Sudan University of Science and Technology
College of Graduate Studies

Effect of Feeding Garlic and Ginger Mixture on Production
Performance and Immune Response of Broiler Chicks.

A thesis Submitted for the Fulfillment of the Requirements for the
Degree of Master of Science in Animal Production
(POULTRY NUTRITION)

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أنا الموقع أدناه أقر بأننيمؤلف الوحيد لرسالة الماجستير المعنونة:

**أثر إضافة جلية العرقة والزنجبيل على الأداء والمناعة**

الإجابة، وإذ مينتسباته المذكورة أدناه، وهي منتج فكري أصيل. وباختياري أعطي حقوق طبع ونشر هذا العمل لكلية الدراسات العليا - جامعة السودان للعلوم والتكنولوجيا، عليه يحق للجامعه نشر هذا العمل للأغراض العلمية.

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قال تعالى:

(و قد قلت لموسي لن نصير على طعام واحد فادع لنا ربك يخرج لنا مما تتبت الأرض من بقلها وقشانها وفومها وعسدها وبصلها قال استبدلون الذي هو أدنىذي هو خير اهبطوا مسرأ فإن لكم ما سألتم وضربت عليهم الذلة والمسكنة وباوء وبغضب من الله ذلك بأنهم كانوا يكفرون بأيات الله ويعتلون النبيين غير الحق ذلك بما عصوا وكانوا يعدون)

صدق الله العظيم

سورة البقرة الآية (61)

قال تعالى:

(ويستانون فيها كما كان مزاجها زنجيلة)

صدق الله العظيم

سورة الإنسان الآية (17)
Dedication

To my Parents,

Brothers,

Sisters,

Wonderful Friends

Hassan Mustafa, Mohamed Yagoub and Mubark Hassan,

And all who gave help
I dedicated this word.
Acknowledgement

First and foremost I thank Allah who gave me health, patience, ability, and strength that enabled me to conduct this work and carry it to the end. Thanks to Dr. Elfadil Ahamed Adam who supervised the research giving his constructive criticism and guidance and great effort throughout the study, also I would like to thank the head of the Department of Poultry Science and Technology, Dr. Badr Hasab EL Rasoul for his assistance during the development of my thesis proposal. Thanks are extended to my family for their support and standing firmly beside me during the research work, especially my sister Nuha. Additional thanks are due to my dear colleagues from the General Administration of Animal Wealth of North Kordofan State for their assistance during the practical work of my study, special thanks to Dr. Mubark Hassan, Dr. Suzan Abd Elraheem, MR. Reem Adam and to the under graduate students of the College of Animal Production, Sudan University of Science and Technology who assisted me during the experiment.

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Abstract

The aim of this study was to evaluate the effect of feeding garlic and ginger mixture on production performance and immune response of broiler chicks. A total of 160 one-day old unsexed (Ross-308) broiler chicks were randomly divided into 4 experimental groups of 40 chicks. Each group was further subdivided into 4 replicates at the rate of 10 chicks per each. The garlic bulbs (*Allium sativum*) and slices of ginger (*Zingiber officinale*), were sundried, ground separately to a fine powder and then mixed at a ratio of (1:1) and then added to the basal diets resulting in four experimental groups. The first group (A) fed on basal diets without feed garlic and ginger mixture (control diet) the other groups (B), (C) and (D) were fed on basal diets supplemented with different ginger and garlic mixture levels (0.2%, 0.3% and 0.4%) respectively. The experimental diets were fed for 6 weeks duration.

The performance of the experimental birds in term of feed intake, live weight gain and feed conversion ratio were recorded. Mortality was recorded when occurred. At the end of the experiment three birds from each replicate were randomly selected and slaughtered, spleen, liver, heart and gizzard were weighed individually and recorded. Blood samples were collected. Antibody titers against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) were measured.
Statistical analyses were made by analysis of variance for a completely randomized design, using Statistical Packages for the Social Sciences. The results revealed that feeding low level (.2%) of garlic and ginger mixture(1:1) to broiler chicks induced a positive effect on feed intake, conversion ratio, live body weight and body weight gain mean, while high rates of inclusion resulted in a negative effect on the same parameters. On the other hand, all tested levels improved immunological profile by increasing antibody titers against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV), without affecting feed conversion ratio, mortality and internal organs weights.
ملخص الدراسة

الهدف من هذه الدراسة تقييم تأثير إضافة مخلوط الزنجبيل والثوم بنسبة (1:1) على الأداء الإنتاجي والاستجابة المناعية للتكاثر اللاحق. تم توزيع 160 كنكةت غير محسنة عمر يوم من سلالة (Rose عشوانيا إلى أربعة معاملات احتوت كل معامل على 40 كنكةت، وكل معاملة تم تقسيمها إلى أربعة مكررات بحيث كل مكرر على 10 كنكةت. تم شراء شرائح الثوم والزنجبيل من السوق المحلية بعدما تم تجفيفها بشكل منفصل عن طريق اشعة الشمس مباشرة ومن ثم تم طحنها للحصول على مسحوق ناعم وخلطها بنسبة (1:1) ومن ثم تم إضافة المخلوط إلى الزيتية الأساسية للحصول على أربعة علائق تجريبية،العلية الأولى (A) كانت بمثابة المجموعة الضابطة وهي خالية من مخلوط الثوم والزنجبيل بينما المجموعات الأخرى (D , C , B) تم إضافة المخلوط إلى الزيتية الأساسية بمعدل (0.2, 0.3 و 0.4%) على التوالي. وقدمت الزيتية لطريقة التجريبية للاستهلاك الحر لمدة 6 اسابيع المتركزات التي تم دراستها تشمل إستهلاك الزيتية، الوزن المكتسب ، معدل التحويل الغذائي. بالإضافة إلى تسجيل النفوذ إن وجد. بنتهاة التجربية تم اختيار 3 طيور من كل كنكة شكل عشويات وذبحها ومن ثم تسجيل وزن القلب، الكبد، الطحال بشكل فردي. تم جمع عينات عشويات من الدم، وكذلك للحصول على مصل الدم. لقياس المعبر الحجمي للجسام المضادة لفيروسات الطيور والكزور. التحليل الإحصائي المستخدم هو تحليل التباين عن طريق نظام التصميم العشوائي الكامل.

ثبت عند إضافة مخلوط الزنجبيل والثوم (1:1) إلى علائقة بداری التسمین بنسبة منخفضة ادت إلى زيادة معنوية في الوزن الحي، الوزن المكتسب واستهلاك اللف، بينما هناك انخفاض معنوي كبير عند ارتفاع معدل الأضافة وجد أن هناك تحسن معنوي (P≤0.05) عالي بالنسبة لقياس العيار الحجمي للجسام المضادة ضد فيروسات أمراض الطيور والكزور. عند كل مستويات الإضافة بينما لم تكن هناك فروق معنوية في معدل التحويل الغذائي والنفوق. وزن الاحساء الداخلي (القلب، الطحال، الكبد والقائص) بين كل المعاملات.
هذه النتائج توضح بأن إضافة مخلوط الزنجبيل والثوم (1:1) إلى علائق بداري التسمين بمعدل منخفض تحسن الإداء الإنتاجي والاستجابه المناعيه من خلال زيادة الأجسام المضادة دون تأثير علي معدل التحويل الغذائي والنفوذ وزن الاحشاء الداخلية.
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CHAPTER ONE

1:0 INTRODUCTION

A number of feed additives including antibiotics have widely employed in the poultry industry for several decades. A manipulation of gut function and microbial habitat of domestic animal with feed additive has been recognized as important tool for improving growth performance and feed efficiency. Several attempts have been made in order to reduce feed cost while improving productivity of high-meat yielding exotic poultry chickens. These include the use of agro-industrial by-products, leafy vegetable protein concentrates and herbaceous human haematinics (Agbede and Aletor, 2003). Poultry producers are commonly apply natural feeding supplements, mainly herbs. The positive effects of herbal supplements on broiler performance and carcass quality have been demonstrated (Onibi, et al., 2009). A variety of herbs could be expected to serve as feed additives due to their suitability and preference, reduced risk of toxicity, minimum health hazards and environment friendliness (Devegowda, 1996). Moreover there is a great phobia in using antibiotics as feed additives because of public concern about antibiotic residues in animal products and the potential evolving of antibiotic resistant bacteria. Recent research works on herbal formulations as feed additives have shown encouraging results as regards weight gain, feed efficiency, lowered mortality and increased liveability in poultry birds (Deepak, et al., 2002; Javandel, et al., 2008 and Onibi, et al.,2009). Herbs
spices like ginger (Zingiber officinale) and garlic (Allium sativum) have been reported to possess useful pharmacological potent chemical substances for use in poultry (Akhtar, et al., 1984 and Najafi and Torki, 2010). Ginger and garlic as natural feed additives in poultry nutrition may be of great benefit and value especially for broiler chicks. The beneficial effects of these herbs in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions. However, their influence on growth performance, immune response of broiler chicks had not been sufficiently documented specially their additive and cumulative properties. Thus the objectives of this study were to evaluate the efficiency of ginger and garlic mixture as feed additives and their subsequent influence on the performance and immune response of broiler chicks. Other objective to minimize the production cost by improving nutrient and broiler performance, because feed cost represents approximately 60-70% of the total cost of Production for the most classes of livestock and the improvement of the feed efficiency should be a major consideration of the breeding and feeding programs.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Feed additives:

Feed additive are material that are not considered to be nutrient and are used in small amount in the ration to improve feed efficiency, promote growth, improve animals health or increase production of animals (James and Gillespie .1989). Other definition feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality animal by product 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). Feed additive was divided into two-part nutritive and non nutritive feed additive.

2.1.1 Nutritive feed additives:

Nutritive feed additive are define as a chemical elements or compounds that aid in support of life and necessary for cells to live, growth and function properly, these nutrients are essential Amio acid (Lysine and Methionine), vitamins and minerals.(James,1989).
2.1.2 Non nutritive feed additives:
Poultry formulations also contain an array of substances known as “feed additives”. These are non-nutritive substances usually added in amounts of less than 0.05 percent to maintain health status, uniformity and production efficiency in intensive production systems. These additives have now become vital components of practical diets. Some of the useful effects of non nutritive feed additive are Pellet binders, Flavoring agent ,enzyme, Antifungals, Broad-spectrum, antibiotics Coccidiostats, worming drugs, antioxidant, Carotenoid, horomon, Reserpine ,asprin and antibiotics (Jams and Gillespie2003)in addition to non nutritive feed used in animal feed, such as herbs and spices (e.g: garlic, anise, cinnamon, coriander, oregano, chili ,pepper, rosemary, ginger and thyme), (Kamel, 2001; Balunas and Kinghorn,2005; Athanasiadou, et al., 2007).

2.2 Growth promoters

2.2.1 Antibiotics as growth promoter

Antibiotics are chemical substances derived initially from certain fungi, bacteria, and other organisms that can inhibit the growth of, and even destroy, harmful microorganisms (Davey, 2000). According to their effect on microorganisms, antibiotics might be classified into bactericidal, which kill bacteria, and bacteriostatic, which only inhibit bacterial growth (Hinton, 1988; Norcia, et al., 1999). Early findings of beneficial effects of AGP were reported in poultry diets by Moore et al.
(1946) and in swine diets by Jukes, et al. (1950). Initial report was also showed by Starr and Reynolds (1951) after feeding trial of streptomycin in turkeys. Other preliminary reports were made by Barnes (1978) and Elliott and Barnes (1959) that showed the use of tetracycline as antibiotics growth promoter in poultry diets. Antibiotics have been added mainly during the grow-out period to protect poultry from pathogenic organisms, maintain health, promote growth, facilitate better feed efficiency, and improve meat quality. For instance, two of the more popular broad spectrum antibiotics utilized within the poultry industry, i.e. virginiamycin and bambermycins, have been reported to improve the growth and performance of broilers and turkeys (Waldroup, et al., 1985; Salmon and Stevens, 1990). Subtherapeutic levels of avoparvin, bacitracin methylene disalicylic acid, efrotomycin, lincomycin, penicillin G procaine, and virginiamycin in the diets have been also reported to improve rate of weight gain and feed efficiency of male broiler chickens (Feighner and Dashkevicz, 1987). In a more recent study, Miles, et al. (2006) showed that addition of virginiamycin to a corn-soybean meal diet stimulated improvement in total body weight and the number of absorptive cells per unit length in the intestine of male and 30 female broiler chickens. This physical improvement facilitates cause better nutrient absorption, thus promotes growth performance stimulation. Moreover, it has been reported that virginiamycin controlled microbial growth within the lumen of the gastrointestinal tract by distributing
bacterial protein synthesis (Parfait, et al., 1978). In other studies, it has been reported by Engberg, et al. (2000) that zinc bacitracin significantly reduced the number of coliform bacteria in the ileum and increased the activities of amylase and lipase in pancreas homogenates. Supplementation with salinomycin and zinc bacitracin, alone or in combination, resulted in significant reduction of the growth of C. perfringens and Lactobacillus salivarius in the gut of broiler chickens. Numerous studies have reported that growth enhancement properties of antibiotics are closely related to interactions with the microbes in the gut. Antibiotics growth promoter can help control disease by selectively modifying and improving the gut microflora, reducing bacterial fermentation and preventing infectious diseases, and results in health status improvement. All these changes lead to an increase in nutrient availability for the animal, allowing enhanced feed efficiency and being able to achieve better growth performance (Dibner and Buttin, 2002; Hernández, et al., 2006). Moreover, Donoghue (2003) showed that the use of antibiotic in poultry diets gives significant economical advantages as it facilitates better production efficiency, thus allowing consumer to purchase high quality poultry products at lower price. Antibacterial substances are used in considerable amounts as growth promoters in animal Husbandry, and carry incalculable risks for human health resulting from the use of particular feed additives (Witte, 2000). The indiscriminate use of antibiotics as feed additives could lead to an
increased number of antimicrobial-resistant bacteria, and ultimately compromise the treatment of bacterial infections in humans (Gersema and Helling, 1986 and Dermott, et al., 2002). Many countries concerned about this problem have restricted and or banned the use of antimicrobial compounds in feed for food animals to slow the development of resistance (McEwen and Fedorka-Cray, 2002). Major changes occurred in the use of antimicrobial agents for growth promotion during the last 6 years in different countries (Aarestrup and Jensen, 2001).

2.2.2 Mechanisms of growth promoter action of antimicrobials:

The mechanism by which antibacterial agents improve growth performance is not known, but several theories have been proposed. Because they thin the small intestinal epithelium, nutrients are more efficiently absorbed (Boyd and Edward, 1967; Fuller, et al., 1984) Nutrients are spared because competing microorganisms are reduced (Eyssen, 1962) The different microorganisms responsible for subclinical infections are reduced or eliminated (Barnes, et al., 1978) There is a reduction in production of the growth-depressing toxins or metabolites by intestinal microflora (Dang and Visek, 1960).

2.3 Alternatives to feed antibiotics growth – promotor

2.3.1 Prebiotics

Prebiotic has been defined as “a non-digestible feed ingredient that give beneficial effects on the host intestinal health by selectively stimulating
the growth and/or activity of one or a limited number of beneficial bacterial species already resident in the large intestine” (Gibson and Roberfroid, 1995). A prebiotic is a dietary supplement that intended to reach the large intestine in an intact form and has a specific metabolism therein. In this target site (large intestine), prebiotics was directed toward, beneficial rather than harmful bacteria. The preferred target organisms for prebiotics are beneficial bacterial species belonging to the genera of Lactobacillus and Bifidobacterium (Gibson and Fuller, 2000). For a feed supplement to be classified as a prebiotic, it must be neither hydrolyzed by digestive enzymes nor absorbed in the stomach or small intestine. Prebiotics must be selective for one or a limited number of potentially beneficial commensal bacteria in the large intestine and induce luminal or systemic effects that are beneficial to the host health (Gibson and Roberfroid, 1995). Prebiotics must be able also to stimulate the growth and metabolism of beneficial bacteria in the gut, and thus shifts the intestinal microflora ecology towards a healthier composition (Collins and Gibson, 1999; Simmering and Blaut, 2001). Non-digestible oligosaccharides are dietary substrates that have been reported to possess good prebiotic potential. These are oligomeric carbohydrates, whose acidic bond allow resistance to digestive enzymes in the upper gastrointestinal tract but can be fermented and metabolized by colonic bacteria (Roberfroid, 1997). Among non-digestible oligosaccharides, fructo oligosaccharides and mannan oligosaccharides are predominantly
used as prebiotics. They have been reported to stimulate the growth of endogenous microflora in the gut (Gibson and Roberfroid, 1995). Similarly, Patterson and Burkholder (2003) stated that fructo oligosaccharide products (FOS, oligofructose, inulin) are mostly used in poultry diets. Fructo oligosaccharides are available naturally as inulin, which is the storage carbohydrate in many thousands of plants, or can be synthesized enzymatically from sucrose (Van Loo, et al., 1995). However, other products such as trans-galacto oligosaccharides, gluco oligosaccharides, glyco oligosaccharides, lactulose, lactitol, malto oligosaccharides, xylo oligosaccharides, raffinose, stachyose, and sucrose thermal oligosaccharides have also been investigated as the candidate of prebiotics (Collins and Gibson, 1999). The proposed mode of action of prebiotics to stimulate growth development and health of poultry is mostly attributed to growth inhibition of potentially harmful intestinal microflora (through competition for substrates and mucosal attachment sites) (Spring, et al., 2000), increased intestinal acidity through production of short-chain fatty acids (Campbell, et al., 1997), growth stimulation of intestinal absorptive cells (Iji and Tivey, 1999), and stimulation of the enteric immune system (Parks, et al., 2001), thus facilitating better performance and health status of the birds. It has been reported in many studies that supplementing diets with probiotics stimulates the growth of beneficial microflora in the gut (Gibson and Roberfroid, 1995).
2.3.2 Probiotics:

Nurmi and Rantala (1974) introduced direct-fed microbial (DFM) or probiotics as a means of maintaining gut health, by controlling endemic and zoonotic agents in poultry (La Ragione and Woodward, 2003). In many studies, the live microbials are known as probiotics (Morishita, et al., 1997). Initially, probiotic, which means ‘for life’ in Greek, has been defined by Parker (1974) as “Organisms and substances which contribute to intestinal microbial balance”. This definition was subsequently refined as “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance of microflora” (Fuller, 1989). Probiotics must be of host origin, withstand of processing and storage, resist of gastric acid and bile, persist in the intestinal tract and adhere to epithelium or mucus (Simmering and Blaut, 2001). Probiotics aimed to produce a beneficial effect on the host by administration of viable organisms (Gibson and Fuller, 2000). For a feed supplement to be classified as probiotic, it must produce inhibitory substances, alter microbial activities, and modulate immune response (Jin, et al., 1997). The direct-fed microbial concept was originally designed to restore microflora population in the gut by stimulating activities of bacteria which have beneficial effects on the host and depressing activities of those bacteria that have adverse effects on the host (Simmering and Blaut, 2001; Schneitz, 2005).
2.3.3 Dietary enzymes

Poultry diets are mostly composed of cereal feedstuffs in large amounts. These cereal feedstuffs contain macro-nutrients like starch, protein and fats, and many other components such as β-glucans, xylans, arabinoxylans, pentosans, pectins, mannans, cellulose, lignin, mucilage and phytic acid, which cannot be digested properly by poultry digestive organs (Adams, 2004) and reduced nutrient digestibility by interfering with interactions between digestive enzymes and their substrates (Bedford and Schulze, 1998). Those of which are mentioned as non-digestible feed components possibly increase intestinal viscosity and generate stress in the digestive tract which in turn cause reduction in nutrient utilization in the gut (Adams, 2004). Intestinal viscosity is a major factor limiting bird performance, especially in cereal based fed birds (Bedford and Morgan, 1996). Increased intestinal viscosity due to high-fiber diets depress feed passage rate (Bedford and Morgan, 1996) and increase pathogenic microbial population in the small intestine (Longhout, et al., 2000). Uncontrolled growth of pathogenic species in the gut, that requires energy and protein from intestinal digesta, can compete to the nutrients available for the host. Excessive amount of these pathogens in gut will lead to depression in enteric development and health of the birds. Since antibiotics growot promoter were no longer allowed in the diets, other substances such as various exogenous enzymes, must be used to overcome these problems (Mathlouthi, et al.,
In many studies, it has been shown that supplementation of various exogenous enzymes improve growth performance of animals (Augspurger, et al., 2003; Adedokun et al., 2004). Since it is likely that cereal diets contain starch that is protected in the cell walls, the use of dietary enzymes has become an important factor to help improve starch digestion (Pettersson and Åman, 1989). It has been suggested by Pluske, et al. (2002) that dietary enzymes fasten nutrient digestion by breaking open endosperm cell walls. The benefits in this enzymatic reaction can be almost entirely expected to decrease digested viscosity, and thus achieve better digestion/diffusion (Choct and Annison, 1992). Evidence shows that the use of supplementary enzymes as alternatives for AGPs in poultry diets modulate the gut microflora and performance of broiler chickens (Choct, 2009).

2.3.4 Organic acids

Organic acids are considered to be any organic carboxylic acid with the general structure of R-COOH, and hence include fatty acids and amino acids. Organic acids are short chained acids (C1–C7) and are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing an hydroxyl such as lactic, malic, tartaric, and citric acids (Dibner and Buttin, 2002). These weak acids are added to the feed in sub-therapeutical dosage and are believed to have the capacity to improve growth performance (Vogt, et al., 1982),
nutrient utilization (Runho, et al., 1997), and health (Canibe, et al., 2001).

2.3.5 Medicinal plants

Natural feed additives of plant origin are believed to be safer, healthier and less regarded than synthetic additives (antibiotics). It was estimated that there are 250000-500000 species of plants on earth (Borris, 1996; Hashemi and Davoodi, 2010). Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers (Greathead, 2003). Many scientists have searched for alternatives to antibiotics through utilization of the extracts or leaves of some of these plants (Wenk, 2000; Kamel, 2001; Alcicek, et al., 2003).

Herbs and their essential oils have been used extensively for many years in food products, perfumerly and dental and oral products due to their different medicinal proprieties (Suppakul, et al., 2003). However, secondary plant metabolites are largely unexploited in conventional animal production systems. In the past, plant metabolites generally consider as sources of antinutritional factors. Herbal medicines are regarded as dietary supplements for humans and are widely used to treat medical illness within the past year in the US (Bent and Ko, 2004). Herbs contain some complicated mixtures of organic chemicals that may vary depending upon many factors related to the growth, production and
processing of the herbal product. Though herbs with antimicrobial properties are reported, their use in broiler diets has not been studied extensively. The supplementation of herbs and spices could have many benefits to broilers health and performance such as having antioxidative potential (Hui, 1996), antimicrobial activity (Dorman and Deans, 2000), enhancing digestion by stimulating endogenous enzymes (Brugalli, 2003). Naidoo, et al., (2008) demonstrated that antioxidant rich plant extracts have potential benefits in treating coccidial infections.

2.4.2 Herbs and Spices as Alternatives to Antibiotics growth promoter (AGP)

Herbs and herbal products are incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight gain, higher production rates and improved feed efficiency. Moreover, active components of herbs may improve digestion and stimulate the immune function in broilers (Ghazalah and Ali, 2008). Steiner (2009) stated that medicinal plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects and the stimulating effects on the animal digestive systems. The microflora of the small intestine is made up mostly of lactic-acid producing bacteria (Engberg, et al., 2000). Lactic acid is the fermentation by product of lactic-acid producing bacteria and the increase in lactic acid concentrations in the poultry gastrointestinal tract that causes the pH to
drop, and thus preventing the colonization of certain pathogens (Zhang, et al., 2003). Also, Rahimi, et al. (2011) observed that plant extracts can increase the number of lactic acid bacteria in the ileal and ceecal contents of broilers. It has been shown that the dietary incorporation of herbs and their associated essential oils may provide beneficial effects on poultry performance and health due to the antimicrobial activity of their phytochemical components (Lee, et al., 2004). However, other studies have not found positive effects of herbs and their related essential oils. These latter findings may be related to experimental conditions, such as hygiene and dietary agents (Lee, et al., 2003). The primary mode of action of phytochemicals as growth-promoter is attributed to the growth inhibition of harmful intestinal microflora in the gastrointestinal tract (GIT) (Lopez, et al., 2005; Islam, et al., 2006) and by stimulating function of digestive organ, for example, the pancreas and small intestine (Jang, et al., 2004). Windisch and Kroismayr (2007) reported that reduction on the population of enteropathogens results in a more stabilized microflora that will indirectly stimulate functions of digestive organs and reduce microbe-host competition for nutrients. The mechanism by which the phytochemicals exert their antimicrobial activity consists of interactions with the microbial cell membranes of microorganisms by changing permeability for cations such as H+ and K+ (Cabuk, et al., 2006). The antimicrobial compounds are quickly exerted by determining structural alterations of the cell envelope.
Population of enteropathogen microbes which are known to less resistant to this antimicrobials activity will decreased, while many beneficial microbes, such as Bifidobacterium spp. and Lactobacillus spp. are relatively resistant (DiPasqua, et al., 2007; Ouwehand, et al., 2010). Another mechanism of actions which proposed for active compounds in herbal products as growth promoters are related to their oxidation-resistant activity (Faix, et al., 2009; Zhang, et al., 2009) and improvement of the immune system (Emadi and Kermanshahi, 2007; Yarru, et al., 2008; Najafi and Torki, 2010). The main phytochemicals contained in common herbs and spices (garlic, ginger, turmeric, oregano, and cinnamon) which are commonly used in poultry diets are presented in vitro studies showed that phytobiotic substances present in herbs and spices have antibacterial and antifungal (Hammer, et al., 1999), antiparasitic (Anthony, et al., 2005), antihelminthic (Chatterje, et al., 1982), and anticoccidial (Giannenas, et al., 2003) properties. There is an evidence to suggest that herbs, spices and various plant extracts have broad properties to improve growth performance and health of poultry (Al-Kassie, 2009).

2.4.2.1 Ginger

Ginger (Zingiber Officinale) is a medicinal plant which is widely used all over the world, ginger is an underground rhizome plant that belongs to the family Zingibeaceae and now it is considered a common constituent of diet worldwide (Sertie, et al., 1991) and widely used as a spice. The
genus Zingiber was named after the Sanskrit word zindschi (horn shaped) by the English botanist William Roscoe (1753-1831), in a report published in 1807 (Roscoe, 1807). Ginger, probably, originates from South-East Asia. The ancient Greeks and Romans brought the rhizome to Southern Europe. Already in the (11th) century, it is mentioned in Anglo-Saxon veterinary pharmacopoeia as and leech books. In the (13th) century, it was well known in all of Europe, and the Spanish established first plantations in the West Indies (mainly Jamaica) and in Mexico in the 16th century. Nowadays ginger is cultivated in the tropical parts of the world, from Asia to Africa, and large parts of South and Central America; mainly in India, in southern China, Indonesia, Nepal, and Nigeria. The best quality is said to come from Jamaica (Köhler, 1887; Wichtl, 2002).

2.4.1.1 Chemical composition of ginger

Ginger is a medicinal plant which is widely used all over the world. The main important compounds in Ginger (Zingiber officinale) are gingerol, gingerdiol and gingerdione which have the ability to stimulate digestive enzymes, affect the microbial activity (Nidaullah, et al., 2010; Dieumou, et al., 2009), when used in broiler diets. The pungent taste of ginger is caused by gingerol (Jolad, et al., 2004; Shariq, et al., 2011) which contains an enzyme called “zingibain” that aids digestion (Adulyatham and Owusu-Apenten, 2005). Also, it act, as an antioxidant (Nakatani, 2000; Rababah, et al., 2004), antimicrobial (Akoachere, et al., 2002;
Jagetia, et al., 2003; Mahady, et al., 2003), and has various pharmacological effects (Chrubasik, et al., 2005; Ali, et al., 2008). Immuno-modulatory, antitumori-genic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and antiemetic properties are among the other therapeutic effects of ginger observed (Badreldin, et al., 2008). Powdered rhizome of ginger has long been used as traditional medicine to alleviate the gastrointestinal illnesses (Afzal, et al., 2001). Ginger extracts have shown to exhibit antibacterial activity in invitro studies (Malu, et al., 2008; Indu and Nirmala, 2010). Ginger has been found to enhance pancreatic lipase activity (Platel and Srinivasan, 2000), intestinal lipase, disaccharidase and maltase activities of rats (Platel and Srinivasan, 1996). All of these have favorable effects on gut function, which is the primary mode of action for growth promoting feed additives (Windisch, et al., 2008).

2.4.2 Garlic

Garlic (Allium sativum) is a perennial herb with a bulb divided into segments (cloves) (Singh and Agarwal, 1998) and belongs to the family Amaryllidaceae and genus Allium (Wikipedia, the free encyclopedia (2013)). Garlic is one of the oldest cultivated plants (Moyers, 1996; Ramaa, et al., 2006). Garlic has been cultivated in all over Middle East and used as flavoring agent and as a medicinal plant beside other several medicinal properties (Zargari, 1997). Different forms of garlic preparations are commercially available in the form of garlic oil, garlic
powder, and pills. These are widely used for certain therapeutic purposes, including, lowering blood pressure and improving lipid profile (Elkayam, et al., 2003). Garlic has been used for many years to prevent health problems including colds, flu, menstrual pain, high blood, coughs, gastrointestinal problems, arteriosclerosis, and bronchitis. Garlic has been proven to kill various fungal infections, viruses, bacteria, and intestinal parasites (Elnima, et al. 1983 and Zenner, et al., 2003). Garlic (Allium sativum L.) is known to have antimicrobial, antioxidative and antihypertensive properties (Sivam, 2001). In vitro studies have shown that garlic extract has antimicrobial effects, such as antibacterial and antifungal properties (Indu, et al., 2006).

2.4.2.1 Chemical composition of Garlic

Garlic contains more than two hundred (200) chemical compounds which include: Sulphur containing volatile oils (allinase, peroxidase and ajoene), and enzymes (allinase, persoxidase and myrosinase) (Earyl, 1994) Allicin gives its antibiotic properties and strong odour. Ajoene contributed to the anticoagulant action of garlic (Earyl, 1994). Garlic also contains cira~ geraniol, linalool, Aphellandrene and B-phellandere. The allyl contained in garlic is also found in other members of the Alliaceae family and is considered a very valuable therapeutic compound (Earyl,1994).In general, the primary sulfur-containing constituents in whole garlic are the B-glutamyl-S-alk(en)yl-L-cysteines and S-alk-(en)yl-L-cysteine sulfoxides, including alliiin. Whole garlic
normally contains approximately 1% alliiin, together with (1)-S-methyl-L-cysteine sulfoxide (methiin) and (1)-S-(trans-1-propenyl)-L-cysteine sulfoxide. Garlic cloves contain S-(2 Carboxypropyl) glutathione, \( \gamma \)-glutamyl-S-allyl-L-cysteine, g-glutamyl- S-(trans-1-propenyl)-L-cysteine and B-glutamyl-S-allyl-mercapto-L-cysteine. A garlic bulb contains approximately 0.9% \( \gamma \)-glutamylcysteines and up to 1.8% allin. The S-allyl cysteine is formed from \( \gamma \)-glutamyl cysteine catabolism and is thought to contribute to the health benefits of some garlic preparations (Amagase, et al., 2001; Amagase, 2006). Several phytochemicals of garlic, mainly polyphenols such as flavonoids and sulfur-containing substances, have been revealed antioxidative properties in meat-type (Kim, et al., 2009) and egg-type (Gorinstein, et al., 2005) chickens. Furthermore, antioxidative activity of garlic depends on the part of the plant, as they contain different type and amount of phytochemicals. For instance, garlic husk had seven times greater total polyphenols than garlic bulb, and the non-edible garlic husk had 1.5 times greater radical scavenging activity than the edible part (Kim, et al., 2009; Choi, et al., 2010).

2.5 Effect of feeding ginger and garlic and their mixture on broiler performance

Body weight is a very important trait in the broiler industry. Many Studies were conducted to investigate the effects of feeding medicinal herbs on the body weight of broilers. Mohamed, et al. (2012) reported
that body weight was significantly (P< 0.05) improved by the supplementation of dietary ginger powder at levels 0.1and 0.2% compared to the control group, being 2020.83, 2075.90 and 1875 g/bird respectively during 42 days of age. Also according to the researchers Moorthy, et al. (2009), adding 0.2% ginger into the broiler diets improved body weight significantly (P< 0.05) compared to the control group, being 1898.66g and 1867.14g respectively over 42 of age. On the other hand, there were no significant differences in body weight when 0.05% of ginger powder used in broiler diets (El-Deek, et al., 2002). The same researchers also in trial 2 added 0.1% of ginger powder to the broiler diet but they did not found any effect on body weight. Ademola, et al. (2009) showed that using 1 and 1.5% of ginger in broiler diets had no significant effect on the body weight, while adding 2% of ginger had significant (P< 0.01) negative impact on the body weight compared to the control group, being 1829.05g and 2000g respectively during (56 days of age). There was no improvement in body weight of broiler chickens fed 2% of dried Zingiber officinale rhizomes supplement when compared to the chickens fed the control (Thayalini, et al., 2011). This is in agreement with the findings of Al-Homidan (2005) who used 2 and 6% of ginger powder into the broiler diets and did not find any effects on the final body weight when compared to the control group.

Garlic showed positive effects on the performance of different animals, the results were obtained when broilers were fed diets containing 1 or
2% garlic (Horton, et al., 1991; Freitas et al., 2001; Bampidis, et al., 2005). Hernandez, et al., (2004) showed that at the end of the second week, the highest body weight was achieved in the control group while in the experiment group with garlic at 2% was slightly lower. He stated that this result may be due to decreased feed consumption resulting from the intense smell of garlic which required a period of adaptation of chicken to this kind of feed. Horton, et al. (1991) reported similar results. Body weight gain is one of the most important measures that can be used to evaluate the performance of a flock of broilers, Herawati and Marjuk (2011) reported that adding 1.5% of ginger powder into the broiler diet significantly (P< 0.05) improved the total weight gain compared to the control, being 1955.5g and 1899.7g respectively. Similar results were obtained when broilers fed diets containing 1.5% of red ginger when compared to the control, 0.5%, 1% and 2% groups, being 1955.53, 1899.71, 1888.44, 1858.25 and 1859.5 g/bird respectively.

The same authors reported that feed consumption was significantly (P< 0.01) decreased in a group fed 2% ginger compared to the control group being (3966.7 and 4180 g/bird) respectively through 5 weeks of experimental period, while feed consumption was significantly (P< 0.01) increased in the group received 0.5% being (4406g), but there were no significant differences in the feed consumption of groups fed 1 and 1.5% of ginger powder when compared to the control group. Mohamed et al.
(2012) stated that feed consumption of broiler chickens was significantly (P< 0.05) decreased by the supplementation of dietary ginger powder at levels 0.1 and 0.2% compared to the control, being (2853, 2792 and 29010g/bird) respectively. Al-Homidan (2005) and Tekeli, et al. (2011) showed that feed consumption increased significantly (P< 0.05) in groups fed on antibiotic and 240 ppm ginger compared to the group which fed control diet throughout the experimental period 1-42 days, (3970, 3910 and 3335g/bird) respectively. Moorthy, et al. (2009) concluded that using 0.2% ginger in the broiler diets did not affect the feed intake. Similar results were found by El-Deek, et al. (2002) when they used 0.05% of ginger powder in broiler diets and they did not find any effect on feed intake. The same researchers in the trial 2 used 0.1% of ginger powder in the broiler diets but there were no differences in the levels of feed intake between treatments and control. These results were in agreement with those of Ademola, et al. (2009) who reported that using 1, 1.5 and 2% of ginger powder in broiler diets had no significant effect on feed intake. Similar results were obtained by Kehinde, et al. (2011) when they used different levels of ginger 1.5, 3 and 4.5% and there were no differences in the levels of feed intake of cockerel chicks. Birds received garlic for the whole of the experiment, had higher feed intake (FI) (Raeesi, et al., 2010). Chowdhury, et al. (2002) when different levels of garlic were added to layers diet.
Feed conversion ratio (FCR) is an index associated with both feed consumption and body weight gain. It's well known that broiler chickens are more efficient in utilisation of feed than other farm animals. Many medicinal herbs used in human diet which are known as "spices" improve digestibility. The same property can be used in poultry to increase the FCR which results in increasing body weight and more profit (Moorthy, et al., 2009). Herawati and Marjuk, (2011) stated that feed conversion ratio was significantly improved ($P< 0.05$) when different levels of dietary ginger powder used in broilers diet at 0.5, 1.5 and 2% compared to the control group, being (2.2, 2.2, 2.2, 2.1 and 2.3) respectively during 35 days, which are in agreement with those of Mohamed, et al. (2012) who found that feed conversion ratio of broilers were significantly improved ($P< 0.05$) by the supplementation of dietary ginger powder 0.2% compared to the group 0.1% and the control, which were (1.90, 1.98 and 2.25) respectively through 42 days of experimental period. On the other hand, Moorthy, et al. (2009) did not observe any differences in the FCR of broilers fed 0.2% ginger powder compared to the control. Also El-Deek, et al. (2002) did not find any differences between FCR of the treatments and the control when used 0.5 and 0.1% ginger powder respectively in broilers diets. Ademola, et al. (2009) noted that using high dose of ginger powder 2% had a highly significant negative impact ($P< 0.01$) on the broilers FCR compared to the other treatments 1%, 1.5% and the control (3.1, 2.7, 2.8 and 2.6) respectively,
while there were no significant differences in FCR of broilers fed 1 and 1.5% ginger compared to the control group. On the other hand, Thayalini, et al. (2011) mentioned that there was no improvement in feed conversion ratio of broiler chickens fed on high dose of dried Zingiber officinale rhizomes when supplemented at 2% compared to those fed the control diet. Using different levels of ginger powder 1.5, 3 and 4.5% statistically did not affect the feed conversion ratio of cockerel chicks (Kehinde, et al., 2011). Supplementation of 1% garlic powder decreased feed conversion ratio compared to 0.5% supplementation and control group Raeesi, et al (2010). The same author stated that bird received 3% garlic powder in their diets had better FCR than control group, control group significantly consumed more feed than the others, except those which were supplemented with 0.5% garlic powder, there were no significant differences between control and 0.5% supplemented group. Al-Hamadani, et al. (2010) used two levels of ginger powder 0.4 and 0.8% and they reported that mortality rate was zero in the group fed 0.8% ginger powder compared to the 0.4% ginger, antibiotic and control groups which were (2.3, 2. 3 and 6.8%). While Thayalini, et al. (2011) mentioned that there was no significant difference in mortality rate of broiler fed 2% dried Zingiber officinale rhizomes compared to the control, these findings agreed with those stated by Mohamed, et al. (2012) they did not observe any mortality case during 42 days of experimental period when 0.1 and 0.2% ginger powder used in broiler
diet. Also Zomrawi, et al.(2012) used different levels of ginger root powder (0.5, 1 and 1.5%) in broilers diets, he did not found significant differences in mortality rate when compared to the control group. It has been reported that garlic as a natural feed additive, improved growth and feed conversion ratio (FCR), and decreased mortality rate in broiler (Tollba and Hassan, 2003). The improvement in weight achieved by ginger and garlic supplementation over the control indicated that they have great impact on the growth of the birds, this explain that this improvement may be due to improve gut environment and micro flora achieved with garlic and ginger supplementation in addition to the fact that the susceptibility of pathogenic Gram positive bacteria to the antibacterial components of garlic and ginger are higher than that of the physiological desirable intestinal bacteria (Reeds, et al 1993 ; Cullen, et al., 2005). This observation is in line with the finding of (Shi, et al., 1999 and Javandel, et al., 2008). However observation contradicts the report of (Omage, et al., 2007, Ademola, et al., 2004 and Horton, et al., 1991) who reported that the inclusion of ginger and garlic mixture did not improve the weight gain of broiler. Safa (2014) studied the response of chicks to diets containing different mixture levels of garlic and ginger powder as natural feed additives and she concluded that the supplementation of mixture of garlic and ginger powder at level 1.75% enhanced growth, productive performance and meat quality of broiler chicks.
2.6 Effect of feeding ginger and garlic mixture on the immune response

Immunological response is influenced by several factors. Among them, the nutritional condition of the animal subjected to antigenic challenge has been studied, including the effects of medicinal plants. Arshad, et al. (2012) used different levels of ginger extract in drinking water of broiler and they observed that adding 50ml/liter of water caused increasing in antibody titers against IBDV and NDV being (4402 and 5.33) compared to the control (2628 and 4.5) respectively. Garlic supplements in broiler chicken have been recognized for their strong stimulating effect on the immune system and the very rich aromatic oils enhance digestion of birds (Gardzielewska et al., 2003) Garlic (Allium sativum), was found to enhance the immune response (Sumiyoshi, 1997). Also Ghazanfari et al (2002) reported that injected mice intraperitoneally with different doses of garlic exteracts and found a significant increase of delayed type hypersensitivity response, but not of antibody response. S-allyl cysteine, (Sumiyoshi, 1997). The allium species show immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo et al, 1998).
2.7 Effect of feeding garlic and ginger mixture on internal organs weight

Research studies have been focusing on improvement of chicken carcasses in order to meet the food industry standards. The best results are achieved through genetic selection, nutrition and breeding technology, which are reflected in a significant increase of overall carcass masses and the share of meat as well as a reduction of the abdominal fat content in 6-week-old chicks. Relative weight of liver was higher in control and 3% supplemented groups (Raeesi, et al. 2010). When birds fed garlic in their starter diets, they showed higher relative liver weight (p<0.001) (Raeesi, et al. 2010). Relative weight of gizzard, spleen, heart and liver were significantly higher in control groups when starter diet was supplemented with garlic, while (Raeesi, et al. 2010). Dieumou, et al. (2009) studied the effects of ginger and garlic essential oils on growth performance reported that all organ weights and carcass characteristics were not affected by the treatments, except for a decrease (P < 0.05) in relative liver weight of birds fed garlic oil treatment compared with those given ginger oil and control. The effects of the supplements on relative weights of internal organs showed that the relative weights of the heart, liver, gizzard, spleen and pancreas were not affected by dietary treatments, which was in agreement with the findings of Hashish, et al. (1995). A similar observation was reported by Ceylan, et al. (1998). Hernandez, et al. (2004) found no differences in gizzard,
liver and pancreas weights of broiler chickens fed wheat-soybean meal based diets supplemented with an antibiotic and ginger and garlic extracts.
CHAPTER THREE
3:0 MATERIALS AND METHODS

3.1 Experimental Side and Duration
The experiment was conducted at the college of Animal Production Science and Technology, Poultry farm, Sudan University of Science and Technology between first May to 21 June 2012 during which the temperature ranged between 35- 42 °C.

3.2 Experiment house
The experiment was conducted in an open sided deep litter, poultry house (10x4 x 2.7m) height, with concrete floor and corrugated sheet roof, the long axis of the house was located in east west direction of the wind, for efficient nature ventilation and it was constructed from red prick covered by soft cement at east west side and wire netting at south and north sides. The house equipment were cleaned and disinfected before starting the experiment using formalin (10%) and black phenol. The house was divided into equal 16 units (1*1 m) each; the floor was covered by 2inch deep wood shaving as litter. Each unit provided with one tubular feeder and one fountain drinker.

3.3 Experimental birds
One hundred and sixty, one day old Chicks were purchased from the Arab Poultry Production and Processing Company (APPP CO.). Chicks were randomly divided into four treatment groups, (40 chicks per group) and each group was furthered replicated (4) times given (10) birds per
replicate. The treatments were distributed in a completely randomized design (CRD).

3.4 Experimental diets:

3.4.1 Processing of garlic and ginger
The garlic bulbs were segmented into cloves then cut into chips. The chips were subjected to sun dry for a period of 3 day. The dried garlic chips were grounded and stored in an air tight container. Slices of dried ginger were grounded with pestol and mortar into powdery form.

3.4.2 Diets formulation
Two types of basal diets (Starter and Finisher) were formulated to meet the nutrients requirement given by the National Research Council (NRC 1994) for starter and finisher broiler chicks. (Table 1) showed the composition and calculated analysis of the experimental basal diets. Determined analysis was conducted by the method of AOAC (1995) (Table 2). Dietary treatments included, diet 1 (TA) basal diet without ginger and garlic mixture which served as control diet, then garlic and ginger mixture (1:1) was added to the basal diet at 0.2, 0.3 and 0.4% resulting in Diet 2 (TB), diet 3 (TC) and diet 4 (TD) respectively. Feed and water were provided ad libitum. The experimental diets (starter and finisher diets) were used over the period of the experiment (42 days).

3.5 Rearing Program:
Chicks were vaccinated against Newcastle (ND) and infectious bronchitis (IB) at arrival by spraying; all other vaccines were
administered through drinking water which included Gumboro vaccine (IBD) at 14 days of age. And ND at 28 days, vitamin was added (1gm/liter) after each vaccination for three consecutive days.
Table (1) Composition and calculated analysis of the experimental starter and finisher basal diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>68</td>
<td>73</td>
</tr>
<tr>
<td>Ground nut meal</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>Concentrate</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>DiCalcium Phosphat</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Lime stone</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Calculated analysis**

<table>
<thead>
<tr>
<th></th>
<th>Starter</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME/MJ/ Kg</td>
<td>13.1</td>
<td>13.2</td>
</tr>
<tr>
<td>CP %</td>
<td>22.7</td>
<td>21.2</td>
</tr>
<tr>
<td>CF %</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Ca %</td>
<td>1.2</td>
<td>1.15</td>
</tr>
<tr>
<td>P %</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>Lysine %</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Methionine %</td>
<td>0.37</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Super concentrate provimi provided: CP 38%, CF 3%, Ca 6%, av.P, 4.2%, lysinse 6%, Methionine 3%, ME 8.37 MJ/Kg.
Table (2) Determined analysis of the experimental starter and finisher basal diets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Starter</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, MJ/Kg*</td>
<td>12.1</td>
<td>12.3</td>
</tr>
<tr>
<td>Crud Protein</td>
<td>24.88</td>
<td>21.58</td>
</tr>
<tr>
<td>Crud Fiber</td>
<td>5.20</td>
<td>5.00</td>
</tr>
<tr>
<td>Calcium%</td>
<td>1.2</td>
<td>1.15</td>
</tr>
<tr>
<td>Phosphorus%</td>
<td>0.37</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*ME: values were calculated according to the equation of Wiseman, (1987)
3.6 The performance trial

3.6.1 Body weight (LBW)
Body weight was measured for all bird at the beginning of the experiment, and it was repeated weekly at the beginning of each week at the same time.

3.6.2 Body Weight gain (BWG)
Weight gain was calculated by subtraction the live weight at the beginning of the week from live body weight of the next week.

3.6.3 Feed intake (FI)
Feed intake is the amount of feed consumed every week; it was calculated for each treatment on weekly basis. At the end of the week the residual amount of feed was weighed and subtracted from the known weight provided through out the the week. The product was divided by the total number of bird to give the g FI/bird/week.

3.6.4 Feed Conversion Ratio (FCR)
Feed conversion ratio (FCR) was calculated on weekly basis by the following equation:
\[
\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}
\]

3.6.5 Mortality Rate (%)
Mortality was recorded when occurred for each replicate and mortality percent was calculated by the following equation:
\[
\text{Mortality (\%)} = \frac{\text{Total dead birds}}{\text{Total number of birds}} \times 100
\]
3.6.6 Internal Organs weight
At the end of the experiment three birds from each replicate were randomly selected and slaughtered, Spleen, Liver, Heart and Pancreas were weighed individually and recorded.

3.6.7 Blood Sample Collection
At the end of the experiment, five birds from each replicate were selected randomly, and blood samples were collected from the wing vein using sterile needle into a non-heparinized test tubes, the blood allowed to clot and centrifuged at 2000 revolutions per minute (rpm) for 15 minutes to obtain the blood serum, which stored in deep freeze (-20)°C until analysis.

3.6.8 Antibody titer
The blood serums of 42 days old broilers were used for humoral immunity test. Antibody titers against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) were measured by using BioChek NDV and IBDV test kits with ELISA Reader Appendix(1).

3.7 Statistical analysis
All data generated from the experiment were carried out in a complete randomized design (CRD). These data were subjected to analysis of variance (ANOVA) according to the general linear model procedure of SPSS soft ware (SPSS, 2001). The significant differences among means were determined by least significant differences (L.S.D) tests (Steel et al.,) (1997).
CHAPTER FOUR
4.0 RESULTS

4.1 Effect of feeding garlic and ginger mixture on weekly body weight (gm/bird)

The effect of feeding garlic and ginger mixture on weekly body weight resulted in no significant differences in body weight during the 1st and 2nd week of age. At 3rd, 4th, 5th and 6th week a highly significant differences (P<0.05) were observed between the treatments. The average weekly body weight is shown in (Table, 3).

4.2 Effect of feeding garlic and ginger mixture on weekly body weight gain (gm/bird)

The study explore that feeding birds the mixture of garlic and ginger resulted in no significant differences in weekly weight gain between the control group and the groups that fed garlic and ginger mixture during the 1st, 3rd, 4th, and the fifth week. At 2nd and 6th week a significant differences were noted between the treatments (Table, 4).

4.3 Effect of feeding garlic and ginger mixture on weekly feed intake (gm/bird)

Table (5) showed the weekly feed intake when broilers fed different levels of garlic and ginger mixture. The results revealed that feeding broilers garlic and ginger mixture had a significant effect on weekly feed intake compared to the control group during the 2nd, 5th and 6th week. No
significant differences were observed between the treatments during the 3rd and 4th week of age.

4.4 Effect of feeding garlic and ginger mixture on weekly feed conversion ratio (gm feed/gm gain)

The effect of Feeding garlic and ginger mixture on weekly feed conversion ratio of broiler chickens had showed a highly significant differences during the 2nd week, no differences were noted during the 1st, 3rd, 4th 5th, and 6th week (Table 6).

4.5 Effect of feeding garlic and ginger mixture on internal organs weight (gm).

The internal organs weights were not significantly (P>0.05) influenced by the dietary treatments (Table 7).

4.1.6 Effect of feeding garlic and ginger mixture on Antibodies titer against Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV).

Table (8) showed the effect of feeding ginger and garlic mixture on the humoral immunity (antibody titers against (NDV) virus and (IBDV) virus of 6 weeks age broiler chicks. Concerning antibody titers against NDV (Newcastle Disease Virus) feeding garlic and ginger mixture had a significant (P<0.05) effect on antibodies titer against NDV compared to the control diet, on the other hand IBDV antibody titer showed the same trend.
4.1.7 Effect of feeding garlic and ginger mixture on production
Performance of 6 weeks old broiler chicks.
The results of the effect of feeding ginger and garlic mixture on the accumulative performance are presented in table (9). Body weight, weight gain, feed intake, mortality percentage and antibody titers against NDV and IBDV showed a significant $P \leq 0.05$ response to dietary treatments, but feed conversion ratio and internal organs weight (Table (8)) weight showed insignificant response.
Table (3): Effect of feeding garlic and ginger mixture on weekly body weight (gm/bird)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>133.75±6.18</td>
<td>212.38±16.83</td>
<td>331.23±11.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>488.18±10.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>760.56±14.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1038.00±26.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2</td>
<td>145.50±11.03</td>
<td>174.88±104.3&lt;sup&gt;2&lt;/sup&gt;</td>
<td>350.50±25.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>542.50±56.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>808.30±75.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1141.00±96.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3</td>
<td>133.00±7.07</td>
<td>196.50±6.98</td>
<td>284.40±18.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>437.03±25.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>663.90±34.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>946.96±71.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4</td>
<td>138.75±2.50</td>
<td>214.00±11.70</td>
<td>324.50±16.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>481.13±30.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>749.25±50.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>991.70±461.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Level of sig</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

*<sup>a</sup>,<sup>b</sup>,<sup>c</sup>: Means with different superscripts in each column differ significantly (P<0.05).

NS: not significant.

** :highly significant (P<0.05)
Table (4) Effect of feeding garlic and ginger mixture on weekly weight gain (gm/bird)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week1</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
<th>Week6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>55.90±0.90</td>
<td>78.62±10.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.35±17.4</td>
<td>158.20±17.2</td>
<td>272.39±11.3</td>
<td>310.0±29.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2</td>
<td>61.75±7.50</td>
<td>92.20±7.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.38±36.3&lt;sup&gt;5&lt;/sup&gt;</td>
<td>192.00±41.6</td>
<td>287.55±55.7</td>
<td>353.08±23.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3</td>
<td>54.25±5.74</td>
<td>72.00±5.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107.88±13.3</td>
<td>152.62±22.0</td>
<td>251.88±17.1</td>
<td>285.56±37.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4</td>
<td>58.75±2.50</td>
<td>75.25±9.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>110.62±08.9</td>
<td>156.50±18.8</td>
<td>243.12±18.5</td>
<td>304.08±48.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Level of sig</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
</tr>
</tbody>
</table>

*<sup>a</sup>, <sup>b</sup>, <sup>c</sup>: Means with different superscripts in each column differ significantly (P≤0.05).

**: highly significant (P≤0.01)
*significant (p≤0.05).

NS: not significant.
Table (5): Effect of feeding garlic and ginger mixtures on weekly feed intake (gm/bird).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week1</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
<th>Week6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>149.75±20.4</td>
<td>306.93±55.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>357.40±42.9</td>
<td>415.08±37.7</td>
<td>671.73±16.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>790.45±21.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2</td>
<td>148.75±36.0</td>
<td>256.88±32.90&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>358.88±11.6</td>
<td>430.00±42.4</td>
<td>648.68±42.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>844.48±67.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3</td>
<td>112.25±19.1</td>
<td>209.13±32.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>271.23±102.6</td>
<td>407.45±72.2</td>
<td>589.80±73.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>718.95±66.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4</td>
<td>128.75±23.3</td>
<td>240.00±13.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>299.50±45.0</td>
<td>347.50±63.1</td>
<td>578.38±27.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>696.60±31.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Level of sig.: NS | * | NS | NS | * | **

*a, b, c: Means with different superscripts in each column differ significantly (P≤0.05).

**: highly significant (P ≤ 0.05).

*significant (P≤0.05).

NS: not significant
Table (6): Effect of feeding garlic and ginger mixture on weekly feed conversion ratio (FCR) (gm feed/gm gain).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>2.65±.3</td>
<td>3.93±.51\textsuperscript{a}</td>
<td>3.08±.5</td>
<td>2.65±.5</td>
<td>2.45±.1</td>
<td>2.56±.2</td>
</tr>
<tr>
<td>0.2</td>
<td>2.38±.6</td>
<td>2.73±.22\textsuperscript{b}</td>
<td>2.93±.8</td>
<td>2.30±.5</td>
<td>2.45±.7</td>
<td>2.33±.0</td>
</tr>
<tr>
<td>0.3</td>
<td>2.05±.4</td>
<td>2.88±.49\textsuperscript{b}</td>
<td>3.00±1.0</td>
<td>2.63±.3</td>
<td>2.33±.2</td>
<td>2.48±.3</td>
</tr>
<tr>
<td>0.4</td>
<td>2.15±.6</td>
<td>3.15±.37\textsuperscript{b}</td>
<td>2.65±.4</td>
<td>2.18±.4</td>
<td>2.35±.1</td>
<td>2.23±.2</td>
</tr>
<tr>
<td>level of sig.</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*\textsuperscript{a, b, c}: Means with different superscripts in each column differ significantly (P<0.05).

**: highly significant (P<0.05)

NS: not significant
Table (7): Effect of feeding garlic and ginger mixture on internal organs weights (gm)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart</th>
<th>Liver</th>
<th>Gizzard</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.79±0.053</td>
<td>3.05±0.015</td>
<td>2.07±0.084</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>0.2</td>
<td>0.70±0.034</td>
<td>2.79±0.162</td>
<td>1.94±0.061</td>
<td>0.09±0.00</td>
</tr>
<tr>
<td>0.3</td>
<td>0.78±0.042</td>
<td>3.05±0.015</td>
<td>2.17±0.100</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>0.4</td>
<td>0.81±0.052</td>
<td>2.87±0.112</td>
<td>2.05±0.112</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Level of sig.</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: not significant different
Table (8) Effect of feeding garlic and ginger mixture on antibodies titer against NDV & IBDV viruses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NDV antibodies titers</th>
<th>IBDV antibodies titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0%</td>
<td>3389.0± 2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>981.25±1.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2%</td>
<td>3905.5±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1682.0±7.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3%</td>
<td>3986.5±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1773.0±8.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%</td>
<td>4067.5±4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2030.2±4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Level of sig</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

*a, b, c: Means with different superscripts in each column differ significantly (p<0.05).

**: highly significant
Table (9): Effect of feeding garlic and ginger mixture on performance of six weeks old broiler chicks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LBW (g/bird)</th>
<th>BWG (g/bird)</th>
<th>FI (g/bird)</th>
<th>FCR (g feed/g gain)</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1035.5±26.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>993.5±40.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2964.2±31.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2</td>
<td>1133.0±96.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1094.0±133.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3077.8±31.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81 ±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3</td>
<td>946.2±71.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>904.2±18.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2621.8±14.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4</td>
<td>990.3±461.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>948.3±38.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2699.3±38.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.85±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Level of sig.</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

<sup>a</sup>, <sup>b</sup>, <sup>c</sup>: Means with different superscripts in each column differ significantly (<i>P</i>&lt;0.05).

****: highly significant <i>P</i>≤0.05

* Significant (<i>P</i>≤0.05)
CHAPTER FIVE

5. DISCUSSION

Medicinal plant supplements are used commonly as dietary additives for humans. They are chosen for their non-toxic chemical composition, relatively low cost and easy availability. Also, over the past few years, medicinal plants and their extracts have been used in animal diets as feed additives in order to improve their performance, health and the quality of their products. This use of aromatic plants is based on their wide range of antimicrobial (SivroPoulou, et al., 1996), antioxidant (Botsoglou, et al., 1997) or even appetite and digestion simulative properties (Kamel, 2001).

5.1 Effect of feeding garlic and ginger mixture on production performance of six weeks old broiler chicks

The overall performance results of six weeks old broiler chicks are presented in table (9). The mean total feed intake (g/bird) showed a significant (P ≤ 0.05) increased for birds fed the control diet (2964.2 g) and 0.2% garlic and ginger mixture (3077.8 g) compared to those fed 0.3% (2621.8 g) and 0.4% (2699.3 g). The lower inclusion rate of garlic and ginger mixture 0.2% showed the highest feed intake. This result indicated that feed intake decrease when the levels of ginger and garlic mixture increase this might be due to the pungent odor of garlic that can adversely affect feed intake. Allicin is an extremely odoriferous compound (Cavallito and Bailey, 1994). This suggest that the
organoleptic properties of garlic are responsible for the decrease feed intake (Cullen, et al., 2005).

The mean weight gain of birds fed 0.2% garlic and ginger mixture was significantly (P≤0.05) higher compared to those birds fed the other experimental diets. The live weight (g/bird) and feed conversion ratio (g feed g/ gain) followed the same trend, these result are in agreement to those reported by Thayalini, et al (2011) who found that high levels of herbal combination in broiler diets have a negative effect on weight gain and feed conversion ratio and they clarified that high dosage of medicinal herbs may have a negative effect on some beneficial microbial populations such as Lactobacillus, preventing the herbs from exhibiting its positive influence on performance indices. Also these findings are in agreement with the results reported by Moorthy, et al (2009); Najafi and Torki (2010) ; Rahimi, et al (2011) ; Sadeghi, et al (2011) and Mohamed, et al (2012) who mentioned that adding herbs to broiler diets with lower inclusion rate resulted in a significant positive effect on live weight, weight gain and FCR of broiler chicks. On the other hand, our results are in disagreement with the results obtained by (Al-Homidan, 2005; Al-Jugifi, 2009; Ademola, et al., 2009; Thayalini, et al., 2011; Kehinde, et al., 2011; Al-Mashhadani, et al., 2011; Mansoub and Myandoab, 2011) who reported that dietary supplementation of broiler with ginger and garlic mixture had no significant negative effect on body weight gain of broilers, This may be attributed to the fact that
immunostimulation may have adverse effects on growth performance, because more nutrients will be repartitioned to synthesize antibodies and develop the immune organs, thereby decreasing the nutrients available for growth (Hevener, et al., 1999; Takahashi, et al., 2000). Although the effect of feeding ginger and garlic mixture on mortality rate was significant, all birds died were apparently healthy. These deaths may be due to heat stress during the experiment.

5.2 The effect of feeding garlic and ginger mixture on internal organs weight.

Adding ginger and garlic mixture at tested levels had no significant effect on internal organs weights (Liver, heart, gizzard and spleen). This result disagreed with those outlined by (Tekeli, et al., 2011; Mansoub and Myandoab, 2011 and safa, 2014) who reported that feeding garlic and ginger supplemented diet had a significant negative effects on visceral organs weights. On the other hand, the results were inconsistent with those obtained by (El-Deek, et al., 2002; Tekeli, et al., 2006; Demir, et al., 2008; Moorothy et al., 2009; Najafi and Torki, 2010; Toghyani, et al., 2010; Sadeghi, et al., 2011; Rahimi, et al., 2011) who did not found any significant effect on internal organs weights when broilers fed on diets contained ginger and garlic mixture.
5.3 Effect of feeding garlic and ginger mixture on Antibody titer against Newcastle disease (ND) and Infectious bursal disease (IBD) viruses.

The results showed that all birds fed (1:1) garlic and ginger mixture (0.2, 0.3 or 0.4%) had a significant (P≤0.05) improvement in antibody titers against IBD and New Castle ND. This effect may be attributed to the fact that active component of herbs (Gingerol and Allicin) may improve digestion and stimulate the immune function in broiler (Ghazalah and Ali, 2008; Kim, et al., 2009; Choi, et al., 2010 and Foroghi, et al., 2011). Josling (2001) reported that garlic mobilized immune system and empowers the defense ability of the body against infectious organism. Our results were in agreement with some studies of (Shahriyar and Durrani, 2006; Najafi and Torki, 2010 and Mansoub and Myandoab, 2011) who reported that these plants improve health status and performance of broiler, in addition to the improvement of antibody titers against ND and IBD viruses. Also the results of the present study are in line with those reported by Foroughi, et al (2011) who stated that supplementation of garlic and ginger mixture in diets increased humeral immune response against (IBD) and (NDV) compared to the control group.
CHAPTER SIX
6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the results of the present study the following conclusions can be addressed:

1- Supplementing broiler diets with (1:1) garlic and ginger mixture particularly at inclusion rate of 0.2% resulted in a positive effect in all production parameters and immunological profile.

2- Higher rates of ginger and garlic mixture (0.3% and 0.4%) had a negative effect on broiler growth performance parameters.

3- Internal organs weight were not affected by all tested levels of garlic and ginger mixture.

4. Although high levels of garlic and ginger mixture did not induce any significant positive effect on the growth related parameters, they were improved the immunological profile of broiler chicken by increasing antibody titers against Newcastle and Infectious Bursal disease viruses.

5. Feeding (1:1) ginger and garlic mixture had no adverse effect on mortality rate.

6.2 Recommendations

1- Based on the results of this study it is recommended to use (0.2%) garlic and ginger mixture (1:1) as feed additive in broiler feed without adverse effect on Production Performance and to enhance immunity response.
2- We suggest further research on the same herbs and their extracts in diverse doses, different housing systems and other types of poultry (rabbit, quail and Ostrich...ect).
References:


Appendix

The following preparations and reagent for the Newcastle Disease Virus titer (NDV) and Infectious Bursal Disease Virus titer (IBDV) antibodies Tests kits were done.
Figure (1) Show ELISA Reader on the left side and ELISA washer on the right side that used for measuring antibodies titers of NDV and IBDV.

- The following preparation and reagent for the Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) antibodies Tests kits were done:

- Conjugate Note: the NDV and IBDV have the same test procedure

Reagents provided:

1. NDV and IBDV coated plates. Inactivated viral antigen on microtitre plates.

2. Conjugate reagent. Anti-chicken: Alkaline Phosphatase in Tris buffer with protein stabilizers, inert red dye and sodium azide preservative (0.1% w/v).

3. Substrate tablets. PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with substrate buffer.


5. Stop solution. Sodium hydroxide in diethanolamine buffer.
6. Sample diluent reagent. Phosphate buffer with protein stabilizers and sodium azide preservative (0.1% w/v).


8. Negative control. Specific pathogen free serum in phosphate buffer with protein stabilizers and sodium azide preservative (0.1% w/v).

9. Positive control. Antibodies specific to NDV and IBDV in phosphate buffer with protein stabilizers and sodium azide preservation (0.1% w/v).

**Materials and Equipments Required**

1. Precision pipettes and disposable tips.

2. 8 or 12 channel pipette/repeater pipette.


4. Distilled or deionised water.

5. Microtitre plate reader with 405 nm filter.

2. Calculation of antibody titer:

The following equations relate the S/P of a sample at a 1:500 dilution to an end point titer.

**For NDV:**
Log 10 Titer = 1.0 * Log (S/P) + 3.52
Antilog = Titer

**For IBDV:**
Log 10 Titer = 1.1 * Log (S/P) + 3.361
Antilog = Titer

Spectrophotometer machine used for analysis of serum biochemical parameters.