumed more feed than those fed through water based infusion. The dressing percentage was also significantly increased and abdominal fat was significantly reduced when ginger and garlic were fed in powder form.

Table 7. Summary of response of broilers to dietary inclusion of different levels of ginger as phytogenics

Dietary dose of ginger (%)	Treatment effects	References
2%	Increased body weight	Onimisi *et al.*(2005) and Ademola *et al.* (2009)
2% and 6%	Increased weight gain	Al-Homidan (2005)
2%	Better FCR	Tollba (2003); Onimisi *et al*. (2005) ; Moorthy *et al*. (2009) and Herawati (2010)
0.5%	Improved growth performance	Sadeghi *et al.* (2013)
1.4%	Higher FI, BWG, dressing percentage, reduced abdominal fat and lower FCR	Oleforuh-Okoleh *et al.* (2014)
2%	Higher final body weight	Herawati (2010)
0.75%	Improved feed efficiency, highest mean weekly BWG and economical	Ahmed *et al*. (2014)
1%	Improved performance, higher slaughter weight and dressing percentage	Zomrawi *et al*. (2013)
0.5%, 1% and 1.5%	Slightly improved growth performance (safe up to 3%)	Ademola *et al*. (2004)
2%	Significantly lower FI and better FCR	Herawati (2010)
0.1% and 0.2%	Lower FI, Higher FBW, better BWG, best FCR, Lowest serum glucose, triglycerides and cholesterol level	Arkan *et al.* (2012)

2.7. Synergetic and Complementary Effects of Feed Additive Mixtures

Synergism occurs when a mixture of two or more compounds produces a greater response than expected (i.e., greater than the sum of their individual effects) (James and William, 2009). Ademola *et al.* (2009) reported that mixtures of garlic (1% and 1.5%) and ginger (0.25 and 0.5%) in broiler diets enabled broiler chickens to achieve maximum final live weights as well as lower triacylglycerol than sole inclusion of dietary garlic and ginger. Based on the findings of Daneshmand *et al.* (2012), it is reasonable to observe that a combination garlic (30 g/kg), oyster mushroom (2 g/kg) and propolis extract (0.2 g/kg) could lower serum total cholesterol. According to Ademola *et al.* (2012), mixtures of garlic and ginger also displayed performance enhancing effects as demonstrated in the growth rate of pullet growers, final live weight, hen day production and egg weight of laying hens. Ahmed and Sharma (1997) reported that the growth rate of rats fed mixture of garlic (2%) and ginger (0.5%) was significantly higher than those fed sole inclusion of dietary garlic and ginger. It had earlier been reported that the mixtures of garlic and ginger significantly promoted the growth of broiler chickens by enhancing digestion of feed nutrients and probably improved feed absorption through the wall of GIT (Ademola *et al.*, 2007). Similarly, Ademola *et al*. (2009) also reported that mixtures of garlic and ginger significantly improved the growth of the broiler chickens than garlic and ginger as sole agent in broiler diets.

In addition to the above, Onu (2010) observed a numerical improvements in weights of birds fed garlic and ginger mixtures (combination of 0.25% garlic and ginger) in comparison with control group. The best FCR was recorded by the diet with 1.75% mixture level of GAP and GIP. The improvement in FCR can be attributed to the antibacterial properties of both GAP and GIP, which resulted in a better absorption of the nutrients in the gut and finally leading to improvement in FCR. The same author also indicated that, birds fed with the (1.75%) GAP and GIP mixture level showed the highest hot and cold percentages in comparison with control group. The supplementation of a mixture of garlic and GIP at level 1.75% enhanced growth performance and meat quality

of broiler chickens (Safa, 2014a). The following Table (Table eight) summarizes the effects of inclusion of different mixtures of mushroom, garlic and ginger in broiler diets.

Table 8. Summary of response of broilers and layers to dietary inclusion of different mixtures of mushroom, garlic and ginger

Dietary dose of mixtures	Treatment effects	References
1.75% GAP and 1.75% GIP	Decreased FCR	Onu (2010)
1.75% GAP and 1.75% GIP	Enhanced growth performance and meat quality	Safa (2014a)
Combination of GAP (3%), OMP (2%) and propolis extract (0.2%)	Decreased serum cholesterol	Daneshmand *et al*. (2012)
0.5% GAP and 0.5% GIP	Increased FBW in laying hens	Ademola *et al.* (2012)
1% GAP and 0.5% GIP	Decreased serum total cholesterol, triacylglycerol and LDL-cholesterol in laying hens	Ademola *et al.* (2012)
1% GAP and 0.5% GIP	Heaviest FBW, lowest serum total cholesterol and triacylglycerol	Ademola *et al*. (2009)
1.5% GAP and 0.25% GIP	Increased hot and cold dressing percentages and share of commercial cuts, decreased abdominal fat percentage	Safa (2014a)

2.8. Implication of Natural Feed Additives on Economics of Broiler Production

A study by Oleforuh-Okoleh *et al*. (2014) indicated that birds given GAP mixed in the feed at 1.4% of basal diet recorded the highest revenue and net return compared to the control and the same level of inclusion of ginger. A lot of research findings also indicated the economic feasibility of incorporation of phytogenic feed additives in broiler diets. Generally, cost of production and net profit per broiler determine the fate of broiler

productivity (Farooq *et al.*, 2001). The return-on-investment for phytogenic feed additives will depend on both the biological impact and the actual market price. It must be taken into account the fact that the feed cost of these alternatives is quite variable. Generally the net economic effect will depend on several factors, including the effects on performance levels and the cost of any technologies adopted to compensate for the termination of AGPs, and may be offset by the benefits of increased consumer confidence (Sadeghi *et al*., 2013).

3. MATERIALS AND METHODS

The study involved two phases of experimental trials, where the first phase dealt with evaluation of the three feed additives, each at two inclusion levels in comparison with a control diet, whereas, the second phase dealt with evaluation of different combinations of the three herbal feed additives (against antibiotic growth promoter) which were formulated based on the results of the first trial.

3.1. Phase I: Response of Broiler Chickens to Diets with Different Levels of Oyster Mushroom, Garlic and Ginger Powders

3.1.1. Experimental site

The study was conducted at the College of Veterinary Medicine and Agriculture's poultry farm in Bishoftu. Bishoftu is situated at 47 km southeast of Addis Ababa at an altitude of 1900 m above sea level, latitude of 8 ^{o}44`N and longitude of 38$^{o}$ 57`E. The average (25 years) annual rainfall was 851mm with an average minimum and maximum temperature of 8.9^{o}C and 28.3^{o}C (mean 19 ^{o}C), respectively. The average relative humidity was 58.6 percent (EIAR, 2016).

3.1.2. Feed ingredients and experimental treatments

The feed ingredients used in the formulation of the experimental diet were corn grain (CG), noug seed cake (NSC), soybean meal (SBM), wheat bran (WB), broiler premix, limestone, salt, DL-methionine, L-lysine HCl, OMP, GAP and GIP. Dried OM was supplied by Menagesha integrated organic farm PLC which was later ground to fine powder. Matured garlic bulbs and ginger rhizomes were collected from vegetable markets in Bishoftu town. The collected garlic was cleaned and the cloves removed manually, followed by the seed coat and the inner seeds were sliced. The sliced seeds were sun dried for three days before grading. The ginger rinds were peeled off using knife. The peeled ginger was washed and sun dried for three days, and later ground to fine powder. The dried materials were stored under natural conditions.

3.1.3. Laboratory analysis of feed samples

Chemical analyses of major feed ingredients and additives were done at the National Veterinary Institute in the nutrition and biochemistry laboratory (Bishoftu). Chemical composition of the major feed ingredients and additives was determined from representative samples of CG, NSC, SBM, WB, OMP, GAP and GIP (Table nine). Samples were analyzed for dry matter, crude fiber (CF), total ash (TA), ether extract (EE) and Kjeldahl nitrogen (N) (AOAC, 1998). The crude protein (CP) content of each of the ingredients was determined as N x 6.25. Calcium and total phosphorus contents were determined by atomic absorption and vanado-molybdate method, respectively (AOAC, 1998). The metabolizable energy values (ME) were calculated indirectly from the EE, CF and ash adopting the equation proposed by Wiseman (1987), as:-

ME (Kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.80 Ash.

Table 9. Chemical composition of feed ingredients and additives used (% DM basis)

Chemical composition	Ingredients						
	CG	SBM	NSC	WB	OMP	GAP	GIP
DM %	90.33	94.17	92.99	89.83	91.80	92.07	90.73
Ash %	1.40	6.34	7.50	3.97	7.84	3.66	6.65
CF %	4.62	5.95	16.03	7.24	9.80	1.30	8.38
CP %	6.88	37.37	36.79	11.03	20.51	7.67	7.78
EE %	2.40	12.55	8.16	2.93	1.28	0.52	6.13
Ca %	1.66	2.12	1.44	1.29	1.27	1.27	1.29
P %	0.30	0.65	0.65	1.11	0.75	0.10	0.12
ME (kcal/kg DM)	3300.0	3847.8	2666.7	3306.6	2831.3	3714.6	3270.1

** Nutrient per DM percentage; DM = Dry Matter; CF = Crude Fiber; CP = Crude Protein; EE = Ether Extract; ME = Metabolisable Energy; CG = Corn Grain; SBM = Soybean Meal; NSC = Noug Seed Cake; WB = Wheat Bran; OMP = Oyster Mushroom Powder; GAP = Garlic Powder; GIP = Ginger Powder.*

Broiler starters' (1 to 28 days of age) and finishers' (29 to 49 days of age) diets were formulated on the basis of the results of the laboratory analysis of the individual

ingredients using a least cost feed formulation software (*feed win*). Control diets were formulated to meet nutrient requirements according to the NRC (1994) for broiler chickens and the other six diets were prepared separately using the same ingredients as in the control diet and feed additives incorporated on top of the basal diets and thoroughly mixed. The ingredients and calculated nutrient composition of the basal diet are presented in Table 10 below.

Table 10. Ingredients and nutrient composition of basal diets (%)

Ingredients	Starter diet (0-28 days)	Finisher diet (29-49 days)
Corn grain	36	39
Noug seed cake	27	21
Soybean meal	20.8	20
Wheat bran	14	18
Broiler premix[1]	1	1
Limestone	0.5	0.5
Salt	0.3	0.3
Methionine	0.2	0.1
Lysine	0.2	0.1
Total	100	100
Calculated nutrients content		
DM %	91.94	91.72
CF %	8.24	7.66
CP %	22.03	20.02
Ca %	1.59	1.54
P %	0.57	0.58
ME (kcal/kg DM)	3183.3	3217.7

* Nutrient per DM percentage [1]Broiler premix 1% per kg contains: Vitamins: Vitamin A, 200 000 IU; Vitamin D_3, 1 000 mg; Vitamin E, 225 mg; Vitamin K3, 125 mg; vitamin B_1, 500 mg; vitamin B_2, 1375 mg; vitamin B_3, 1250mg; vitamin B_6, 2 mg; vitamin B_{12}, 4000 mg; Vitamin PP (niacin), 100 mg; folic acid, 37500 mg;. Trace elements: Iron, 0.4%; Copper,0.05%; Manganese, 0.6%; Zinc,0.7%; Selenium, 0.004 %; Minerals: Calcium, 29.7%. Other Additives: Anti-oxidant (BHT, Ethoxyquin, propyl Gallate) 0.05%.

These basal diets (either starter or finisher) were considered as controls for both phase I and phase II. Varyingly leveled tested feed additives were added on controls to make dietary treatments.

The treatments for both starter and finisher were designed as:

T_1: Basal diet only (control group)
T_2: Basal diet + 1% OMP
T_3: Basal diet + 2% OMP
T_4: Basal diet + 1% GAP
T_5: Basal diet + 2% GAP
T_6: Basal diet + 1% GIP
T_7: Basal diet + 2% GIP

Note: Basal diet is either starter or finisher.

3.1.4. Management of experimental birds

A total of 315 unsexed commercial hybrid day-old broiler chicks of Cobb 500 strain, which were received from Alema farm-Koudijs Feed P.L.C., with initial body weight of 40.8 ± 0.76 (mean ± SD) were randomly divided into seven dietary treatments and three replications per treatment in a CRD with 15 chicks per replicate or pen. A floor system housing, each with a dimension of 1 * 1.5 m was prepared. The chicks were penned at a stocking density of 10 chicks per m^2. Chicks were vaccinated against New Castle Disease (HB1 on day 1 and Lasota at day 21 through ocular routes) and Infectious Bursal Disease (Gumboro) at day 14 and day 28 with drinking water. Before placing the experimental birds into the pens, the whole unit was cleaned, disinfected and littered with properly dried and disinfected *teff* straw. The wet litter was changed with dry, disinfected, and clean teff straw whenever necessary. A plastic drinker and a hanging type plastic feeder were placed in each experimental pen after being thoroughly cleaned and disinfected. Other health precautions and sanitary measures were also taken throughout the study period. A 200 W lamp was used for each unit as a source of heat and light with gradual

height adjustment. Feed and clean tap water were offered *ad libitum* throughout the experimental period. The number of dead birds encountered was recorded and the cause of death was identified by veterinarian throughout the experimental period.

3.1.5. Measurements

3.1.5.1. Performance evaluation

Feed was weighed every morning, using a sensitive balance with a sensitivity of 0.01g and offered to the respective group. Feed refusals were collected every morning and weighed and FI was calculated as the difference between offers and refusals for each replication. Birds were weighed at the beginning of the experiment and then weekly in a group per pen using sensitive balance and pen average was calculated. Body weight gain was calculated as the difference between the final and initial body weights. Average daily gain (ADG) was calculated as the ratio of BWG to the number of experimental days. Feed conversion ratio was computed as the ratio of daily feed consumption to ADG.

3.1.5.2. Carcass evaluation

At the end of the experiment, two birds were purposely selected from each replication based on average group weight for carcass evaluation. The birds were starved for overnight (except for water), weighed individually and leg-banded for identification immediately before slaughtering. After taking the slaughter weight measurement data, each bird was killed and bled hanging from a bleeding cone for about three minutes. Following bleeding, the birds were de-feathered, manually after scalding in hot water for approximately two minutes. Dressed and eviscerated weights were calculated following the method of FAO (2001): Dressed carcass weight was measured after removal of blood and feather. Dressing percentage was calculated as the proportion of dressed carcass weight to slaughter weight multiplied by one hundred. Eviscerated carcass weight was determined after removing blood, feather, kidney, lungs, pancreas, crop, proventriculus, small intestine, large intestine, caeca and urogenital tracts from dressed carcass. Eviscerated percentage was determined as the proportion of the eviscerated weight to

slaughter weight multiplied by one hundred. From eviscerated carcass drumsticks with thighs, breast meat, wings, neck and back were separated and weighed and their weights were divided by slaughter weight and multiplied by 100 to determine percentage weight of each component. The commercial carcass yield was calculated by including drumsticks with thighs, wings, breast, back and neck. The edible carcass yield was calculated by including the edible offal to the commercial carcass parts considering the common practices in Ethiopia.

The different values for the measurements of interest were computed employing the following formulae (FAO, 2001):

Dressed weight = Drumsticks with thighs + Wings + Breast + Back + Neck + Heart + Liver + Gizzard + Feet + Head + Viscera (inedible offal)
Eviscerated weight = Dressed weight - Viscera (inedible offal)
Commercial carcass weight (**Ready-to-cook) =** Drumsticks with thighs + Wings + Breast + Back + Neck
Edible carcass yield = Commercial carcass weight + Heart + Liver + Gizzard (Edible offal)

Fat around the proventriculus and gizzard and against the abdominal wall and the cloacae was collected and weighed using sensitive balance to the nearest 0.01 g and its percentage was calculated as the proportion of slaughter weight multiplied by 100**.** The edible offal (giblets) which includes the heart, gizzard and liver and also the inedible offal was weighed and expressed in relation to slaughter weight.

3.1.5.3. Hematological evaluation

Blood collection was carried out at the 7^{th} week of the experiment for the determination of blood hematological and biochemical parameters. Two birds per replicate were randomly selected and bled via wing veins, using a 5 ml sterile disposable syringe of 22 gauge for both hematological and biochemical evaluation. About 2 ml of blood was collected in to bottle tubes containing ethylenediaminetetraacetic acid, for each replicate.

The RBC and TWBC counts were counted using hemocytometer (Schalm *et al.*, 1975). The PCV was determined by microhematocrit method (Schalm *et al.*, 1975). The Hb concentration was measured by Sahli's method (Sahli's, 1905). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated from Hb, PCV and erythrocyte concentration of blood (RBC) (Jain, 1986). MCV is the expression of the average volume of individual erythrocytes calculated with the following formula:

$$MCV = \frac{PCV(\%)}{RBC} \text{ X } 10$$

MCH is the expression of the average Hb content of a single erythrocytes calculated with the following formula:

$$MCH = \frac{Hb\ (g/dL)}{RBC} \text{ X } 10$$

MCHC is the expression of the volume within the erythrocytes occupied by the hemoglobin and is calculated with the following formula:

$$MCHC = \frac{Hb\ (g/dL)}{PCV\ (\%)} X100$$

3.1.5.4. Biochemical evaluation

About 2 ml of blood was collected into plain bottles (i.e. without anticoagulant) for serum separation. Serum was obtained by centrifugation and the serum samples were stored in a deep freezer at (-20^{0}C) until analyzed. Serum total protein and albumin were determined according to Doumas (1971); Witt and Trendelenburg (1982). Globulin concentration was calculated as the difference between total protein and albumin, and then the ratio of albumin/globulin was also calculated. Serum glucose was determined using glucose oxidase method prescribed by Trinder (1969). Total cholesterol (TC) was determined

according to Watson (1960). ALT and AST follows the proposed optimized formulation of the international federation of clinical chemistry (Bergmeyer *et al*., 1986), while, measurement of alkaline phosphatase (ALP) follows the GSCC (1972). Sample analysis was conducted using the instrument HumaStar 80 automated chemistry analyser (HUMAN Gesellschaft fur biochemical und Diagnostica GmbH, Germany). The instrument was calibrated using calibrator (Autocal) and quality control samples normal (Humatrtol N) and pathological (Humatrol P) were run for validation before running samples for tests.

3.1.5.5. Economic appraisal

The following parameters were evaluated to estimate the economics of production: the costs (cost/kg) for the different diets at the starter, finisher and entire period of feeding were considered. The cost per kg of weight gain was calculated according to the procedures of Sonaiya *et al*. (1986) and Ukachukwu and Anugwa (1995), which involved taking the product of cost per kg of feed and feed-to-gain ratio of birds. The average selling prices of broilers was obtained by using the average carcass weight of birds multiplied by the price of broiler carcass in the supermarkets located in Bishoftu town on selling time. Total income (TI) generated/bird, net returns (NR)/bird, economic efficiency (ECE) {(NR/total cost of production)*100} were computed. Relative economic efficiency (%) (REE) was also calculated with the formula {(ECE of treatment/EE of control)*100}. The economic analysis was made assuming that purchasing price of birds, labor and other expenses are constant at Birr 26.1/bird. Income was based on Birr 90/kg of carcass (supermarket) and Ethiopian currency (Birr) 22.5 was equivalent to 1 USD at the time of the research.

3.2. Phase II: Response of Broiler Chickens to Diets with Different Mixtures of Oyster Mushroom, Garlic and Ginger Powders as Substitutes for Antibiotic Growth Promoter

3.2.1. Feed ingredients and experimental treatments

The feed ingredients used in the formulation of the different treatments and preparation of the test ingredients were similar to the first experiment. The broad spectrum antibiotic oxytetracycline powder which is commonly used in the area, was purchased from the local veterinary drug store. The antibiotic was thoroughly mixed with the basal diet (positive control) using wheat bran as a carrier based on the levels described in the producers manual. Preliminarily feed additives with determined amount of combinations of any of the three test ingredients were prepared and mixed thoroughly. The five diets were prepared separately using the same ingredients as in the control diet and later mixtures of feed additives incorporated on top of the basal diets and thoroughly mixed. The levels of inclusions of each of the three test ingredients in the mixtures was determined from the results of the first trial. The lowest inclusion level of OMP (1%) was chosen following the negative response of birds to the OMP containing diet in the first trial interms of growth performance and EE. Since inclusion of 2% of GAP and GIP did not negatively affect the performance characteristics of bird, this treatment level was chosen as the basis to formulate the mixtures. To minimize the cost of the starter and finisher diets, the mixtures were combined using half of the chosen levels of the additives for T_3, T_4 and T_5. Likewise, for T_7, the three additives were mixed using one third of the chosen inclusion levels in order to maintain the proportion of each ingredients and minimize the cost of the feed.

The treatments for both starter and finisher were designed as:

T_1: Basal diet only (negative control)
T_2: Basal diet + 0.30 g oxytetracycline/kg (positive control)
T_3: Basal diet + 0.5% OMP + 1% GAP
T_4: Basal diet + 0.5% OMP + 1% GIP
T_5: Basal diet + 1% GAP + 1% GIP
T_6: Basal diet + 0.33% OMP + 0.66% GAP + 0.66% GIP

Note: Basal diet is either starter or finisher.

3.2.2. Management of experimental birds

A total of 270 unsexed commercial hybrid day-old broiler chicks of Cobb 500 strain with initial body weight of 38.9 ± 0.52 (mean ± SD) were randomly divided into six dietary treatments and three replications per treatment in a CRD with 15 chicks per replicate or pen. All the other management practices are similar as mentioned in "3.1.3" above.

3.2.3. Measurements

Methods followed and data collection procedures were the same as described in the first experiment in aspects of performance characteristics, carcass characteristics, hematological parameters, biochemical parameters, and economic appraisal.

Cecum microbial load determination

To measure microbial load, collection of samples of intestinal content was carried out from the same birds used for carcass evaluation. Starting from the Meckel's diverticulum to 4 cm above the ileo-cecal junction was quickly dissected and the cecal contents of each bird pooled on universal bottle placed on icebox and transport to National Veterinary Institute 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 was poured on Eosin Methylene Blue agar for *Escherichia coli* bacteria counts, 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 after being incubated at 37 °C for 24 h (Cheesbrough, 1985). Then plates were counted between 24 and 48 h after inoculation (Cheesbrough, 1985; Collee *et al.,* 1989). The counted coliform forming bacteria values were transformed to $\log^{10}$ cfu/ml.

3.3. Statistical Analysis

The data collected were analyzed using the general linear model procedures of Statistical Analysis Systems software (SAS, 2002). Duncan's multiple range test was used to detect the differences among the treatment means and significance was considered at $p<0.05$ (Duncan, 1955).

The model used was:

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

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

μ = overall mean

α_i = the i^{th} treatment effect

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

4. RESULTS

4.1. Phase I: Response of Broiler Chickens to Diets with Different Levels of Oyster Mushroom, Garlic and Ginger Powders

4.1.1 Performance

Average FI, BWG and FCR of broiler chickens fed with 1% and 2% levels of oyster mushroom, garlic and ginger are presented in Table 11. Compared to the control diet, inclusion of different levels of natural feed additives did not significantly ($P>0.05$) affect FI during the starter phase. Among treatment feeds containing feed additives, birds kept on T_5 consumed significantly ($P<0.05$) lower amount of feed in as comparison to those kept on T_2. During the finisher phase, birds kept on T_7 had significantly ($P<0.05$) higher FI as compared to T_1 ($P<0.05$). On the contrary, birds kept on T_2 and T_3 had significantly ($P<0.05$) lower FI compared to T_6 and T_7 ($P<0.05$). Average FI for the entire experiment was significantly higher ($P<0.05$) for T_7 as compared to T_1, T_2, T_3 and T_5. Results of FBW, BWG and ADG during the starter phase were not significantly ($P>0.05$) affected by the additives. During the finisher phase, birds fed on the higher level of OMP had significantly ($P<0.05$) lower BWG and FBW compared to all other treatments except, T_2 and T_4. The recorded BWG was significantly lower ($P<0.05$) for T_3 compared to T_1, T_4, T_6 and T_7 during the finisher phase. Birds kept on T_3 had significantly ($P<0.05$) lower BWG and ADG during the entire experimental period compared to all the other treatments except T_2 and T_4. Birds kept on T_3 had significantly ($P<0.05$) higher FCR compared to T_5 and T_6 during the first four weeks of the trial whereas no significant difference ($P>0.05$) was observed among the other treatments. Similarly, during the overall experimental period, T_3 had the highest FCR ($P>0.05$).

Table 11. The effects of feeding different levels of oyster mushroom, garlic and ginger on performance of broilers during the starter and finisher phase and the entire experiment

Treatments	Parameters														
	FI (g/bird)			IBW	FBW (g/bird)		BWG (g/bird)			ADG (g/day)			FCR (g FI/g BWG)		
	0-28 Days	29-49 Days	0-49 Days		28th Day	49th Day	0-28 Days	29-49 Days	0-49 Days	0-28 Days	29-49 Days	0-49 Days	0-28 Days	29-49 Days	0-49 Days
T_1	1874^{ab}	3372^{bc}	5246^{bc}	40.9	870	2027^{a}	830	1156^{a}	1986^{a}	29.6	55.1^{a}	40.5^{a}	2.26^{ab}	2.92	2.64
T_2	1918^{a}	3261^{c}	5179^{c}	40.2	869	1950^{ab}	829	1081^{ab}	1910^{ab}	29.6	51.5^{ab}	39.0^{ab}	2.31^{ab}	3.02	2.71
T_3	1896^{ab}	3302^{c}	5198^{c}	41.1	833	1878^{b}	792	1045^{b}	1837^{b}	28.3	49.8^{b}	37.5^{b}	2.40^{a}	3.16	2.83
T_4	1889^{ab}	3435^{abc}	5324^{abc}	40.7	867	2014^{ab}	826	1147^{a}	1973^{ab}	29.5	54.6^{a}	40.3^{ab}	2.29^{ab}	2.99	2.70
T_5	1808^{b}	3419^{abc}	5227^{bc}	40.9	879	2025^{a}	838	1146^{ab}	1984^{a}	29.9	54.6^{ab}	40.5^{a}	2.16^{b}	2.98	2.64
T_6	1879^{ab}	3582^{ab}	5461^{ab}	41.1	886	2063^{a}	845	1177^{a}	2022^{a}	30.2	$56.^{a}$	41.3^{a}	2.22^{b}	3.07	2.71
T_7	1882^{ab}	3617^{a}	5499^{a}	41.1	878	2053^{a}	837	1174^{a}	2012^{a}	29.9	55.9^{a}	41.0^{a}	2.27^{ab}	3.09	2.74
SEM	11.7	36.2	37.3	0.2	7.3	20.1	7.3	14.8	20.1	0.3	0.7	0.4	0.02	0.03	0.02
P-value	0.035	0.032	0.01	0.83	0.63	0.028	0.627	0.007	0.029	0.627	0.013	0.029	0.025	0.588	0.358

[a-c] Means with different superscripts within the same column are significantly different ($P<0.05$); SEM: Standard error of mean; FI: Feed intake; IBW: Initial Body Weight; FBW: Final Body Weight; BWG: Body Weight Gain; ADG: Average daily gain; FCR: Feed conversion Ratio; T_1 = No feed additive inclusion. $T_2 = T_1$+ 1% OMP. $T_3 = T_1$+ 2% OMP. $T_4 = T_1$+ 1% GAP. $T_5 = T_1$+ 2% GAP. $T_6 = T_1$ + 1% GIP. $T_7 = T_1$ + 2% GIP.

Trends in the average weekly FI of broiler chickens fed with ration containing 1 and 2% levels of oyster mushroom, garlic and ginger powders are presented in Figure one. The trends were similar for all the treatments during the starter phase. During the final two weeks of the trial, a marked difference in feed intake was observed between the control and the other treatments where, the birds' preference to the OMP containing diets declines during this period of the study.

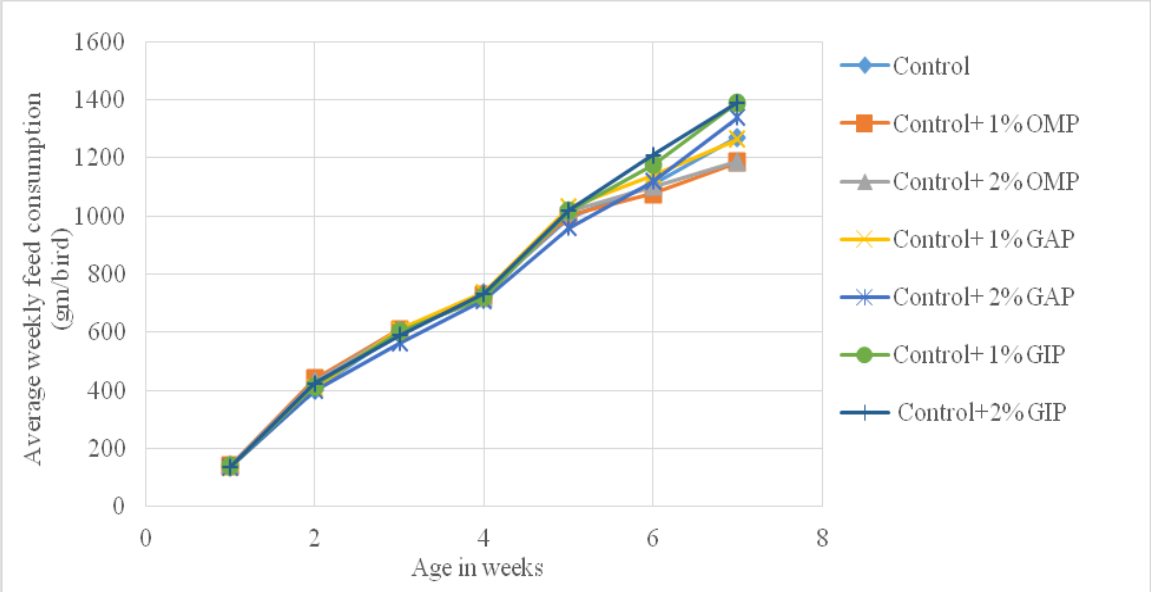


Figure 1. Trends in the average weekly feed consumption of broilers fed with different levels of oyster mushroom, garlic and ginger during the entire experiment

Trends in the average weekly BWG of birds fed with ration containing different levels of oyster mushroom, garlic and ginger powders is presented in Figure two. During the first three weeks of the trial, similar trend in the average weekly BWG was observed among all the treatments which is consistent with the FI results, whereas during 4^{th}, 6^{th} and 7^{th} weeks there was a decline in the average weekly BWG of birds fed with diet containing 2% OMP compared to the control.

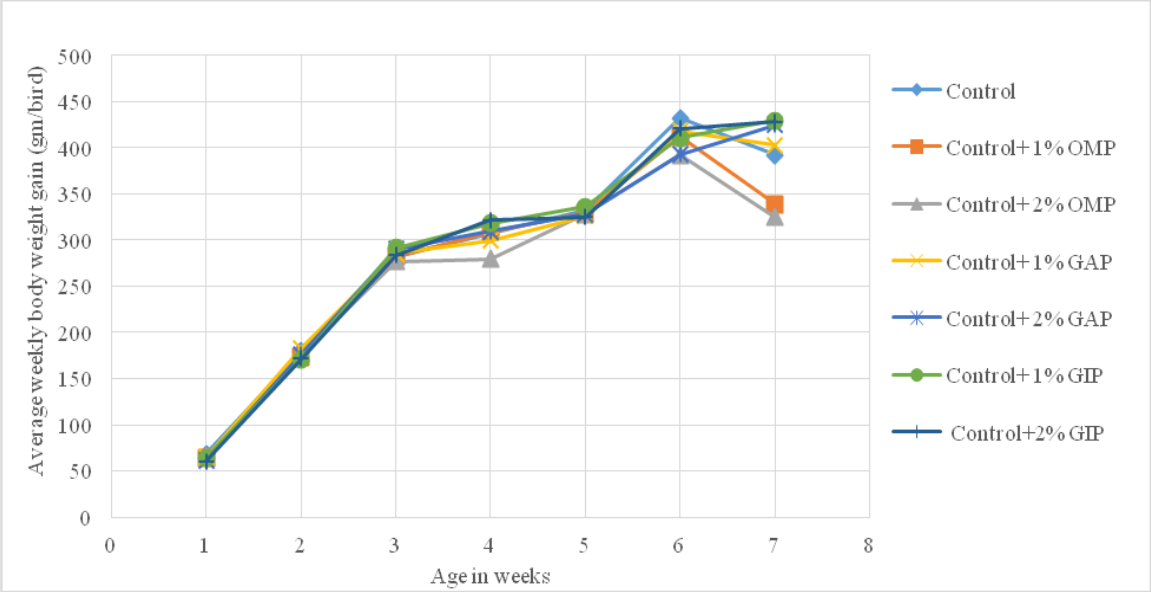


Figure 2. Trends in the average weekly body weight gain of broilers fed with different levels of oyster mushroom, garlic and ginger during the entire experiment

Trends in the average weekly FCR of birds fed with diets containing different levels of oyster mushroom, garlic and ginger is shown in Figure three. Uniform trends were observed for all the treatments, whereas the diet containing 2% OMP was the least efficient during the whole experimental period.

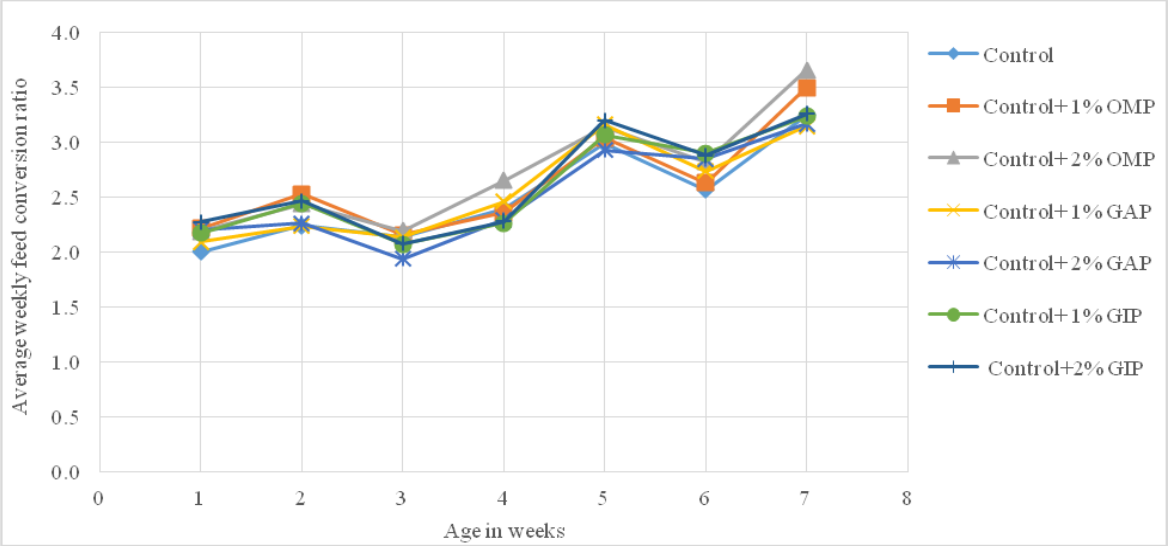
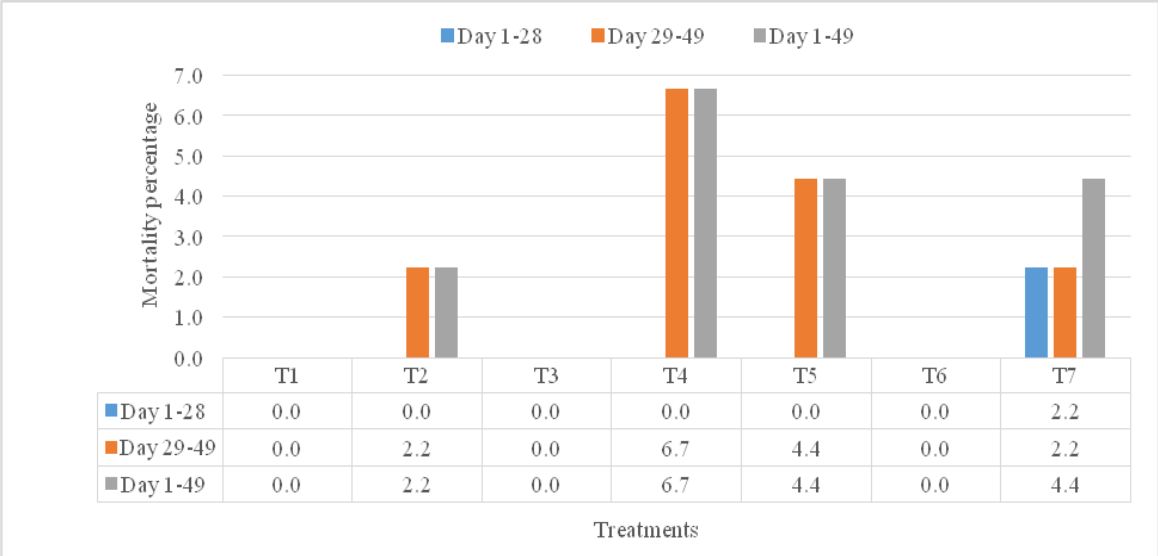


Figure 3. Trends in the average weekly feed conversion ratio of broilers fed with different levels of oyster mushroom, garlic and ginger during the entire experiment

Mortality rate for birds fed with diets containing different levels of oyster mushroom, garlic and ginger during the different experimental periods is shown in Figure four. Mortality rates assessed during the duration of this experiment were relatively high for T_4 whereas hundred percent livability was observed for birds kept on T_1, T_3 and T_6.



	T1	T2	T3	T4	T5	T6	T7
Day 1-28	0.0	0.0	0.0	0.0	0.0	0.0	2.2
Day 29-49	0.0	2.2	0.0	6.7	4.4	0.0	2.2
Day 1-49	0.0	2.2	0.0	6.7	4.4	0.0	4.4

T_1 = No feed additive inclusion. T_2 = T_1+ 1% OMP. T_3 = T_1+ 2% OMP. T_4 = T_1+ 1% GAP. T_5 = T_1+ 2% GAP. T_6 = T_1 + 1% GIP. T_7 = T_1 + 2% GIP.

Figure 4. Mortality rate for broilers fed with different levels of oyster mushroom, garlic and ginger during the different experimental periods

4.1.2. Caracas yield

The effects of feeding different levels of OMP, GAP and GIP on the carcass yield and relative organ weight of broiler was evaluated. Statistical results of the study are shown in Tables 12-15. The slaughter weight was significantly lower ($P<0.05$) for T_3 as compared to other treatments except T_2 and T_4, which were consistent with final body weight recorded at the end of the experiment. The dressed weight was also significantly lower ($P<0.05$) for T_3 compared to T_5, while, dressing percentage was non-significantly affected by dietary inclusion of herbal feed additives. Eviscerated weights of various treatment groups of broiler were non-significant ($P>0.05$), whereas eviscerated percentage was significantly ($P<0.05$) higher for T_3 (74.4%) compared to T_4 (70.8%) (Table12).

Table 12. The effects of feeding different levels of oyster mushroom, garlic and ginger on slaughter weight, dressed weight and eviscerated weight of broilers

Parameters	Treatments							SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
Slaughter weight (g)	2035^{a}	1937^{ab}	1882^{b}	2005^{ab}	2037^{a}	2050^{a}	2040^{a}	19.72	0.049
Dressed weight (g)	1822^{ab}	1755^{ab}	1694^{b}	1823^{ab}	1855^{a}	1850^{ab}	1832^{ab}	19.10	0.013
Dressing (%)	89.5	90.6	90.0	90.9	91.1	90.2	89.8	0.32	0.884
Eviscerated weight (g)	1479	1415	1400	1420	1462	1486	1478	14.82	0.595
Eviscerated (%)	72.7^{ab}	73.0^{ab}	74.4^{a}	70.8^{b}	71.8^{ab}	72.5^{ab}	72.5^{ab}	0.37	0.017

[a-b] Means with different superscripts within the same row are significantly different ($P<0.05$); SEM: Standard error of mean; T_1 = No feed additive inclusion. $T_2 = T_1$+ 1% OMP. $T_3 = T_1$+ 2% OMP. $T_4 = T_1$+ 1% GAP. $T_5 = T_1$+ 2% GAP. $T_6 = T_1$ + 1% GIP. $T_7 = T_1$ + 2% GIP. SEM = Standard error of the mean, P = probability

As shown in Table 13, no significant difference ($P>0.05$) was observed in the weight and percentage of commercial cuts among all the treatments except for the wings and neck, whereas OMP containing diet had significantly lower wings weight compared to 2% GIP as well as 2% OMP had also significantly lower percent of neck compared to similar inclusion level of GAP. No significant differences ($P>0.05$) were observed for commercial carcass weight, commercial carcass percentage and edible carcass weight. Edible carcass percentage was significantly higher ($P<0.05$) for T_3, compared to T_4 whereas no significant difference was observed among the other treatments.

Table 13. The effects of feeding different levels of oyster mushroom, garlic and ginger on the commercial cuts of broilers

Parameters	Treatments							SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
Breast weight (g)	576.0	547.0	527.4	521.3	576.9	567.7	574.9	11.11	0.741
Breast (%)	28.25	28.24	28.02	25.99	28.31	27.63	28.20	0.43	0.832
Drumsticks with thighs(g)	421	402	395	422	427	421	424	4.91	0.536
Drumsticks with thighs (%)	20.7	20.7	22.0	21.0	21.0	20.6	20.8	0.17	0.993
Back weight (g)	213	203	208	210	201	212	207	2.94	0.954
Back (%)	10.4	10.5	11.1	10.5	9.9	10.3	10.1	0.14	0.430
Wing weight (g)	79^{ab}	77^{b}	76^{b}	79^{ab}	80^{ab}	79^{ab}	82^{a}	0.69	0.017
Wing (%)	3.88	3.96	4.02	3.96	3.91	3.87	4.04	0.03	0.838
Neck weight (g)	68.6	69.3	75.0	73.2	57.0	77.9	72.1	2.42	0.383
Neck (%)	3.39^{ab}	3.59^{ab}	3.99^{a}	3.64^{ab}	2.80^{b}	3.79^{ab}	3.53^{ab}	0.13	0.017
Commercial carcass weight (g)	1357	1298	1281	1306	1341	1358	1360	14.34	0.666
Commercial carcass (%)	66.7	67.0	68.1	65.1	65.8	66.2	66.7	0.37	0.549
Edible carcass weight (g)	1450	1385	1372	1399	1433	1453	1448	14.64	0.651
Edible carcass (%)	71.2^{ab}	71.5^{ab}	72.9^{a}	69.7^{b}	70.3^{ab}	70.8^{ab}	71.0^{ab}	0.35	0.012

[a-b] Means with different superscripts within the same row are significantly different ($P<0.05$); SEM: Standard error of mean; T_1 = No feed additive inclusion. T_2 = T_1+ 1% OMP. T_3 = T_1+ 2% OMP. T_4 = T_1+ 1% GAP. T_5 = T_1+ 2% GAP. T_6 = T_1 + 1% GIP. T_7 = T_1 + 2% GIP. SEM = Standard error of the mean, P = probability

The data on the weight and share of the internal organs are presented in Table 14. The result showed the absence of a significant difference (P>0.05), except for the weight and share of the heart which was significantly lower for T_2 compared to T_1 and T_4.

Table 14. The effects of feeding different levels of oyster mushroom, garlic and ginger on the giblets weight and percentage of broilers

Parameters	Treatments							SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
Liver weight (g)	46	44	46	45	44	45	42	1.11	0.977
Liver (%)	2.25	2.25	2.46	2.23	2.19	2.20	2.04	0.05	0.668
Gizzard weight (g)	35	34	35	36	36	39	36	0.83	0.773
Gizzard (%)	1.73	1.74	1.84	1.82	1.77	1.92	1.75	0.04	0.941
Heart weight (g)	12.4^{a}	9.3^{b}	10.2^{ab}	11.9^{a}	10.6^{ab}	11.1^{ab}	10.9^{ab}	0.31	0.021
Heart (%)	0.60^{a}	0.48^{b}	0.54^{ab}	0.59^{a}	0.52^{ab}	0.54^{ab}	0.53^{ab}	0.01	0.042
Giblets weight (g)	93.2	86.6	91.3	93.0	91.3	95.4	88.5	1.49	0.816
Giblets (%)	4.58	4.47	4.84	4.63	4.48	4.66	4.32	0.07	0.605

[a-b] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = No feed additive inclusion. T_2 = T_1+ 1% OMP. T_3 = T_1+ 2% OMP. T_4 = T_1+ 1% GAP. T_5 = T_1+ 2% GAP. T_6 = T_1 + 1% GIP. T_7 = T_1 + 2% GIP. SEM = Standard error of the mean, P = probability

In order to examine the effects of various levels of OMP, GAP and GIP on non-edible parts, the weights of feet, head, abdominal fat and GIT were recorded at the end of the experiment and presented in Table 15. The results indicated that the relative weight of non-edible parts were not significantly affected (P>0.05) due to the inclusion of OMP, GAP and GIP in the diets of broilers in all groups in comparison with the control.

Table 15. The effects of feeding different levels of oyster mushroom, garlic and ginger on weight of non-edible parts of broilers

Parameters	Treatments							SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
Feet weight (g)	78^{ab}	76^{ab}	71^{b}	86^{a}	78^{ab}	80^{ab}	78^{ab}	1.37	0.018
Feet (%)	3.82	3.92	3.80	4.31	3.82	3.89	3.81	0.07	0.446
Head weight (g)	56	54	56	58	58	57	61	0.90	0.614
Head (%)	2.76	2.78	2.96	2.89	2.84	2.80	2.98	0.05	0.847
Abdominal fat weight (g)	27.2	15.4	22.1	21.9	25.8	24.8	17.0	1.45	0.231
Abdominal fat (%)	1.33	0.79	1.16	1.09	1.26	1.22	0.83	0.07	0.245
Weight of GIT (g)	83	83	76	82	82	78	81	1.44	0.883
GIT (%)	4.09	4.27	4.06	4.08	4.00	3.80	3.95	0.07	0.744

[a-b] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = No feed additive inclusion. T_2 = T_1+ 1% OMP. T_3 = T_1+ 2% OMP. T_4 = T_1+ 1% GAP. T_5 = T_1+ 2% GAP. T_6 = T_1 + 1% GIP. T_7 = T_1 + 2% GIP. SEM = Standard error of the mean, P = probability

4.1.3. Hematological values

The effects of inclusion of different levels of OMP, GAP and GIP on hematological parameters were presented in Table 16. There was a significant difference (P<0.05) on erythrocyte concentration of blood between the different treatment groups, where the birds fed on T_1 and T_3 showed the lowest erythrocyte concentration of blood and those fed on T_6 showed the highest. There were no significant differences in erythrocyte concentration of blood (P>0.05) between the other groups fed on T_2, T_5 and T_7. Inclusion of 1% GAP resulted in a significantly higher TWBC counts. No significant differences (P>0.05) were also observed in Hb concentration among the blood samples from all the treatment groups. Similarly birds kept on T_1 showed significantly (P<0.05) lower PCV value compared to T_5. Birds kept on T_3 and T_5 showed significantly (P<0.05) higher MCV value compared to all the other treatments, except the control. Birds on T_3 also showed significantly (P<0.05) higher MCH value compared to all the others except T_1, while, T_1 had significantly (P<0.05) higher MCHC value compared to T_5 and no

significant difference (P>0.05) was observed among all the other treatment groups for the values of MCHC.

Table 16. The effects of feeding different levels of oyster mushroom, garlic and ginger on hematological parameters of broilers

Parameters	Treatments							SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
RBCs ($x10^6/\mu L$)	3.90^{d}	4.22^{c}	3.77^{d}	4.56^{b}	4.11^{c}	5.01^{a}	4.25^{c}	0.09	<.0001
TWBC ($x10^3/\mu L$)	2.43^{bc}	2.67^{bc}	1.90^{c}	3.80^{a}	3.27^{ab}	2.30^{bc}	2.47^{bc}	0.16	0.009
Hb (g/dL)	11.0	10.1	11.4	10.3	10.2	10.0	10.9	0.21	0.531
PCV (%)	29.7^{b}	32.8^{ab}	32.8^{ab}	33.4^{ab}	35.2^{a}	31.6^{ab}	31.0^{ab}	0.58	0.011
MCV (fL)	76.0^{ab}	77.8^{ab}	86.9^{a}	73.3^{bc}	85.7^{a}	63.2^{c}	72.9^{bc}	2.02	0.005
MCH (pg/cell)	28.2^{ab}	23.8^{bcd}	30.2^{a}	22.7^{cd}	24.8^{bc}	20.0^{d}	25.6^{bc}	0.82	0.002
MCHC (%)	37.0^{a}	30.9^{ab}	34.7^{ab}	31.1^{ab}	29^{b}	31.6^{ab}	35.2^{ab}	0.89	0.029

[a-d] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = No feed additive inclusion. T_2 = T_1+ 1% OMP. T_3 = T_1+ 2% OMP. T_4 = T_1+ 1% GAP. T_5 = T_1+ 2% GAP. T_6 = T_1 + 1% GIP. T_7 = T_1 + 2% GIP. 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, SEM = Standard error of the mean, P = probability.

4.1.4. Biochemical values

Although most of the serum biochemical parameters were not significantly (P>0.05) affected by different dietary treatments, but, AST/Glutamic Oxaloacetic transaminase (GOT) activity was significantly affected (P<0.05) (Table 17), where birds kept on T_7 had significantly (P<0.05) lower AST/GOT activity compared to those kept on T_3 and T_5.

Table 17. The effects of feeding different levels of oyster mushroom, garlic and ginger on serum biochemical parameters of broilers

Parameters	Treatments							SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
Glucose (mg/dL)	216.1	245.9	237.4	245.9	256.5	246.8	248.1	4.71	0.411
Total cholesterol (mg/dL)	110.2	148.6	139.3	120.8	126.7	135.4	114.8	5.17	0.381
Total protein (g/dL)	3.15	3.48	3.53	3.60	3.25	3.20	3.33	0.07	0.630
Albumin (g/dL)	1.80	2.40	2.55	2.28	2.23	2.27	1.98	0.11	0.667
Globulin (g/dL)	1.35	1.08	0.98	1.32	1.02	0.93	1.35	0.08	0.620
A/G ratio	1.52	3.43	3.23	1.93	2.49	2.63	2.14	0.29	0.601
ALP (IU/L)	1021	1055	1129	1030	1051	1046	1038	33.49	0.993
ALT (IU/L)	16.3	11.4	13.4	13.6	14.2	10.3	11.6	0.93	0.730
AST (IU/L)	467^{abc}	450^{abc}	531^{a}	430^{abc}	504^{ab}	405^{bc}	352^{c}	16.9	0.039

[a-c]Means with different superscripts within the same row are significantly different ($P<0.05$); SEM: Standard error of mean; T_1 = No feed additive inclusion. $T_2 = T_1$+ 1% OMP. $T_3 = T_1$+ 2% OMP. $T_4 = T_1$+ 1% GAP. $T_5 = T_1$+ 2% GAP. $T_6 = T_1$ + 1% GIP. $T_7 = T_1$ + 2% GIP. A/G = Albumen to Globulin ratio, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, One deciliter (dL) = 10^{-10} Liter, SEM = Standard error of the mean, P = Probability.

4.1.5. Economic appraisal

Data for economic evaluation summarized in Table 18 revealed that almost all the economic parameters considered differed widely compared to the control. Cost per kg of starter and finisher diets, the total cost of feed consumed and feed cost/kg of live weight gain was the cheapest for the control diet. On the contrary feed cost/kg of gain was the highest for T_3. Similarly T_3 recorded the highest total feed cost, total cost of production and feed cost/kg carcass weight. Feed cost/kg live weight gain increased with an increase in the level of inclusion of additives. The highest NR was obtained from birds kept on the control diet. T_6 (1% GIP) had the highest EE and REE (62.9 and 68.5%, respectively), while, T_3 (2% OMP) recoded the lowest EE and REE being (29.0 and 31.6%, respectively) compared to all the other treatments.

Table 18. Effects of using feed additives in broilers on selected economic parameters during the starter and finisher phase and the entire experiment.

Parameters	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Cost per kg of starter diet (Birr)	7.6	10.1	12.6	9.6	11.6	9.4	11.2
Cost per kg of finisher diet (Birr)	6.9	9.4	11.9	8.9	10.9	8.7	10.5
Total cost of feed/bird (Birr)	37.6	50.1	63.2	48.8	58.3	48.9	59.1
Feed cost/kg of gain (0-28 th day)	17.2	23.4	30.3	21.9	25.0	20.9	25.2
Feed cost/kg of gain (29-49 th day)	20.2	28.4	37.7	26.7	32.6	26.7	32.5
Feed cost/kg of gain (1-49th day)	18.9	26.2	34.5	24.7	29.4	24.2	29.4
Total cost of production /bird (Birr)	63.7	76.2	89.3	74.9	84.4	75.0	85.2
Feed cost/kg carcass/bird (Birr)	27.7	38.6	49.4	37.3	43.5	36.0	43.5
Total income/bird (Birr)	122.1	116.8	115.3	117.5	120.7	122.2	122.4
Net return/bird (Birr)	58.5	40.6	25.9	42.7	36.3	47.2	37.2
Economic efficiency (ECE)	91.8	53.3	29.0	57.0	43.1	62.9	43.6
Relative economic efficiency (REE)%	100.0	58.1	31.6	62.1	46.9	68.5	47.5

T_1 = No feed additive inclusion. T_2 = T_1+ 1% OMP. T_3 = T_1+ 2% OMP. T_4 = T_1+ 1% GAP. T_5 = T_1+ 2% GAP. T_6 = T_1 + 1% GIP. T_7 = T_1 + 2% GIP.

4.2. Phase II: Response of Broiler Chickens to Diets with Different Mixtures of Oyster Mushroom, Garlic and Ginger Powders as Substitutes for Antibiotic Growth Promoter

4.2.1. Performances

The effects of feeding different mixture levels of OMP, GAP and GIP on FI, BWG and FCR are presented in Table 20. Comparison among treatments showed that, birds kept on T_2 and T_3 consumed significantly ($P<0.05$) higher amounts of feed during the starter phase as compared to those kept on all the other treatments, except T_5. During the finisher phase birds kept on T_6 had significantly ($P<0.05$) higher FI compared to T_5, whereas, no significant differences ($P>0.05$) were observed among all the other treatment feeds. Total FI for the entire experiment was significantly higher ($P<0.05$) for T_2 and T_3 compared to T_4 and T_5. FBW, BWG and ADG during the starter, finisher and entire experimental periods were not significantly ($P>0.05$) affected by the dietary treatments. No significant differences ($P>0.05$) in FCR were also observed among the treatments during the starter phase. During the finisher phase birds kept on T_5 had the lowest FCR ($P<0.05$) compared to all the other treatment feeds, except the negative control. For the overall experimental period T_5 had a significantly ($P<0.05$) lower FCR compared to T_3 and T_6, whereas no significant difference were observed among all the other treatment feeds.

Table 19. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on performance of broilers

Treatments	Parameters														
	FI (g/bird)			IBW	FBW (g/bird)		BWG (g/bird)			ADG (g/day)			FCR (g FI/g BWG)		
	0-28 Days	29-49 Days	0-49 Days		28^{th} Day	49^{th} Day	0-28 Days	29-49 Days	0-49 Days	0-28 Days	29-49 Days	0-49 Days	0-28 Days	29-49 Days	0-49 Days
T_1	1965^{b}	3760^{ab}	5726^{ab}	39.1	878	2028	839	1150	1989	29.9	54.7	40.6	2.35	3.27^{ab}	2.88^{ab}
T_2	2236^{a}	3785^{ab}	6022^{a}	39.1	965	2108	926	1143	2069	33.1	54.4	42.2	2.42	3.33^{a}	2.91^{ab}
T_3	2186^{a}	3774^{ab}	5961^{a}	38.4	860	1974	822	1113	1935	29.4	53.0	39.5	2.68	3.40^{a}	3.10^{a}
T_4	1948^{b}	3539^{ab}	5487^{b}	38.9	839	1901	800	1062	1862	28.6	50.6	38.0	2.44	3.33^{a}	2.95^{ab}
T_5	2058^{ab}	3453^{b}	5512^{b}	38.9	884	2080	845	1196	2041	30.2	56.9	41.6	2.44	2.89^{b}	2.70^{b}
T_6	1960^{b}	3863^{a}	5823^{ab}	39.3	858	1956	819	1098	1916	29.2	52.3	39.1	2.40	3.52^{a}	3.05^{a}
SEM	34.22	49.83	64.51	0.12	16.46	29.97	16.41	17.44	29.92	0.59	0.83	0.61	0.05	0.07	0.05
P-value	0.014	0.011	0.037	0.449	0.313	0.358	0.311	0.336	0.356	0.311	0.336	0.356	0.465	0.011	0.020

a-b Means with different superscripts within the same column are significantly different ($P<0.05$); SEM: Standard error of mean; FI: Feed intake; IBW: Initial Body Weight; FBW: Final Body Weight; BWG: Body Weight Gain; ADG: Average daily gain; FCR: Feed conversion Ratio; T1 = Negative control. T2 = Positive control. T3 = T1 + 0.5% OMP + 1% GAP. T4 = T1 + 0.5% OMP + 1% GIP. T5 = T1 + 1% GAP + 1% GIP. T6 = T1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. SEM = Standard error of the mean, P = Probability.

Trends in the average weekly FI of broiler chickens are presented in Figure five. The graph showed that during the last two weeks of the experiment bids kept on ration containing a mixture of 1% GAP 1% GIP had lower feed intake compared to the others.

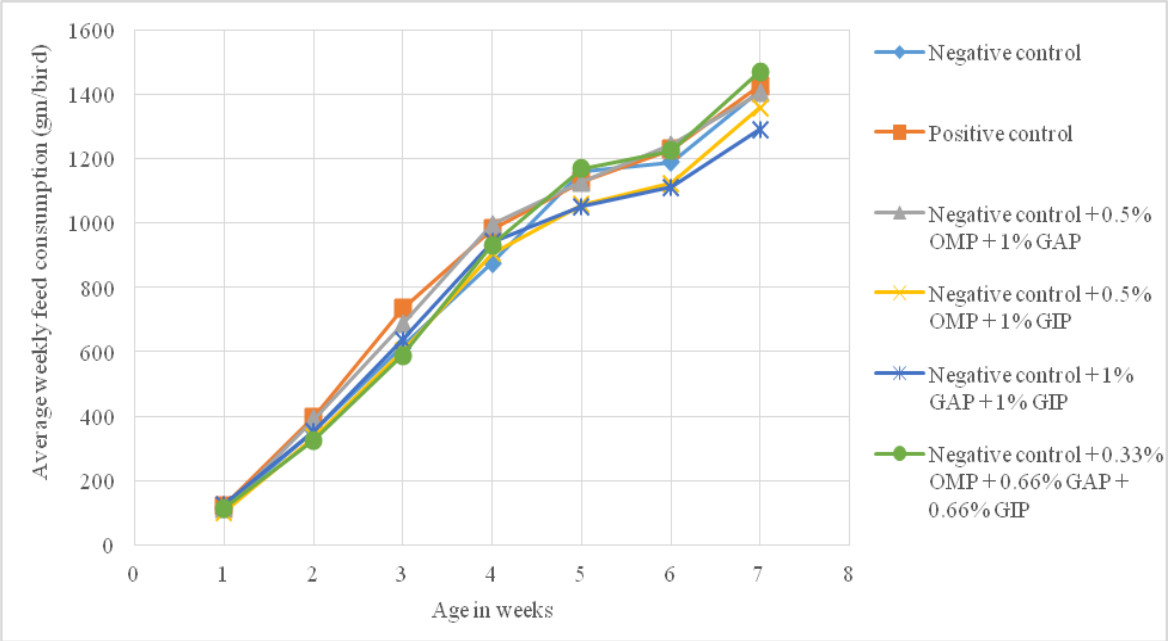


Figure 5. Trends in the average weekly feed consumption of broilers fed with different mixtures of oyster mushroom, garlic and ginger during the entire experiment

Figure six showed the trends in the average weekly BWG of birds fed with ration containing different mixtures of oyster mushroom, garlic and ginger. During the first four weeks of the experimental period broiler chickens kept on the antibiotic containing diet had comparatively higher BWG whereas bids fed on T_5 showed slight improvement in the weekly trends of BWG during week five and week six of the trial.

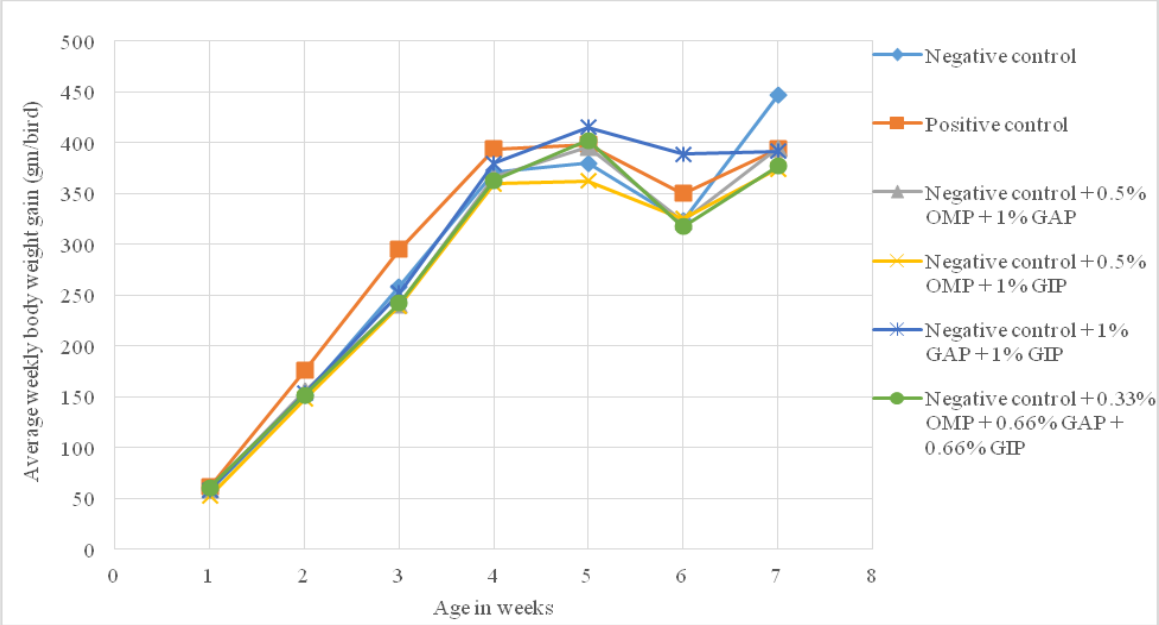


Figure 6. Trends in the average weekly body weight gain of broilers fed with different mixtures of oyster mushroom, garlic and ginger during the entire experiment

Trends in the average weekly FCR of birds fed with ration containing different mixtures of oyster mushroom, garlic and ginger are shown in Figure seven. During weeks two, week three, week four and week six, T_3 was the least efficient diet in terms of a unit of body weight gain for every unit of feed consumed, whereas during the final three weeks of the trial, diet containing mixture of garlic and ginger powders was the most efficient diet except for the last week.

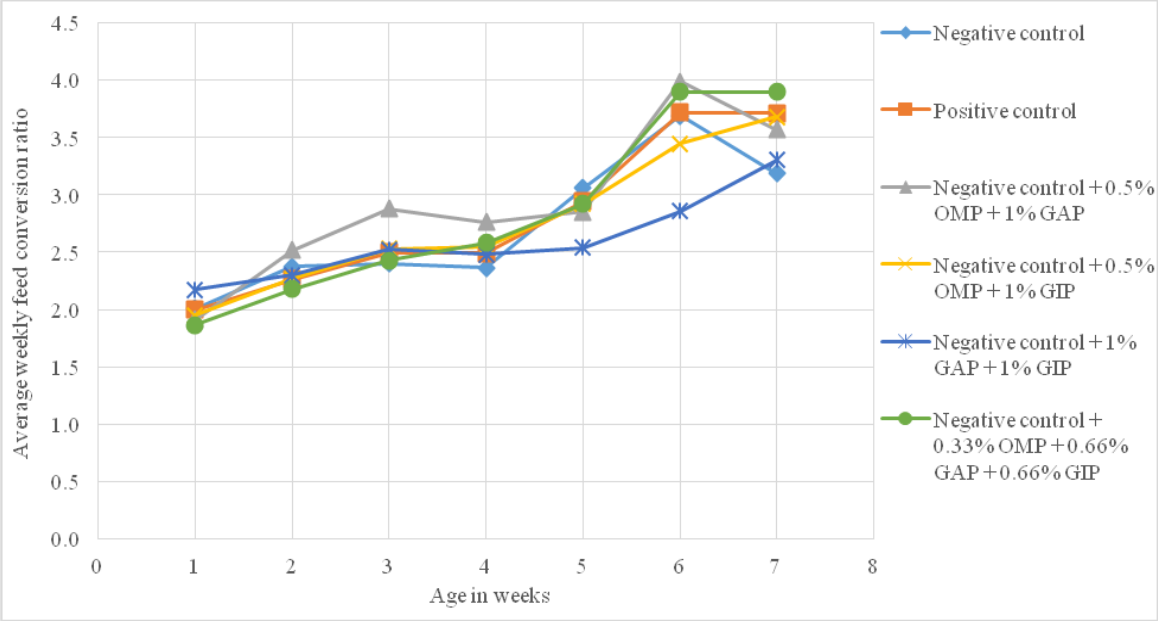
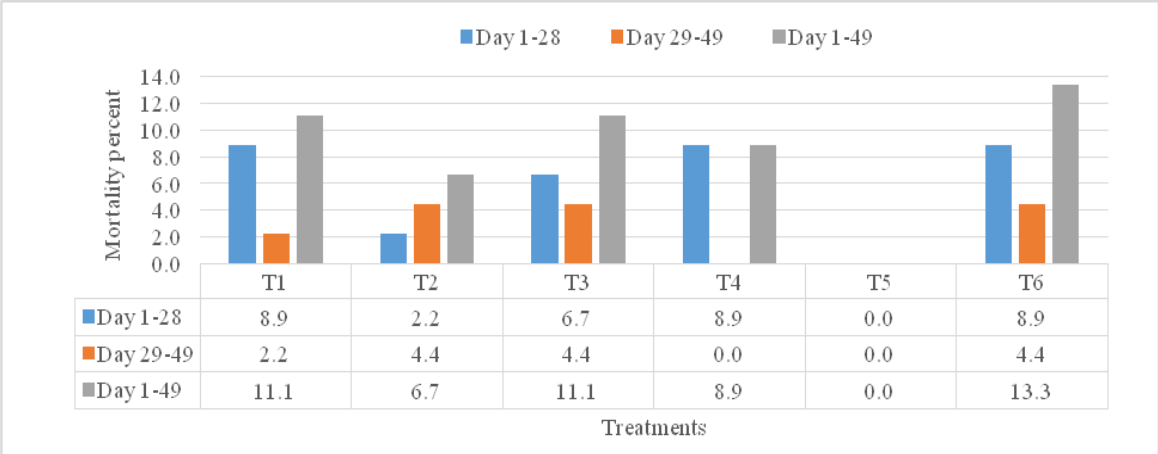


Figure 7. Trends in the average weekly feed conversion ratio of broilers fed with different mixtures of oyster mushroom, garlic and ginger during the entire experiment

The mortality rates for the different treatments are shown in Figure eight. Mortality rate assessed during the duration of this experiment was the highest with T_6 (13.3%) whereas hundred percent livability was observed with inclusion of combination of GAP and GIP in broiler diets.



	T1	T2	T3	T4	T5	T6
Day 1-28	8.9	2.2	6.7	8.9	0.0	8.9
Day 29-49	2.2	4.4	4.4	0.0	0.0	4.4
Day 1-49	11.1	6.7	11.1	8.9	0.0	13.3

T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP

Figure 8. Mortality rate for broilers fed with different mixtures of oyster mushroom, garlic and ginger during the different experimental periods.

4.2.2. Carcass yield

The effects of feeding different mixture levels of OMP, GAP and GIP as substitutes for AGP on carcass characteristics of broiler are presented in Tables 20-24. The slaughter weight was non-significantly lower (P>0.05) for T_4 as compared to the other treatments, whereas, birds kept on the antibiotic containing diet had the highest slaughter weight (P>0.05). Dressed weight was also significantly lower (P<0.05) for T_4 and T_6 compared to the positive control. Numerically the dressed weight was higher for T_2 and T_5 than the negative control. The lowest (P<0.05) dressing percentage and eviscerated weight was recorded for T_6 compared to the positive control, whereas, no significant difference (P>0.05) was observed among the other treatments. Eviscerated percentage was also the lowest (P<0.05) for T_6 as compared to both the negative and positive controls (Table 20).

Table 20. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on slaughter weight, dressed weight and eviscerated weight of broilers

Parameters	Treatments						SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Slaughter weight (g)	2020	2112	1968	1893	2078	1958	30.88	0.353
Dressed weight (g)	1818^{ab}	1935^{a}	1763^{ab}	1698^{b}	1852^{ab}	1704^{b}	28.49	0.023
Dressing (%)	90.1^{ab}	91.7^{a}	89.6^{ab}	89.7^{ab}	89.1^{ab}	87.1^{b}	0.51	0.036
Eviscerated weight (g)	1468^{ab}	1491^{a}	1388^{ab}	1348^{ab}	1475^{ab}	1325^{b}	22.91	0.015
Eviscerated (%)	72.7^{a}	70.6^{a}	70.5^{a}	71.2^{a}	70.9^{a}	67.7^{b}	0.45	0.013

[a-b] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. SEM = Standard error of the mean, P = Probability.

The treatment group values of commercial cuts expressed as percentage from slaughter weight are given in Table 21. No significant (P>0.05) effect was observed between the groups except for treatment diet with herbal mixture containing all the three test ingredients (OMP, GAP and GIP), which significantly (P<0.05) reduced the weight and share of breast meat compared to the antibiotic containing diet. There was no significant

(P>0.05) treatments effects in commercial and edible carcass percentage values and mean values of all treatment groups were similar.

Table 21. The effects feeding of different mixtures of oyster mushroom, garlic and ginger on commercial cuts of broilers

Parameters	Treatments						SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Breast weight (g)	579^{ab}	619^{a}	532^{ab}	537^{ab}	586^{ab}	521^{b}	12.55	0.026
Breast (%)	28.65^{ab}	29.34^{a}	26.96^{bc}	28.33^{abc}	28.19^{abc}	26.59^{c}	0.31	0.023
Drumstick with thigh (g)	445	452	416	412	436	428	8.37	0.757
Drumstick with thigh (%)	22.04	21.38	21.11	21.78	20.96	21.86	0.22	0.701
Back weight (g)	192.2	190.5	200.8	174.0	179.0	170.7	4.91	0.498
Back (%)	9.55	9.02	10.18	9.24	8.65	8.72	0.25	0.526
Wing weight (g)	79.8	81.3	75.5	75.2	77.2	72.7	1.58	0.706
Wing (%)	3.96	3.85	3.83	3.97	3.71	3.71	0.05	0.589
Neck weight (g)	53.0	48.9	47.5	43.3	44.4	46.2	1.36	0.402
Neck (%)	2.63	2.32	2.41	2.28	2.14	2.36	0.06	0.394
Commercial carcass weight (g)	1350	1392	1272	1242	1323	1239	23.92	0.346
Commercial carcass (%)	66.8	65.9	64.5	65.6	63.7	63.24	0.53	0.242
Edible carcass weight (g)	1435	1469	1360	1323	1412	1325	24.46	0.419
Edible carcass (%)	71.0	69.6	68.9	69.9	67.9	67.67	0.49	0.272

[a-c] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. SEM = Standard error of the mean, P = Probability.

As shown in Table 22, treatment effects on the weight and share of liver, gizzard and heart were not significant (P>0.05). The only significant effect (P<0.05) was observed for the share of the giblets, which indicated that the antibiotic containing diet resulted in a significantly (P<0.05) lower share of the giblets than that of T_3 and T_6.

Table 22. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on giblets weight and percentage of broilers

Parameters	Treatments						SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Liver weight (g)	42.8	35.1	42.3	36.7	43.6	41.1	1.69	0.664
Liver (%)	2.11	1.66	2.17	1.93	2.09	2.09	0.08	0.494
Gizzard weight (g)	31.9	33.3	35.1	35.2	36.0	36.4	0.74	0.538
Gizzard (%)	1.58	1.58	1.78	1.86	1.73	1.86	0.04	0.107
Heart weight (g)	10.3	9.3	10.2	9.1	9.4	9.3	0.32	0.882
Heart (%)	0.51	0.44	0.52	0.48	0.45	0.48	0.02	0.750
Giblets weight (g)	85.0	77.7	87.6	80.9	89.1	86.8	1.97	0.596
Giblets (%)	4.19^{ab}	3.68^{b}	4.47^{a}	4.27^{ab}	4.29^{ab}	4.43^{a}	0.09	0.048

[a-b] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. SEM = Standard error of the mean, P = Probability.

In order to examine the effects of various mixture levels of oyster mushroom, garlic and ginger on non-edible parts, the weight of feet, head, abdominal fat and GIT were recorded at the end of the experiment and the results are shown in the Table 23. Weight and percentage of abdominal fat were the highest for T_1 compared to T_2 and T_3, whereas, no significant difference were observed in the weight of feet, head and GIT.

Table 23. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on weight of non-edible parts of broilers

Parameters	Treatments						SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Feet weight (g)	71.9	77.8	83.3	79.8	71.4	74.9	1.60	0.209
Feet (%)	3.57^{b}	3.67^{ab}	4.26^{a}	4.22^{a}	3.43^{b}	3.83^{ab}	0.10	0.042
Head weight (g)	43.0	47.0	53.0	45.7	42.7	42.8	1.49	0.319
Head (%)	2.14^{ab}	2.23^{ab}	2.70^{a}	2.41^{ab}	2.05^{b}	2.19^{ab}	0.08	0.021
Abdominal fat weight (g)	20.4^{a}	13.2^{b}	11.9^{b}	15.3^{ab}	16.0^{ab}	15.0^{ab}	0.86	0.014
Abdominal fat (%)	1.01^{a}	0.63^{b}	0.60^{b}	0.81^{ab}	0.77^{ab}	0.77^{ab}	0.05	0.018
Weight of GIT (g)	73.5	81.2	76.3	79.8	80.8	78.8	1.33	0.583
GIT (%)	3.62^{b}	3.84^{ab}	3.89^{ab}	4.22^{a}	3.89^{ab}	4.03^{ab}	0.07	0.027

[a-b] Means with different superscripts within the same row are significantly different ($P<0.05$); SEM: Standard error of mean; T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. SEM = Standard error of the mean, P = Probability.

4.2.3. Hematological values

The effects of AGP and five mixtures of natural feed additives in broiler diets on some hematological parameters are summarized in Table 24. In the current study, birds on T_2, T_5, T_3, T_4, T_5 and (T_5 and T_6) had the highest values of (RBC and TWBC), Hb, PCV, MCV, MCH and MCHC, respectively. There were no significant differences ($P>0.05$) on RBC, TWBC, PCV, MCV, MCH and MCHC between different groups. Blood samples from T_5 and T_6 had significantly ($P<0.05$) higher Hb content compared to the negative control group.

Table 24. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on hematological parameter of broilers

Parameters	Treatments						SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6		
RBCs ($x10^6/mm^3$)	1.67	2.35	1.91	1.46	1.62	1.66	0.14	0.555
TWBC ($x10^3/\mu L$)	2.3	3.6	3.0	2.9	2.1	2.6	0.21	0.450
Hb (g/dL)	8.9^{c}	10.3^{abc}	10.5^{abc}	9.7^{bc}	11.7^{a}	10.7^{ab}	0.27	0.033
PCV (%)	24.8	27.0	28.7	27.0	28.3	27.0	0.82	0.859
MCV (fL)	151.2	127.0	157.9	191.5	183.9	178.9	12.41	0.737
MCH (g/dL)	54.8	49.6	57.1	68.2	74.3	68.9	3.76	0.393
MCHC (%)	36.0	38.5	36.8	36.3	41.4	41.1	1.19	0.709

[a-c] Means with different superscripts within the same row are significantly different ($P<0.05$); SEM: Standard error of mean; T_1 = Positive control. T_2 = Negative control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. 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, SEM = Standard error of the mean, P = probability.

4.2.4. Biochemical values

Results of the biochemical analysis of serum samples reveals that blood glucose, globulin, albumin to globulin ratio and the enzyme ALT/GPT were not significantly affected by dietary inclusion of feed additives (Table 25). The total cholesterol contents of serum samples from T_3, T_5 and T_6 were significantly reduced due to inclusion of the herbal mixtures. Birds on T_6 had rather significantly ($P<0.05$) lower total protein content compared to the antibiotic containing diet. On the other hand, birds kept on T_5 and T_6 had significantly ($P<0.05$) lower albumin contents in comparison to the control diets. Significantly higher ($P<0.05$) ALP enzyme activity was observed in birds kept on T_6 compared to the negative control diet. Serum sample from T_3 had also significantly ($P<0.05$) higher AST/GOT enzyme content in comparison to the negative control.

Table 25. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on serum biochemical parameters of broilers

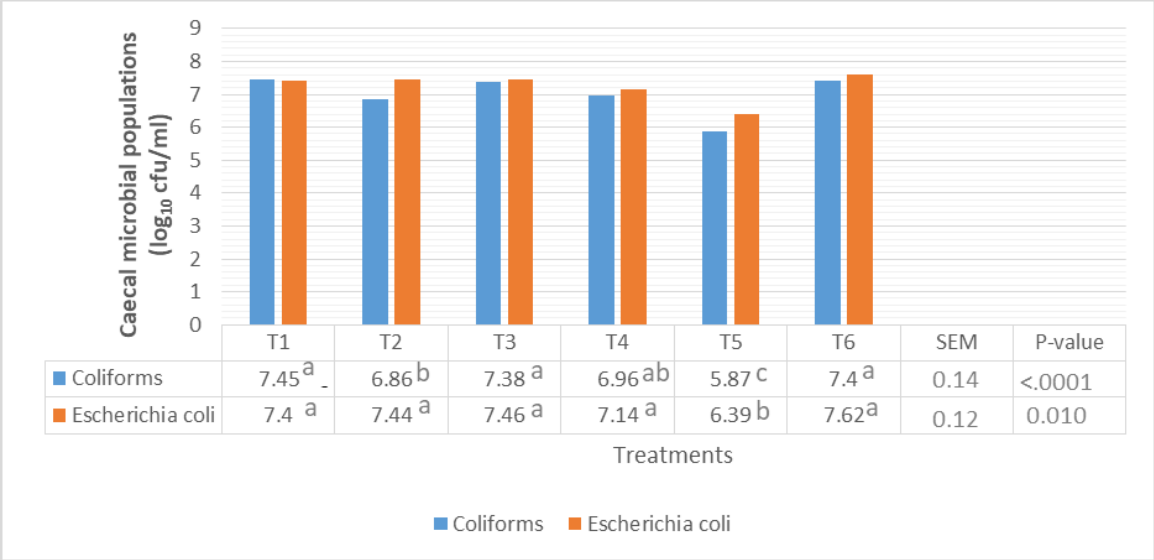
Parameters	Treatments						SEM	P-value
	T_1	T_2	T_3	T_4	T_5	T_6		
Glucose (mg/dl)	268.0	258.2	247.5	249.3	284.0	238.2	6.39	0.386
Total cholesterol (mg/dl)	161.8^{a}	181.3^{a}	128.0^{b}	155.0^{a}	127.2^{b}	113.5^{b}	6.35	0.001
Total protein (g/dl)	3.87^{ab}	4.03^{a}	3.53^{ab}	3.67^{ab}	3.50^{ab}	3.33^{b}	0.09	0.048
Albumin (g/dl)	2.47^{a}	2.50^{a}	2.25^{ab}	2.00^{ab}	1.83^{b}	1.83^{b}	0.09	0.023
Globulin (g/dl)	1.40	1.53	1.28	1.37	1.67	1.50	0.05	0.332
A/G ratio	1.80	1.67	1.79	1.50	1.10	1.22	0.10	0.157
ALP (IU/L)	790.2^{b}	941.7^{ab}	1170.8^{ab}	957.4^{ab}	1111.4^{ab}	1239.1^{a}	56.6	0.014
ALT (IU/L)	15.3	18.3	19.8	17.0	15.8	16.8	1.26	0.946
AST (IU/L)	413.1^{b}	459.7^{ab}	477.0^{a}	414.8^{b}	468.6^{ab}	460.7^{ab}	8.64	0.043

[a-b] Means with different superscripts within the same row are significantly different (P<0.05); SEM: Standard error of mean; T_1 = Positive control. T_2 = Negative control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP. A/G = Albumen to Globulin ratio, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, One deciliter (dL) = 10^{-10} Liter, SEM = Standard error of the mean, P = Probability.

4.2.5. Cecal microbial count

The effects of inclusion of mixtures of the three natural feed additives and antibiotic on the counts of total coliform bacteria and *Escherichia coli* in the distal illeal part of the small intestine of broiler chickens at 49 days of age are shown in Figure nine. It is noted that the treatments which contained mixture of GAP and GIP led to a significant reduction (P<0.05) in the number of colonies of *Escherichia coli* and other enterobacteria in digesta of ileo-cecum compared with all the other treatments. Similarly, the antibiotic

had also a significant effect on the total coloni forming units (CFU) of bacterial loads in comparison to birds kept on T_1, T_3 and T_6. The counts of *Escherichia coli* did not differ between the controls and treated groups of birds, except for T_5 where it was significantly (P<0. 05) reduced. On the other hand, the antibiotic had not any significant effect on the number of colonies of *Escherichia coli* enumerated (p>0.05).



	T1	T2	T3	T4	T5	T6	SEM	P-value
Coliforms	7.45 a	6.86 b	7.38 a	6.96 ab	5.87 c	7.4 a	0.14	<.0001
Escherichia coli	7.4 a	7.44 a	7.46 a	7.14 a	6.39 b	7.62 a	0.12	0.010

T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP.

Figure 9. The effects of feeding different mixtures of oyster mushroom, garlic and ginger on counts of total coliform bacterial and *Escherichia coli*

4.2.6. Economic appraisal

Results of the economics of production of feeding the treatment feeds from 1-49 days of age are shown in Table 26. The price figures are based on the local market prices for feed ingredients, feed additives and broiler carcass in Bishoftu town, Ethiopia, during the time of the research. The results showed that cost per kg of starter and finisher diets and the total cost of feed consumed were the least for the control diets, whereas, both starter and finisher diets of T_5 were considered as the most expensive diet. The amount of money (Birr) spent for the purchase of feed required to bring about 1kg live weight gain was higher for all the diets containing different mixtures of feed additives as compared with

the controls. Total cost of production and feed cost/kg carcass/bird was also the cheapest for the control diets. The NR was also high for the control diets. EE for the negative control was equivalent to that of the antibiotic containing diet. Comparison among treatments showed that T_5 had the highest value of REE, while, T_6 recoded with the lowest REE value.

Table 26. Effects of using mixtures of feed additives in broilers on selected economic parameters

Parameters	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Cost per kg of starter diet (Birr)	7.60	7.64	10.85	10.65	11.40	10.96
Cost per kg of finisher diet (Birr)	6.92	6.96	10.17	9.97	10.72	10.28
Total cost of feed/bird (Birr)	40.96	43.44	62.1	56.0	60.48	61.18
Feed cost/kg of gain (0-28 th day) (Birr)	17.87	18.47	29.13	26	27.79	26.34
Feed cost/kg of gain (29-49 th day) (Birr)	22.67	23.16	34.62	33.23	30.96	36.23
Feed cost/kg of gain (1-49th day) (Birr)	20.64	21.02	32.28	30.11	29.64	32.0
Total cost of production /bird (Birr)	67.06	69.54	88.21	82.13	86.58	87.28
Feed cost/kg carcass/bird (Birr)	30.43	31.23	49.36	45.13	45.77	49.56
Total income/bird (Birr)	121.47	125.25	114.49	111.78	119.04	111.48
Net return/bird (Birr)	54.41	55.70	26.28	29.64	32.45	24.19
Economic efficiency (ECE)	81.0	80.1	29.7	36.0	37.6	27.8
Relative economic efficiency (REE)%	100.0	98.9	36.7	44.4	46.4	34.3

T_1 = Negative control. T_2 = Positive control. T_3 = T_1 + 0.5% OMP + 1% GAP. T_4 = T_1 + 0.5% OMP + 1% GIP. T_5 = T_1 + 1% GAP + 1% GIP. T_6 = T_1 + 0.33% OMP + 0.66% GAP + 0.66% GIP

5. DISCUSSION

5.1. Phase I: Response of Broiler Chickens to Diets with Different Levels of Oyster Mushroom, Garlic and Ginger Powders

5.1.1. Nutrient content

The proximate properties of OMP reveal that it is a rich protein source. The CP content of OMP (20.51) was in accordance with the results reported by Aishah and Rosli (2013) where sun dried OMP contains 20.89% CP. Crisan and Sands (1978) also showed that, on a dry weight basis, OMP normally contains 10.5-30.4% CP. However, the CP content of GAP reported in this study was by far lower than the results reported by Okolo *et al*. (2012) and Ali *et al*. (2014). GIP was close to the results reported by scholars (Ademola *et al*., 2009; Shirin and Jamuna, 2010 and Okolo *et al*., 2012). CF and ME values for OMP and GIP were equivalent, whereas, GAP had the lowest CF content with a rather high ME value.

5.1.2. Performances

5.1.2.1. Feed intake

The inclusion of herbal feed additives did not affect FI of the birds during the starter phase, except for treatment diet containing 2% GAP, where FI was significantly decreased compared to birds kept on diet containing 1% OMP. During the same period, the birds responded positively to the OMP, whereas, an opposite trend was observed in FI for the same treatment during the finisher phase. Birds preferred the feed containing GIP at 2% level over the control diet, whereas, positive effects were accomplished only during the finisher period. According to Zhang *et al*. (2009) most of the researchers reported that, the better performance of the birds fed ration containing ginger was due to an improvement in palatability and the quick digestion improvement effect of this natural product. They further postulated that due to the effect of this natural product, the digestive tract would have been emptied earlier and feed consumption will have been promoted. Ginger had also been found to increase secretion of gastrointestinal enzymes including

lipase, disaccharidase and maltase (Zhang *et al*., 2009). The improved performance in terms of FI and BWG may be attributed to the two types of digestive enzymes in ginger; protease and lipase, which are present as part of the plants natural protective mechanisms (Zhang *et al*., 2009). Zhao *et al*. (2011) also reported that ginger enhances animals' nutrient digestion and absorption because of its positive effects on gastric secretion, enterokinesia and digestive enzyme activities. The increment in feed consumption by the birds kept on diets containing GIP 2%, which was illustrated in this study, may be also due to the pungent taste or aroma and flavor imparted to the feed by ginger. The characteristic odor and flavor of ginger which were observed in this study may be due to a mixture of volatile oils like shogaols and gingerols (Sasidharan and Nirmala, 2010). The lack of significant effects of GAP on FI, BWG and FCR of broilers observed in this study may be attributed to the intense smell of the local garlic variety, which required a period of adaptation of chickens to this kind of additive.

The above results were in agreement with Hossain *et al*. (2014a) who also reported a significant improvement in FI for birds fed with diet containing GIP 1% compared to OMP 1%. Oleforuh-Okoleh *et al*. (2014) also reported an improved FI of birds fed diet containing 1.4% GIP. The work by Daneshmand *et al*. (2011) also confirms that inclusion of 0.2% OMP significantly decreases FI which was in accordance with this study. Similar results were also reported by Rahmatnejad *et al*. (2009) on the absence of a significant difference in FI while using GAP1% as well as Zomrawi *et al*. (2011) and Mohammed *et al*. (2014) at GIP 1%. Ademola *et al*. (2009) also confirms the absence of a significant difference in FI among diets containing 2% of GAP and GIP.

Contrary to this finding (Arkan *et al*., 2012) at 0.1% and 0.2%, (Zomrawi *et al*., 2013) at 1.5% and 2% and (Oleforuh-Okoleh *et al*., 2014) at 1.4% reported a significant reduction of FI on birds kept on diet containing the indicated levels of GIP. Also not in line with the current results, Oleforuh-Okoleh *et al*. (2014) reported improved FI of birds fed 1.4% GAP. The current finding is not also in agreement with the report of Herawati (2010) who stated that broilers fed 2% dried supplementary red ginger meal had significantly lower FI than those on the control diet.

5.1.2.2. Body weight gain

Feeding OMP to broilers at 2% inclusion level generally negatively affected the ADG, BWG and FBW of birds, whereas, the other treatment feeds containing GAP and GIP did not significantly improve growth performance. Treatments using OMP generally displayed the lowest body weights, which indicate that this important performance parameter is adversely affected. The decrease in BWG for 2% OMP was partly accounted for by the lowest FI of birds in this treatment group. This may be due to the unpleasant flavor of OMP used in the study which may negatively affect the palatability of the treatment diet. Ivana *et al*. (2017) also indicated that as the mushroom ages, the flesh becomes tough and the flavor becomes acrid and unpleasant. On the other hand oyster mushroom may also possess an active compound responsible for reducing the body weight *in vivo*. The results of inclusion of oyster mushroom in this study provide a strong basis for further research in obese subjects to reduce body weight.

In the current work, comparison among treatment feeds containing herbal feed additives indicates that, GAP 2% and GIP at both inclusion levels, were superior over OMP at 2% inclusion level. This observation agrees with the earlier reports of Hossain *et al*. (2014a) where no significant differences were observed between mushroom (*Agaricus bisporus*), control diet and GAP at (1%) inclusion level. The same author also reported that GIP and GAP resulted in a significantly better BWG than inclusion of mushroom at (1%) inclusion level, which is in line with this result. The absence of a significant improvement in daily BWG and FBW of birds fed two different levels of GIP was also confirmed by scholars (Ashayerizadeh *et al*., 2009 and Rahmatnejad *et al*., 2009 at 1%; Kehinde *et al*., 2011 at 1.5, 3.0 and 4. 5%; Zomrawi *et al*., 2011 at 1.5%; Sadeghi *et al*., 2013 at 0.5%; Zomrawi *et al*., 2013 at 1 and 2% as well as Ahmed *et al*., 2014 and Mohammed *et al*., 2014 at 0.125, 0.175 and 0.225%.) Unlike the above, Arkan *et al*. (2012) reported significantly higher FBW at 0.1% and 0.2% inclusion levels of GIP and Oleforuh-Okoleh *et al*. (2014) also reported a significant improvement in BWG at 1.4% inclusion levels of GAP and GIP.

Contrary to the current finding, Onu (2010) also reported a significant improvement in BWG at 0.25% inclusion levels of GAP and GIP. Giannenas *et al*. (2010a) also showed improved performance of broiler chickens while adding 1% and 2% of an edible mushroom (*Agaricus bisporus*) to the diet which is not in accordance with the current result.

5.1.2.3. Feed conversion ratio

Higher or lower FI and BWG do not indicate the good or bad performance of birds rather FCR indicates the actual performance. Lower FCR is an indicator of a better performance. Lower FCR explains lower FI accompanied with higher BWG. In the current study diet containing OMP at 2% inclusion level was the least efficient in terms of the response of birds as explained by FCR**.** These results also suggest that there is dose-dependent variation in the FCR due to inclusion of OMP. Increasing the dose of OMP decreases the feed efficiency.

These results are consistent with the findings of Ademola *et al*. (2009), who reported the absence of a significant difference in FCR between the control diet and diets containing GAP and GIP at 1, 1.5 and 2% inclusion levels. Similarly Hashemi *et al*. (2008), also reported the absence of a significant difference in FCR following the inclusion of GIP 1%, GAP1% and OMP1%. Scholars (Moorthy *et al*., 2009 ; Kehinde *et al*., 2011 ; Zomrawi *et al*., 2011; Sadeghi *et al*., 2013; Zomrawi *et al*., 2013; Mohammed *et al*., 2014 and Ahmed *et al*., 2014) also reported the absence of a significant difference in FCR due to the inclusion of GIP at different levels in broiler diets. Similarly, scholars (Ashayerizadeh *et al*., 2009 and Rahmatnejad *et al*., 2009) also reported the absence of a significant difference in FCR due to the inclusion of GAP1%. Similar to the current result, Daneshmand *et al*. (2011) also reported that inclusion of OMP at a very low inclusion level of 0.2% did not bring about a significant effect on FCR.

Contrary to our finding, Arkan *et al*. (2012) showed that inclusion of a very low amount of GIP (0.2%) improved the FCR while a rather lower FCR of birds fed on diets

containing 0.25% GAP and GIP (Onu, 2010) as well as 0.2% GIP were also reported (Oleforuh-Okoleh *et al*., 2014).

5.1.3. Carcass yield

5.1.3.1. Slaughter weight

In the present study, adding OMP to broiler diet at 1% and 2% inclusion levels brings about a reduction in slaughter weight. A significant reduction was observed at 2% inclusion level, whereas, the inclusion of GAP and GIP did not significantly affect slaughter weight of birds compared to the control diet. This result is in agreement with Mohammed *et al*. (2014), who reported that, GIP inclusion at 1% and 1.5% levels had not any significant effect on slaughter weight. On the other hand, Nikola *et al*. (2016) (GAP at 0.5 and 1%), Karangiya *et al*. (2016b) (GAP and GIP at 1%) reported a significant increase in slaughter weight, which is not in accordance with the current result.

5.1.3.2. Dressed weight and its percentage

Neither OMP, GAP nor GIP significantly affected dressed weight and dressing percentage at both inclusion levels in this study. Birds kept on diets containing OMP had the lowest dressed weight, and with increased level of OMP (2%) inclusion the amount of dressed weight recovered decreased significantly compared to birds kept on ration containing 2% GAP. Numerically better dressing percentage was obtained from birds kept on diet containing 2% GAP (91.1%).

These results could be supported by the findings of scholars Rani *et al*. (2016) and Hossain *et al*. (2014a) who reported the absence of a significant change in dressed weight and dressing percentage using OMP at inclusion levels of 0.5%, 1% and 1.5% as well as mushroom powder (*Agaricus bisporus*) at inclusion levels of 1%, respectively. The absence of a significant difference in dressing percentage due to inclusion of GAP was also supported by scholars (Huda *et al*., 2015 (GAP at 1, 2 and 3%) and Nikola *et al*., 2016 (GAP at 0.5%)). Kharde *et al*. (2014) also reported that dressing yield was not

significantly affected by inclusion of 0.5g and 1g/kg of GAP. Similar to the effects of GAP, the current results for effects of GIP on dressing percentage are also supported by scholars (Zomrawi *et al*., 2013 (GIP at 0%, 1% and 1.5%); Huda *et al*., 2015 (GIP at 0.25, 0.5 and 0.75%) and Karangiya *et al*., 2016b (GIP at 1%)).

Not in line with our finding, different authors reported contradictory results. Among these, Karangiya *et al*. (2016b) and Nikola *et al*. (2016) reported a significant reduction in dressing percentage through inclusion of GAP at 1% inclusion level. Oleforuh-Okoleh *et al*. (2014) and Safa (2014b) also found significantly higher dressing percentage using GIP and GAP at 14g/kg as well as GIP at 1% and 1.5% of the diet, respectively. An improvement in dressed weight was also reported by Karangiya *et al*. (2016b) while using GAP and GIP at 1%. According to Ahmed and Kloor (2009), Carcass weight, carcass cuts, dressing percentage without giblets and dressing percentage with giblets were not also significantly influenced by inclusion of GIP at 0.5 and 1% level.

5.1.3.3. Eviscerated weight, eviscerated percentage and commercial cuts

The inclusion of all the three feed additives did not significantly affect eviscerated weight of birds. These results were equally in harmony with the finding of Ebrahimnezhad *et al*. (2014) who reported the absence of a significant difference in percentage of eviscerated carcass while using diets containing GIP at 0, 5, 10, 15, 20, and 25 g/kg of diet.

Treatment effects of the three additives at two levels described in this study were not significant on commercial cuts and giblets weight except for the wing weight where lower yield was recorded for OMP inclusion compared to that of 2% GIP. Onu (2010) and Javandel *et al*. (2008) also reported the absence of a significant difference in all carcass characteristics among birds fed on diets containing 0.125%, 0.25, 0.5, 1 and 2% GAP and control diet, respectively. The findings of Raeesi *et al*. (2010) showed that inclusion of 1% and 3% GAP in the broiler diet had no significant effects on relative weights of carcass among different treatments. Ashayerizadeh *et al*. (2009) also showed that 0.1% GAP does not affect thigh and breast weight compared to the control diet. Similarly, Nikola *et al*. (2016) reported the absence of a significant difference in the share of breast,

drumstick with thigh and back while using 0.5 % and 1% GAP. Huda *et al*. (2015) also confirmed the absence of a significant treatments effects in relative weight of drumstick, breast and thigh while using GAP 1, 2 and 3%. According to Huda *et al*. (2015) inclusion of GIP at 0.25, 0.5 and 0.75% did not also bring about a significant change in relative weight of breast, thigh and drumstick. Habibollah *et al*. (2013) mixed the basal diets of Ross broiler chickens with GIP at 0.5 and 1% inclusion levels and observed no significant effects on relative weight of thigh and breast muscles.

The current findings are contradictory with those of different scholars (Ashayerizadeh *et al*., 2009 (0.1% GAP improves carcass yield); Kharde *et al*., 2014 (0.5g/kg and 1g/kg GAP significantly reduced the thigh muscle and breast muscle); Valiollahi *et al*., 2013 0.02% GIP (increased relative weight of drumstick) and Nikola *et al*., 2016 (0.5 and 1% GAP increased breast weight, reduced relative weight of wing, increased ready to grill weight).

5.1.3.4. Weight of giblets

Among the organs measured in this category, it was only the weight and share of the heart that was negatively affected, which was observed for the dietary treatment which contained 1% OMP. Supporting our results, Karangiya *et al*. (2016b) also reported the absence of a significant difference on weight of the liver and heart through inclusion of 1% GAP. Huda *et al*. (2015) also used sun dried GAP at 1, 2 and 3% of the basal diet and made the same conclusion regarding the relative weight of heart, liver and gizzard. Likewise Huda *et al*. (2015) also used GIP at 0.25, 0.5 and 0.75% inclusion levels and reports the absence of a significant difference in relative weight of heart, liver and gizzard. No significant effects on relative weight of liver, heart and gizzard was also observed through inclusion of GIP at 125 mg/kg inclusion level (Zeweil *et al*., 2016). On the contrary, inclusion of 1% and 1.5% GIP was reported to have decreased the relative weights of liver and gizzard (Safa, 2014b).

5.1.3.5. Non edible parts

The results of the present study showed non-significant differences in all the treatments groups in the weight of non-edible parts measured. These results are in line with finding of Rani *et al*. (2016) who stated that, feeding OMP at 0.5%, 1% and 1.5% does not affect internal organ weights of broilers. Huda *et al*. (2015) and Karangiya *et al*. (2016b) also concluded the absence of a significant difference on weight of GIT through inclusion of GAP at 1, 2 and 3% and GAP at 1%, respectively. Raeesi *et al*. (2010) (GAP at 1% and 3%) as well as Huda *et al*. (2015) (GIP at 0.25, 0.5 and 0.75%) in the broiler diet had no significant effects on relative weights of digestive organs among different treatments. Similarly, Javandel *et al*. (2008) reported that there was no significant effect on intestine percentage of broilers fed GAP at level up to 2%.

With respect to the effect of dietary inclusion of OMP, GAP and GIP in the diet on the abdominal fat percentage, all the three herbal feed additives did not also significantly reduce the trait mentioned, although birds kept on diet containing 1% OMP had the lowest abdominal fat percentage (0.79%). The cell wall of oyster mushroom contains chitin as a compound of cell structure that can bind with fat. Chitin is an N-asetilglucosamine polymer that contains negatively charged amino group, while, fat compound is positively charged. Both of these compounds will be bound by electrostatic interactions (Muzzareli, 2006), next fat that has been bound with chitin will be taken out of the body since chitin cannot be digested by poultry. This will reduce the amount of absorption and deposition of fat in the body and eggs of the poultry (Maezaki *et al*., 1993). The abdominal fat percentage reported in the current study (0.79-1.33 %) is in the range described by Becker *et al*. (1979) (0.73 to 3.86% of live weight).

Similar results were also reported by Rani *et al*. (2016). Raeesi *et al*. (2010) also reported that inclusion of 1% and 3% GAP in the broiler diet had no significant effects on relative weights of fat pad among different treatments. Scholars (Habibollah *et al*., 2013 (GIP at 0.5 and 1%); Ebrahimnezhad *et al*., 2014 (GIP at 0, 5, 10, 15, 20, and 25 g/kg of diet); Huda *et al*., 2015 (GIP at 0.25, 0.5 and 0.75%) and Zeweil *et al*., 2016 (GIP at 125 mg/kg) also reported the absence of a significant effect on abdominal fat percentage while

using GIP at the above mentioned inclusion levels. The results of the study by Huda *et al*. (2015) also showed that the abdominal fat values were not affected significantly by the dietary ginger at 0.25%, 0.50% and 0.75% levels of inclusion. The absence of a significant difference in abdominal fat percentage may be because of the fact that each treatment diet is formulated to be nearly iso-protein and iso-caloric, and abdominal fat percentage is influenced by the balance of energy and protein in diet.

Not in line with the current study, different authors (Ashayerizadeh *et al*. (2009) (GAP at 0.1%); Habibollah *et al*. (2013) (GIP at 1.5%); Oleforuh-Okoleh *et al*. (2014) (GIP and GAP at 14g/kg); Valiollahi *et al*. (2013) (GIP at 0.02%); Safa (2014b) (GIP at 1% and 1.5%) and Huda *et al*. (2015) (GAP at 1, 2 and 3%)) inclusion of the respective test ingredients at the given levels in the diets of broilers reportedly reduced abdominal fat percentage in a significant manner.

5.1.4. Hematological values

Feeding 1% OMP improves the RBCs count but didn't have any significant effect on all the other hematological parameters. Hematological parameters in birds had been shown to be influenced by various factors including physiological condition (age, sex) (Alodan and Mashlay, 1999), environmental conditions (as season) and diet contents (Seiser *et al*., 2010). According to researchers (Pollack *et al*., 2005 and Cray, 2010), the results of blood analysis from the current experiment for RBC (3.77-5.01($\times 10^6$ cells/μL), TWBC (1.9-3.8 ($\times 10^3$ cells/μL)), Hb (10.0-11.4g/dL), PCV (29.7-35.2%), MCV (63.2-86.9 fL) and MCHC (29-37%) were within the safety limits for healthy chicken.

These results are supported by Onu (2010), who also inferred that the hematological indices were within safety limits for broilers fed diets containing garlic and ginger. In the current study birds kept on diet containing 1% GIP, 2% OMP, 2% GAP, 2% OMP and the control diet had the highest values of RBC, Hb, PCV, (MCV and MCH) and MCHC, respectively. The results are also in agreement with Abdalla *et al*. (2009) where, results of erythrogram revealed a significant increase in RBCs count in mushroom treated group in

a dose of 1.17%. Supporting our result, Wasser *et al*. (2000) reported that biologically active substances from higher basidiomycetes of mushroom stimulate hematogenesis.

In the same aspect the data on hematological indices associated with GAP inclusion indicates that the RBCs count values were improved at both inclusion levels and the same result was observed for PCV at 2% inclusion levels. This may be explained as garlic plant is considered an active oxygen scavenger. It is thus possible that garlic components compete with hemoglobin in the red blood cell for oxygen resulting in hypoxia which then stimulates hemoglobin synthesis and red blood cell production (Iranloye, 2002). It has been also found that garlic has some constituents that may play a role in the function of organs related to blood cell formation such as thymus, spleen and bone marrow (Shalaby et al., 2006).

These results are supported by Yasser *et al*. (2014), who conducted an experiment on broiler chickens by feeding basal diet containing 1% garlic and observed a significant increment in the values of RBCs and PCV. Also, decreasing the level of GAP to 1% caused a significant increase in total white blood cell (TWBC). These data therefore support the earlier reports by Sumiyoshi (1997) that garlic extracts stimulate immune functions. This observation may partly explain the role of garlic in activating the natural killer cells, the function of T-lymphocytes and the level of interleukin-2 (Tang *et al*, 1997). The non-significant effect of feeding garlic containing diet on Hb values in broilers and layers was also supported by Prasad *et al*. (2009b) and Zeryehun *et al*. (2017). According to Siegmund (1973), the degree of anemia is determined by Hb, PCV and RBCs count, while, the characterization of anemia is aided by calculated red cell indices (i.e. MCV, MCH and MCHC). In the present study, following the addition of 1% and 2% GAP, MCH and MCHC values were negatively affected in a significant manner, while, the other hematological parameters remain unaffected. According to Guyton (1986) MCHC, indicates the concentration of hemoglobin per unit volume of RBCs, it provides similar information as the MCH but considered to be more accurate. A low MCHC is usually accompanying hypochromic microcytosis which is seen in iron deficiency (Davidson, 1969). Augusti (1996) indicated that prolonged feeding of high

levels of raw garlic in rats have resulted in anemia. The other possible explanation is that garlic may interfere with copper absorption which is involved in the formation of red blood cells, the absorption and utilization of iron, which result an iron deficiency anemia (Aiello, 1998).

Not in line with the current study Elagib *et al*. (2013a) and Yasser *et al*. (2014) reported the absence of significant effects of feeding GAP on the PCV and RBCs count. Similarly Onu (2010) and Hossain *et al*. (2014b) indicated no significant difference in the hematological indices due to garlic inclusion in different forms. Elagib *et al*. (2013a) also shows no difference in RBCs count and PCV among the groups fed on 3% and 5% GAP. Other contradicting reports include Javed *et al*. (2012) and Elagib *et al*. (2013a) where a decreased mean Hb value were reported.

The current study identified that feeding GIP did not affect the different hematological parameters except for the RBCs counts, MCV and MCH where significant improvement was observed at both inclusion levels for RBCs counts and the latter two parameters were negatively affected by 1% inclusion of GIP. This finding is in agreement with Kehinde *et al*. (2011) who also observed slight improvements in RBCs counts following inclusion of sun dried GIP in broiler diets. Also in line with the current finding, Marth *et al*. (2012) indicated that Hb and PCV of the broiler finisher chickens fed ginger by-product meal did not vary statistically, suggesting that the feed had no negative effects on levels of PCV and Hb. In addition to the above supporting findings, Zomrawi *et al*. (2011) showed that GIP at levels of 0%, 0.5%, 1% and 1.5% no significant effects on Hb and PCV. In addition to the above, Fatema *et al*. (2014) also indicated that, observation of hematological parameter (Hb, PCV) did not show any significant difference between the control and ginger extract treated group.

Ogbe *et al*. (2010) stated that fluctuations in hematological values of avian blood are a normal phenomenon, and in most instances the variations in hematological values depend on the physiological state of the birds. Since the results of hematological analysis were inconsistent in this study, the hematological data did not make it clear whether the type of

feed additive or level of inclusion provided any significant and conclusive changes in relation to the above mentioned parameters.

Generally inclusion of the test ingredients at all levels didn't have any adverse effects on the blood constituents of broiler chickens. The normal hematological values portray the nutritional status of the broiler chickens and thus indicating adequate nourishment of the birds (Church *et al*., 1984). The improvements observed for some treatments in the RBC and PCV values of birds fed the phytogenics containing diets suggest that the diets were better utilized and assimilated into the blood stream for use by the birds and the medicinal plants have enhancing effect on the blood profile of broiler chickens when offered at the current inclusion level in the dried form.

5.1.5. Biochemical values

In the current study the treatments did not have any significant effect on all the biochemical parameters considered. It can be inferred that the results of blood analysis from the current experiment glucose (216.1-256.5 mg/dL), total cholesterol (110.2-148.6 mg/dL), total protein (3.15-3.60 g/dL), albumin (1.8-2.55 g/dL), globulin (0.93-1.35 g/dL), albumin/globulin ratio (1.52-3.43), ALP (1021-1129 IU/L), ALT/GPT (10.3-16.3 IU/L) and AST/GOT (352-531 IU/L) were within safety limits for healthy chickens (Campbell, 2012).

The results of the current finding regarding the effects of OMP on the different biochemical parameters is supported by Hossain *et al*. (2014a) (1%), Abdalla *et al*. (2009) (17.5 g/kg and 23.5 g/kg) where no significant effect was reported on blood glucose level. Similarly different authors also reported that inclusion of OMP had no significant effect on blood plasma cholesterol level, and some of these include, Toghyani *et al*. (2012) at (10 and 20g/kg); Maria *et al*. (2013) at (1, 2, 3, and 4%) and Hossain *et al*. (2014a) at (1%) inclusion levels. The non-significant effect of OMP inclusion on serum total protein, albumin and globulin is supported by Toghyani *et al*. (2012) at (10 and 20g/kg) inclusion levels. Abdalla *et al*. (2009) reported that broiler chickens fed with OMP in a dose of 17.5g/kg and 23.5g/kg of the diet had no significant effects on albumin/globulin ratio and

AST level. Not in line with the current study Abdalla *et al*. (2009) indicates that inclusion of 17.5 g/kg and 23.5 g/kg OMP increased total protein, albumin and globulin content while it results in a reduced blood plasma cholesterol level.

The current findings on the non-significant effect of inclusion of GAP on serum glucose levels is supported by Hossain *et al*. (2014a) at 1% and Huda *et al*. (2015) at 1 and 2% inclusion levels. Kim (2010) also showed that garlic did not affect glucose level significantly in blood of birds. According to Mansoub (2011), basal diet plus 1 gr/Kg GAP had no significant effect on serum cholesterol. Similarly, Onu (2010) and Huda *et al*. (2015) reported similar findings showing that inclusion of GAP at 0.25%, 1% and 2% had no significant effect on serum protein levels. According to Onu (2010), diets containing 0.25% garlic did not also significantly affect serum albumin and globulin contents. These results show that the quality and quantity of the dietary proteins were nutritionally adequate and there was no alteration of normal systemic protein utilization (Onu, 2010). According to Awosanya *et al*. (1999) blood protein depends on the quality and quantity of dietary protein. The absence of a significant effect on serum ALP levels in the current study is also supported by Huda *et al.* (2015) using 1% and 2% GAP.

Not in line with the current results, a lot of research works have reported a reduction in blood cholesterol concentration due to inclusion of GAP, among these, Chowdhury and Smith (2002) showed that serum cholesterol decreased significantly with increasing levels of sun-dried dietary garlic paste in laying hens. Similarly, significant hypocholostrolemic effects were observed through inclusion of GAP at 0.2 and 0.4% (Jamal and Omar, 2011); 0.1% (Rahimi *et al*., 2011); 0.2 and 0.4% Issa and Omar, 2012); 2, 6 and 8% (Khan *et al*., 2012a); 1% (Hossain *et al*., 2014a); 0.5, 1 g/kg (Kharde *et al*., 2014); 0.5% and 1.0% (Puvača *et al*., 2014) as well as 1% and 2% (Huda *et al.*, 2015).

Inclusion of 1% and 2% GIP to broiler diet did not also affect most of the different biochemical parameters considered in the current study. Most research works also support these results. Among these reports by scholars (0.5, 1 and 1.5% (Zomrawi *et al*., 2011); 0.5 and 1% (Habibollah *et al*., 2013); 7.5 or 15 g/kg (Ahmed *et al*., 2014); 5, 10, 15, 20,

25 g/kg (Ebrahimnezhad *et al*., 2014); 1% and 2% (Huda *et al*., 2015) and 2% (Sayeed *et al*., 2016)) shows that the indicated inclusion levels of GIP had no significant effect on serum glucose levels. The absence of a significant effect on serum cholesterol level due to inclusion of GIP is also in agreement with the reports of Habibollah *et al*. (2013) at (0.5 and 1%); Ahmed *et al*. (2014) at 0.5, 0.75 and 1%; Ebrahimnezhad *et al*. (2014) at 5, 10, 15, 20, 25 g/kg and Hossain *et al*. (2014a) at 1%. Regarding the non-significant effects of GIP inclusion at different inclusion levels on total protein content of serum different scholars (Onu, 2010 at 0.25%; Zomrawi *et al*., 2011 at 0.5, 1 and 1.5%; Ebrahimnezhad *et al*., 2014 at 5, 10, 15, 20 and 25 g/kg as well as Huda *et al*., 2015 at 1% and 2%) also reported similar results which are in agreement with the current study. GIP included at 0.25% (Elagib *et al*., 2013b) and 5, 10, 15, 20, 25 g/kg (Ebrahimnezhad *et al*., 2014) inclusion levels also did not have significant effects on albumin and globulin content of serum from broiler chickens. The reports by Huda *et al*. (2015) (1%) also matched to the current finding regarding the non-significant effect of GIP inclusion on serum ALP levels. Inclusion of 2% OMP caused numerically higher AST activity which may be due to the fact that level of this substance may have made some inflammation in liver. On the other hand numerically reduced liver enzyme (AST) activity was observed for birds kept on 2% GIP which is equally in harmony with the results reported by Huda *et al*. (2015) who used the same level of GIP in broiler diet. It seems that one of the main reasons for this protective feature is the effect of ginger on the expression of proliferating cell nuclear antigen (PCNA). PCNA is a nuclear protein that is involved in regulating cell proliferation (Abdulaziz *et al*., 2013).

On the contrary birds kept on diet containing 1 and 1.5% GIP had higher cholesterol level and higher protein and albumin content at 0.5 and 1% inclusion level (Ahmed and Kloor., 2009). Rather reduced serum cholesterol level associated with GIP inclusion was also reported by Ahmed and Kloor (2009) at 0.5 and 1%; Zomrawi *et al*. (2011) and Wafaa *et al*. (2012) both at 0.5 and 1%) and Huda *et al*. (2015) at 1 and 2%. Reduced blood glucose level due to inclusion of GIP was also reported by Ahmed and Kloor (2009) at 0.5% and 1% and Hossain *et al*. (2014a) at 1% inclusion levels.

5.1.6. Economic appraisal

The results of this trial indicates that birds kept on T_7 gave the highest TI followed by birds kept on T_6, T_1, T_5, T_4, T_2 and T_3, respectively. Birds kept on T_1 gave the highest NR and ECE, whereas, the lowest NR and ECE was obtained from birds kept on T_3; implying that the control diet is the best diet from the economic point of view; since reaching the highest body weight or maximum egg production in return for each unit of FI is the aim of raising commercial poultry (Raeesi *et al*., 2010). More than 70 per cent of expenses in broiler management are in the form of feed management. So, the cost of feed in broiler management can be reduced by using less expensive feed additives (Senthilkumar *et al.*, 2015). The return on investment for alternatives to AGPs will depend on both the biological impact and the actual market price. It must be taken in to account the fact that the feed cost of these alternatives was quite high. This suggests that inclusion of 1% and 2% level of OMP, GAP and GIP has substantially increased the feed cost per unit live BWG and is practically uneconomical.

5.2. Phase II: Response of Broiler Chickens to Diets with Different Mixtures of Oyster Mushroom, Garlic and Ginger Powders as Substitutes for Antibiotic Growth Promoter

5.2.1. Performances

5.2.1.1. Feed intake

The overall FI in the current study tended to be higher in groups fed with antibiotic as well as diet containing mixture of OMP and GAP, but these differences were not statistically significant compared to negative control diet and the diet containing mixture of OMP, GAP and GIP. This result is in harmony with the findings of different authors who also reported that inclusion of AGPs like ampicillin, enramycin, oxytetracycline, virginamycin, flavomycin and enramycin in broiler diets did not significantly improve FI compared to the negative control diet (Daneshmand *et al*., 2012; Majid *et al*., 2013; Hossain *et al*., 2014ab; Safa, 2014ab and Huda *et al*., 2015). It was also indicated that inclusion of different AGPs did not significantly improve FI of birds compared to herbal

feed additives like garlic and ginger (Hossain *et al*., 2014ab; Safa *et al*, 2014; Huda *et al*., 2015; Sarica *et al*., 2005). Ademola *et al*. (2009); Safa (2014a) as well as Bamidele and Adejumo (2012) also concluded that FI of growing pullets and broilers was not also significantly improved by feeding diets containing varying mixtures levels of garlic and ginger. Similarly, Onu (2010) also indicated that diet containing a combination of 0.25% of garlic and ginger did not have any significant effect on FI. In line with the current study, Oleforuh-Okoleh *et al.* (2015) showed that, using mixture of ginger and garlic extract in drinking water did not significantly improve weekly FI. Our results were not in agreement with Daneshmand *et al*. (2012) who reported that the combination of garlic, oyster mushroom and propolis extract significantly reduce daily FI compared to the positive and negative control diets. Also unlike with the current finding, Karangiya *et al*. (2016a, b), reported that a combination of 1% of garlic and ginger significantly increased FI. Thus, the assumption that mixtures of herbs improve the feed palatability does not seem to be justified in the current study.

5.2.1.2. Body weight gain

Birds kept on treatment diet containing antibiotic (0.30 g oxytetracycline / kg) achieved numerically the highest FBW, whereas, birds on T_6 achieved the lowest FBW. Weight improvement of the birds fed on the mixture of garlic and ginger containing diets was comparable with birds fed on the antibiotic-containing diets. Trends of BWG for the different treatments were also similar to the FBW achieved. These results are equally in harmony with Bamidele and Adejumo (2012), who reported the absence of a significant difference in FBW and BWG in growing pullets using diet containing different mixture levels of air dried GAP and GIP. Similarly, Karangiya *et al*. (2016a, b) also showed that basal diets containing a combination of 1% of GAP and GIP had no significant effects on FBW and BWG of broilers. Similar to our result, many authors also indicated that basal diet containing antibiotic (Doxystin, Ampicillin, Oxytatracycline, Flavomycin, Virginamycin, Neomycin, Enramycin) had no significant effect on BWG of broiler chickens (Elagib *et al*., 2013b; Majid *et al*., 2013; Hossain *et al*., 2014ab; Safa *et al*, 2014; Sarica *et al*., 2005;).

Not in line with these results, Daneshmand *et al*. (2012) reported that birds kept on basal diet containing a combination of garlic, oyster mushroom and propolis extract achieved a significantly lower FBW and ADG compared to positive and negative control diets. On the other hand, contrary to the current results, some reports show that diet containing different combinations of garlic and ginger significantly improved FBW of broilers (Ademola *et al*., 2007; Onu, 2010; Safa, 2014a; Oleforuh-Okoleh *et al*., 2015). Also not in line with this result, Huda *et al.* (2015) reported that birds kept on diet containing antibiotic (Neomycine) had significantly higher BWG.

5.2.1.3. Feed conversion ratio

In the current study it appears that treatments did not have any significant effect on FCR compared to the controls, whereas, within treatment comparisons indicates that diets containing GAP and GIP mixture significantly improves feed efficiency of birds compared to mixtures of OMP + GAP as well as OMP + GAP + GIP resulting high nutrients availability due to changes in the intestinal ecosystem. This was attested by a reduced FCR in animals of the GAP and GIP mixture group 0.25% lower than positive control group and 0.17% lower than the negative control group). In the current study, synergistic effect of mixtures of garlic and ginger gave a strong antibacterial activity against CFU and *Escherichia coli* bacterial loads and creates a healthier gut microflora, aiding optimum digestion and improving bird performance in terms of improved FCR during the finisher phase as compared to the antibiotic containing diet. This finding is important basis for the herbal mixture studies of GAP and GIP to find an alternative to AGP since improved feed efficiency will lower the cost of production. The negative effect of OMP on FCR in the first study is also reflected in this study, where OMP containing mixture (0.5% OMP + 1% GAP) significantly increased FCR in comparison to the non OMP containing mixture (1% GAP + 1% GIP). The current results reflecting the non-significant effect on FCR due to the inclusion of different mixtures of herbal feed additives as compared to the control diet are supported by Bamidele and Adejumo (2012) who used GAP and GIP mixture diets as growth promoter. In a published study by Ademola *et al*., (2007), diets containing different mixture levels of garlic and ginger did not also improve FCR except for 1% garlic and 0.25% ginger mixture. Similarly, basal

diet containing the antibiotics doxystin, ampicillin, oxytetracycline, flavomycin, virginamycin, neomycin and enramycin had no significant effect on FCR of broilers compared to negative control diet (Sarica *et al*., 2005; Elagib *et al*., 2013b; Majid *et al*., 2013; Hossain *et al*., 2014ab and Safa *et al*, 2014). Also in line with the current results, inclusion of different antibiotic feed additives did not bring about a significant change in FCR compared to diets containing garlic or ginger in powder, essential oil and extract forms (Sarica *et al*., 2005; Hossain *et al*., 2014ab and Safa *et al*, 2014).

The lack of a significant growth improvement due to mixtures of the three herbal feed additives as well as the antibiotics observed in this study could be attributed to the sanitary or clean conditions (good management, strict biosecurity and properly disinfected experimental facility) under which birds were raised. The beneficial effects of antimicrobial feed additives on growth performance would be apparent under poor hygienic conditions or when feeding poorly digestible diets (Garcia *et al*., 2007). The current study was conducted under good rearing conditions. Under good conditions, broilers did not need any feed additives for maximum growth performance (Baurhoo *et al*., 2007). It is possible that the birds in our study were not stressed and their health status was good. Therefore, experimental conditions can be considered a possible reason for the absence of a significant performance enhancement following the inclusion OMP, GAP, and GIP to the basal diet in the current study.

Opposing results have been also published by different authors regarding the effects of additive mixtures on FCR of broiler chickens. Among these Oleforuh-Okoleh *et al*. (2015) stated that, using mixture of ginger and garlic extract in drinking water significantly improve FCR compared to sole inclusion of garlic and ginger. Onu (2010) also indicated that diets containing a combination of 0.25% of garlic and ginger resulted in a significantly lower FCR compared to control diet without antibiotic. In addition to the above, Safa (2014a) also showed that diets containing varying mixture levels of GAP and GIP in powder form resulted in a significantly lower FCR.

Contradicting results about effects of antibiotics inclusion on FCR was also reported by Daneshmand *et al*. (2012) where control diet containing Virginamycin (0.25 g/kg) as antibiotic diet significantly improve FCR of birds compared to diet containing the combination of the garlic, oyster mushroom and propolis extract as well as diet without antibiotic. Huda *et al*. (2015) also indicated that birds kept on diet containing Neomycine 16mg/kg significantly improve FCR compared to diet without antibiotic.

The variations between the results of the present study and those obtained by some other researchers could be attributed to differences in the variety and nutrient composition of herbal plants used, doses used, preparation process, the overall diet composition, environmental stress factors and duration of the experiments.

5.2.2. Carcass yield

5.2.2.1. Slaughter weight, dressed weight and its percentage

In the present study, inclusion of antibiotics or herbal mixtures did not bring about a significant change in slaughter weight. Numerically the highest slaughter weight was observed in broiler chickens fed with antibiotic containing diet. Dressed weight, dressing percentage and eviscerated weight of birds in T_6 was significantly reduced compared to the antibiotic containing diet, but not significantly different from the negative control diet. Similarly Hossain *et al*. (2014a) also reported that inclusion of antibiotics (1g ampicillin/L and 1g oxytetracycline/L) had no significant effect on average dressing percentage compared to negative control as well as diets containing garlic, ginger and mushroom as sole agents. The non-significant effect of inclusion of antibiotic (neomycin) on dressing percentage was also supported by Huda *et al*. (2015).

Not in line with the current study Elagib *et al*. (2013b) indicated that antibiotic (Doxystin) significantly increased dressing percentage compared to negative control diet. Daneshmand *et al*. (2012) also showed that Virginamycin (0.25 g/kg) as AGP had higher carcass yield compared to control and combination of garlic, oyster mushroom and propolis extract. Also not in line with the current finding Sarica *et al*, (2005) indicated

that slaughter weight and hot dressing percentages showed significant improvement with the inclusion of different mixture levels of GAP and GIP in broiler diets in comparison with control diet.

5.2.2.2. Eviscerated weight, eviscerated percentage and commercial cuts

Similar to the results of the dressing percentage, eviscerated weight and percentage of birds in T_6 was significantly reduced compared to the antibiotic containing diet but not significantly different from the negative control diet for the eviscerated weight. This result is also a reflection of the lowest performance of birds kept on T_6.

The weights of prime cuts were recorded at the end of the experiment. Results regarding relative organ weights were non-significant except breast muscle and relative weights of giblets which were in accordance with Denli *et al*. (2003) and Daneshmand *et al*. (2012). Elagib *et al*. (2013b) also reported that antibiotic (Doxystin) had no significant effects on weights of thigh muscle and wing. Moreover, Hossain *et al*. (2014b) also described that inclusion of antibiotics; enramycin did not affect relative organ weight of broilers. Similarly, Sarica *et al*. (2005) indicated that AGP (1g Flavomycin/kg) did not affect carcass yields and organ weights. Huda *et al*. (2015) showed that relative weights of breast, thigh and drumstick were not affected by inclusion of antibiotic (neomycin). Ademola *et al*. (2009) also fed different mixture levels of garlic and ginger and reported no significant effects on weight of head, neck, thigh, breast and back. The commercial and edible carcass components are the most important parameters which indicate the actual edible or marketable yield of carcass from broiler chickens. The absence of a significant difference due to inclusion of different mixtures of OMP, GAP and GIP as a substitute for AGP indicates that the inclusion of herbal feed additives did not adversely affected the yield of commercial and edible carcass components though numerically lower results were observed compared to both the negative and positive controls.

Contrary to the current study, Sarica *et al*. (2005) reported that the relative weight of commercial cuts (breast, thigh and drumstick); showed significant improvement with the inclusion of different mixture levels of GAP and GIP in broiler diets in comparison with

control diet. In the current study, the addition of mixtures of oyster mushroom, garlic and ginger did not affect the weights of the commercial cuts. This implies that the test diets did not contain any appreciable toxin.

5.2.2.3. Weight of giblets

The weight of giblets was not significantly affected by the dietary treatments except for the relative weight of giblets. Supporting our results, Elagib *et al*. (2013b) also reported that antibiotic (Doxystin) addition had no significant effects on weight of heart, liver and gizzard. Huda *et al.,* 2015 showed that relative weight of heart, liver, gizzard and intestine were not affected by inclusion of antibiotic (neomycin). Further findings of Karangiya *et al*. (2016b) described that weight of heart and liver was not significantly affected through feeding 1% mixture level of garlic and ginger.

5.2.2.4. Non edible parts

Fat deposition in the abdominal area of broilers is considered as waste in the poultry industry; since it represents a loss in the market and consumer acceptability, and enhances expense during the treatment of effluent produced when processing broilers (Willis *et al*., 2008). The results of this study indicate that the inclusion of different mixture levels of OMP, GAP and GIP in broiler diets reduced the weight and percentage of abdominal fat with a significant effect on antibiotic as well as oyster mushroom + garlic mixture containing diets matched to negative control which may be due to the action of both oyster mushroom and garlic which have been reported to possess lipid lowering effects (Konjufca *et al*., 1997; Gunde and Plemenitas, 2001 and Hossain *et al*., 2003). No clear mechanisms have been reported responsible for the reduction of lipid synthesis by herb oligosaccharides. It might in part be due to increasing beneficial bacteria such as Lactobacillus that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis. The results reported by Zhou *et al.* (2009) also agree with our findings, these scientists reported that application of chitooligosaccharide in diet reduced abdominal fat pad of broiler chickens. In addition, it has been reported that inclusion of mushroom extract plus probiotic in the diet result in a marked reduction

of fad pad in male and female broilers slaughtered at 49 days (Willis et al., 2007). Further findings of Ademola *et al*. (2009) described that garlic in a diet decreased production of abdominal fat pads of broiler chickens. Studies in animal models indicate that garlic depresses the hepatic activities of lipogenic and cholesterogenic enzymes, including malic enzyme, fatty acid syntheses and glucose-6 phosphate dehydrogenase (Qureshi *et al.*, 1983).

The non-significant effect of feeding diets containing different mixture levels of garlic and ginger on the weight of GIT was also similar with the results reported by Ademola *et al*. (2009) and Karangiya *et al*. (2016b). Unlike the current results Sarica *et al*. (2005) showed that adding different mixtures of GAP and GIP to broiler diet reduced the abdominal fat pad. Not in line with the current study, Huda *et al*. (2015) also reported the absence of a significant effect on abdominal fat percentages following the inclusion of antibiotic (neomycin).

5.2.3. Hematological values

It can be inferred that the results of blood analysis from the current experiment that RBC (1.46-2.35 ($\times 10^6$ cells/μL)), TWBC (2.1-3.6 ($\times 10^3$ cells/μL)), Hb (8.9-11.7g/dL), PCV (24.8-28.7%), MCV (127.0-191.5 fl) and MCHC (36.0-41.4%) were within safety limits for healthy chickens (Pollack *et al*., 2005 and Cray, 2010), hence, the values obtained could be considered normal.. The antibiotic (oxytetracycline) used in the present study had no significant effect on the hematological parameters considered. This is in harmony with Hossain *et al*. (2014b) where basal diet containing antibiotics (5 ppm enramycin) had no significant effects on RBC and TWBC counts. Based on these findings, it is also reasonable to observe that the different combinations of OMP, GAP and GIP had no significant effects on the hematological parameters considered except for Hb which was significantly higher for the garlic and ginger containing diet. These results are also in line with Bamidele and Adejumo (2012) where basal diet containing different mixture levels of garlic and ginger had significantly higher serum hemoglobin content. Oleforuh-Okoleh *et al*. (2015), also added 50 ml ginger and garlic in drinking water and observed no significant effect on PCV values. Similarly Onu (2010) also reported that control diet

containing a combination of 0.25% of garlic and ginger had no significant effects on RBCs, TWBC, Hb, PCV, MCV, MCH and MCHC. The non-significant effects of feeding broiler chickens with diet containing mixtures of garlic and ginger on RBC, TWBC, PCV and Hb is also supported by Ademola *et al*. (2009) who used different mixture levels (1.0% garlic + 0.25% ginger, 1.5% garlic + 0.25% ginger, 1.0% garlic + 0.5% ginger and 1.5% garlic+ 0.5% ginger) of these herbal feed additives.

In the current study treatments didn't affect the TWBC count. Similarly Bamidele and Adejumo, 2012 also argues that inclusion of 0.50% garlic + 0.50% ginger, as well as 0.75% and 2.00% Garlic + 0.75% ginger did not affect TWBC counts.

Contrary research findings had been also reported by Bamidele and Adejumo (2012) where basal diet containing 1.00% garlic + 0.50% ginger, 1.50% garlic + 0.75% ginger and 2.00% garlic + 0.75% ginger resulted in a significantly higher PCV values. Similarly Oleforuh-Okoleh *et al*, 2015 also showed that using 50 ml ginger + garlic extract in drinking water resulted in higher RBC and TWBC counts.

The results for the TWBC count, implies that the mixtures of feed additives at the current levels of inclusion could be tolerated without compromising the welfare or immunity of the birds. The normal PCV, Hb and other hematological values portray the nutritional status of the broiler chickens and thus indicating adequate nourishment of the birds (Church *et al.,* 1984). The numerical increments observed in the Hb, PCV and MCH values for birds fed the different herbal mixture containing diets suggest that the diets were better utilized and assimilated into the blood stream for use by the birds (Onu, 2010). In addition to the above, the increase in PCV, Hb, and RBC contents of blood samples from birds fed the test ingredients is an indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrients to the birds consequently affecting their well-being.

5.2.4. Biochemical values

The results of blood analysis for glucose (238.2-268.0 mg/dl), total cholesterol (113.5-181.3 mg/dl), total protein (3.33-3.67 g/dl), albumin (1.83-2.50 g/dl), globulin (1.28-1.67 g/dl), albumin/globulin ratio (1.10-1.80), ALP (941.7-1239.1 IU/L), ALT/GPT (15.8-19.8 IU/L) and AST/GOT (413.1-477.0 IU/L) were within safety limits for healthy chickens (Campbell, 2012).

The antibiotics containing diet did not affect the biochemical parameters considered in this study. This is in agreement with Majid *et al*. (2013) who reported that antibiotic (15 mg Virginiamycin/kg) had no significant effects on serum glucose and total cholesterol levels. Daneshmand *et al*. (2012) also reported that control diet containing Virginamycin (0.25 g/kg) as antibiotic diet also did not have any significant effect on total cholesterol. According to Demir *et al*. (2005), commercial feed containing AGP (1 g Flavomycin/kg diet) had no significant effects on total cholesterol, total protein, albumin, AST and ALT. A study by Huda *et al*. (2015) using neomycine 16mg/kg as AGP also revealed a non-significant effects on glucose, cholesterol, total protein, ALP and AST. Contrary to the current finding, Hossain *et al*. (2014a), reported that inclusion of antibiotics (1g/l ampicillin and 1g/l oxytatracycline) to the basal diet significantly reduced average blood cholesterol level.

In the current study, herbal mixtures significantly affected serum total cholesterol, total protein and albumin as well as the enzymes ALP and AST/GOT. Serum cholesterol level was significantly reduced for all herbal mixtures except for T_4. The exact mechanisms through which blood metabolites are altered are not known (Khan *et al*., 2012b). It was postulated that (E)-8 β, 17-epoxyllabed-12-ene-15, 16-dial, a compound isolated from ginger, interferes with cholesterol biosynthesis in liver homogenates of hypercholesterolaemic mice causing its reduction (Tanabe *et al*., 1993). Srinivasan and Sambaiah (1991) reported that feeding rats with ginger significantly elevated the activity of hepatic cholesterol 7-alpha hydroxylase which is a rate limiting enzyme in the biosynthesis of bile acids and stimulates the conversion of cholesterol to bile acids leading to the excretion of cholesterol from the body. The mechanism by which garlic or

garlic preparations reduce plasma lipids has not been fully investigated. Animal studies, however, have shown that garlic inclusion in the diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase (Chi *et al*. 1982 and Qureshi *et al*., 1983) and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (Qureshi *et al*., 1983). Oyster mushroom also contains a number of substances with potential effects on the absorption of cholesterol or of other lipids. Particularly the water-soluble gel-forming components of the fiber matter (β-l, 3 D-glucan) with a low degree of polymerization, (forming 15-20% of dry mass) can interact with bile acids and affect the formation of micelles. In this way such substances could interfere with the absorption of cholesterol (Vahouny *et al*., 1980). The mushroom sterols (0.2% of DM) can reduce cholesterol absorption by competitive inhibition (Ikeda *et al*., 1988). These results shows that the combined use of the three phytogenics have beneficial effects in improving serum biochemical profile of chickens through their hypocholostromic effect than their separate use. The current result is supported by Oleforuh-Okoleh *et al*. (2015) who used 50 ml ginger + garlic extract in drinking water which resulted in a significantly reduced serum cholesterol level. Similarly ademola *et al*. (2009) also showed that control diet containing 1.0% garlic + 0.25% ginger, 1.5% garlic + 0.25% ginger, 1.0% garlic + 0.5% ginger and 1.5% garlic + 0.5% ginger reduced serum cholesterol level. Rehman *et al*. (2011) studied the effect of dosing broilers (10 ml/l of drinking water) with an aqueous extract of a mixture of medicinal plants (garlic, berberine and aloe vera) along with ginger, which resulted in a significantly reduced total cholesterol level. On the contrary a study by Daneshmand *et al*. (2012) using control diet containing a combination of garlic (30 g/kg), oyster mushroom (2 g/kg) and propolis extract (0.2 g/kg) indicates the absence of a significant difference on average blood cholesterol level. Opposing results were also reported by Bamidele and Adejumo (2012) who indicated the absence of a significant effect on average blood cholesterol level due to inclusion of 2.00% Garlic and 0.75% ginger to the control diet. On the contrary, Bamidele and Adejumo, 2012 also reported that basal diet containing 0.50% garlic + 0.50% ginger, 1.00% garlic + 0.50% ginger and 1.50% garlic + 0.75% ginger significantly increased serum cholesterol level.

In the current study final mixture of OMP, GAP and GIP significantly reduced serum total protein in comparison to the antibiotic containing diet and lowers albumin content of serum compared to both controls. The combination of garlic and ginger also resulted in significantly lower albumin in comparison to the controls. This observation tells that, spice mixture containing oyster mushroom, garlic and ginger powders caused a marked reduction in serum total proteins and albumin, indicating possible adverse effect on the liver. This could compromise protein synthetic ability under the prevailing circumstance. The difference in values may also suggest a lower protein intake by them, which is also reflected in the lowest final body weight achieved by the same treatment group (T_6). The similar values of globulin as well as albumin to globulin ratio determined in all the treatments suggest that the birds presented the same health status.

Similarly results were also reported by Onu (2010) where control diet containing a combination of 0.25% of garlic and ginger had no significant effect on total protein content of serum, whereas, Oleforuh-Okoleh *et al*. (2015) reported that adding 50 ml ginger + garlic to drinking water resulted in a rather higher albumin concentration.

Results of the liver function enzymes shows that the highest enzyme ALP and AST activity was observed for T_6 and T_3, respectively, whereas, the ALT level remains unaffected. Even though the accumulations of these enzymes in the liver are indicators of liver damage, the values are within the normal reference range as indicated by Campbell (2012).

5.2.5. Cecal microbial count

Although the antibiotic inclusion had no effect on *Escherichia coli* loads, it significantly reduced the CFU. Antibiotics may control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chickens gut (Ferket, 2004). These effects may be due to interference in cell wall synthesis, changes in the permeability of the cytoplasmic membrane, interference in chromosome replication and interference in cell protein synthesis (Mellor, 2000). Similarly the active constituent of spices may also exhibit their antimicrobial effect either by degradation of microbial cell

wall, disruption of cytoplasmic membrane, leakage of cellular components, damage protein, interfere with the enzymatic activities inside cell, affect synthesis of DNA and RNA, affect electron transport and nutrient uptake, leakage of cellular components, impair the energy production inside cell, change fatty acid and phospholipid constituents (Shan *et al*., 2007). According to Wallock *et al.* (2014), the antimicrobial activities of garlic and other plant alliums are primarily based on allicin, a thiosulphinate present in crushed garlic bulbs and the chemical mechanisms involved in the bactericidal action of allicin are poorly understood. Ginger rhizome also contains several constituents which have antibacterial and anti-fungal effects. The gingerol and shagelol are identified as more active agents (Atai *et al*., 2009). According to Aliyu *et al*. (2015) the result of the phytochemical screening of garlic and ginger revealed the presence of alkaloids, saponins, cardiac glycosides, steroids and flavonoids in both plants while tannins was absent in garlic but present in ginger and it was indicated that the synergistic effect of both extracts gave a strong antibacterial activity against the isolates of *Escherichia coli*. The antibacterial effect of mixture of GAP and GIP in the current study is consistent with those of Tekeli *et al*. (2010) who stated that adding ginger into the broiler`s diet significantly decreased the number of coliform bacteria in the small intestine compared to the control group. According to Habsah *et al*. (2000) and Srinivasan *et al*. (2001), *Zingiber officinale* has been also shown to have antimicrobial activity. Ethanolic extract of the rhizomes of *Zingiber officinale* showed significant inhibition of growth of both certain gram-positive and gram-negative bacteria (Mascolo, 1998), similarly the essential oils of *Zingiber officinale* also showed antimicrobial activity against gram-positive and gram negative bacteria using the agar diffusion method (Martins *et al*., 2001). Findings of our trial showed that feeding broilers with diets containing mixtures of 1% GAP and 1% GIP led to a favorable shift into micro biota composition in the ileum of birds. The mixtures of phytogenic growth promoter (GAP and GIP) in the diet of broiler birds have a promising biological effect on their growth performance to reduce the pathogenic bacteriological load in the ileum. A more balanced biota population in the GIT of poultry could lead to a greater efficiency in digestibility and utilization of feed, resulting in an enhanced growth and improved FCR (Ferket, 2004), which is also reflected in the current study. The other mixtures of feed additives might be losing their antibacterial activity

because of action of different enzymes in the process of its digestion and absorption (Mohan, 2004).

5.2.6. Economic appraisal

The highest TI was obtained from the treatment of antibiotics followed by T_1, T_5, T_3, T_4, and T_6. According to Gerard *et al*. (2011), antimicrobial growth promoters have made a tremendous contribution to profitability in intensive husbandry. The highest NR was also recorded for the control diets, whereas, the lowest NR was obtained from birds kept on T_6. All herbal treatments had an ECE which was far below compared to the controls which in turn have equivalent ECE, implying that the treatments were costly from the economic point of view. This was directly related to the actual market price of test ingredients in the area and the lower performance achieved through herbal inclusion in the basal diet. It must be taken into account the fact that the feed cost of these alternatives (herbs) is quite variable and high for herbs in comparison with feed antibiotics (Gerard *et al*., 2011). Unlike this result, Safa (2014a) reported that 1.75% mixture level (1.50% garlic + 0.25% ginger) in powder form resulted in the highest profitability compared to the control. According to Gerard *et al*. (2011), the net economic effect will depend on several factors including the effects on performance levels and the cost of any technologies adopted to compensate for the termination of AGPs, and may be offset by the benefits of increased consumer confidence.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Antibiotic growth promoters have made a tremendous contribution to the profitability of the poultry industry. However, as a consequence of the increasing concern about the potential public health problems because of antibiotic resistant strains of bacteria, poultry nutritionists are being challenged to develop an alternative for AGP. If herbal alternative to AGP can be found, poultry nutritionists could formulate a diet that would meet the needs of the commercial broiler industry without using AGP. This study showed that incorporation of the test ingredients, GAP and GIP in the diets of broilers did not improve either BWG or FCR; whereas, BWG was negatively affected in all birds subjected to 2% OMP. The significant weight gain reduction in birds fed 2% OMP implies that dietary additives like OMP have their limitations too. All levels of garlic, ginger and oyster mushroom powders added to the diets made no change to most of the carcass characteristics of broiler chickens. Inclusion of garlic, ginger and oyster mushroom powders at two different levels improved the RBC counts, whereas, the level of serum glucose, cholesterol, proteins and the enzyme activity remained unaffected.

From the results, it can also be inferred that neither of the mixtures improved the performance of birds in terms of FI, BWG or FCR. Broiler chickens fed on diets to which antibiotics as well as mixtures of OMP and GAP were added exhibited hypolipidemic effects on abdominal fat pad of chickens, whereas, the other carcass parameters were not adversely influenced. It can also be concluded that the herbal mixtures except T_4 had hypocholestrolemic effect. Inclusion of mixtures of GAP and GIP had reduced the *Escherichia coli* and total CFU bacterial loads which can be taken as a result of beneficial synergetic effects of the test ingredients in reducing the pathogenic bacteria in the digestive tract of broiler chickens. Generally sole treatments (treatment either with ginger or garlic) were not as beneficial as the mixture of the two herbs in reducing feed conversion ratio, mortality rates as well as in terms of its hypocholestrolemic and antimicrobial effects. From an economic point of view, the inclusion of either individual or mixtures of test ingredients increased the cost of feeding which significantly affected

the REE and as such could not be economical to use them as growth promoters for broilers at the levels of inclusion used in this study.

6.2. Recommendations

Based on the results of the current study oyster mushroom, garlic and ginger powders each at 1% inclusion level as well as blends of the three medicinal plants at 0.33% OMP, 0.66% GAP and 0.66% GIP could be considered as potential growth promoters that may replace the antibiotic in broiler diets. The results of the economic appraisal showed that the use of these phytogenic feed additives instead of antibiotics at all inclusion levels was costly with a significant implication that their uses in the current form cannot be recommended for wider commercial applications. Therefore, there is a need to test their effects at lower inclusion levels and other preparation as well as administration methods for producing the phytogenics at a cheaper price/form.

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8. APPENDICES

8.1. Supplementary Tables

Appendix Table 1. Price of feed ingredients and feed additives

Ingredients/additives	Price/Kg (Birr)
Maize	4.60
Noug seed cake	6.00
Soybean meal	11.60
Wheat bran	5.80
Broiler premix	30.00
Limestone	0.20
Salt	4.50
DL-Methionine	178.00
L-Lysine HC1	220.00
Oyster mushroom powder	250.00
Garlic powder	200.00
Ginger powder	180.00
Antibiotic (oxytetracycline)	140.00

Appendix Table 2. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on performance of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
FI (0-28)	Between Groups	21037.04	6	3506.173	3.17	0.035
	Within Groups	15506.04	14	1107.57		
	Total	36543.08	20			
FI (29-49)	Between Groups	319797.07	6	53299.511	3.250	0.032
	Within Groups	229357.43	14	16382.674		
	Total	549154.50	20			
FI (0-49)	Between Groups	297454.96	6	49575.826	4.470	0.010
	Within Groups	155162.83	14	11083.059		
	Total	452617.79	20			
IBW	Between Groups	1.90	6	0.317	0.450	0.830
	Within Groups	9.78	14	0.698		
	Total	11.68	20			
FBW(28^{th} Day)	Between Groups	5317.94	6	886.324	0.730	0.630
	Within Groups	16885.88	14	1206.134		
	Total	22203.82	20			
FBW(49^{th} Day)	Between Groups	76679.12	6	12779.852	3.37	0.028
	Within Groups	53062.599	14	3790.185		
	Total	129741.716	20			
BWG(0-28)	Between Groups	5329.21	6	888.201	0.740	0.627
	Within Groups	16805.75	14	1200.411		
	Total	22134.96	20			
BWG(29-49)	Between Groups	44885.48	6	7480.914	4.84	0.007
	Within Groups	21632.721	14	1545.194		
	Total	66518.204	20			
BWG(0-49)	Between Groups	76504.75	6	12750.791	3.34	0.029
	Within Groups	93148.99	14	3816.267		
	Total	129932.485	20			
ADG(0-28)	Between Groups	6.80	6	1.133	0.740	0.627
	Within Groups	21.44	14	1.531		
	Total	28.23	20			
ADG(29-49)	Between Groups	101.78	6	16.964	4.150	0.013
	Within Groups	57.19	14	4.085		
	Total	158.97	20			
ADG(0-49)	Between Groups	31.86	6	5.311	3.34	0.029
	Within Groups	22.25	14	1.589		
	Total	54.11	20			
FCR(0-28)	Between Groups	0.10	6	0.018	3.51	0.025
	Within Groups	0.07	14	0.005		
	Total	0.18	20			
FCR(29-49)	Between Groups	0.11	6	0.019	0.800	0.588
	Within Groups	0.33	14	0.024		
	Total	0.44	20			
FCR(0-49)	Between Groups	0.08	6	0.013	1.210	0.358
	Within Groups	0.15	14	0.011		
	Total	0.23	20			

Appendix Table 3. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on slaughter weight, dressed weight and eviscerated weight of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Slaughter weight	Between Groups	74028.57	6	12338.095	2.87	0.049
	Within Groups	60200.00	14	4300.000		
	Total	134228.57	20			
Dressed weight	Between Groups	62364.12	6	10394.020	4.18	0.013
	Within Groups	34787.40	14	2484.814		
	Total	97151.52	20			
Dressing (%)	Between Groups	5.91	6	0.984	0.370	0.884
	Within Groups	36.84	14	2.631		
	Total	42.75	20			
Eviscerated weight (g)	Between Groups	23257.46	6	3876.243	0.790	0.595
	Within Groups	68973.51	14	4926.679		
	Total	92230.96	20			
Eviscerated (%)	Between Groups	22.14	6	3.689	3.87	0.017
	Within Groups	13.35	14	0.954		
	Total	35.49	20			

Appendix Table 4. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on the commercial cuts of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Breast weight	Between Groups	10319.44	6	1719.906	0.580	0.741
	Within Groups	41532.43	14	2966.602		
	Total	51851.87	20			
Breast (%)	Between Groups	12.46	6	2.076	0.450	0.832
	Within Groups	64.38	14	4.599		
	Total	76.84	20			
Drumsticks with thighs(g)	Between Groups	2765.32	6	460.887	0.880	0.536
	Within Groups	7355.36	14	525.383		
	Total	10120.68	20			
Drumsticks with thighs (%)	Between Groups	0.54	6	0.091	0.110	0.993
	Within Groups	11.32	14	0.808		
	Total	11.86	20			
Back weight	Between Groups	342.99	6	57.164	0.240	0.954
	Within Groups	3282.69	14	234.478		
	Total	3625.68	20			
Back (%)	Between Groups	2.48	6	0.414	1.060	0.430
	Within Groups	5.46	14	0.390		
	Total	7.95	20			
Wing weight	Between Groups	83.35	6	13.891	3.870	0.017
	Within Groups	50.22	14	3.587		
	Total	133.57	20			
Wing (%)	Between Groups	0.08	6	0.013	0.440	0.838
	Within Groups	0.41	14	0.029		
	Total	0.49	20			
Neck weight	Between Groups	815.28	6	135.880	1.150	0.383
	Within Groups	1649.09	14	117.792		
	Total	2464.37	20			
Neck (%)	Between Groups	2.55	6	0.424	3.87	0.017
	Within Groups	1.53	14	0.109		
	Total	4.08	20			

Appendix Table 5. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on commercial and edible carcass weight of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Commercial carcass weight	Between Groups	19585.95	6	3264.325	0.680	0.666
	Within Groups	66828.78	14	4773.484		
	Total	86414.73	20			
Commercial carcass (%)	Between Groups	15.68	6	2.613	0.860	0.549
	Within Groups	42.72	14	3.051		
	Total	58.39	20			
Edible carcass weight	Between Groups	20899.12	6	3483.186	0.710	0.651
	Within Groups	69115.84	14	4936.846		
	Total	90014.96	20			
Edible carcass (%)	Between Groups	19.65	6	3.27	4.21	0.012
	Within Groups	10.89	14	0.78		
	Total	30.55	20			

Appendix Table 6. Analysis of the effect of feeding of different levels of oyster mushroom, garlic and ginger on the giblets weight of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Liver weight	Between Groups	37.61	6	6.268	0.180	0.977
	Within Groups	480.16	14	34.297		
	Total	517.77	20			
Liver (%)	Between Groups	0.27	6	0.045	0.680	0.668
	Within Groups	0.93	14	0.067		
	Total	1.20	20			
Gizzard weight (g)	Between Groups	53.34	6	8.891	0.530	0.773
	Within Groups	232.75	14	16.625		
	Total	286.09	20			
Gizzard (%)	Between Groups	0.08	6	0.014	0.270	0.941
	Within Groups	0.70	14	0.050		
	Total	0.78	20			
Heart weight	Between Groups	18.93	6	3.155	3.64	0.0217
	Within Groups	12.13	14	0.866		
	Total	31.06	20			
Heart (%)	Between Groups	0.03	6	0.005	3.01	0.042
	Within Groups	0.02	14	0.002		
	Total	0.05	20			
Giblet weight	Between Groups	158.35	6	26.391	0.470	0.816
	Within Groups	777.96	14	55.569		
	Total	936.31	20			
Giblet (%)	Between Groups	0.50	6	0.083	0.770	0.605
	Within Groups	1.51	14	0.108		
	Total	2.01	20			

Appendix Table 7. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on weight of non-edible parts of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Feet weight	Between Groups	357.54	6	59.589	3.81	0.018
	Within Groups	219.01	14	15.643		
	Total	576.55	20			
Feet (%)	Between Groups	0.59	6	0.098	1.030	0.446
	Within Groups	1.33	14	0.095		
	Total	1.92	20			
Head weight	Between Groups	82.89	6	13.815	0.760	0.614
	Within Groups	255.29	14	18.235		
	Total	338.18	20			
Head (%)	Between Groups	0.14	6	0.023	0.430	0.847
	Within Groups	0.74	14	0.053		
	Total	0.87	20			
Abdominal fat weight	Between Groups	351.44	6	58.573	1.560	0.231
	Within Groups	526.49	14	37.606		
	Total	877.93	20			
Abdominal fat (%)	Between Groups	0.79	6	0.132	1.510	0.245
	Within Groups	1.22	14	0.087		
	Total	2.01	20			
Weight of GIT	Between Groups	121.04	6	20.173	0.380	0.883
	Within Groups	751.75	14	53.696		
	Total	872.79	20			
GIT (%)	Between Groups	0.36	6	0.060	0.580	0.744
	Within Groups	1.47	14	0.105		
	Total	1.83	20			

Appendix Table 8. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on hematological parameters of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
RBCs	Between Groups	3.09	6	0.516	76.430	<.0001
	Within Groups	0.09	14	0.007		
	Total	3.19	20			
TWBC	Between Groups	7.37	6	1.22	4.590	0.009
	Within Groups	3.74	14	0.26		
	Total	11.11	20			
Hb	Between Groups	5.19	6	0.865	0.890	0.531
	Within Groups	13.68	14	0.977		
	Total	18.87	20			
PCV	Between Groups	56.82	6	9.469	4.37	0.011
	Within Groups	30.35	14	2.168		
	Total	87.16	20			
MCV	Between Groups	1186.63	6	197.771	5.260	0.005
	Within Groups	526.84	14	37.632		
	Total	1713.47	20			
MCH	Between Groups	208.43	6	34.738	6.390	0.002
	Within Groups	76.15	14	5.440		
	Total	284.58	20			
MCHC	Between Groups	149.02	6	24.837	3.36	0.029
	Within Groups	103.54	14	7.395		
	Total	252.56	20			

Appendix Table 9. Analysis of the effect of feeding different levels of oyster mushroom, garlic and ginger on serum biochemical parameters of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Glucose	Between Groups	2980.70	6	496.784	1.100	0.411
	Within Groups	6343.29	14	453.092		
	Total	9323.99	20			
Total cholesterol	Between Groups	3860.03	6	643.339	1.160	0.381
	Within Groups	7776.53	14	555.467		
	Total	11636.57	20			
Total protein	Between Groups	0.56	6	0.093	0.730	0.630
	Within Groups	1.77	14	0.126		
	Total	2.32	20			
Albumin	Between Groups	1.14	6	0.190	0.680	0.667
	Within Groups	3.90	14	0.279		
	Total	5.04	20			
Globulin	Between Groups	0.61	6	0.102	0.750	0.620
	Within Groups	1.91	14	0.137		
	Total	2.53	20			
Albumin/globulin ratio	Between Groups	8.52	6	1.420	0.780	0.601
	Within Groups	25.60	14	1.828		
	Total	34.12	20			
ALP	Between Groups	22472.48	6	3745.41	0.120	0.993
	Within Groups	448712.99	14	32050.92		
	Total	471185.47	20			
ALT/GPT	Between Groups	73.73	6	12.289	0.590	0.730
	Within Groups	289.39	14	20.671		
	Total	363.12	20			
AST/GOT	Between Groups	65431.23	6	10905.204	3.070	0.039
	Within Groups	49727.89	14	3551.99		
	Total	115159.12	20			

Appendix Table 10. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on performance of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
FI (0-28)	Between Groups	235719.41	5	47143.882	4.610	0.014
	Within Groups	122651.15	12	10220.929		
	Total	358370.56	17			
FI (29-49)	Between Groups	398843.730	5	79768.746	2.520	0.0116
	Within Groups	196933.780	12	16411.148		
	Total	595777.511	17			
FI (0-249)	Between Groups	750435.30	5	150087.061	4.86	0.037
	Within Groups	523067.38	12	43588.948		
	Total	1273502.68	17			
IBW	Between Groups	1.38	5	0.277	1.020	0.449
	Within Groups	3.26	12	0.272		
	Total	4.64	17			
FBW(28^{th} Day)	Between Groups	29684.34	5	5936.867	1.340	0.313
	Within Groups	53230.85	12	4435.904		
	Total	82915.19	17			
FBW(49^{th} Day)	Between Groups	92662.33	5	18532.466	1.220	0.358
	Within Groups	182193.47	12	15182.789		
	Total	274855.80	17			
BWG(0-28)	Between Groups	29582.96	5	5916.592	1.340	0.311
	Within Groups	52806.56	12	4400.547		
	Total	82389.53	17			
BWG(29-49)	Between Groups	32292.15	5	6458.430	1.280	0.336
	Within Groups	60743.94	12	5061.995		
	Total	93036.09	17			
BWG(0-49)	Between Groups	92551.46	5	18510.291	1.220	0.356
	Within Groups	181431.65	12	15119.304		
	Total	273983.11	17			
ADG(0-28)	Between Groups	37.73	5	7.547	1.340	0.311
	Within Groups	67.36	12	5.613		
	Total	105.09	17			
ADG(29-49)	Between Groups	73.22	5	14.645	1.280	0.336
	Within Groups	137.74	12	11.478		
	Total	210.97	17			
ADG(0-49)	Between Groups	38.55	5	7.709	1.220	0.356
	Within Groups	75.57	12	6.297		
	Total	114.11	17			
FCR(0-28)	Between Groups	0.20	5	0.041	0.990	0.465
	Within Groups	0.50	12	0.041		
	Total	0.70	17			
FCR(29-49)	Between Groups	0.70	5	0.139	4.89	0.011
	Within Groups	0.34	12	0.343		
	Total	1.04	17			
FCR(0-49)	Between Groups	0.29	5	0.058	4.16	0.020
	Within Groups	0.17	12	0.014		
	Total	0.46	17			

Appendix Table 11. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on slaughter weight, dressed weight and eviscerated weight of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Slaughter weight	Between Groups	98916.67	5	19783.333	1.230	0.353
	Within Groups	192783.33	12	16065.278		
	Total	291700.00	17			
Dressed weight	Between Groups	126113.71	5	25222.743	3.99	0.023
	Within Groups	75942.83	12	6328.569		
	Total	202056.54	17			
Dressing (%)	Between Groups	33.13	5	6.627	3.47	0.036
	Within Groups	22.93	12	1.911		
	Total	56.06	17			
Eviscerated weight (g)	Between Groups	76259.78	5	15251.956	4.49	0.015
	Within Groups	40731.66	12	3394.305		
	Total	116991.44	17			
Eviscerated (%)	Between Groups	40.26	5	8.051	4.740	0.013
	Within Groups	20.40	12	1.700		
	Total	60.66	17			

Appendix Table 12. Analysis of the effect of feeding of different mixtures of oyster mushroom, garlic and ginger on commercial cuts broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Breast weight	Between Groups	22025.83	5	4405.167	3.86	0.026
	Within Groups	13688.16	12	1140.680		
	Total	35714.00	17			
Breast (%)	Between Groups	16.29	5	3.257	3.97	0.023
	Within Groups	9.84	12	0.820		
	Total	26.13	17			
Drumsticks with thighs(g)	Between Groups	3706.78	5	741.356	0.520	0.757
	Within Groups	17120.00	12	1426.667		
	Total	20826.78	17			
Drumsticks with thighs (%)	Between Groups	2.88	5	0.576	0.600	0.701
	Within Groups	11.52	12	0.960		
	Total	14.40	17			
Back weight	Between Groups	2048.57	5	409.714	0.920	0.498
	Within Groups	5316.83	12	443.069		
	Total	7365.40	17			
Back (%)	Between Groups	4.93	5	0.986	0.870	0.526
	Within Groups	13.53	12	1.128		
	Total	18.46	17			
Wing weight	Between Groups	151.96	5	30.391	0.590	0.706
	Within Groups	614.17	12	51.181		
	Total	766.12	17			
Wing (%)	Between Groups	0.18	5	0.037	0.770	0.589
	Within Groups	0.57	12	0.048		
	Total	0.76	17			
Neck weight	Between Groups	180.69	5	36.137	1.120	0.402
	Within Groups	388.07	12	32.339		
	Total	568.76	17			
Neck (%)	Between Groups	0.39	5	0.079	1.140	0.394
	Within Groups	0.83	12	0.069		
	Total	1.23	17			

Appendix Table 13. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on commercial and edible carcass weight of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Commercial carcass weight	Between Groups	57693.93	5	11538.785	1.250	0.346
	Within Groups	110824.58	12	9235.381		
	Total	168518.50	17			
Commercial carcass (%)	Between Groups	29.11	5	5.821	1.570	0.242
	Within Groups	44.59	12	3.716		
	Total	73.70	17			
Edible carcass weight	Between Groups	54919.89	5	10983.977	1.080	0.419
	Within Groups	121969.09	12	10164.090		
	Total	176888.97	17			
Edible carcass (%)	Between Groups	23.83	5	4.765	1.460	0.272
	Within Groups	39.10	12	3.258		
	Total	62.93	17			

Appendix Table 14. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on weight of giblets of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Liver weight	Between Groups	187.19	5	37.438	0.650	0.664
	Within Groups	686.65	12	57.221		
	Total	873.84	17			
Liver (%)	Between Groups	0.54	5	0.108	0.930	0.494
	Within Groups	1.38	12	0.115		
	Total	1.92	17			
Gizzard weight (g)	Between Groups	44.48	5	8.897	0.850	0.538
	Within Groups	125.00	12	10.417		
	Total	169.48	17			
Gizzard (%)	Between Groups	0.25	5	0.050	2.330	0.107
	Within Groups	0.26	12	0.022		
	Total	0.51	17			
Heart weight	Between Groups	3.80	5	0.759	0.330	0.882
	Within Groups	27.23	12	2.269		
	Total	31.03	17			
Heart (%)	Between Groups	0.01	5	0.003	0.530	0.750
	Within Groups	0.06	12	0.005		
	Total	0.08	17			
Giblet weight	Between Groups	284.45	5	56.889	0.760	0.596
	Within Groups	898.63	12	74.886		
	Total	1183.08	17			
Giblet (%)	Between Groups	1.22	5	0.245	3.140	0.048
	Within Groups	0.92	12	0.08		
	Total	2.14	17			

Appendix Table 15. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on weight of non-edible parts of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Feet weight	Between Groups	325.25	5	65.049	1.700	0.209
	Within Groups	458.76	12	38.230		
	Total	784.01	17			
Feet (%)	Between Groups	1.77	5	0.354	3.290	0.042
	Within Groups	1.29	12	0.107		
	Total	3.06	17			
Head weight	Between Groups	240.44	5	48.089	1.320	0.319
	Within Groups	436.67	12	36.389		
	Total	677.11	17			
Head (%)	Between Groups	0.83	5	0.167	1.670	0.021
	Within Groups	1.20	12	0.100		
	Total	2.03	17			
Abdominal fat weight	Between Groups	125.85	5	25.170	4.610	0.014
	Within Groups	65.49	12	5.458		
	Total	191.34	17			
Abdominal fat (%)	Between Groups	0.33	5	0.066	4.240	0.018
	Within Groups	0.18	12	0.015		
	Total	0.51	17			
Weight of GIT	Between Groups	132.26	5	26.452	0.780	0.583
	Within Groups	406.75	12	33.896		
	Total	539.01	17			
GIT (%)	Between Groups	0.59	5	0.118	3.79	0.027
	Within Groups	0.37	12	0.031		
	Total	0.96	17			

Appendix Table 16. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on hematological parameter of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
RBCs	Between Groups	1.48	5	0.295	0.820	0.555
	Within Groups	4.29	12	0.358		
	Total	5.77	17			
TWBC	Between Groups	3.97	5	0.79	1.020	0.450
	Within Groups	9.38	12	0.78		
	Total	13.35	17			
Hb	Between Groups	13.10	5	2.619	3.560	0.033
	Within Groups	8.83	12	0.736		
	Total	21.93	17			
PCV (%)	Between Groups	27.40	5	5.481	0.370	0.859
	Within Groups	177.50	12	14.792		
	Total	204.90	17			
MCV	Between Groups	8771.52	5	1754.303	0.550	0.737
	Within Groups	38391.05	12	3199.254		
	Total	47162.57	17			
MCH	Between Groups	1388.94	5	277.787	1.140	0.393
	Within Groups	2932.47	12	244.372		
	Total	4321.40	17			
MCHC	Between Groups	85.09	5	17.018	0.590	0.709
	Within Groups	347.22	12	28.935		
	Total	432.31	17			

Appendix Table 17. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on serum biochemical parameters of broilers

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Glucose	Between Groups	4060.24	5	812.047	1.150	0.386
	Within Groups	8448.50	12	704.042		
	Total	12508.74	17			
Total cholesterol	Between Groups	9903.24	5	1980.647	9.750	0.001
	Within Groups	2437.00	12	203.083		
	Total	12340.24	17			
Total protein	Between Groups	1.20	5	0.239	3.14	0.048
	Within Groups	0.91	12	0.076		
	Total	2.11	17			
Albumin	Between Groups	1.37	5	0.273	3.98	0.023
	Within Groups	0.82	12	0.068		
	Total	2.19	17			
Globulin	Between Groups	0.28	5	0.056	1.290	0.332
	Within Groups	0.52	12	0.043		
	Total	0.80	17			
Albumin/globulin ratio	Between Groups	1.32	5	0.263	1.960	0.157
	Within Groups	1.61	12	0.134		
	Total	2.93	17			
ALP	Between Groups	421757.85	5	84351.571	4.60	0.0141
	Within Groups	219920.71	12	18326.726		
	Total	641678.56	17			
ALT/GPT	Between Groups	41.24	5	8.247	0.220	0.946
	Within Groups	446.83	12	37.236		
	Total	488.07	17			
AST/GOT	Between Groups	11619.00	5	2323.800	3.270	0.043
	Within Groups	8528.16	12	710.68		
	Total	20147.16	17			

Appendix Table 18. Analysis of the effect of feeding different mixtures of oyster mushroom, garlic and ginger on counts of coliform bacterial and *Escherichia coli*

Parameters	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Coliforms count	Between Groups	5.44	5	1.088	16.330	<.0001
	Within Groups	0.80	12	0.067		
	Total	6.24	17			
Escherichia coli counts	Between Groups	2.96	5	0.592	5.020	0.010
	Within Groups	1.42	12	0.118		
	Total	4.38	17			

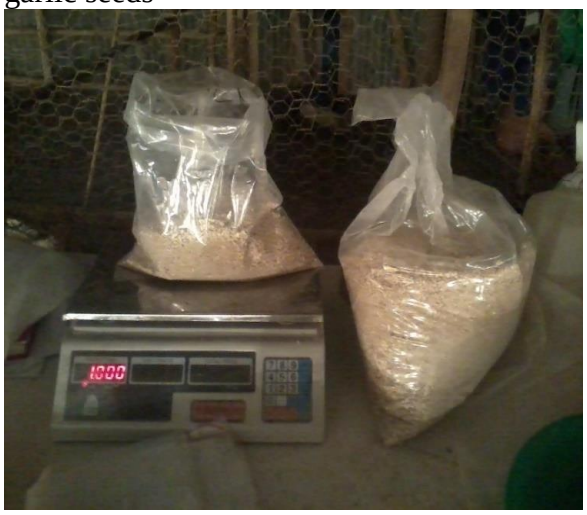
8.2. Supplementary Figures

Appendix figure 1. Sun dried oyster mushroom

Appendix figure 4. Oxytetracycline powder

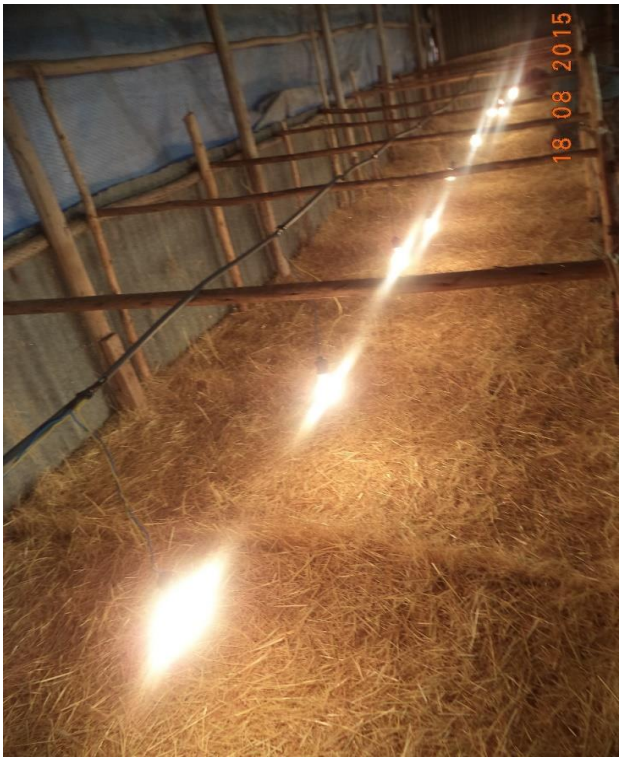
Appendix figure 2. Sun drying of sliced garlic seeds

Appendix figure 5. Ginger powder containing diet set for hand mixing



Appendix figure 3. Weighing of ginger powder before being mixed with the basal diet

Appendix figure 6. Treatment feeds arranged in labeled polyethyline bags

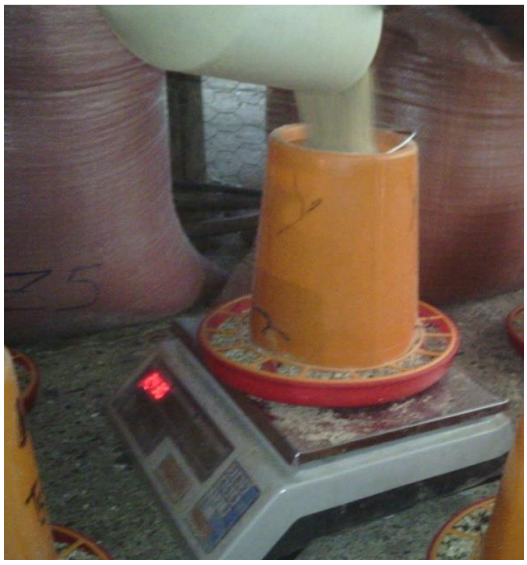


Appendix figure 7. Experimental pens before arrival of day-old chicks

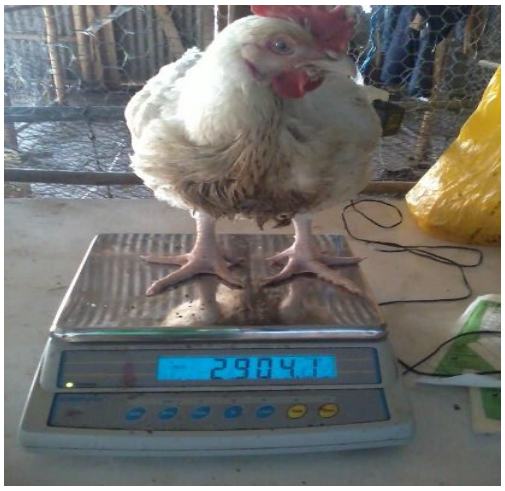
Appendix figure 9. Chicks on day ten

Appendix figure 8. Day-old chicks upon their arrival

Appendix figure 10. Chickens on day forty five



Appendix figure 11. Weighing of feed to be offered

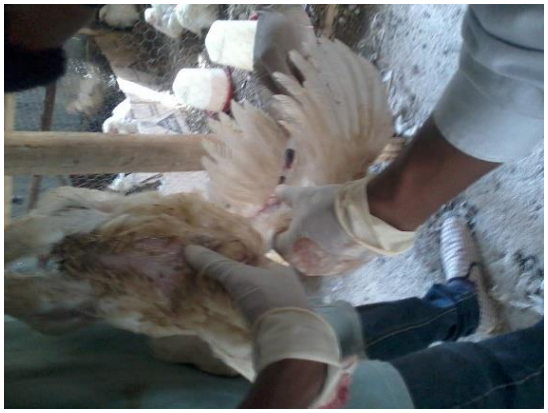


Appendix figure 14. Slaughter weight measurement

Appendix figure 12. Feed being distributed to respective pens

Appendix figure 13. Measuring group body weight

Appendix figure 15. Slaughtering using bleeding cone and de-feathering of birds



Appendix figure 16. Colleting blood sample from wing vein

Appendix figure 17. Determination of PCV

Appendix figure 18. Determination of hemoglobin

Appendix figure 19. Serum biochemistry analyzer

Appendix figure 20. Serum samples being set for biochemical analysis

8.3. Published Articles

1. *Zena Kidane, Ashenafi Mengistu and Harpal Singh (2017).* Effect of Different Levels of Oyster Mushroom, Garlic and Ginger Powder as Feed Additives on Carcass Traits of Broilers. *Advances in Biological Research 11 (2): 100-108.*
2. *Zena Kidane, Ashenafi Mengistu and Harpal Singh (2017).* Effect of Different Mixture Levels of Oyster Mushroom, Garlic and Ginger Powder as Substitutes for Antibiotic Growth Promoter on Carcass Traits of Broilers. *Advances in Biological Research 11 (4): 183-189.*