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Effect of Dried Mushroom (*Agaricus bisporus*) Powder, *Bacillus subtilis* and their combination of Performance and Some Physiological traits in Broiler Chickens

Thesis

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Supervisor Certificate

I certify that this thesis ***((Effect of Dried Mushroom (Agaricus bisporus) Powder, Bacillus subtilis and their combination of Performance and Some Physiological traits in Broiler Chickens))*** has been prepared by **Ameer Hameed Kadhim Al-Sheikh** under my supervision at the College of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the Degree of Master in the Sciences of physiology, biochemistry and pharmacology.

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DEDICATION

TO THE SOUL OF MY PARENTS
(MOTHER AND FATHER)

WITH MY ENDLESS SORROW

TO THE ALL MY FAMILY

TO THE MARTYRS OF THE
IRAQI SOLDIERS

WITH MY ENDLESS LOVE

AMEER AL-SHEIKH

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Abstract

The immune response and broiler performance may be affected by physical health and challenge levels, broiler production is a dynamically developing industry, with most feeds based on probiotics and prebiotics that becoming nutritional supplements. Therefore, this study aims to explore the effect of probiotics (*Bacillus subtilis*), prebiotics mushroom (*Agaricus bisporus*) and combination between them on improving humeral and cellular immunity, in addition to the health and performance of broilers, the expermental period is from from 4/1/2020 to 7/2/2020, and lasts for 5 weeks. A total of 160 chicks for one day, Ross 308, were randomly assigned in to four equal groups and designed to be four replicates for each group. The control group (Con) was fed a basic diet without any additives, second group the Mushroom group (Mush) was fed the basic diet (1% dried mushroom powder). The *Bacillus subtilis* group (BS) was fed a staple diet basal diet with 4×10^9 cfu/gm Bacillus subtilis as a probiotic, while forth was fed on mixed of (Mush and BS) with 1% dried mushroom powder with basal diet have 4×10^9 cfu/gm Bacillus. Blood samples were collected at 9, 18 and 35 days of the study for immunological and hematological investigation, on the other hand 3 ml of blood Samples collected from wing veins into EDTA tubes, , A minimum of 200 leukocytes per slide were sorted into categories: small or medium lymphocytes, heterophils to determinated H/L ratio, as well as 2 birds from each group were killed by cervical dislocation, following a thorough visual appraisal, the bursa and spleen were immediately removed, dry and individually weighed (g) for each individual and the ratio of bursa, spleen weight (%) was calculated, 0.5 cm in length from middle of the duodenum, and ileum were excised and opened longitudinally at the ant mesenteric attachment, then rinsed and stored in 70% ethanol at 4°C for histological examination, Villi and crypts were carefully individualized under a dissecting microscope . The results showed that the concentrations of the liver enzyme AST, ALT, and ALP for the (Mush-BS), (Mush) and (BS) groups were significantly lower ($P\leq0.05$) compared to (CON). Otherwise, triglycerides TG, cholesterol and very low density lipoprotein

(LDL-C, VLDL-C) concentrations of the given groups (Mush-BS), (Mush) and (BS) were less significant. However, compared to (CON), the high-density lipoprotein (HDL) concentration of the (Mush-BS), (Mush) and (BS) groups increased significantly ($P \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle disease (ND) and infectious bronchitis (IB) were improved significantly ($P \leq 0.05$) in the (Mush-BS), (Mushroom), and (BS) groups respectively compare with the (Control) in the ninth and eighteenth age days.The growth performance of broilers were varies greatly among (Mush-BS), (Mush) and (BS) in the feed intake, body weight, feed conversion ratio and body weight gain have significant differences ($P \leq 0.05$) groups compared with (CON).On the other hand, the morphological parameters for depth of the small intestine crypts, and villus height show highly increase in the Mush-BS groups compare with control groups. The results showed non-significant differences in the control group for spleen and bursa index compare with (MSH) and (BS) groups and combination groups (Mush+BS), in general, The results showed significant decrease in values of pH in illium and duodenum in group teceived Mush+BS as compared with other experimental groups. It is also found significant differences compared with the control group in the H/L ratio between (MSH) and (BS) groups and combination groups (Mush+BS).In conclusion, adding a mixture of probiotics(*Bacillus subtilis*) and prebiotics(mushroom) to their staple diet can improve broiler performance, immune response, fat distribution liver enzymes in broilers chickens and immune response by enhancing both cellular and Humeral immunity.

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List of abbreviation

Abbreviation	
Con	control
Mush	mushroom
BS	Bacillus subtilis
BW	Body Weight
WG	Weight gain
FI	Feed intake

FCR	Feed conversion ratio
TC	Total cholesterol
TG	Triglyceride
HDL-C	High density lipoprotein
LDL-C	Low density lipoprotein
vLDL-C	Very low density lipoprotein
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ELISA	Enzyme Immunoassay Technique
TBH	Tert-Butyl hydr quinone
TCA	Trichioro acetic acide
HBD	Hydrogen bond donors

Chapter One

Introduction

Introduction:

Mushroom has been used as a source of food medicine (Asatiani *et al*.,2010).It has been used as a dietary food and medicinal supplement in china for over 2000 years ago . Cultivation of *Agarius bisporus* in Europe was achieved in France on 17th century (Savoie and Largeteau,2011). Mahajna *et al* (2009) showed the beneficial effect of fungi from the Basidiomycota due to contain numerous of active compound like antibiotic, glycoproteins, triterpenes and polysaccharides (wasser, 2002). Polysaccharide has been used as immunomodulating by enhancing lymphocyte and antibody production (El Enshasy & Hatti-Kaul, 2013). In recent years, researchers have been looking for safe natural growth enhancers, such as organic acids, probiotics, prebiotics, and Plant toxins(Arowolo and He, 2018).

Beneficial effects of probiotics on growth performance, nutrient digestion, and flora cyclase formation (Awad *et al*., 2009; Mountzouris *et al*., 2010) and the antibody response to Newcastle disease virus and infectious bursa vaccination against the disease has been reported (Talebi *et al*., 2008).phytochemicals and natural products For example, herbal medicine may be a successful additive in poultry production. As we all know, mushrooms have great antibacterial effects and immune booster (Jiaannas *et al*., 2010)

The use of antibiotics has been the cornerstone of the poultry industry, enabling producers to reduce management costs by raising livestock. New strains that are resistant to drugs as pathogens after their emergence, the routine use of antibiotics is in animal feed unfamiliar(Hoelzer et al., 2017). Many non-therapeutic uses for antibiotics poultry farming is being phased out. In light of this trend،

alternative methods are needed to prevent and treat the common bacterial infection in poultry. Probiotics are a promising alternative. Probiotics It is a live microbial food supplement that benefits the host to achieve the purpose of animals by improving the balance of intestinal microbes (Holmes *et al*.,2016). These health-promoting bacteria are It is increasingly used in poultry feed in place of antibiotics as an alternative method of controlling growth Unfavorable microorganisms. Other health-promoting compounds are also being studied of particular interest is the extract from Shiitake (Reis *et al*., 2017).

Mushrooms have major health-promoting effects due to various antioxidants and antibacterials The features it contains. Wang *et al*., (1998) on the antibacterial activity, immune strengthening and stress reduction of farm animals using natural medicines. From fungi and herbs. Hashemi& Davoodi (2011) ,reported the antibacterial effect of Agarius bisporus Mushrooms in different experiments. Willis *et al*., (2007)reported a population of bifidobacteria The addition of mushrooms (Agarius bisporus) significantly increased the content of lactic acid bacteria and lactic acid bacteria.

Based on these results, it can be reasonably assumed Including some probiotics and Mushroom extract may have significant health benefits for poultry. Given this potential, an important step for poultry is determining whether probiotics can Synergize with mushroom extract to enhance The health and growth of broilers (Lillehoj *et al*., 2018).

Several studies have established the importance of probiotic as dietary supplements to improve the performance of the animals and sometime as preventative and curative (Ezema., 2013). Dietary of probiotic has become accepted to promote health for both human and

animal, also, many studies suggested that using probiotic cultures in the poultry industry as a natural mean to pathogen (Markowiak, & Śliżewska, 2018)

Gastrointestinal bacteria compete with the host for other nutrients, induce rapid turnover of absorbable epithelial cells, increase the rate of mucus secretion by intestinal goblet cells, and cause inflammation through the immune system (Dibner and Richards, 2005). On the other hand, the intestinal flora assists the host by digesting easily digestible food components and producing short-chain fatty acids (Gibson & Fuller, 2000), which lowers the pH of the intestine and creates a barrier that prevents the growth of some microorganisms. Environmental conditions; bacterial pathogens in the intestine (NEPA *et al*., 2009).

Likewise, the production of acetate from non-hydrolyzed oligosaccharides, short-chain polysaccharides fatty acids, propionates, and butyrates will increase the proliferation of intestinal epithelial cells, thus increasing the weight of intestinal tissue and altering the general shape of the intestinal mucosa (Reisinger *et al*., 2012). whereas broiler chickens are fed with 0.2% yeast cell wall feeding, villi length increases, and this increase in villi length is thought to increase the intestinal absorption surface (Reisinger *et al*., 2012). When adding yeast cell walls to the diet increases intestinal mucin, the density of goblet cells is also higher, which is very important for elimination of intestinal pathogens (Reisinger *et al*., 2012).

Another way of working prebiotics is to prevent colonization of pathogens (Haldar *et al*., 2011). These studies found that when consuming a diet containing prebiotics, the concentration of intestinal

bacteria in the cecum changed significantly, resulting in a reduced number of salmonella in mixed digestion samples and faeces. When prebiotics attach themselves to type 1 pathogenic bacteria, they reduce the ability of the bacteria to colonize and multiply in the gastrointestinal mucosa (Jacobs, 2011).

The aims of this study are to identify the following:

This study aims to in investigate the effect of of supplementation mushroom as prebiotic with or without *Bacillus subtilis* as probiotic on performance, and some physiological, biochemical aspects in addition to study the immune response in broilers chickens.

Chapter Two

Literature Review

2. literature review:

2.1 Mushroom:

Mushroom cultivation fungus *Auricularia* has a long tradition in East Asian countries, especially in China, where mushroom cultivation began around 600 AD.In Europe, *Agaricus blazei* mushrooms were first cultivated in France in the seventeenth century (Kues & Liu, 2000) it was originated from the following scientific classification

Kingdom: Fungi

Division: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

Family: Agaricaceae

Genus: Agaricus

Species: A. bisporus.(Manzoor *et al*., 2019) (Table 1). At least 12,000 species of fungi are considered mushrooms, and at least 2,000 of them are edible species on our list (Zhang, 1999). Sanchez, (2004), was collected more than 200 species of the wild and used them for various traditional medicinal purposes, most of them in the Far East. Worldwide, they have 35 types of commercial cultivation, and 20 species are cultured on an industrial scale. The most widely grown mushrooms in the world are *A. bisporus* (button mushroom), followed by *Volvariella volvacea* (mushroom straw), *Lentinus edodes* (shiitake), *Pleurotus spp* (oyster mushroom), *Fluffina velutipes* (winter mushroom) and *Auricula auricula* (mushroom mushroom) .

Table 1: Global production of mushrooms and agricultural organizations United Nations (2009).

Country	Production (tonnes) 2007	Percentage(%)
China	1605000	65.0
United states	390000	5.9
Canada	81500	16.5
Indonesia	30000	36.7
Republic of Korea	28500	53.8
Islamic republic of Iran	28000	64.3
Vietnam	18000	44.4
Thailand	10000	10
Israel	9500	86.7
Jordan	700	28.6
Kazakhstan	500	100
Singapore	10	100
*FAO estimate. Source: (FAO, 2009.)		

2.1.1. The benefits of Mushroom.

Mushrooms are not only used as a food source but also as a medicinal resource (Wasser, 2002). The medicinal properties of the fungus have been confirmed by in-depth research on a global scale. According to (Chang, 2001), medicinal mushrooms have been used as nutritional supplements or medicinal foods in China for more than 2000 years. The mushroom's extractable components are incorporated into the products, which are said to improve the biological functions of the human body. Since the late (1980s), it has garnered much attention. According

to Mahajna *et al*., (2009), basal mushroom have attracted a lot of attention because they contain a large number of bioactive compounds such as triterpenes, glycoproteins, polysaccharides, and antibiotics (Wasser, 2002). (however, among all the bioactive compounds), *Mannentake* or "*Ganoderma lucidum*" (*Ganoderma lucidum)* antibodies of fruits, germs and fungi (Liu et al., 2002). Previous studies have shown that these polysaccharides have immune properties, including enhancing antibody production and lymphocyte proliferation Bao *et al*.,(2001).

Generatesd activities that enhance the control of tumors and genotoxicity (Wasser, 2002). Tao *et al*., (2006) was observed anti-tumor activity in Pleurotus spp. On the other hand, the polysaccharide isolated from the fruiting bodies of ostreatus also showed activity against Hela tumor cells (Tong *et al*., 2009). Reducing toxicity and side effects of chemotherapy and radiotherapy in some patients (Bao *et al*., 2001).

These polysaccharides have different chemical combinations, and most of them belong to the B-glucan group.In order to demonstrate anti-tumor activity, dextran B- (1_3) vertebral column must be connected with other B- (1_6) sub points (Wasser, 2002).The mechanism of action of basidomyces mushroom includes stimulation of apoptosis, inhibition of aggressive behavior, inhibition of proliferation, induction of interruption of the cell cycle, and inhibition of angiogenesis in several experimental programs including prostate cancer (Mahajna *et al*., 2009). The most recent discovery by Hearst *et al*., (2009) reveals another benefit of mushrooms. *Lentinula edodes* and P. *ostreatus* have antibacterial and antifungal properties were analyzed. Surprisingly, it was found that mushroom extract was more effective as an antibacterial and antibacterial agent rather than ciprofloxacin. Mushroom extracts have also been reported to have antioxidant properties.

Bao *et al*., (2008) discovered the antioxidant properties of *Flamolina phyllotibs*. *Jayakumar*, also Thomas and Geraldine (2009) also studied the antioxidant properties of oyster mushrooms (*P. ostreatus*). At a maximum concentration of 10 mg / mL, an ethanol extract from oyster mushrooms showed significant reducing power compared to the commercial antioxidant butyl-toluene-butyl hydroxylated (BHT). This indicates that the fungus may become a food supplement or even a drug.

2.1.2 The nutritional importance of mushroom:

Doyon and Labrecque (2008) started from groups of experts in North America and Europe have used the Delphi technique to redefine functional foods. Nowadays, functional foods are defined as foods that are similar to or similar to conventional foods. It should be part of a standard diet and should be taken regularly and in normal amounts. Additionally, it must be demonstrated that it can reduce the risk of developing certain chronic diseases or beneficially affect for target function, beyonds its primary nutritional function.

The vital oligosaccharides containing prebiotics have sparked interest in food research in recent decades, and recently have attracted more and more attention. Prebiotics such as oligosaccharides and insulin have become very important as functional food ingredients because they can manipulate coliform flora formation in the human gut by inhibiting external pathogens (Recroft *et al*., 2001), thus improving host health (Roberfroid , 2002), Cummings *et al*.,(2001), are classified prebiotics as relatively short chain carbohydrates.

Gibson,(2004) was considered dietary carbohydrates such as fiber as a candidate beverage, and found that oligosaccharides are more promising. Currently available prebiotics, such as inulin and its derivatives, and the polysaccharides galactoligo-oligosaccarites (GOS), are relatively inexpensive to manufacture and have been widely used as functional ingredients in food (Macfarlane *et al*., 2006).

It is believed that taking prebiotics can improve immune function, improve colon integrity, reduce the incidence and duration of intestinal infections, negatively regulate allergic reactions, and improve digestion and elimination of stool (Douglas and Sanders, 2008).

Bifidobacterium and Lactobacillus are beneficial bacteria that can be used as biological targets (Macfarlane *et al*., 2008). The positive effects of prebiotics in vitro reflect a significant increase in the number of bifidobacteria and lactobacilli, while the development of this subgroup delayed tissue lysis (Palframan *et al*., 2003). Macfarlane *et al*. (2006), were reported a Bifidobacteria can stimulate the immune system, inhibit the growth of pathogens, produce vitamin B, reduce the level of ammonia and cholesterol in the blood, and help restore normal flora after antibiotic treatment, while lactobacilli help digest and convert lactose. Became lactose. Individual intolerance, diarrhea and constipation in children help fight salmonella and other infections and relieve irritable bowel syndrome (Manning and Gibson, 2004)**.**

2.1.3 Composition of Mushrooms.

Mushrooms appear to be a potential candidate for prebiotics because they contain carbohydrates such as alpha-glucan chitin, mannane hemicellulose, galactan and xylan sticks.

Chitin is a water insoluble polysaccharide, which accounts for 80% to 90% of the dry matter of fungal cell walls. Chitin contains N as one of the structural fungal polysaccharides responsible for the stiffness and shape of the wall. Chitin occurs only in certain taxonomic groups of Basidio, Asco, Zygo, and Deuteromycetes, but not in other groups such as Oomycetes (Vetter, 2007).

Most of the polysaccharides exist in the form of linear and branched glucans with different types of glycosidic bonds. However, some of them are truly

heterogeneous capsules, which contain arabinose, mannose, fucose, galactose, xylose, glucose, and glucuronic acid as major components of the side chain or are present in various combinations. Although the polysaccharides in fungi have different chemical compositions, most of them belong to the b-glucan group (Wasser, 2002). The digestive enzymes secreted by the pancreas or the brush borders of vertebrates, especially mammals, are unable to break down the *β*-glycosidic bonds, this makes it resistant to acid hydrolysis in the stomach and cannot be digested by human digestive enzymes (Van Lo, 2006).

The indigestible nature of the carbohydrates in mushrooms makes it a potential source of prebiotics because it meets part of the definition of prebiotics. However, a lot of research is needed, which said not all carbohydrates in the diet are biological materials (Gibson *et al*., 2004). Prebiotics cannot be easily digested or resistant to the upper intestine. The first criterion in fact is to ensure that prebiotics can withstand the digestive process before reaching the colon, thus stimulating beneficial bacteria; Lactobacillus and bifidobacteria are effective (Macfarlane *et al*., 2008).

Criteria that allow food ingredients to be classified as prebiotics also include selective fermentation of potentially beneficial bacteria in the colon (Wang, 2009). The effects of this fermentation can lead to a decrease in end products containing nitrogen and enzymes, an increase in fecal weight, an increase in the expression of short-chain fatty acids or a change in composition, and a slight decrease in the pH of the luminal colon,as well as Increased expression of binding proteins or active transporters related to mineral intake and changes in the immune system (Douglas and Sanders, 2008).

2.1.4 Effect of mushroom as antioxidant

For a long time, the mushroom has been an important source of biologically active compounds of medicinal value (Breene, 1990). Certain types of mushrooms have been used in many parts of the world to combat disease outbreaks, and are still used in veterinary ethno medicine in countries in Asia and the Mediterranean (Chang and Buswell, 1996).

It has been reported that the combined use of probiotics and mushroom extracts can be used as an alternative to chicken-derived antibiotics (Guo *et al*., 2004a, b). Grant Health - Many compounds in farm animals have immunostimulating, anti-bacterial, anti-oxidant and stress-reducing properties, so their benefits are enhanced (Dalloul and Lillehoj, 2006; Dalloul *et al*., 2006). Mushrooms possess a wide range of activities (Guo *et al*., 2003).

Oxidative stress is related to the formation of free radicals, and leads to poor health. Hydroxyl radicals, hydrogen peroxide and superoxide, which are modulated by radiation, are also by-products of normal metabolism (Wagner *et al*., 1992). The poultry industry will highly appreciate the natural antioxidants, which can replace synthetic antioxidants and fulfill consumer requirements for food without leaving substances that can harm human health. In addition to deteriorating health, lipid peroxidation is also the main cause of reduced quality, nutritional value and texture of meat, affecting color and flavor (Halliwell and Gutteridge, 1999).

Synthetic antioxidants were used to stabilize the meat. The most commonly used synthetic antioxidants are TBH, TCA, and HBD, which are added to fatty and oily foods to prevent oxidative degradation (Lolliger, 1991). Synthetic antioxidants are currently approved to control lipid oxidation in foods, but consumers are concerned about their use (Botterweck *et al*., 2000). Ergothionine has been recognized and quantified in various types of mushrooms as the main antioxidant compound (Dubost *et al*., 2007), Dioboquinone, a variable acid, and phenolic

antioxidants were also found in fungi (Kasuga *et al*.,1995). The antioxidant activity of fungi has been shown to be a scavenger of free radicals activity in vitro (Hartmann, 1998), and it has been shown to be a cell protective agent in vivo against oxidative damage to rat liver microsomes (Chaudiere and Ferrari-Iliou, 1999).

However, there is no evidence that supplementation of dried mushrooms can improve the oxidative stability of chicken tissues. *Agaricus bisporus* is also a good source of selenium (Vetter and Lelley, 2004). Studies have shown that eating *bisporus* mushrooms is the most widely studied edible mushroom, which delays the production of free radicals (Falandysz, 2008). On this basis, some of studies using *Aspergillus bisporus* to chickens has antioxidant and growth-promoting effects on chicken tissue stability (Giannenas *et al*., 2010 and Rana *et al*., 2019).

2.1.5 Effect of mushroom as antimicrobial

The development of antibiotics has been one of the most important scientific achievements of the past 70 species of fungi. These compounds act in different ways, and interfere with the metabolic processes or structure of the human body (Fuchs FD *et al*., 2004). The mechanism of action is mainly related to cell wall synthesis, changes in plasma membrane permeability, and interference with chromosome replication or protein synthesis (Tenover FC *et al*., 2003).

The cell wall is responsible for the shape and stiffness of bacterial cells, and acts as a barrier to permeation (Koch AL. Bacterial *et al*., 2003). For both Gram-positive and Gram-negative bacteria, the content of peptidoglycan in the cell wall is between 10% and 60%, respectively (Ginsburg I, 2002). Beta lactam antibiotics act in the stage of peptidoglycan synthesis and are classified according to this stage. Some examples of this group are *d-cyclocerin, fosfomycin, glycopeptides* (bacitracin, vancomycin, ticoplanin) and beta-lactam (penicillin, cephalosporin, carbapenem, monobactam) (Silvera G, 2006). Several fungal extracts have been reported to have antibacterial activity against Gram-positive bacteria.

Most studies of mushrooms with antibacterial activity described the effects of their extracts, but the compound that caused this effect was not identified. However, some compounds have been described as effective agents against Gram-positive bacteria. Five of these compounds are terpenes. Grifola frondosa, Confluentin, and neoformin in reeds are active against Bacillus cereus and Enterococcus faecalis. The results for *Agaricus bisporus* are contradictory (Öztürk *et al*., 2011).

At the same time, it has no activity against Gram-negative bacteria (Ozen *et al*., 2011). It is reported to primarily target Escherichia coli, but also has active activity against *Pseudomonas aeruginosa*, *Proteus*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Salmonella typhimurium, and Salmonella typhimurium*. However, these differences may be due to the different methods and concentrations used. The methanol extract of *Agaricus blazei* Murrill has specific effects on three Gram-negative bacteria, namely Yersinia e*nterocolitica*, *Klebsiella pneumoniae*, and *Proteus vulgaris* (ÖztürkM *et al*., 2011). *Agaricus blazei*, *Agaricus blazei*, *Agaricus blazei* cf, *Agaricus blazei*. Blacks have no antibacterial activity against Gram-negative bacteria (ÖztürkM *et al*., 2011).

2.1.6 Effect of Mushroom on intestinal lumen :-

The role of fermented carbohydrates in the microbial environment of the poultry intestine remains unclear (Knarreborg *et al*., 2002). Although mannose oligosaccharide in the diet can reduce salmonella colonization in chicken plants (Fernandez *et al*., 2002). Additionally, in order to prevent Escherichia coli that causes intestinal diseases from attaching to the intestinal mucosa of mice with high levels of supplementation (Peuranen *et al*., 2004), at present, fungi have a significant effect on intestinal bacteria and intestinal chickens (Chuang et al., 2020).

2.2 Prebiotics:

2.2.1 Mechanisms of action of Prebiotic

Prebiotics can selectively stimulate beneficial microorganisms in the intestine, which is beneficial for the physiological reaction of animals. This may play an important role in reducing the occurrence of intestinal pathogens. The exact mechanism for minimizing prebiotic pathogen infection remains unclear. By competitive elimination of pathogens by more and more bacteria associated with healthy hosts, different types of bacteria can be produced. These bacteria can have adverse effects on pathogens by enhancing macrophages, stimulating production of antibodies, and anti-tumor effects (Vamano & Famano, 2010). It has been suggested that the main mechanism of prebiotics is immune regulation, including selective cultivation of lactic acid-producing bacteria, resulting in elevated concentrations of short chain fatty acids (SCFA) (such as acetate, propionate, and especially butyrate), the preferred energy source for cell colonization, and revitalizes the intestines. The honesty, high fermentation activity, and high concentration of SCFA are associated with lower pH, and it is related to inhibition of pathogens and increased solubility of certain nutrients. Józefiak *et al*., (2004), the increased SCFA and immune cells, direct contact in the gastrointestinal tract, and changes in mucin production help reduce the incidence of bacteria passing through the intestinal barrier (Lee and Salminen, 2009). This phenomenon can inhibit some pathogenic bacteria and reduce colonization of certain species, such as salmonella and campylobacter (Charalampopolus and Rastall, 2009). Other beneficial effects of adding prebiotics may be reflected in increased secretion of intestinal enzymes, decreased ammonia and phenol products, and increased resistance to the growth of pathogenic bacteria in the intestine (Yusrizal and Chen, 2003b). The advantage of prebiotics over probiotics is that they can promote the growth of beneficial bacteria, which are found everywhere in the host organism and can survive in all environmental conditions.

Fructooligosaccharides (FOS) selectively promote the growth of beneficial bacteria by acting as a source of nutrients. FOS can be found in many foods, such as onions, garlic, bananas, and asparagus (Charalampopolus and Rastall, 2009). FOS will not be degraded or absorbed in the upper gastrointestinal tract, and act as a food source for beneficial host bacteria, while competitively eliminating pathogenic bacteria. Mannitol polysaccharides provide alternative binding sites for disease-causing bacteria.

These usually come from components of the outer cell wall of yeast (Saccharomyces cerevisiae), according to Virkit, (2003), a diet supplemented with SOM can improve animals' resistance to intestinal diseases and promote their growth in six different ways: it improves the mucous barrier of brush margin; it reduces intestinal cell turnover, it limits colonization of intestinal pathogens by inhibiting the adhesion of bacteria to the intestinal wall; altering bacterial fermentation leads to host acquisition; improve immunity and enhance intestinal lining unit.

Supplementing the vital oligosaccharides containing dietary prebiotics, such as FOS, may improve the number of intestinal microbes, including reducing salmonella colonization,this indicates that the dietary FOS supplement in salmonella control and antibiotic-free programs may be a viable option. SOMs have been used to regulate the host's intestinal flora, thus reducing the incidence of pathogen colonization by binding and removing pathogens from the intestine and stimulating the immune system (Fernandez *et al*., 2002).

2.2.2 Types of prebiotics

Most of the prebiotics that have been identified are classified as low carbohydrate sugars with different molecular structures common to most animal diets. All dietary fibers are candidates for prebiotics, but the most effective are the indigestible low-chain sugars (Gaggia *et al*., 2010). Several compounds can be used as biological substances, and some have been extensively studied in vivo and in vitro (Zakeri and Kashefi, 2011).

Fructose may be the most studied prebiotic (Swanson *et al*., 2002), which includes all of the less digestible sugars that are not digestible.

For digestion, in addition to fructose and glucose monomers. Fructooligo is naturally found in onions and certain types of cereal crops, such as wheat, barley, and rye (Bailey *et al*., 1991). It has been suggested that feeding low in fructose increases the concentration of short-chain fatty acids, which in turn aid the growth of lactobacilli and bifidobacteria. These short-chain fatty acids lower the pH, leading to a decrease in anaerobic pathogens (such as bacteria, Clostridium, and clostridia) (Baurhoo *et al*., 2007b).

2.2.3 Effects of prebiotics on productivity

In some studies, the effects of prebiotics on poultry productivity at different production stages and changing environmental conditions have been studied (Bozkurt *et al*., 2012b). When broiler chickens were fed diets containing 0.1% yeast cell wall derivatives, their body weight will increased and their daily gains significantly (Reisinger *et al*., 2012). It was concluded that the weight gain was a result of higher feeding efficiency. In another study, another study in broiler chickens said fed a diet supplemented with mannose polysaccharide and then subjected to heat stress, which improved weight gain and reduced nutrient conversion (Sohail *et al*., 2012).

They are hypothesized that mannose-oligopolysaccharide improves intestinal nutrient absorption and counteracts the harmful effects of heat stress. When these prebiotics were added to the feed for more than 28 days, it was observed that a feed containing 0.05% oligosaccharide mannose or 0.25% low fructose could improve broiler performance (Kim *et al*., 2011). In other Japanese studies, when quail was fed diets containing organic acids and mannose-oligosaccharides, the nutrient conversion rate and yield index were significantly increased (Ghosh *et al*., 2007),while adding 2-4% lactose to broiler meals for 1 to 21 days can lead to weight gain ($P < 0.08$) (Douglas *et al*., 2003). In addition, the addition of hot caramelized oligosaccharide to sucrose to

feedlots significantly increased body weight gain (P <0.001), feed consumption and feed conversion rate (Orban *et a*l., 1997).

2.2.4 Effect prebiotic on the cholesterol levels

Dietary prebiotics have been reported to increase the concentration of short-chain fatty acids and enhance intestinal production that controls lipid metabolism (Wolver *et al*., 1995) (Samanta *et al*., 2003) has shown that the decrease in serum cholesterol is due to Propionic acid in the colon.

Additionally (Delzenne and Kok, 1999) was reported that insulin reduces the lipid content of the triglycerides in the blood and reduces its profile through short-chain fatty acids. The mechanism of action of MOS in reducing cholesterol has not been fully demonstrated, but MOS is a bacterial substrate of lactic acid bacteria (such as Bifidobacterium bifidum) (Van Loo, 2004). An increase in the SOM level also leads to an increase in CFU from lactic acid bacteria.

Xu *et al*., (2003) Gilleland *et al*., (1985) were speculated that certain types of lactobacilli could bind cholesterol. Enter the human cell membrane, so the absorption of cholesterol by lactobacilli will reduce the absorption of cholesterol into the system. Low blood triglyceride levels may be due to increased levels of lactic acid bacteria in the intestines of broiler chickens. Other studies from Santoso *et al*., (1995) was recorded by adding Bacillus subtilis to broiler feed can reduce blood triglycerides.

2.2.5 Effect prebiotics on immunity

Inflammation is the immune system's natural response to damage caused by physical, chemical, and pathogen factors. Acute inflammation is a short-lived process and usually resolves spontaneously. However, in some cases, the inflammation can turn into a chronic disease (Dennis and Norris, 2015). Edible fungi are a source of many compounds with biological activity and health-promoting effects, and this fact supports the nutritional and medicinal value of edible fungi. Secondary metabolites in

mushrooms contain a variety of beneficial properties, such as anticancer, antioxidant, antiviral, antibacterial, anti-inflammatory and ability to improve cardiovascular function (Kalač, 2010a).

Stimulation of thymus, follicle, and spleen cell proliferation increases the concentration of immune proteins in the blood, thus improving the overall immune system function (Park and Park, 2011), through increasing serum concentrations of IgG, IgA, and IgM were significantly increased in the prebiotic-fed group compared to the antibiotic treatment and control groups. Lactic acid and prebiotic bacteria have an indirect positive effect on the immune system. The bacteria act on different levels of the immune system by producing immune stimulants. They enhance certain immune system cells, cytokines, stimulate the synthesis of large amounts of immunoglobulins and enhance macrophages (Maefarlance and Cummings, 1999).

Other studies have found that prebiotics show higher levels of serum IgG in inulin-treated broiler chickens, so they can effectively boost humoral immunity (Park, 2008). It has been reported that prebiotics have an indirect positive effect on the immune system of a host organism. The presence of bacteria act on different levels of the immune system by producing immune stimulants and cytokines that can boost the immune system, stimulate the formation of a large number of immunoglobulins, and enhance macrophages (Maefarlance and Cummings, 1999). Nabizadeh, (2012a) was reported that insulin supplementation can significantly increase IgG.

Maintaining a healthy chicken flock is very important to produce profitable poultry and providing consumers with safe poultry products. There are several foodborne pathogens in poultry products, such as salmonella, that can cause serious diseases to humans (Scallan *et al*., 2011).

Intestinal flora may be a nutrient deficiency in fast-growing broiler chickens (Dibner and Richards, 2005), as it may lead to increased energy required for maintenance and reduced nutrient use efficiency (Yang *et al*., 2009), By adding

different types of food additives (such as growth-promoting antibiotics, probiotics, prebiotics and commensal foods) was add to the diet to regulate the growth of beneficial microorganisms and inhibit pathogenic bacteria (Jacobs, 2011; Kim *et al*., 2011). Symbiosis is a combination of prebiotics and probiotics, in which prebiotics and probiotics are used as fermentable substrates to extend their life, thus improving the health of the host (Yang *et al*., 2009).

Prebiotics have been shown to significantly improve the immune status of birds by regulating the intestinal flora and reducing the burden of pathogenic microorganisms in the intestine, thus improving bird health (Zakeri and Kashefi, 2011). Heat-producing low-density sucrose confections significantly increased the number of cleaved bacteria circulating in the cecum ($P < 0.03$), compared to birds fed on basic control systems (Fructooligo the one-day diet at percentage 0.25%, which increases the number of Clostridium perfringens (E. Kim *et al*., 2009).

Mano oligosaccharides can activate macrophages through specific mannose receptors, thus enhancing the immune status of birds, thus making macrophages more active in killing disease-causing bacteria (Zakeri and Kashefi, 2011). These authors found that when fed a diet containing 1000 mg / kg mannose oligosaccharides, the antibody titer in the Newcastle Disease vaccine serum increased for 25 day old broilers ($P < 0.05$).

2.3 Probiotic :

The unique definition of a probiotic is “a live microbial food supplement that can beneficially affect a host animal by improving the intestinal microbial balance” (Fuller, 1989).

Probiotics is defined as “the ingestion or topical application of a sufficient number of live microorganisms (bacteria or yeast) to confer one or more proven health

benefits to the host" (Anil and Harjinder, 2007). It is a natural component of food (Al-Barwary *et al*., 2012).

The history of feed supplements can only be traced back to 1974, but the history of microbial feed supplements can be traced back thousands of years (Hawrelak and Nat, 2013). It was believed that Lactobacillus in milk may have a positive effect on the intestinal flora, which is of great importance for human health and longevity (Li *et al*., 2014).

Moreover (Hamasalim, 2015). The reported beneficial effects of probiotics which include treating and preventing diseases, as well as improving digestion and absorption of nutrients, through the Nutrition with probiotics combination helps prevent pathogens from colonizing the intestine and producing specific enzyme-like substances (Lee *et al*., 2007).

Probiotic have been shown to inhibit pathogenic bacteria in vivo and vitro in through several different mechanisms (Cabir *et al*., 2005). Competitive rejection and hostility through binding side consider the main important roles of probiotics as feed additives in poultry rations, and the maintenance of normal intestinal flora (Kizerwetter and Binek, 2009). Probiotics and competitive rejection methods have been used as one of the methods for controlling epidemics and zoonotic diseases in poultry (Edens, 2003).

Fritz *et al*. (2000) was studied classic probiotics by inoculating (one-day-old) chicks and estimate as a good example of competitive rejection, because the one-day sensitivity of these chicks can determine the modus operandi of these beneficial microorganisms, competitive exclusion is a highly effective measure that can protect newly hatched chicks, turkeys, quail, pheasants and other wild birds from salmonella and other intestinal pathogens (Schneitz, 2005).

2.3.1 Importance of probiotic :

Animal nutrition researchers are paying special attention to probiotics through the use of traditional growth promoters (antibiotics) (Ignatova *et al*., 2009). Therefore, the use of probiotics in animal production is considered an alternative to antibiotics, and several scientific studies have shown the positive effect of adding strains of probiotic organisms in the diets of chickens, turkeys, fish and other animals (Veizaj-Delia *et al*. 2010; Sulaimani *et al*. 2010).

The beneficial effects of probiotics depend on several factors, including the selected strain, level of consumption, time and frequency of exposure, and the individual's physiological status (Koop-Hoolihan, 2001).

Santos (2010) stated that the efficacy or inefficiency of probiotic products may be related to the types of microorganisms, their viability, methods and frequency of use, age of poultry, health status of the facility, feed composition and environmental stress factors. .

Qi *et al*. (2009) was noticed that photosynthetic bacteria can improve water quality, promote growth, and prevent any host infection. On the one hand, probiotics have been used as feed to increase feed conversion efficiency, improve growth performance and enhance the immune response of poultry and livestock (Brashears *et al*., 2003). It can stimulate the immune system of animals and birds to resist infectious agents and other stressors (Li *et al*., 2007).

Much evidence shows that probiotics are often used as feed additives in poultry production systems to improve production performance, immune response and improve health. (Bansal *et al*., 2011). Probiotic bacteria promote the digestion and absorption of carbohydrates, proteins and proteins by providing digestible proteins, enzymes, vitamins and other important cofactors, such as factories of active enzymes (lipase, amylase, and protease), which help improve metabolism, digestion and utilization of

nutrients. Fats can also improve the feed conversion efficiency. On the other hand, probiotics contribute to mineral metabolism and synthesis of vitamins (biotin, B1, B2, B12 and K), which are responsible for normal growth and metabolism (Dhama and Singh, 2010).

Quinin *et al*. (2004) reported the benefits of probiotics, namely through enhanced digestibility and utilization of nutrients, inhibition of organisms, administration of antibiotics and vaccines, reduced post-transfer stress and stimulation of immune responses to increase productivity, and increase growth rate. .

Tahri *et al*., (1995) suggested that as a beneficial bacterium from the genus Lactobacillus, it contains anti-cholesterol and lipid-lowering agents and helps lower cholesterol, some of studies revise it the People who consume the probiotics have lower cholesterol in their blood (Fuller, 1992).

2.3.2 Mode of action of probiotic:

Although probiotics have been shown to be beneficial in improving poultry production and growth, their mode of action is not always clear (Ajuwon, 2015).They are different probiotics work through different mechanisms, which are not yet fully understood and it is hypothesized to be due to their role in the gastrointestinal lumen or gastrointestinal wall. Although probiotics have been upgraded to replace AGP, the mechanism of action of these feed additives appears to be different (Fajardo *et al*., 2012).

Probiotics assist in preventing and controlling gastrointestinal pathogens and / or improving the performance and productivity of production animals through various mechanisms (Lodemann, 2010).

The classic mechanism of action of bacteria used in probiotics is described as follows: Competition for binding sites: also called "competitive rejection", where probiotics bind to intestinal mucosal binding sites to form a physical barrier and prevent

attachment to pathogenic bacteria. It will produce anti-bacterial material. The probiotic bacteria synthesize compounds such as bacteriocins and hydrogen peroxide, which have antibacterial effects against pathogenic bacteria (De Keersmaecker *et al*., 2006). Another probiotic mechanism for improving food conversion efficiency includes altering the intestinal flora and promoting the growth of non-pathogenic facultative anaerobes, the formation of lactic acid and hydrogen peroxide by Gram-positive bacteria will inhibit the growth of intestinal pathogens, it is also Promote digestion and utilization of nutrients (Calafathy *et al*., 2008).

Although some people have suggested some mechanisms for the action of probiotics, these have not been fully elucidated. However, it is known that inhibition of the growth of pathogenic microorganisms will be achieved through the production of antimicrobial compounds, which will compete with pathogens at sites. (Oelschlaeger, 2010). This probiotic effect will prevent the colonization of pathogenic bacteria along the intestinal wall, thus preventing disease progression (Fuller, 2001).

2.3.3 Type of probiotic :

There are many probiotics on the market, which have different commercial types, different modes of action and different metabolic activities (Tariq *et al*., 2005). In addition, they also show differences in the ability to colonize the intestine (Mountzouris *et al*., 2007). Probiotics can be divided into colonial species (*Streptococcus, Lactobacillus, and Enterococcus*) or free non-colonized species (Saccharomyces cerevisiae) (Patterson and Burkolder, 2003).

Gupta and Garg (2009) referred to the List of recognized public safety (GRAS) issued by the FDA and the US quality control Organization ,which lists permissible dietary effects and no Side effects in human diets. It should be resistant to bile, pancreatic juice and hydrochloric acid, have anti-cancer activity, stimulate the immune system, and be able to survive under acidic conditions in the stomach and and duodenum (Vimala and Dileep, 2006).

It works as an antibacterial agent by secreting products as organic acids (butyric acid, lactic acid, and acetic acid) and hydrogen peroxide (De Keersmaecker *et al*., 2006). Kabeer, (2009) reports that Lactobacillus acidophilus has been shown to produce two compounds, the bacteriocin β-lactosin and two acids. It has been demonstrated that Lactobacillus B can inhibit Lactobacillus in vitro, and that acid glycerides can inhibit intestinal disease-causing bacteria.

Probiotics can benefit the host directly or indirectly, including enhancing barrier function, regulating the immune system of the mucosa, producing antibacterial agents, promoting digestion and absorption of food and altering the intestinal flora (Fioramonti *et al*., 2003).

There are two types of microbial collections in the digestive system of poultry, one of which is the opportunistic bacteria that settle in the intestine, from the environment due to feeding behavior or other activities, and the second is the bacteria that arise inside and outside the intestine, or through dietary supplement, it is introduced into the digestive system through feed or drinking water as direct Feed Microorganisms (DFM) or probiotics. These types of bacteria are called hemophilic bacteria (Chichlowski *et al*., 2007).

3.3.4 Effect of probiotic in growth promotion :

Production attributes can be used not only to monitor economic performance, but also to assess animal health (Kritas and Morrison, 2005). The effect of substitute materials (such as probiotics) on promoting growth may depend on the feeding regime which results in differences in breeding conditions when added to poultry feed (Pirgozliev *et al*., 2014).

Willis and Reid (2008) reported that nutrient-added broilers had a significant increase in live weight and higher car body production, when adding probiotics to animal feed was feed conversion rate increase, gain weight and improve poultry growth

performance, improve the immune system by resisting intestinal pathogens, and thus affect intestinal morphology and functions (Vila *et al*., 2009).

Nike *et al* (2000) evaluated the effects of various probiotics Lactobacillus acidophilus and Saccharomyces cerevisiae and their combinations on broiler performance, and reported that broiler chickens added 0.05% of the base diet compared to incomplete controls could improve feed efficiency.

Endens, (2003) reported that probiotics improve digestion, absorption and availability of nutrients, while at the same time they have a positive effect on increasing intestinal activity and digestive enzymes. On the other hand, in different studies, it was found that measuring live weight gain in poultry treated with probiotics showed variable results, and also indicated a dose-dependent response after application of probiotics (Flint and Garner, 2009).

In the previous study by Burkholder *et al*. (2005), the increased weight of chicks fed probiotics, which may be due to the increased digestibility and utilization of several nutrients (such as proteins, fats and carbohydrates, as well as some minerals and vitamins). Nagy *et al* (2009) was indicated that several beneficial bacteria can enhance the action of naturally produced endogenous enzymes in the digestive system. Several studies have shown that the effect of probiotics enhances the digestion and absorption of most nutrients and is the main mechanism leading to improved performance of broilers (Li *et al*., 2008).

Probiotics can effectively stimulate the growth of beneficial bacteria in the small and large intestine, thus achieving a better balance of bacteria (Capcarová *et al*., 2011). The beneficial bacteria, such as Lactobacillus, have an The enzymes was produce digestive enzymes can help improve the digestibility of the host animal and increase the feed conversion rate (Jen *et al*., 2000.)

Additionally, Zhang *et al* (2001) reported that probiotics have several advantages, such as promoting animal growth. Probiotics break down hydrocarbons, which means separating the essential elements in the food, and this effect will allow for complete absorption through the digestive system. Probiotics can also greatly increase nutrition and promote rapid cell growth and development.

The probiotic potential of non-spore was evaluated in pre-spore and yeast, which may increase the rate of growth in commercial poultry production (Afsharmanesh and Sadaghi, 2014). In many cases, the increased growth rate of probiotic-treated poultry is associated with increased feed intake (Lei *et al*., 2015). And improving feed use efficiency (Zhang and Kim, 2014) compared to untreated birds. Therefore, improving feed digestibility and thus feed utilization efficiency may be a means of increasing the growth rate. Likewise, the difference in performance between treated and untreated birds may be due to increased Short-chain fatty acids production and immune regulation leading to changes in microbial assemblies in the gastrointestinal tract (Zhao *et al*., 2013).

An increase in growth rate is also associated with increased villi height, which increases the intestinal absorption of nutrients (Awad *et al*.,2009)

2.3.5 Effect of probiotic on immunity :

Probiotics are mainly used in bird food to prevent competitive rejection of binding site of potentially pathogenic bacteria (such as *Clostridium perfringens, Salmonella, and E. coli*), thus preventing gastrointestinal diseases. The secretion of antibiotics (bacteriosins) can stimulate the immune response, thus helping to maintain or reinstall intestinal health (Stern *et al*., 2006).

Probiotics can effectively promote broiler growth and increase feed conversion rate. These results may be due to the immunomodulatory activity of probiotics and the enhancement of the beneficial effects of the intestinal flora to improve the efficiency of

the host's digestion and nutrient absorption (Mountzouris *et al*., 2010; Alkhalf *et al*., 2010b). In addition, stimulation of the immune system by probiotics has been shown to increase the production of immunoglobulins, stimulate the activity of macrophages and lymphocytes, and increase the production of gamma interferons (Yang & Choct, 2009). Adaptive immune response, such as epithelial cells, dendritic cells, monocytes / macrophages, B cells, T cells including T cells with regulatory characteristics and natural killer cells (Zhang *et al*., 2007.)

Several studies indicate that some types of probiotics can adequately stimulate protective immune responses, thus enhancing resistance to microbial pathogens (Noverr and Huffnagle, 2004). Probiotics have been shown to have the ability to boost the immune response by facilitating the elimination of many economically important pathogens, such *as Eimeria. , Salmonella, Escherichia coli, and Clostridium perfringens*, further claiming that they can be used as alternatives to antibiotics (Pender *et al*., 2017). The benefits of probiotics have two main functions, which are to stimulate the growth of beneficial bacteria and inhibit the growth of pathogenic bacteria. The potential health benefits associated with using probiotics include improved digestion, stimulation of gastrointestinal immunity, and enhanced natural resistance to bowel disease (Tellez *et al*., 20010).

Cross (2002), was indicated that certain types of probiotics can induce a protective immune response, thus enhancing resistance to microbial pathogens. Haji *et al* (2005) reported that birds treated with probiotics had higher antibodies in the blood than birds not treated with probiotics. Intestinal bacteria have a profound effect on the immune development of the digestive system. They are the main source of stimulation of lymphoid tissue associated with the intestine, and produce antimicrobial peptides and immunoglobulin A antigens (Flint & Garner, 2009). It has been shown that lactic acid-producing bacteria can activate nonspecific immune responses by enhancing macrophage activity (Perdigon *et al*., 2001).

Probiotics may stimulate the immune system due to increased levels of T lymphocytes, phagocytes and serum proteins (Hedayati *et al*., 2015). Christensen *et al* (2002) suggested that probiotics could stimulate immune cells to secrete cytokines. In addition, the indirect effects of probiotics may occur by changing the number of microorganisms in the lumen of the digestive tract. Gram-positive bacteria (Lactobacillus and bifidobacteria) such as probiotics will improve the immune response (Hedayati *et al*., 2015).

The immune function of probiotics is related to an important part of their beneficial effects, because the probiotics taken initially will interact with the intestinal epithelial cells and then stimulate the production of anti-inflammatory cytokines. Probiotics (such as lactic acid bacteria) may stimulate epithelial cells to secrete some cytokines, and thus regulate the intestinal immune response (Delcenserie *et al*., 2008). Probiotics stimulate innate immune activity (eg. phagocytosis), increase natural killer cell activity and immune globulin A secretion to stimulate anti-inflammatory effects and strengthen the mucosal barrier (Delcenserie *et al*., 2008).

Quinin *et al*. (2004) was Compared with other uninfected chicks during his work he found, strains of oral lactic acid bacteria in the first week of chicks showed a lower mortality rate.In addition to boosting immune stimulation by cellular immunity and increasing immunoglobulin production, probiotics can also increase production of interferons, increase macrophages, lymphocytes, and a natural killer. The modification and heterogeneity of the oxidative burst have been studied, It is suggested that Lactobacillus bacteria increase intraepithelial lymphocytes in intestinal lymphoid tissue, and these bacteria respond to microorganisms by secreting immunoglobulin A (IgA) and providing local immunity. IgA secreted immunoglobulin A plays an important role in mucosal immunity and helps fight pathogens.

Stimulation of the immune system by probiotics may be due to increased levels of T-lymphocytes, phagocytes, and serum proteins (Hedayati *et al*., 2015). Christensen *et al* (2002) suggested that probiotics could stimulate immune cells to secrete cytokines. In addition, the indirect effects of probiotics may occur by changing the number of microorganisms in the lumen of the digestive tract. Gram-positive bacteria (lactobacilli and bifidobacteria) such as probiotics will improve the immune response (Hedayati *et al*., 2015).

The immune effect of probiotics is associated with an important part of their beneficial effects, as the probiotics ingested initially interact with the intestinal epithelial cells and then stimulate the production of anti-inflammatory cytokines. Probiotics (such as Lactobacillus) may stimulate epithelial cells to secrete some cytokines and then regulate the intestinal immune response (Delcenserie *et al*., 2008). Probiotics stimulate innate immune activity (eg phagocytosis), increase natural killer cell activity and immune globulin A secretion to stimulate anti-inflammatory effects and strengthen the mucosal barrier (Delcenserie *et al*., 2008.)

Koenen *et al*. (2004) that the strains of oral lactobacilli in the first week of chicks showed a lower mortality rate compared to other chicks not infected with this bacterium. It is reported that probiotics enhance immune stimulation by stimulating cellular immunity and increasing the production of immunoglobulins in addition to that it also enhances the production of interferon and increases macrophages, lymphocytes and natural killers. Cellular activity. It is suggested that Lactobacillus bacteria increase intraepithelial lymphocytes in intestinal lymphoid tissue, and these bacteria respond to microorganisms by secreting immunoglobulin A (IgA) and providing local immunity. Secreted immunoglobulin A plays an important role in mucosal immunity and helps fight disease-causing bacteria and viruses (Waneck, 2011).

Probiotics (Lactobacillus) have a positive effect on the cellular immune response to Aimeria. As assessed by cytokine and egg production, in infected broiler chickens (Dalloul *et al*., 2005). Ramarao *et al*. (2004) found that antibody titres against Newcastle disease and infectious bursa disease were higher in broilers consuming probiotics between three to thirty-five days. Feeding probiotics can improve the antibody titer against Newcastle disease and infectious bursa disease. Likewise, probiotics can also help reduce the immune response to secondary infections common in birds with viral diseases or immunosuppressive conditions (Talebi *et al*. 2008).

Dama and Singh (2010) & Hajati and Rezaei (2010) indicated that probiotics can enhance bird resistance and partially protect against negative growth of pathogenic microorganisms, and it can also significantly reduce the load of pathogenic microorganisms in the intestine. Therefore, it helps prevent various infectious agents, including bacteria, fungi, protozoa, and viral agents (Singh and Chauhan, 2002). Lactobacillus strains evaluated have a regulatory effect on the immune systems of laying hens and broilers, while Lactobacillus has a positive effect on humoral and cellular immune responses (Koenen *et al*., 2004). The epithelial cells of the gastrointestinal mucosa form a selectively permeable barrier between the intestinal lumen (which contains harmful substances, such as microorganisms, foreign antigens, toxins, and beneficial nutrients) and the body's internal environment (Groschwitz and Hogan, 2009). This barrier is the first line of defense against microorganisms in the digestive system (Peterson and Artis, 2014). Has an all-around defensive function; Including anatomical structure, immune secretions composed of mucus, immunoglobulin (as IgA), antimicrobial peptides and the epithelial junction adhesion complex (Ohland and MacNaughton, 2010). Pathological conditions that lead to impaired immunity destroy this barrier (Turner, 2009). It causes inflammation of the intestinal wall (Sartor, 2006). Probiotics can prevent chronic inflammation in the gastrointestinal tract by stimulating the innate immunity to the gastrointestinal epithelium (Pagnini *et al*., 2010).

2.3.6 Effect of probiotic on anti-microbial -:

Some probiotics produce antibacterial substances, which may inhibit the growth of pathogenic microorganisms in the intestine. Several bacterial species including Lactic Acid (LAB) bacteria (Flynn *et al*., 2002), Bifidobacterium (Cheikhyoussef *et al*., 2008) and Bacillus (Le Marrec et al., 2000) can produce several types of stable bacteria Thermally (Cutter *et al*.,(2005), which has antibacterial activity against a variety of potential animal pathogens including Bacillus, Staphylococcus, Enterococcus, Listeria, and Salmonella (Rea *et al*., 2007; Corr *et al*., 2007).

The bacteria produced by LAB (such as Nisin) inhibit the growth of pathogenic microorganisms by inhibiting cell wall synthesis and the formation of pores on the surface of the bacteria (Hassan *et al*., 2012). For this reason, bacteriosins bind to the precursor fat cell wall to form a complex that can form pores in the bacterial cell membrane and cause bacterial death (Berbaum & Sahl, 2009). Several probiotics, especially SCFA produced by LAB-producer, especially lactic acid and acetic acid, can inhibit pathogenic bacteria (Commane *et al*., 2005). SCFA will reduce the pH value in the microenvironment of the intestinal lumen, which is then absorbed by the intestinal microorganisms in broilers, thus reducing the intracellular pH of some bacteria to lethal levels (Daskiran *et al*., 2012). Probiotics produce other anti-bacterial compounds, which may inhibit harmful microorganisms in the digestive tract (Brashears *et al*., 1998). Bacillus subtilis PB6 is a bacterial strain isolated from the digestive system of chickens, and can produce thermostable Clostridium antioxidants, which can inhibit Clostridium perfringens, the pathogen of necrotizing enteritis in poultry, Campylobacter, and Clostridium difficile. Bacteria, Campylobacter jejuni and Streptococcus pneumoniae (Tiwo & Tan, 2005).

2.3.7 Effect of probiotic on Intestinal Histomorphology

The structure of the intestinal mucosa is an important determinant of intestinal function (digestion and absorption) that affects the growth performance of poultry. In general, due to the larger surface area, villus height and villus height: an increased basement ratio will increase nutrient absorption (Afsharmanesh and Sadaghi, 2014).

The probiotics in poultry food can affect intestinal mucosal tissue,for exmple *Bacillus subtilis* increases villus and villus height: crypt ratio of intestinal mucosa (Afsharmanesh and Sadaghi, 2014). Bacillus coagulans (Hung *et al*., 2012), Lactic acid production Lactobacillus salivarius, Pichia parvum (Biloni *et al*., 2013) and Enterococcus faecalis (Cao *et al*., 2013, Abdel-Rahman *et al*.,2013)

The villi rise in poultry treated with probiotics (Bacillus coagulans ATCC 7050) at 6 weeks of age is greater than the villi rise in poultry treated with AGP (bacitracin zinc) (Hong *et al*., 2012). Likewise, the probiotic *Bacillus subtilis* PB6 rebuilds the normal structure of the intestinal villi of deformed and destroyed chickens due to necrotizing enteritis induced by Cl. Perfringens (Jayaraman *et al*., 2013).

Chapter Three

Materials and Methods

Chapter three

3.1 Materials and Methods

3.1.1 Animal and managment:

This study was carried out in the Collage of Agricuiture, Kerbala University, from 4/1/2020 to 7/2/2020. Chicks were taken from commercial hatchery of Karbala governerate (hatchery Al-Rahma). Feed and water was given *ad libitum*. All broiler chicks received starter diet in (1-10 days) and grower diet in (11-35 days). The starter and finisher diet of the experiment were prepared as powder from according to mash and were met the NRC requirements (NRC, 1994). Table 3-1

Table 3.1. Ingredients and nutrients composition of starter and grower diets.

Ingredients /gram	***Starter % (1-10 days)* Kg**	***Grower % (11-35 days)*Kg**
Corn oil Soybean **Corn**	1.5 33.5 57.5	2.5 33 60
Flour	5	2
Provimi Premix	2.5 (starter premix 3088)	2.5 (finisher premix 3110)
Calculation composition	***100%***	***100%***
Crude protein CP%	21	20.27
Crude fiber CF%	2.77	2.74
Calcium Ca%	0.961	0.919
AV-phosphorus	0.42	0.371
ME kcal/kg	2800	3100
AV-methionine	0.47	0.42
AV-TSAA	0.74	0.68
AV-threonine	0.63	0.61
AV-Lysine	1.18	0.98
Electrolytes	263	241.12

3.1.2 The feed additives used in the experiment:

A. The Prebiotic in the present study is mushroom (*Agaricus bisporus*) . It was taken from the local markets in the markets of Karbala, It was classified in the laboratory of Food analysis in the College of Agriculture, Kerbala University.

B. Probiotics (*Bacillus subtilis*) from (Biochem, Germany), 1/2 gram per kilogram of body weight

3.1.3 Preparation of poultry farm:

The floor , ceiling and wall of farm were cleaning and disinfectant multi-purpose disinfectant virkon (Potassium_peroxymonosulfate, sodium dodecylbenzenesulfonate, sulfamic acid, and inorganic buffers) and left 2days later with closed condition. All windows and all ventilation were switched on to complete removal of toxic gases residues before chick's preparation. waterers and Feeders were disinfected and cleaned, then distributed to the all birds groups. All finite ground was provided with suitable litter (chist paper) and other factors like lighting and ventilation were controlled according to instrnctions.Of Aviagen.Co(Aviagen,2014).

3.1.4 Vaccination programs:

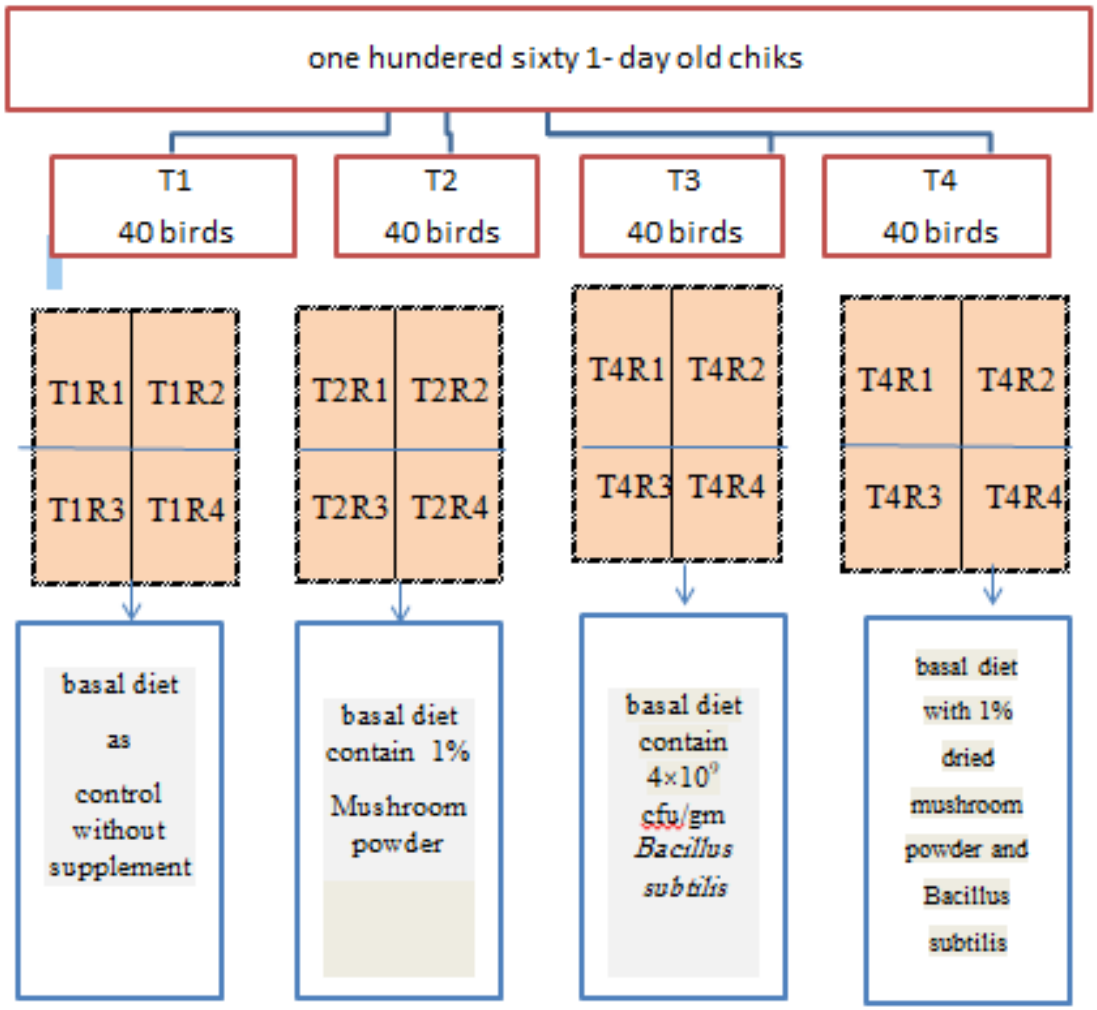
Because of drinking water vaccination is very common and useful procedure in commercial poultry,the vaccination program was accomplished via drinking water. All vaccines programs that using in vaccination program were manufactured by Volvac® (Boehringer Ingelheim- HQ Germany). All broilers chicks were vaccinated as shown in (table 3-4)

Table (3-4) show vaccinated program

Age of chicks	Disease	Type of vaccine	Administration rout
9	Newcastle and Infectious bronchitis	Volvac® ND-IB MLV (Boehringer Ingelheim- HQ Germany)	Via Drinking water
18	Newcastle and Infectious bronchitis	Volvac® ND-IB MLV (Boehringer Ingelheim- HQ Germany)	Via Drinking water
13	Infectious bursal disease	IBD Germany	Via Drinking water

3.1.5 Experimental design

A160 with 1-day-old broiler chicks (Ross 308) were randomly distributed to four groups (forty birds/per group) with 4 replicates and experimental period were extended to 5 weeks. Birds in the first group control (CON) was fed basal diets without supplements(Figure 5-3). The second group was fed the basal diets with 1% dried mushroom powder(MSH). The third group was fed the basal diets with 4×109 cfu/gm Bacillus subtilis(BS). The last was fed the basal diets with 4×109 cfu/gm Bacillus subtilis and 1% dried mushroom(MSH+BS)



(Figure (3-5) experimental design: (All birds in this study were divided randomly into four groups. Each group contain40 chicks with four replicate 10 chicks).

3.1.5.1 Parameters studied

1. Blood lipid profile: by estimating of cholesterol, Triglyceride, HDL-C, LDL-C, vLDL-C concentrations.

2. Broiler immune response: by estimating of antibody titer against ND and IB vaccines at the end of the experiment. Also, study of H/L ratio. bursa index, spleen index.

3. Liver enzymes activity (ALT, AST, and ALP).

4. Weekly broiler performance: (body weight, body weight gain, feed intake, and feed conversion ration) during experimental period 35 days old.

5. Histological changes of intestine: by studying the villi height, and crypt width.

3.1.6. Blood sampling:

All blood samples were collected at day 9, 18 and 35 of age from two birds from each replicate randomly were obtained from the wing vein in a test tube with anticoagulant to asstamet the H/L ratio and without anticoagulant. The tubes were allowed to clot at 18 ^{o}C temperature and centrifuged for 10 minute/ 3000 rpm. Serum was isolated, collected and stored in freeze (-18 ^{o}C) for further analysis.

3.1.7 equipment and Instruments:

Tools and apparatuses used in this study are listed below in (Table 3-4) with their company and origin.

Table (3-7) shows instruments and equipment with their sources.

Instruments and Equipment's	Company (Origin)
Disposable syringes (1, 3, 5) cc	China
Disposable gloves	Malaysia
Medical cotton	Turkey
Cooler box	China
Eppendrof tubes and tips	China
Test tube rack (stainless steel)	Germany
Centrifuge rotofix 32	Hettich® / Germany
Spectrophotometer	Bansh Lomb® /Germany.
Graduated glass pipettes size (2,5,10) cc	Silber® -Brand / Germany.
Multi channel pipettes type -12	Transferpette® -BRAND /Germany
Single channel pipette (micropipettes 1- 50 microliter)	Transferpette® -BRAND /Germany
Sterile glass test tubes with anti-coagulant EDTA (K_3)	Venoject®Terumo /Belgium.
Sterile glass test tubes without anti-coagulant	Venoject® Terumo /Belgium.
Sterile polysterene tube 10 ml gel and clotactivator	Afaco-Dispo /Jordan.
Graduated glass pipettes size (2,5,10) cc	Silber® -Brand / Germany.
Digital computing scale capacity (30) kg	Dragon Limited Co. /China.

Electrical incubator	**Incucell® / Germany.**
Autoclave (121°-131°) C	**SELECTA /Spain.**
Refrigerator	**Beko® /Turkey.**
Deep freeze refrigerator (-20°C)	**GFL Burgwedel / Germany.**
Cuvette tube	**China**
Hematology analyzer (Mythic 18 vet)	**Orphee SA company /Switzerland.**
Centrifuge	**Japan**

3.1.8. Laboratory chemicals and reagents:

All laboratory chemicals and reagents were used in this study are listed in table (3-8) with their supplier.

Table (3-8) Laboratory chemicals and reagents

Reagent	**Suppliers**
Serum cholesterol Kit	**biobase Kit**
Serum triglyceride Kit	**biobase Kit**
Serum HDL-Cholesterol Kit	**biobase Kit**
Serum LDL-Cholesterol Kit	**biobase Kit**
Infectious bursal disease virus antibody test kit	**USA Kit**
Newcastle disease virus antibody test kit	**USA Kit**
Serum aspartate aminotransferase (AST) Kit	**biobase Kit**
Serum alkaline phosphatase (ALP) Kit	**biobase Kit**

Serum alanine aminotransferase (ALT) Kit	**biobase Kit**

3.2.Methods

3.2. Hematological Parameters:

3.2.1 Biochemical parameters:

3.2.1.1 Cholesterol estimation (mg/dl):

The Cholesterol concentration was measured by using Biobase cholesterol kit produced by BIOBASE. company. after enzymatic hydrolysis and oxidation, the cholesterols are determined in the presence of phenol and peroxidase, the hydrogen peroxide and 4-aminoantipyrine forming quino. Appendices(1)

3.2.1.2 Triglyceride estimation (mg/dl):

The Triglyceride concentration was measured by triglyceride kit produced by BIOBASE. company. Its hydrolyzed to glycerol enzymatically according to the following procedure (Fossati and Prencipe, 1982). Appendices (2)

3.2.1.3 HDL-Cholesterol estimation (mg/dl).

The Plasma lipoproteins is spherical particles containing varying amounts of triglycerides, cholesterol, proteins ,phospholipids. The lipid determine and relative protein the density of these lipoproteins and provide the basis on which to begin their classification. The classes are: low-density-lipoprotein (LDL), very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). The principle role of HDL in lipid metabolism are the uptake and transport of cholesterol from peripheral tissues to the liver. Low HDL cholesterol (HDL-C) levels are strongly associated with an

increased risk of coronary artery disease. HDL-Cholesterol concentration measured by HDL kit produced by BIOBASE. the supernatant contains high density lipoprotein (HDL). The HDL-cholesterol are then spectrophotometrically measured by means of the coupled reaction described below (Grove, 1979). Appendices (3)

3.2.1.4 LDL-Cholesterol estimation (mg/dl).

The LDL Cholesterol is synthesized in the liver by the action of various lipolytic enzymes on triglyceride rich VLDL. LDL-cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis. Accurate measurement of LDL-cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture. Measured by LDL kit produced by BIOBASE. company (Alan, 2006). Appendices (4)

3.2.1.5: VLDL- Cholesterol estimation (mg/dl).

Serum VLDL concentration was measured according to the formula of **(Friedewald *et.al.*, 1972)**.

$$\text{VLDL concentration (mg/dl)} = \frac{\text{Triglyceride}}{5}$$

3.2.2 Liver enzymes concentration:

3.2.2.1 Alkaline phosphatase (ALP) activity determination:

Serum Alkaline phosphatase (ALP), measured by Cormay ALP Kit produced by PZ CORMAY S.A company (Soldin, 2003). Appendices (5):

3.2.2.2 serum glutamic-oxaloacetic transaminase SGOT (AST) activity determination:

Aspartate aminotransferase activity is (ASAT, AST, GOT) measured by

cormay GOT kit produced by PZ CORMAY S.A. company (Tietz, 1995). Appendices (6):

3.2.2.3 Serum Glutamic-Pyruvic Transaminase SGPT(ALT) activity determination:

The Serum Alanine aminotransferase activity ALT was determined by using Cormay ALT kit produced by BIOBASE company (Burtis, 1999). Appendices (7):

3.3 Immunological tests:

3.3.1 Serological test: Enzyme Linked Immunosorbent Assay (ELISA)

Antibody titers against Infectious brouchitis and Newcastle Disease Virus in broiler chicks the serum samples were measured at (9 and 18) days of age by using Enzyme Linked Immunosorbent Assay for each broiler strains and for different groups. Appendices (8):

All procedure in the NDV was used with the same infectious bronchitis and infectious bursal disease (IBD) Gumboro

3.4.1 Broiler performance:

3.4.1.1 Mean weekly body weight (BW) (gm/birds):

The weight of broiler body was calculated every week by weighing chicks individually at one day old and at end of each week by using sensitive balance. Mean body weight was measured from the total weight of all chicks divided on the number of chicks (Al-fayadh and Naji, 1989).

3.4.1.2 Weekly mean weight gain(WG) (gm/birds):

The mean body weight gain was measured weekly for each group by recording the weight gain at the beginning of the week and at the end depending on the following equation

Mean weekly weight gain=body weight at the end of the week-body weight at the beginning of the week (Al-fayadh and Naji, 1989).

3.4.1.3 Weekly feed intake (F.I.) (gm):

The feed intake has been measured each week depending on weighting the remaining feed at each end of the week and substrate from the feed that offered at the beginning of the same week, taking with concern the number of the dead chicks and number of feeding days. According to this equation which was mentioned by (AL-fayadh and Naji, 1989). For calculated the food intake of chicks.

Weekly feed intake (gm/chick) = $\frac{W}{L+D}$

W= Quality of feed intake through the week (gm).

L= number of live chicks fed through the week.

D= number of dead chicks × number of their feeding days.

3.4.1.4 Feed conversion Ratio (F.C.R):

Feed Conversion Ratio was calculated weekly for each group up to the end of experiment. (AL-fayadh and Naji, 1989) was reported the equation for measurement of FCR.

$$\textbf{FCR} = \frac{\textbf{mean weekly feed intake (gm)}}{\textbf{mean weekly body weight gain (gm)}}$$

3.5. Measuring the ratio of heterophils and lymphocytes (H/L)

Samples collected from wing veins into EDTA tubes, 1 to 3 mL, A minimum of 200 leukocytes per slide were sorted into categories: small or medium lymphocytes, heterophils. Morphological criteria for sorting were as described by(Eladl *et al*.,2019). Atypical lymphocytes were included in the differential counts, their locations noted, and later photographed.

The division of the sum of all heterophil types by the number of small "resting" lymphocytes gives H/L. Division of the same heterophil value by the sum of all lymphocyte types, (resting, reactive, and atypical) gives H/L. Representative cells used for each H/L calculation are in where cell size is estimated by the long axis of erythrocytes, averaging 9 μM.

3.6 Spleen and Bursa index

Before being sacrificed the body weight (g) were evaluated for each individual and 2 birds from each group were killed by cervical dislocation, following a thorough visual appraisal, the bursa and spleen were immediately removed, dry and individually weighed (g) for each individual and the ratio of bursa, spleen weight: body weight (%) was calculated. The results were expressed for each experimental group as the arithmetic mean and standard deviation (Mattsson *et al*., 20019).

$$\textbf{Spleen: body weight ratio} = \frac{\textbf{Spleen weight in grams}}{\textbf{Body weight in grams}} \textbf{ X 100}$$

$$\text{Bursa: body weight ratio} = \frac{\text{Bursa weight in grams}}{\text{Body weight in grams}} \times 100$$

3.7. Histological examination

Histological Sampling For birds killed at 35 days, sections from the middle of the duodenum, and ileum (about 0.5 cm in length) were excised and opened longitudinally at the antimesenteric attachment and gently flushed with NaCl (9 g·L−1). These samples were then fixed in a solution of formalin buffer (90 mL·L−1) for 12 to 24 h at 4°C, then rinsed and stored in 70% ethanol at 4°C until analysis, Villi and crypts were carefully individualized under a dissecting microscope. The preparations were then mounted between slides and coverslips, with the addition of an aqueous agent for microscopy (Aquamount improved gun, VWR, West Chester, PA). Ten villi and 10 crypts of Lieberkühn from each segment of each bird were measured using an optical microscope. The samples of duodenum, jejunum, and ileum of 2 birds from each line, representative of the population on the basis of BW, were rehydrated with PBS and stored at 4°C until analysis.

Each sample was then embedded in embedding medium in liquid nitrogen, cut at −20°C into 10-µm-thick cross-sections using a cryostat, and placed on gelatine-treated glass slides. Three cross-sections were obtained from each sample for further staining and observation. A routine staining procedure was carried out using Meyer hemalun and eosin (Sigma Chemical Company). The preparations were then mounted between slides and coverslips with the addition of an aqueous agent for microscopy

The slides were examined using an optical microscope . Fitted with a video camera and the images were analyzed using image analysis software (FiJi version 2.0,). Two images

of each section were captured for each sample with a final magnification of 10×. The thickness of the muscularis layer was measured on all sections(Boroojeni *et al*.,2019).

3.8. Intestinal pH measurement :

the pH of the digesta from the duodenum and ileum was used to measure acidity . Birds on samples from cecal digesta (2 g) were taken for pH measurement by using PH meter system (Ripon et al., 2019).

3.9. Statistical analysis:

Data was analyzed as one- Way analysis (ANOVA) using the general linear model (GLM) procedure to SPSS 22.0 software (Corp, 2011). Four treatment means were separated using a “protected” Duncan`s analysis in level (0.05).

Chapter Four

Results

Chapter four

Results

4.1 Biochemical tests:

4.1.1 Mean ± SE of mushroom and *Bacillus subtilis* and their combination on lipid profile concentration in the broilers:

Our results in the table (4-1) found the significant decrece ($p<0.05$) between mushroom and combination differences of control and compered with control *Bacillus subtilis* in the colostrol levels, the results were recorded as 139.4±4.88 , 148.20±4.46, 160±4.59 and 155±5.81 respectively.

On the other hand we noticed a significant decrease of TG levels in the group 4 (combination) and group 2 (mushroom) against control group, the result were recorded as 54±2.55, 52.8±2.28 and 70.2±5.11 respectively.

Whereas the result was different by in the HDL levels, we found significant increase of HDL levels (97.4±4.03) rather than control group (80.7±4.57). other lipid profile showed a non-significant decrease($P \leq 0.05$) in the LDL and vLDL levels in the last three groups rather than control group , the results were showed as 21.20±1.24, 19.60±.678 and 20.00±1.14 for T2 MUSH, T3 Pro and T4 Mush+pro, respectively for vLDL levels , and 17.40±1.50 , 20.40± .678 and 19.00±.894 T2 MUSH, T3 Pro and T4 Mush+pro, respectively for LDL levels in the same time the control group have 23.00 ±3.42 and 25.40±2.01for LDL and vLDL respectivelly (table 4-1).

Table (4-1) Effect of Mushroom and *Bacillus subtilis* and their combination on lipid profile concentration in the broilers at the age 35 days:

Treatment Parameters*	T1 CON	T2 MUSH	T3 BS	T4 Mush+BS
Chole mg/dl	160 ±4.59 A	139.4±4.88 B	155.00 ±5.81 AB	148.20 ± 4.46 B
TG mg/dl	70.20 ±5.11 A	52.80±2.28 B	65.40±4.11 AB	54.00±2.25 B
HDL-C mg/dl	80.60 ±4.57 B	97.00±4.03 A	85.80±2.15 AB	85.20 ± 2.57 AB
LDL-C mg/dl	23.00 ±3.42 A	17.40±1.50 A	20.40± .678 A	19.00± .894 A
vLDL-C mg/dl	25.40 ±2.01 A	21.20±1.24 B	19.60± .678 B	20.00±1.14 B

***Different letters in the same row showed a significant difference at ($p<0.05$)**

CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×10^9 cfu/kg *bacillus subtilis*. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×10^9 cfu/kg *Bacillus subtilis*.

4.1.2 Mean ± SE of mushroom and probiotic and their combination on liver enzymes concentrations in the broilers:

The concentrations of AST, ALT, and ALP in all groups were significantly different ($P\leq0.05$). Compared to the (CON), (MSH) and (BS) groups (Table 4-2), the (MSH-BS) group had significantly lower AST, ALT, and ALP concentrations ($P<0.05$).

The result was found decrease of liver enzymes in the combination group as 14.4 ± 1.24, 13.8 ± .735 and 219.6 ± 34.49, in the AST, AST and ALP, respectively.

Table (4-2) Effect of mushroom and *Bacillus subtilis* and their combination on liver enzymes concentrations in the broilers at the age 35 days.

treatment parameters*	T1 con	T2 MSH	T3 BS	T4 Msh+BS
AST (IU/L)	24.4 ±1.80 A	23.4± .748 AB	20.4 ±1.56 AB	14.4 ± 1.24 B
ALT (IU/L)	21 ±1.48 A	16.4±1.03 BC	17.8± .917 B	13.8± .735 C
ALP (IU/L)	227.2 ±19.02 A	228.8±37.2 8 A	214.2±16. 32 B	219.6 ± 34.49 B

***Different letters in the same row showed a significant difference at ($p<0.05$)**

CON = fed basal diet without supplement. MSH fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×10^9 cfu/kg *Bacillus subtilis*. Mush-BS fed basal diet with 1% dried mushroom powder and 4×10^9 cfu/kg *Bacillus subtilis*.

4.2 Broiler performance:

4.2.1 study of probiotic and prebiotic and their combination on weekly live body weight, feed intake, weight gain, and feed conversion ratio of broilers:

Tables (4-3,4,5,6) Live body weight, feed intake, weight gain and feed conversion rate. The results showed a significant increase ($P\leq0.05$) compared with (the control group) the live body weight, feed intake, the weight gain and the rate of food conversion for the (MSH) and (BS) groups and combination groups(Mush+Bs).

Table (4-3) Effect of mushroom and probiotic and their combination on weekly live body weight (gm/bird) of broilers at the age of 35 days.

Groups **Age**	CON	**Mush**	**BS**	**Mush-BS**
1st day (gm)	40.4 ± 0.244 A	40.6 ± 0.244 A	40.4 ± 0.244 A	40.6 ± 0.244 A
7th . (gm)	157.8 ± 2.59 B	156.6 ± 2.15 AB	161 ± 2.024 AB	164.2 ± 1.496 A
14th. (gm)	386 ± 1.04881 A	390.4 ± 3.155 A	391.2 ± 2.74 A	405.2 ± 2.74 B
21th (gm)	808.2 ± 3.1209 C	822.2 ± 7.05 BC	829.6 ± 6.48 AB	840.8 ± 1.39 A
28th. (gm)	1264.8 ± 8.85 C	1304.8 ± 4.53 B	1321.4 ± 7.2 B	1365.8 ± 4.8 A
35th. (gm)	1857.4±3.1241 D	1909 ± 14.6 C	1959.4 ± 10.77 B	1996.4 ± 11.7 A

Different letters in the same row showed a significant difference at (P≤0.05) CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg *Bacillus subtilis*.

Table (4-4) Effect of mushroom and *Bacillus subtilis* and their combination on weekly feed conversion ratio of broilers at the age of 35 days.

Group Week	CON	Mush	BS	mush-BS
1st wk. (gm)	1.33± 0.03 A	1.35± 0.03 A	1.29± 0.02 A	1.26± 0.02 A
2nd wk. (gm)	1.54± 0.02 B	1.53± 0.02 A	1.52± 0.03 C	1.41± 0.01 D
3rd wk. (gm)	1.63± 0.01 A	1.53± 0.02 C	1.45± 0.03 D	1.54± 0.01 B
4th wk. (gm)	1.76± 0.02 B	1.69± 0.04 A	1.63± 0.04 B	1.56± 0.02 A
5th wk. (gm)	1.91± 0.03 A	1.89± 0.04 B	1.77± 0.02 B	1.79± 0.03 B
Cumulative (gm)	1.72± 0.02 B	1.68± 0.01 A	1.6± 0.01 D	1.59± 0.01 C

***Different letters in the same row showed a significant difference at (P≤0.05) CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.**

Table (4-5):) Effect of mushroom and *Bacillus subtilis* and their combination on weekly weight gain (gm/birds) in broiler at the age of 35 days

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Group Age	CON	Mush	BS	Mush+BS
1st wk. (gm)	117.4 ±2.73 B	116 ±2.32 AB	120.6 ±2.11 AB	123.6 ±1.47 A
2nd wk. (gm)	228.2 ±3.32 B	233.8 ±2.31 B	230.2 ±4.04 AB	241 ±1.58 A
3rd wk. (gm)	422.2 ±3.44 A	431.8 ±4.89 A	438.4 ±8.73 A	435.6 ±3.4 A
4th wk. (gm)	456.6 ±6.03 C	482.6 ±8.98 B	491.8 ±10.65 B	525 ±5.45 A
5th wk. (gm)	592.6 ±8.33 C	604.2 ±12.23 BC	638 ±8.75 AB	630.6 ±10.83 A
Cumulative (gm)	1817 ±3.18 D	1868.4 ±14.42 C	1919 ±10.78 B	1955.8 ±11.83 A

***Different letters in the same row showed a significant difference at ($p<0.05$)**

CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

Table (4-6): Effect of mushroom and *Bacillus subtilis* and their combination on weekly feed intake of broilers at the age of 35 days.

Group Week	CON	Mush	BS	Mush-BS
1st wk. (gm)	155.6± 0.24 AB	156.6± 0.81 A	155.6± 0.81 AB	155.4± 0.51 B
2nd wk. (gm)	350.8± 0.37 A	357± 0.32 A	348.6± 0.51 A	341± 0.71 B
3rd wk. (gm)	687.4± 0.51 A	659.4± 0.51 B	633.6± 1.21 C	669.6± 1.4 B
4th wk. (gm)	803± 1.14 A	816.6± 5.76 AB	800.8± 1.62 BC	819.8± 0.86 C
5th wk. (gm)	1128± 1.7 A	1141.4± 1.29 A	1129.4± 0.81 B	1130.8± 0.86 B
Cumulative (gm)	3121.6± 1.21 A	3134± 1.14 B	3066.4± 1.66 C	3115.6± 1.21 C

***Different letters in the same row showed a significant difference at ($p<0.05$)**

CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

4.3 Effect of Mushroom and *Bacillus subtilis* and their combination on Crypt width and villus height of broilers at the age of 35 days:

The results showed a significant (P<0.05) increase in with crypt in duodenum and ileum in birds treated with Mush+BS as compare to other groups. While, non-significant (P>0.05) differences in the mean villus highly and crypt width between Mush and BS treated group when compared between each other's.

Table (4-7): showed of mushroom and probiotic and their combination on Crypt width and villus height of broilers at the age of 35 days.

Group Intestine (µm)	CON	Mush	B.S	Mush-BS
Crypt width (µm)	293.65± 12.5 C	325.63± 15.21 B	320.55± 26.82 B	351.52± 7.83 A
Villus height (µm)	859.73± 5.69 C	1168.04± 2.65 B	1209.21± 2.02 AB	1395± 5.59 A

***Different letters in the same row showed a significant difference at (p<0.05)**

CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×10^9 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

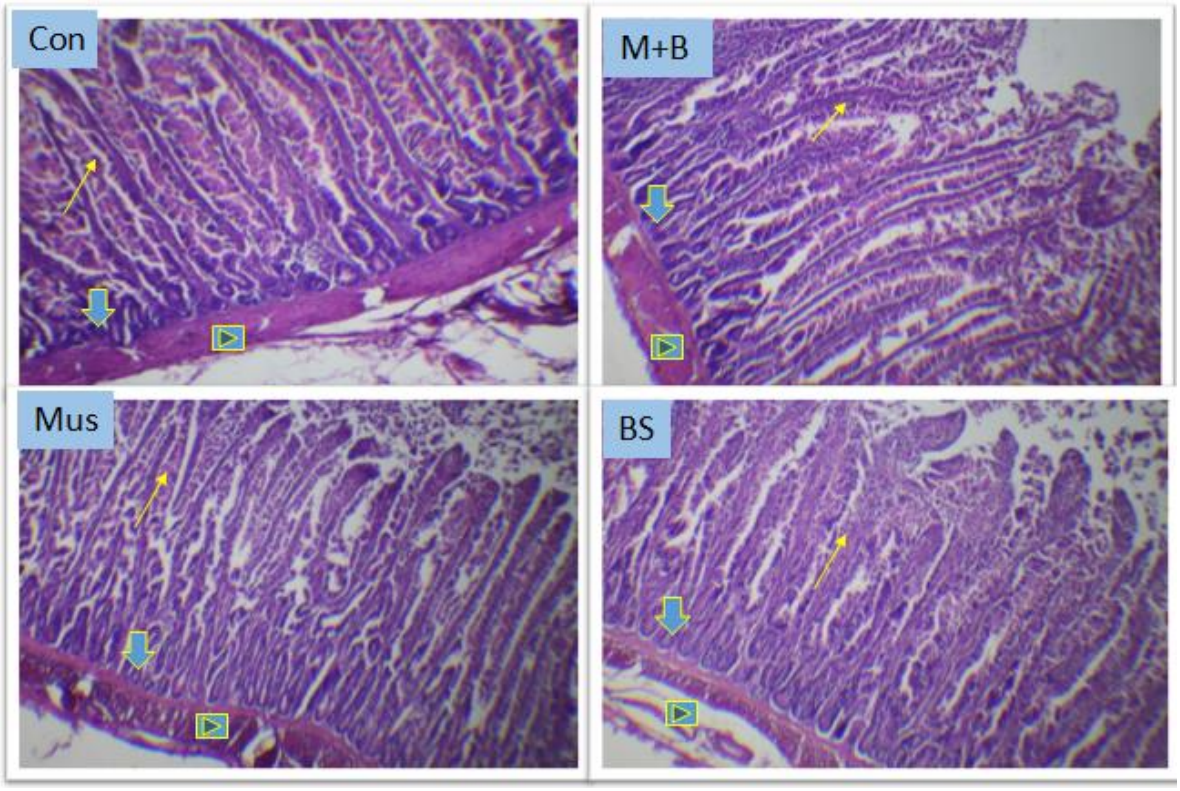

Figure 4-1: Photomicrograph of intestine chicken Con it look like normal slightly length of villi,. Mus and BS revealed moderate length of villi while high length which include in Mush+BS groups.

The arrow (→) showed villus hight , the arrow (➡) showed crypt width and (➡) showed intestinal wall with magnification power 4X (E & H staining).

4.4. Study of Mushroom and *Bacillus subtilis* and their combination on spleen and bursa index of broilers:

Tables (4-3) showed spleen and bursa index . The results showed non-significant differences ($P \geq 0.05$) in spleen and bursa index between four experimental group when competed between each other's.

Table (4-8): Effect of mushroom and *Bacillus subtilis* and their combination on spleen and bursa index of broilers at the age of 35 days.

Group Organs	con	mush	BS	mush-BS
Spleen	1.42± 0.22 A	1.54± 0.37 A	1.34± 0.25 A	1.26± 0.85 A
Bursa	3.6± 0.54 A	4.41± 0.36 A	3.9± 0.47 A	3.75± 0.31 A

***Different letters in the same row showed a significant difference at (p<0.05)**

CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

4.5. study of mushroom and *Bacillus subtilis* and their combination on pH measurement of broilers:

Tables (4-9) showed significant differences (P≤0.05) decrease in valve of pH in illum and doudenum in group Mush+BS as compared to other experimental groups. Besides, no significant (P>0.05) were observed between control, Mush and BS group when compared between each other.

Table (4-9): showed mushroom and *Bacillus subtilis* and their combination on pH measurement of broilers at the age of 35 days.

Group Organs	con	mush	BS	mush-BS
illum	6.1± 0.98 A	6.25± 0.73 A	5.91± 0.34 B	4.92± 0.62 C
doudenum	5.7± 0.83 AB	6.3± 0.42 A	6.5± 0.27 A	5.1± 0.735 C

*Different letters in the same row showed a significant difference at (p<0.05)

CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×10^9 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×10^9 cfu/kg Bacillus subtilis.

4-6. study of mushroom and *Bacillus subtilis* and their combination on H/L ratio of broilers:

Tables (4-10) showed H/L ratio separately. The results showed significant differences (P≤0.05) compared with (the control group) H/L ratio between (MSH) and (BS) groups and combination groups (Mush+BS).

Table (4-10): showed mushroom and *Bacillus subtilis* and their combination on H/L ratio of broilers at the age of 35 days.

Group Intestine	CON	Mush	B.S	Mush-BS
H/L ratio	0.982±0.23 C	0.68±0.09 B	0.72±0.12 B	0.41±0.06 A

*Different letters in the same row showed a significant difference at (p<0.05)
CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

4.7 Study of mushroom and *Bacillus subtilis* on humeral immunity in broiler chicken against Newcastle disease and Infectious bronchitis:

The figure results (figure 4-1) of the humeral immunity response were showed significantly differences (p≤0.05). The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly (P≤0.05) in the (Mush-BS), (Mushroom), and (BS) groups respectively compare with the (Control) in the ninth age days.

On the other hand, The figure results (figure 4-2) of the humeral immunity response were showed significantly differences ($p \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle Disease ND nd Infectious bronchitis IB were improved significantly ($P \leq 0.05$) in the (Mush-BS), (Mushroom), and (BS) groups respectively compare with the (Control) in the eighteenth age days.

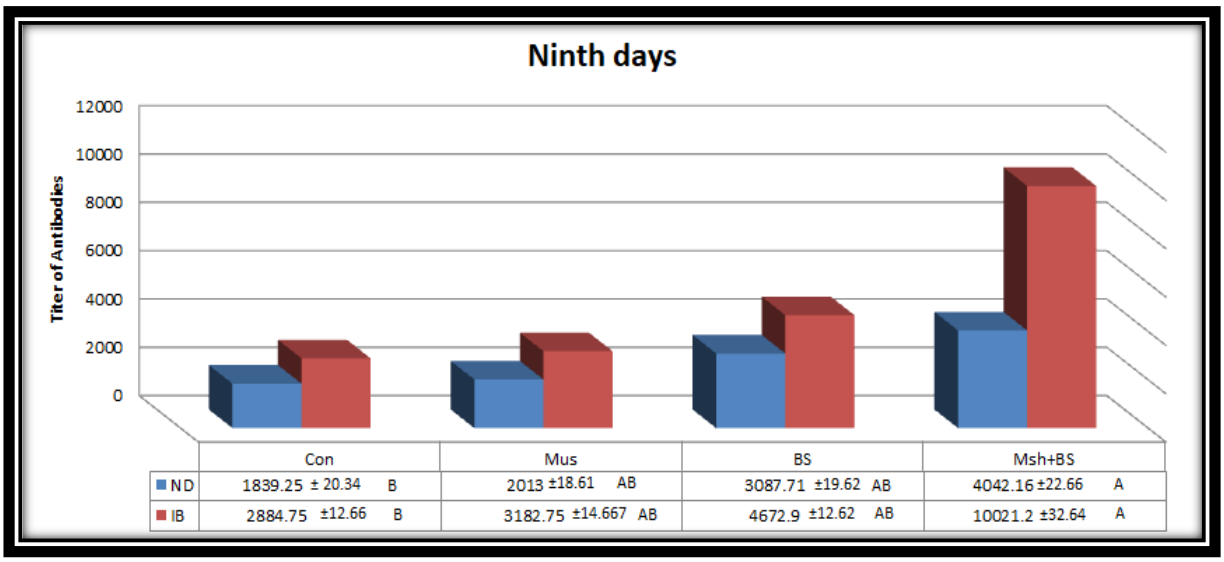

Figure (4-7.1):Effect of mushroom ,BS and their combination on antibody titer against Newcastle and Infectious bronchitis vaccine after nine days of age in broiler chickens the different letters in the same row showed a significant difference at ($p<0.05$) CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

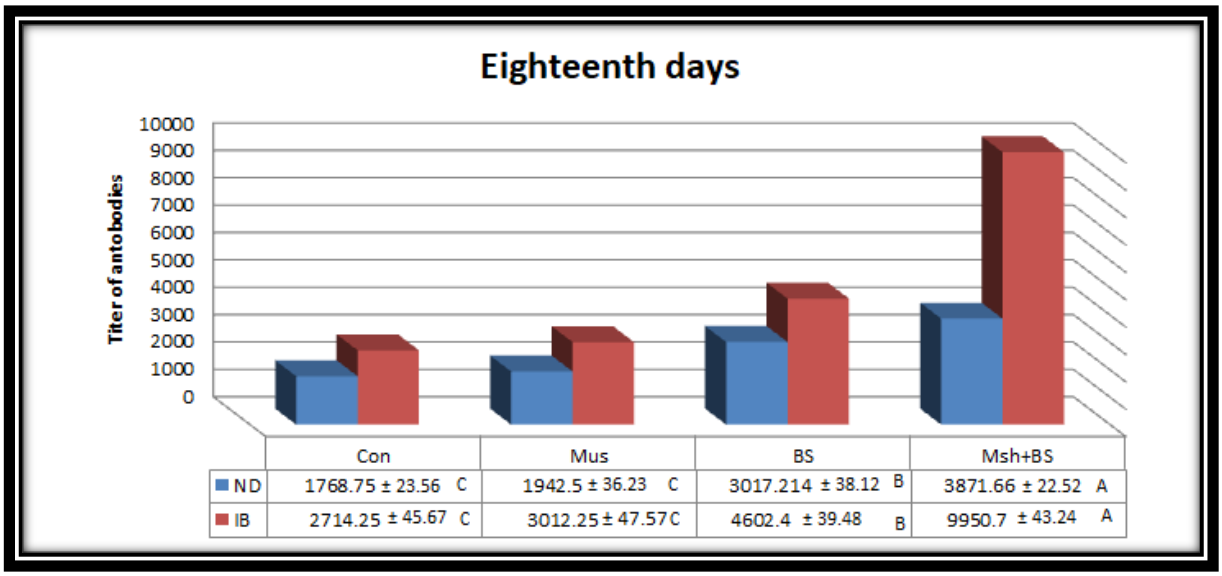

Figure (4-7.2):Effect of mushroom ,BS and their combination on antibody titer against Newcastle and Infectious bronchitis vaccine after eighteenth days of age in broiler chickens the different letters in the same row showed a significant difference at ($p<0.05$) CON = fed basal diet without supplement.MSH = fed basal diet with 1% dried mushroom powder. BS = fed basal diet with 4×109 cfu/kg bacillus subtilis. Mush-BS = fed basal diet with 1% dried mushroom powder and 4×109 cfu/kg Bacillus subtilis.

Chapter Five

Discussion

Chapter five

Discussion

5.1 Biochemical parameters:

5.1.1 Cholesterol, triglyceride, HDL-cholesterol , LDL-cholesterol and vLDL-cholesterol concentrations:

The results of our current research show that serum cholesterol, triglycerides, vLDL-C and LDL-C in the (MSH-BS), (MSH) and (BS) groups were significantly decreased ($P \leq 0.05$). However, in the same group, HDL increased significantly ($P \leq 0.05$) (Table 4-1). Blood triglycerides, low-density lipoproteins, and very low-density lipoproteins decrease with increased levels of high-density lipoprotein, and the reason may be that probiotics produce acetic acid and lactic acid to reduce the intracellular pH to enhance antibacterial substances. Produce. The low pH may be due to the conjugation of bile and lipids during emulsification of the small intestine. Reducing emulsification may decrease the absorption of dietary fats and reduce the reabsorption of bile salts. May lower cholesterol levels. These results are consistent with Begley et al., (2006) and Jeong *et al*.,(2010).

Some authors noted that, compared to controls for broiler chickens, the probiotic diet reduced cholesterol concentration (Mehr *et al*., 2007). Others have found a decrease in blood triglyceride levels (Kalavathy *et al*., 2003; and Mansoub, 2010). Santoso *et al*., (1995) reported that Bacillus subtilis supplementation reduces belly fat may be due to decreased acetyl-CoA carboxylase activity resulting in decreased blood triglycerides.

Pietras *et al*., (2006) reported that probiotic treatment reduced blood cholesterol, and found that probiotic chicken meat lowered blood cholesterol compared to the control group (Cenesz *et al*., 2008 and Yeh *et al* .,2014).

Additionally, the reduction in cholesterol can be attributed to the synthesis of bile acids in the liver of broiler with probiotic supplementation, and thus cholesterol excretion, and the gradual decreased in the environments (Wilson *et al*., 1998). Corvcoran *et al*., (2005) was found that the digestibility of fats is related to the ratio of cholecystic acid in latex digested and the subsequent lipid concentration compared with the control group, the probiotic supplement showed a significant reduction in triglyceride and cholesterol levels. They observed a marked increase in HDL levels (Panda *et al*., 2006), and found that when taking probiotic supplements containing Bacillus subtilis, the significant reduction in blood triglycerides and cholesterol may be attributed to cholesterol absorption in the gastrointestinal tract and / or the synthesis is reduced.

It has been found that, compared to the control group, mushroom supplementation can significantly reduce levels of cholesterol, triglycerides, and low-density lipoprotein and cholesterol, while the level of HDL cholesterol was increased. The decrease of cholesterol and triglycerides in (Mush) group prebiotics may be due to the increased secretion of inulin through the vent drops, which reduces cholesterol absorption. Short chain fatty acids (SCFA) are produced by selective fermentation of intestinal bacterial flora (e.g. propionate, butyrate and acetate). But butyrates are known to inhibit cholesterol synthesis in the liver. Propionate might block fatty acid synthesis in the liver. Therefore, the excretion rate of triglycerin is reduced. Inulin reduces blood cholesterol levels by inhibiting HMG-CoA reductase activity (hydroxymethylglutaryl A coenzyme). The mushroom may have altered the lipase gene expression. Our results are consistent with (Rossi *et al*., 2005 and Samanta, 2013). who reported prebiotics contains mushroom as a source of prebiotics and has a cholesterol-lowering effect in broiler chickens.

The reason for the low blood cholesterol observed in mushroom supplements may be that mushroom is rich in flavonoids , tannins ,glycosides, phenolic compounds and alkaloids which have a cholesterol-lowering effect (Pandimeena *et al*.,2015). Recent

studies have shown that prebiotic supplementation can reduce blood cholesterol levels caused by chicory in broiler chickens (Khoobani *et al*. 2020).

Other potential mechanisms include bacteria uptake of cholesterol and binding of cholesterol to the cell wall of Lactobacillus bacteria (Liong and Shah, 2005). Mannan-oligosaccharide (MOS) is also a substrate of lactic acid bacteria (such as Lactobacillus). and Bifidobacterium bifidum (Van Lo, 2004). The increase in MOS content also resulted in an increase in CFU for lactic acid-producing bacteria (Jazi *et al*., 2018).

5.1.2 Liver enzymes concentrations AST, ALT and ALP:

The present study shows that compared with the control group, the AST, ALT, and ALP of the (Mush-BS), (BS) and (Mush) groups were significantly reduced (Table 4-2).Which may be due to their anti-free radical and antioxidant properties; they have very good antioxidant effects and can inhibit cell membrane lipids. Peroxide. Our results are consistent with findings (Tan and Sun, 2017 and Balakrishnan *et al*.,2018), as the latter indicates that the decrease in ALT and AST activities in chickens fed probiotics may be due to their major role in reducing liver damage.

In this study, mushroom supplementation found that rats had less ALT and AST in plasma than the control group due to their protective effect on liver function (Nworu *et al*.,2014).

Hossain *et al* .,(2019) was reported that mushroom supplementation has hepatotoxic effects protection that may be due to the presence of vitamin thiamine ,A and C in addition to triterpene ,riboflavin, sesquiterpenes, Flavonoids (apigenin and luteolin), lactones, carotenoids (lutein) and fatty acids (myristic acid) as biologically active compounds. It has been reported that the bitter compounds present in mushroom can increase the production of bile in the gallbladder, thus improving liver function (Singhal *et al* .,2020).

5.2 Broiler performance:

5.2. Study of Mushroom and *Bacillus subtilis* and their combination on weekly live B.W., F.I., W.G., and F.C.R.of broilers:

The results of the present study were showed the live body weight, feed intake, weight gain, and feed conversion ratio in tables (3,4,5,6) have nhyju increased significantly ($P \leq 0.05$) in the (Mush-BS), (Mushroom), and (BS) groups, respectively, compared with the control. The results in (table 4-5) indicated that the (Mush-BS) group have the same mean body weight among treatments with no significant differences ($P \geq 0.05$) in the first day, while in the age 35^{th} days we found a significant increase of live body weight in the (Mush-BS) group rather than control group The results in (table 4-3) indicated that the (Mush-BS) group recorded a significant ($P \leq 0.05$) increased in feed intake and improved in the feed conversion ratio as compared with the control group the results were recorded as mean ± SE (1130.8± 0.86), greater than (1128± 1.7) in the control group.

Probiotic supplementation improves BW, WG, FI, and FCR. Probiotics may enhance the health of the body by enhancing mucin secretion, thus improving barrier function and competitively eliminating pathogenic bacteria. Probiotics can enhance the production of antibacterial substances such as lactic acid bacteria and acetic acid, thus reducing the pH of bacterial cells. These results are consistent with (Salmerón *et al*., 2015 and Vieco-Saiz *et al*., 2019), which reported that probiotic supplementation can improve the growth performance and health of broilers. These results are consistent with (Dawood *et al*.,2019 and Rahman *et al*., 2009), who stated that probiotic bacteria can enhance the catalytic activity of endogenous enzymes, thus enhancing hydroxyl and energy production, helping to increase FI FCR improved by proteases, lipases, and amylases. In addition, other people have reported (Gonzalez *et al*., 2001) that high levels (5-10 g / kg) of probiotics can significantly improve nutrition efficiency. , Which corresponds to maintaining the normal level. Relevant level. By altering metabolism,

increasing the activity of digestive enzymes, improving digestibility of nutrients, competing with competitive rejection and reducing intestinal flora of opportunistic pathogens and subclinical infections in bird meals. A probiotic diet replaces antibiotics as an accelerator to promote FCR growth (Dibener and Richard, 2005).

Compared with the control group, probiotic supplementation showed a significant increase in daily weight gain at 2, 3, 4, and 5 weeks (Yeo and Kim, 1997). Gunal *et al.* , (2006) was illustrated the study by showed that compared to the control group, the mean body weight and daily gain of chickens fed probiotics were higher. Other studies have found that adding probiotics (Lactobacillus and Bacillus subtilis) to broiler meals can stimulate the intestinal microbial balance and improve growth performance (Hossain *et al*. 2015). It has been reported that compared to broiler chickens without a supplement diet, a diet fortified with probiotics has higher weight gain and higher feeding efficiency (Chiang and Hsieh, 1995).

Due to the ability of Bacillus subtilis to form spores, Bacillus subtilis has been widely used as a probiotic product in a variety of commercial applications, as it contains both Gram-positive and non-pathogenic bacteria (Griggs and Jacob, 2015). Probiotic supplementation had a positive effect on broiler meal growth performance (Marshall & Levy, 2011). Likewise, probiotics can improve broiler performance by improving immune regulation (Yang *et al*., 2012). Addition of bacilli-based probiotics could improve broiler growth performance (Zhoa *et al*., 2012; Zhang *et al*., 2012, 2013).

Some previous studies contrasted with no effect of probiotics on broiler growth performance (Ergun *et al*., 2000). Other researchers found that increased elevation of micro-villi led to improved growth performance (Kabir and Ahmed, 2004; Panda *et al*., 2006).

Probiotics improve nutrient absorption by altering glycolysis and / or biosynthesis, which affects intestinal function (Feng *et al*., 2019). Probiotics can also alter the structure of the bacterial community in the digestive system of birds (Dawood *et al*.,2019 and Wang *et al*.,2020).

The results of prebiotic supplementation to improve growth performance may be due to the fermentation of insulin in the colon. Inulin fermentation can lead to the production of short-chain fatty acids such as, butyric acid, acetic acid and propionic acid, thereby lowering the pH of the intestine and promoting competitive rejection of pathogens. Therefore, it positively affects intestinal activity and increases digestive enzymes to enhance digestion and intestinal absorption. These results are in line with (Kabir *et al*.,2009 and Samanta *et al*., 2012). They found that the observed improvement in live weight in mushroom supplementation may be due to the presence of bioactive ingredients, such as those found in the mushroom plants. Alkaloids and flavonoids in broiler feeds (Çağlarirmak *et al*., 2011). Mushrim is rich in minerals (K, Mg, Fe and Ca), and vitamins (A and C, B1 thiamine, B2 riboflavin), which play a major role in the metabolism of birds (Chye *et al*.,2008). The antibacterial effect of the mushroom has been attributed to the presence of triterpene, saponins, phenylpropane and other compounds, which may improve the growth performance of broilers (Qureshi, 2015).

Other researchers in the previous study demonstrated that probiotics and prebiotics have significant weight gain and weight gain, thus improving growth performance (Millidi and Tuncer, 2001; and Piray., 2007). Probiotics and prebiotics have better nutrient digestibility, so the FCR is significantly improved ($P \leq 0.05$) (Dowarah *et al* ., 2018). It has been suggested that increasing the overall growth performance indicates that herbal additives can improve the flavor and palatability of the feed (Wenk, 2003; Wang et al., 2007). However, due to the choice of specific herbal medicines, the different herbal substances and methods of their administration, the use of herbal additives can lead to high levels of inconsistency (Windisch et al., 2008).

5.3 Study of Mushroom and *Bacillus subtilis* and their combination on Crypt width and villus height of broilers:

Our result in the table (4-7) found a significant differences compared with (the control group) the Crypt width and villis height between in the (Mush+BS) combination, compare with control group The improvement in growth performance attributed to herbal products had an effect on improving the digestibility of dietary protein in the small intestine (Abdel-Wareth *et al*., 2012). Supplementation of the herbal products reported that an increase in villus height helped increase the surface area of absorption, thus improving the intestinal nutrient utilization of broilers (Abdel-Rahman *et al*., 2014). Damaged villi or short hair may impair intestinal absorption, which may impair the performance of poultry (Xue *et al*., 2018).

Several researchers have found that probiotics can significantly increase villi height (Tsirtsikos *et al*., 2012). Therefore, the intestinal villi play an important role in enhancing the digestion and absorption of nutrients, and increasing the surface area of the small intestine, and the nutritional content in the first tissues is high (Gartner and Hiatt, 2001). Intestinal villi are long and thin and the shape of the finger and the intestinal mucosa are shown anatomically animal changes in all groups (CON, Mush, BS and Mush+BS) It was different from

Giannenas *et al*., (2010) who was confirmed that mushroom food supplement has no effect duodenal height, jejunum, ileum, or villi depth compared to the untreated control group this result was agreement with (Hashemipour *et al*., 2014) who was noticed that an effect of antibiotic alternatives on ileal microflora and intestinal histomorphology foe villus hight and villus width for broiler chickens fed wheat based diet

5.4 Study of Mushroom and *Bacillus subtilis* and their combination on spleen and bursa index of broilers:

The results showed non-significant differences ($P \geq 0.05$) compared with (the control group) for spleen and bursa index between experimental groups. An adding probiotics to the chicken diet will increase the relative weight of the spleen, but has no effect on the relative weight of the bursa, these results disagreement with (Sadeghi *et al*., 2015) who was found immune response of salmonella challenged broiler chickens fed diets containing bacillus subtilis Probiotic and he was said that no difference in the chicken antibody titers between the negative control group and the probiotic treatment group.

It is also agreed with (Wang *et al*., 2020) whom was found after 4 and 8 weeks, there was no significant difference between the chickens fed 10^7 CFU / kg *Lactobacillus salivarius* and the control group, while he found after 4 weeks at 10^8 and 10^9 CFU / kg of Lactobacillus salivarius, the immune system index of spleen and bursa was significantly increased.

5.5 Study of Mushroom and *Bacillus subtilis* and their combination on PH measurement of broilers:

pH measurement separately, Comparing to control the results showed significant differences for pH measurement between (MSH) and (BS) groups and combination groups (Mush+BS), our result were found Mush+BS have most tolerance for pH measurement reach to 4.92± 0.62 and 5.1± 0.735 for ileum and duodenum ,respectively. This result was agreement with (Reis *et al*., 2017) who was used addition of the probiotic decrease the pH of the intestinal content

Dec *et al*., (2018) was illustrated that *lactobacillus spp*. showed excellent survival rates at pH values as low as 1.5 and 2.0. At this pH, 44 strains of Lactobacillus strains can live for 1 hour at a pH of 1.5, and 39 strains can live for 3 hours at 3 pH. At pH 2.0, all

Lactobacillus isolates survived a 2-hour incubations when he used this strain against *Campylobacter jejenum* .

5.6 Study of Mushroom and p *Bacillus subtilis* and their combination on H/L ratio of broilers:

Tables (4-6) showed H/L ratio separately. The results showed significant differences ($P \leq 0.05$) compared with (the control group) H/L ratio between (MSH) and (BS) groups and combination groups (Mush+BS).

Poultry health status is expressed in this study asH / L ratio. Previous workers reported the heterotrophic / lymphocyte (H / L) ratio is an excellent indicator of chicken stress (Kassab et al., 2000).

On the other hand, the results of the (Mush+BS) showed that the H / L ratio mean±SD (0.41±0.06) decreased significantly compared to the control group mean±SD (0.982±0.23). Paryad and Mahmoudi (2008) reported that when adding 0, 0.5, 1.5 and 2% *Saccharomyces cerevisiae* to broiler chicken feed, the H / L ratio decreased significantly at 42 days. Thongsong et al. (2008) that when adding probiotics to the drinking water of male broiler chickens, there was no significant effect on H / L ratio at 28 and 42 days compared to the control group.

5.7 Study of Mushroom and *Bacillus subtilis* on humeral immunity in broiler chicken against Newcastle disease and Infectious bronchitis:

The figure results (figure 4-1) of the humeral immunity response were showed significantly differences ($p \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle Disease (ND) and Infectious bronchitis (IB) were improved significantly ($P \leq 0.05$) in the Mush-BS mean (4042.16) for Newcastle disease virus vaccination and (10021.2) for Infectious bronchitis virus vaccination , Mushroom, and (BS) groups respectively compare with the (Control) with mean 1839.25 and 2884.75 for Newcastle and infectious bronchitis, respectively, in the ninth age days.

On the other hand,

The figure results (figure 4-2) of the humeral immunity response were showed significantly differences ($p \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly ($P \leq 0.05$) in the Mush-BS (3801.16) for Newcastle disease virus vaccination and (9880.2) for Infectious bronchitis virus vaccination , (Mushroom), and (BS) groups respectively compare with the (Control) with mean 1698.25 and 2643.75 for Newcastle and infectious bronchitis, respectively in the Eighteeth age days (Mahfuz et al., 2019).

However, all the chicks of this study recorded significantly ($P \leq 0.05$) increment in antibody titers against Newcastle Disease vaccine with progress the age, This increment in Ab appear obviously in the treatment groups as compared with the control at (9 and 18) days of age.

This results was disagreement with (Fard *et al*.,2014) who was concluded that adding 1% of mushroom waste to the diet can enhance some immune parameters of chickens to a certain extent, but the effect on Newcastle disease virus is weak. Intestinal morphology is influenced by mushroom waste, especially in the ileum and jejunum(Khan *et al*., 2019).

Chapter Six

Conclusions and Recommendations

Chapter six

Conclusions and Recommendations

6.1. Conclusions:

Based on the current research findings, the conclusions are as follows:

1. It was found Reducing of triglycerides ,cholesterol, low-density lipoprotein LDL-C and Very low-density lipoprotein cholesterol VLDL-C, and greatly increasing the HDL-C concentration, especially in the fourth group of chicks fed on Mushroom with *Bacillus subtilis* .
2. AST, ALT and ALP were decreased in the some group compared with the other treatment groups
3. From the second week of experiment to the end of the study, the live weight, weight gain and feed quantity of the chicks fed simultaneously were increased in the fourth treatment, and the same group showed improvement in the rate of feed conversion to the end of the experiment.
4. Adding a mixture of probiotics and prebiotics to their staple diet can improve the immune response by enhancing both cellular and Humeral immunity, otherwise it was no significant in the bursa index, spleen index among study groups.
5. Dietary mushroom with probiotics supplement may improve the affect intestinal health of chicken broiler through increasing the efficacy of crypt width and villus height and natural Ph acidity levels and H/L ratio.

6.2 Recommendations:

1. Estimate the effect of highly purified probiotics on production traits and immune status by improving resistance.

2. Study the effect of mushroom and probiotics(*Bacillus subtilis*) on other chicken breeds.

3. Use of different levels of mushroom (prebiotics) and probiotics(*Bacillus subtilis*) and their combinations in broiler meals.

4. Use a mixture of different types of mushroom (prebiotics) and probiotics in the diet, and demonstrate changes in the performance of broiler chickens.

5. In order to improve growth performance and blood profile and immune system efficiency , use BS in water and diets

References

References

Abdel-Rahman, H. A.; Fathallah, S. I. and Helal, A. A. (2014) . Effect of turmeric (Curcuma longa), fenugreek (Trigonella foenum-graecum L.) and/or bioflavonoid supplementation to the broiler chicks diet and drinking water on the growth performance and intestinal morphometeric parameters. Glob. Vet., 12(5): 627-635.

Afsharmanesh, M. & Sadaghi, B.(2014). Effects of dietary alternatives (probiotic, green tea powder, and Kombucha tea) as antimicrobial growth promoters on growth, ileal nutrient digestibility, blood parameters, and immune response of broiler chickens. Comparative Clinical Pathology, 23, 717-724.

Asatiani, M. D., Elisashvili, V., Songulashvili, G., Reznick, A. Z., & Wasser, S. P. (2010). Higher basidiomycetes mushrooms as a source of antioxidants. In Progress in mycology (pp. 311-326). Springer, Dordrecht.

Abdel-Wareth, A. A. A., Kehraus, S., Hippenstiel, F., & Südekum, K. H. (2012). Effects of thyme and oregano on growth performance of broilers from 4 to 42 days of age and on microbial counts in crop, small intestine and caecum of 42-day-old broilers. Animal feed science and technology, 178(3-4), 198-202.

Aida, F. M. N. A., Shuhaimi, M., Yazid, M., & Maaruf, A. G. (2009). Mushroom as a potential source of prebiotics: a review. Trends in Food Science & Technology, 20(11-12), 567-575.

Ajuwon, K. 2015. Toward a better understanding of mechanisms of probiotics and prebiotics action in poultry species. Journal of Applied Poultry Research, 25, 277-283.

Alan, H. B. (2006). Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 564.

Al-Fayadh, H. A. and Naji, S. A. (1989). Poultry Production Technology. 1st vol., Directorate of higher education printer, Baghdad.

Alloui, M. N., Szczurek, W., & Świątkiewicz, S. (2013). The usefulness of prebiotics and probiotics in modern poultry nutrition: A review/Przydatność prebiotyków i probiotyków w nowoczesnym żywieniu drobiu–przegląd. Annals of Animal Science, 13(1), 17-32.

Al-Malki, A. L.; Abo-Golayel, M. K.; Abo-Elnaga, G.; Al-Beshri, H. (2013). Hepatoprotective effect of dandelion (Taraxacum officinale) against induced chronic liver cirrhosis. J. of Med. Plants Res., 7:1494-505

Arowolo, M. A., & He, J. (2018). Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. Animal Nutrition, 4(3), 241-249.

Arrebola, E., Jacobs, R. & Korsten, L. (2010). Iturin A is the principal inhibitor in the biocontrol activity of Bacillus amyloliquefaciens PPCB004 against postharvest fungal pathogens. Journal of Applied Microbiology, 108, 386-395.

Arul, V. (2016). Isolation and Probiotic Characterization of Lactic Acid Bacteria Enterococcus Faecalis from country Chicken, and Duck and its in vivo Attributes in Broiler Chicken (Doctoral dissertation, Department of Biotechnology, Pondicherry University)

Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry science, 88(1), 49-56.

Bailey, J. S., Blankenship, L. C., & Cox, N. A. (1991). Effect of fructooligosaccharide on salmonella colonization of the chicken

intestine. Poult Sci, 70(12), 2433-2438.

Bailey, J. S., Blankenship, L. C., & Cox, N. A. (1991). Effect of fructooligosaccharide on salmonella colonization of the chicken intestine. Poult Sci, 70(12), 2433-2438

Bao, H. N. D., Ushio, H., & Ohshima, T. (2008). Antioxidative activity and antidiscoloration efficacy of ergothioneine in mushroom)Flammulina velutipes) extract added to beef and fish meats. Journal of Agricultural and Food Chemistry, 56, 10032e10040.

Bao, X., Liu, C., Fang, J., & Li, X. (2001). Structural and immunological studies of a major spores of Ganoderma lucidum (Fr.) Karst. Carbohydrate Research, 332, 67e74

Bao, X., Liu, C., Fang, J., & Li, X. (2001). Structural and immunological studies of a major spores of Ganoderma lucidum (Fr.) Karst. Carbohydrate Research, 332, 67e74.

Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol 2008; 46: 2742–2747.

Baurhoo, B., Letellier, A., Zhao, X., & Ruiz-Feria, C. A. (2007b). Cecal populations of lactobacilli and bifidobacteria and escherichia coli populations after in vivo escherichia coli challenge in birds fed diets with purified lignin or mannanoligosaccharides. Poult Sci, 86(12), 2509-2516. doi: 10.3382/ps.2007-00136.

Balakrishnan, P., & Loganayagi, C. T. (2018). Antihyperglycemic activity of Agaricus bisporus mushroom extracts on alloxan induced diabetic rats. Int J Pharma Res Health Sci, 6(2), 2475-79.

Baurhoo, B., Phillip, L., & Ruiz-Feria, C. A. (2007a). Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult Sci, 86(6),

1070-1078.

Bernaś, E., Jaworska, G., & Kmiecik, W. (2006). Storage and processing of edible mushrooms. Acta Scientiarum Polonorum Technologia Alimentaria, 5, 5–23.

Beski, S. S. M., & Al-Sardary, S. Y. T. (2015). Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. International Journal of Poultry Science, 14(1), 31.

Bierbaum, G. & Sahl, H.-G. 2009. Lantibiotics: mode of action, biosynthesis and bioengineering. Current Pharmaceutical Biotechnology, 10, 2-18.

Begley, M.; Hill, C.; Gahan, C. G. M. (2006) Bile Salt Hydrolase Activity in Probiotics. Appl. Environ. Microbiol., 72: 1729-1738.

Boroojeni, F. G., Männer, K., Rieger, J., Calvo, E. P., & Zentek, J. (2019). Evaluation of a microbial muramidase supplementation on growth performance, apparent ileal digestibility, and intestinal histology of broiler chickens. Poultry science, 98(5), 2080-2086.

Burtis, C. A.; Ashwood, E. R. (1999). ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: Moss D. W., Henderson A. R., 652.

Biloni, A., Quintana, C., Menconi, A., Kallapura, G., Latorre, J., Pixley, C., Layton, S., Dalmagro, M., Hernandez-Velasco, X. & Wolfenden, A. 2013. Evaluation of effects of EarlyBird associated with FloraMax-B11 on Salmonella Enteritidis, intestinal morphology, and performance of broiler chickens. Poultry Science, 92, 2337-2346.

Botterweck, A. A. M., H. Verhagen, R. A. Goldbohm, J. Kleinjans، and P. A. Van Den Brandt. 2000. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk. Results from analyses in the Netherlands Cohort Study. Food Chem. Toxicol. 38:599–605

Bozkurt, M., Kucukyilmaz, K., Catli, A. U., Cinar, M., Bintas, E., & Coven, F. (2012b). Performance, egg quality, and immune response of laying

hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poult Sci, 91(6), 1379-1386. doi: 10.3382/ps.2011-02023.

Bozkurt, M., Kucukyilmaz, K., Catli, A. U., Cinar, M., Bintas, E., & Coven, F. (2012b). Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poult Sci, 91(6), 1379-1386. doi: 10.3382/ps.2011-02023.

Brashears, M. M., Reilly, S. S. & Gilliland, S. E. 1998. Antagonistic action of cells of Lactobacillus lactis toward Escherichia coli O157: H7 on refrigerated raw chicken meat. Journal of Food Protection, 61, 166-170.

Breene, W. M. 1990. Nutritional and medicinal value of specialty mushrooms. J. Food Prot. 53:883–894.

Cao, G. T., Zeng, X. F., Chen, A. G., Zhou, L., Zhang, L., Xiao, Y. P. & Yang, C. M. 2013. Effects of a probiotic, Enterococcus faecium, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with Escherichia coli K88. Poultry Science, 92, 2949-2955.

Chang, H. H., Chien, P. J., Tong, M. H., & Sheu, F. (2007). Mushroom immunomodulatory proteins possess potential thermal/freezing resistance, acid/alkali tolerance and dehydration stability. Food Chemistry, 105, 597–605.

Chang, R. (1996). Functional properties of edible mushroom. Nutr.Rev. 54:91-93

Chang, S. T., and J. A. Buswell. (1996). Mushroom nutriceuticals. World J. Microbiol. Biotechnol. 12:473–476.

Charalampopoulos, D., & Rastall, R. A. (Eds.). (2009). Prebiotics and probiotics science and technology (Vol. 1). Springer Science & Business Media.

Chaudiere, J., and R. Ferrari-Iliou. 1999. Intracellular antioxidants. From chemical to biochemical mechanisms. Food Chem. Toxicol. –37:949 .962.

Cheikhyoussef, A., Pogori, N., Chen, W. & Zhang, H. 2008. Antimicrobial proteinaceous compounds obtained from bifidobacteria: From production to their application. International Journal of Food Microbiology, 125, 215-222.

Chen, T. C. (2003). Effect of adding chicory fructans in feed on fecal and intestinal microflora and excreta volatile ammonia.

Cenesz, S.; Yaman, H.; Ozcan, A. and Karademr, G. (2008). Effect of kefir as probiotic on serum cholesterol, total lipid, aspartate amino transferase and alanine amino transferase activities in broiler chicks. 72(2): 61-67.

Chiang, S. H. and Hsieh, W. M. (1995). Effect of direct-fed microorganisms on broiler growth performance and litter ammonia level. Asian. Aust. J. Anim. Sci., 8(2):159-162.

Chuang, W. Y., Liu, C. L., Tsai, C. F., Lin, W. C., Chang, S. C., Shih, H. D., ... & Lee, T. T. (2020). Evaluation of Waste Mushroom Compost as a Feed Supplement and Its Effects on the Fat Metabolism and Antioxidant Capacity of Broilers. Animals, 10(3), 445.

Corp, I. (2011). IBM SPSS statistics for windows, version 22.0. IBM Corp Armonk, NY.

Chen, X., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., Piel, J. & Borriss, R. 2009. Genome analysis of Bacillus amyloliquefaciens FZB42 reveals its potential for biocontrol of plant pathogens. Journal of Biotechnology, 140, 27-37.

Chen, Y. C., Nakthong, C., & Chen, T. C. (2005). Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin.

International Journal of Poultry Science, 4(2), 103 – 108

Choct, M., 2009. Managing gut health through nutrition. British Poultry Science 50،.15–9.

Choi, U.; Lee, O.; Hyuk, J. Y.; Cho, C.; Kyung, Y. R.; Lim, S.; and Kim, Y. (2010). Hypolipidemic and Antioxidant Effects of (mushroom) Root and Leaf on Cholesterol-Fed Rabbits. Inter. J. Mol. Sci., 11: 67-78.

Chorvathoba, V., Bobek, P., Ginter, E. and Klvanova, J. 1993. Effect of the oyster fungus on glycemia and cholesterolemia in rats with insulin depended diabetes. Physol. Res. 42:175-179.

Commane, D. M., Shortt, C. T., Silvi, S., Cresci, A., Hughes, R. M. & Rowland, I. R. 2005. Effects of fermentation products of pro-and prebiotics on trans-epithelial electrical resistance in an in vitro model of the colon. Nutrition and Cancer, 51, 102-109.

Chávez-Talavera, O., Tailleux, A., Lefebvre, P., & Staels, B. (2017). Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. Gastroenterology, 152(7), 1679-1694.

Coppo J A. 2001. Fisiología comparada del medio inter-no, dunken، buenos, aires 297 compounds from mushrooms I: a lanostane-type triterpene and prenylphenol derivates from Jahnoporus hiritus and Albatrellus flettii and their activities against Bacillus cereus and Enterococcus faecalis. Planta Med 2010; 76: 182–185.

Corr, S. C., Li, Y., Riedel, C. U., O'toole, P. W., Hill, C. & Gahan, C. G. 2007. Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. Proceedings of the National Academy of Sciences, USA, 104, 7617-7621.

Corrigan, A., Horgan, K., Clipson, N., & Murphy, R. A. (2011). Effect of dietary supplementation with a saccharomyces cerevisiae mannan

oligosaccharide on the bacterial community structure of broiler cecal contents. Appl Environ Microbiol, 77(18), 6653-6662. doi: 10.1128/AEM.05028-11.

Cotter, P. D., Hill, C. & Ross, R. P. 2005. Bacteriocins: developing innate immunity for food. Nature Reviews: Microbiology, 3, 777-788.

Craig WA. Antibacterial therapy. In: Goldman & Ausiell, editor. Cecil textbook of medicine, 22nd edition. Philadelphia: Saunders; 2004: 1926–1853

Cummings, J. H., Macfarlane, G. T., & Englyst, H. N. (2001). Prebiotic digestion and fermentation. American Journal of Clinical Nutrition،)73Suppl.), 415e420.

Chye, F. Y., Wong, J. Y., & Lee, J. S. (2008). Nutritional quality and antioxidant activity of selected edible wild mushrooms. Food Science and Technology International, 14(4), 375-384.

Cağlarirmak, N. E. C. L. A. (2011, October). Edible mushrooms: an alternative food item. In Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7) (Vol. 5, pp. 548-54). Arcachon, France: World Society for Mushroom Biology and Mushroom Products.

Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis. Recent advancements in control measures and vaccine development. Expert Rev. Vaccines 5:143–163.

Dawood, M. A., Koshio, S., Abdel-Daim, M. M., & Van Doan, H. (2019). Probiotic application for sustainable aquaculture. Reviews in Aquaculture, 11(3), 907-924

Dalloul, R. A., H. S. Lillehoj, J. S. Lee, S. H. Lee, and K. S. Chung. .2006 Immunopotentiating effect of a Fomitella fraxinea-derived lectin on chicken immunity and resistance to coccidiosis. Poult. Sci. 85:446–451.

Dibener, J. J. and Richards, J. D. (2005). Antibiotic growth promoters in agriculture: History and mode action. Poult. Sci., 84:634-643.

Dowarah, R., Verma, A. K., Agarwal, N., Singh, P., & Singh, B. R. (2018). Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. PloS one, 13(3), e0192978.

Daskiran, M., Onol, A. G., Cengiz, O., Unsal, H., Turkyilmaz, S., Tatli, O. & Sevim, O. 2012. Influence of dietary probiotic inclusion on growth performance, blood parameters, and intestinal microflora of male broiler chickens exposed to posthatch holding time. Journal of Applied Poultry Research, 21, 612-622

Delzenne, N. M.; Kok, N. (1999). Biochemical basis of oligofructose-induced hypolipidemia in animal models. J. of Nutrition, 129, 14675–14705.

Dembitsky, V. M., Terent'ev, A. O., & Levitsky, D. O. (2010). Amino and fatty acids of wild edible mushrooms of the genus Boletus. Records of Natural Products, 4, 218–223.

Denbow, D. M. (2000). Gastrointestinal anatomy and physiology. In G. C. Whittow (Ed.), Sturkie‟s avian physiology (5th ed., pp. 299-625). San Diego, CA: Academic Press.

Dennis, E. A., & Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. Nature Reviews Immunology, 15(8), 511-523.

Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. [Review]. Poult Sci, 84(4), 634-643.

Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. [Review]. Poult Sci, 84(4), 634-643.

Donalson, L. M., McReynolds, J. L., Kim, W. K., Chalova, V. I., Woodward, C. L., Kubena, L. F., Nisbet, D. J., & Ricke, S. C. (2008). The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets

on the gastrointestinal tract fermentation, salmonella enteritidis infection, and intestinal shedding in laying hens. Poult Sci, 87(7), 1253-1262. doi: 10.3382/ps.2007-00166.

Douglas, L. C., & Sanders, M. E. (2008). Probiotics and prebiotics in dietetics practice. Journal of the American Dietetic Association، 510 ،108e521.

Douglas, M. W., Persia, M., & Parsons, C. M. (2003). Impact of galactose, lactose, and grobiotic-b70 on growth performance and energy utilization when fed to broiler chicks. Poult Sci, 82(10), 1596-1601.

Doyon, M., & Labrecque, J. A. (2008). Functional foods: a conceptual definition. British Food Journal, 110(11), 1133e1149.

Dec, M., Nowaczek, A., Urban-Chmiel, R., STĘPIEŃ-PYŚNIAK, D., & Wernicki, A. (2018). Probiotic potential of Lactobacillus isolates of chicken origin with anti-Campylobacter activity. Journal of Veterinary Medical Science, 18-0092.

Drori, A., Shabat, Y., Ben Ya'acov, A., Danay, O., Levanon, D., Zolotarov, L., & Ilan, Y. (2016). Extracts from Lentinula edodes (Shiitake) edible mushrooms enriched with vitamin D exert an anti-inflammatory hepatoprotective effect. Journal of medicinal food, 19(4), 383-389.

Dubost, N. J., B. Ou, and R. B. Beelman. 2007. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. Food Chem. 105:727735

Durdi Q and Aliakbarpour H R. 2005. Serum activities of enzymes in broiler chickens that died from sudden death syndrome. Pakistan Journal of Biology Science 8: 1078–80.

Eladl, A. H., Arafat, N., El-Shafei, R. A., Farag, V. M., Saleh, R. M., & Awadin, W. F. (2019). Comparative immune response and pathogenicity of the H9N2 avian influenza virus after administration of Immulant®, based on Echinacea and Nigella sativa, in stressed chickens. Comparative

immunology, microbiology and infectious diseases, 65, 165-175.

Elrayeh, A. S.; Yildiz, G. (2012). Effects of inulin and b-glucan supplementation in broiler diets on growth performance, serum cholesterol, intestinal length, and immune system. Turk. J. of Vet. & Animal Sci., 36: 388–394.

Ergun, A.; Yalcin, S. & Sacakli, P. (2000). The usage of probiotic and zinc bacitracin in broiler rations. Ankara Universitesi Veteriner Fakultesi Derg., 47: 271_280.

Ezema, C. (2013). Probiotics in animal production: A review. Journal of Veterinary Medicine and Animal Health, 5(11), 308-316.

El Enshasy, H. A., & Hatti-Kaul, R. (2013). Mushroom immunomodulators: unique molecules with unlimited applications. Trends in Biotechnology, 31(12), 668-677.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., ... & Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet, 387(10014), 176-187.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28:2077-2080.

Fard, S. H., Toghyani, M., & Tabeidian, S. A. (2014). Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. International Journal of Recycling of Organic Waste in Agriculture, 3(4), 141-146

Feng, P., Ye, Z., Kakade, A., Virk, A. K., Li, X., & Liu, P. (2019). A review on gut remediation of selected environmental contaminants: possible roles of probiotics and gut microbiota. Nutrients, 11(1), 22.

Fan, S., Breidt, F., Price, R., & Pérez-Díaz, I. (2017). Survival and growth of probiotic lactic acid bacteria in refrigerated pickle products. Journal of

food science, 82(1), 167-173.

Fernandez, F., Hinton, M., & Gils, B. V. (2002). Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella Enteritidis colonization. Avian Pathology, 31(1), 49-58.

Fajardo, P., Pastrana, L., Mendez, J., Rodriguez, I., Fucinos, C. & Guerra, N. P. 2012. Effects of feeding of two potentially probiotic preparations from lactic acid bacteria on the performance and faecal microflora of broiler chickens. The Scientific World Journal, 2012, 562635.

Fard, S. H., Toghyani, M., & Tabeidian, S. A. (2014). Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. International Journal of Recycling of Organic Waste in Agriculture, 3(4), 141-146

Ferket, P. R. (2003). Controlling gut health without the use of antibiotics. In Proceedings of the 30th Annual Carolina Poultry Nutrition Conference (pp. 57-68).

Ferket, P. R. (2004). Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. Paper presented at the Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 20th Annual Symposium: re-imagining the feed industry, Lexington, Kentucky, USA.

Fernandez, F., Hinton, M., Van Gils, B., 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella enteritidis colonization. Avian Pathology 31, 49–58.

Flynn, S., Van Sinderen, D., Thornton, G. M., Holo, H., Nes, I. F. & Collins, J. K. 2002. Characterization of the genetic locus responsible for the production of ABP-118, a novel bacteriocin produced by the probiotic bacterium Lactobacillus salivarius subsp. salivarius UCC118.

Microbiology, 148, 973-984.

Fuchs, F. D., & Kuchenbecker, R. (2004). Princípios gerais do uso de antimicrobianos. Farmacologia clínica: fundamentos da terapêutica racional. 3ª ed. Rio de Janeiro: Guanabara Koogan, 342-9.

Fukushima, M., Nakano, Y., Morii, Y., Ohashi, T., Fujiwara, Y. and Sonoyama, K. 2000. Hepatic receptor mRNA in rats is increased by dietary mushroom (Agaricus bisporus) fiber and sugar beet fiber. J. Nutr. 130:2151-2156.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of nutrition, 125(6), 1401-1412.

Gaggia, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology, 141 Suppl 1, S15-28. doi: 10.1016/j.ijfoodmicro.2010.02.031.

Geier, M. S., Torok, V. A., Allison, G. E., Ophel-Keller, K., & Hughes, R. J. (2009). Indigestible carbohydrates alter the intestinal microbiota but do not influence the performance of broiler chickens. J Appl Microbiol, 106(5), 1540-1548. doi: 10.1111/j.1365-2672.2008.04116x.

Gartner, L. P. and Hiatt, J. L. (2001). Color textbook of histology. 2nd ed. Philadelphia: W. B. Saunders Co.

Gonzalez, N. J.; Cornejo, S.; Jimenez, F. and Lorenzoni, G. (2001). Effect of dietary supplementation with and enzyme complex (Avizyme 1500) containing amylase, xylanase and protease on performance and egg quality of commercial layers. Poult. Sci., 80(7):151.

Griggs, J. P. and Jacob, J. P. (2015). Alternatives to antibiotics for organic poultry production. J. Appl. Poult. Res., 14:750–756.

Grove, T. H. (1979). Effect of reagent pH on determination of high density

lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin. Chem., 25: 560-564.

Gunal, M.; Yayli, G.; Kaya, O.; Karahan, N. & Sulak, O. (2006). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Inter. J. of Poult. Sci., 5: 149_155.

Ghoddusi, H. B., Grandison, M. A., Grandison, A. S., & Tuohy, K. M. .(2007)In-vitro study on gas generation and prebiotic effects of some carbohydrates and their mixtures. Anaerobe, 13, 193e199.

Ghosh, Baurhoo, B., Ferket, P. R., & Zhao, X. (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poult Sci, 88(11), 2262-2272. doi: 10.3382/ps.2008-00562.

Ghosh, H. K., Halder, G., Samanta, G., Paul, S. K., & Pyne, S. K. (2007). Effect of dietary supplementation of organic acid and mannan oligosaccharide on performance and gut health of japanese quail. Asian Journal of Poultry Science, 1, 1-7.

Giannenas ,*1 I. S. Pappas ,† S. Mavridis ,† G. Kontopidis ,‡ J. Skoufos ,§ and I. Kyriazakis. 2010. Poultry Science 89 :303–311 doi: 10.3382/ps.2009-00207.

Giannenas, I., Tontis, D., Tsalie, E., Chronis, E. F., Doukas, D., & Kyriazakis, I. (2010). Influence of dietary mushroom Agaricus bisporus on intestinal morphology and microflora composition in broiler chickens. Research in Veterinary Science, 89(1), 78-84.

Gibson, G. R. (2004). From probiotics to prebiotics and healthy digestive system. Journal of Food Science, 69(5), 141e143.

Gibson, G. R., & Collins, M. (1999). Concepts of balanced colonic microbiota, prebiotics and synbiotics. In L. A. Hanson and R. H. Yolken (Eds.), Probiotics: Other nutritional factors and intestinal microflora. Philadelphia: Raven Publishers.

Gibson, G. R., & Fuller, R. (2000). Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. J Nutr, 130(2), 391s-395s.

Gibson, G. R., Probert, H. M., Rastall, R. A., & Roberfroid, M. B (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews, 17، 259e275.

Gibson, R. G., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Appl. Bacteriol. 1401–1412.

Gilliland, S. E., Nelson, C. R., & Maxwell, C. (1985). Assimilation of cholesterol by Lactobacillus acidophilus. Applied and environmental microbiology, 49(2), 377-381.

Ginsburg I. Role of lipoteichoic acid in infection and inflammation. Lancet Infect Dis 2002; 2: 171–179.

Gong, J., Froster, R.J., Yu, H., Chambers, J.R., Sabour, P.M., Wheatcroft, R., Chen, S،.., .2002Diversity and phylogenetic analysis of bacteria in the mucosa of chicken caeca and comparison with bacteria in the caecal lumen. FEMS Microbiology Letters 208, 1–7.

Guo, F. C., B. A. Williams, R. P. Kwakkel, H. S. Li, X. P. Li, J. Y Luo, W. K. Li, and M. W. A. Verstegen. 2004b. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic on the cecal microbial ecosystem in broiler chickens. Poult. Sci.182–83:175.

Guo, F. C., H. F. J. Savelkoul, R. P. Kwakkel, B. A. Williams, and M. W. A. Verstegen. 2003. Immunoactive, medicinal properties of mushroom

and herb polysaccharides and their potential use in chicken diets. World's Poult. Sci. J. 59:427–440.

Giannenas, I., Tontis, D., Tsalie, E., Chronis, E. F., Doukas, D., & Kyriazakis, I. (2010). Influence of dietary mushroom Agaricus bisporus on intestinal morphology and microflora composition in broiler chickens. Research in Veterinary Science, 89(1), 78-84.

Guo, F. C., R. P. Kwakkel, B. A. Williams, H. K. Parmentier, W. K. Li, Z. Q. Yang, and M. W. A. Verstegen. 2004a. Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of Eimeria tenella-infected chickens. Poult. Sci.1132–83:1124.

Gurbuz, E., Balevi, T., Kurtoglu, V., & Oznurlu, Y. (2011). Use of yeast cell walls and yucca schidigera extract in layer hens' diets. Italian Journal of Animal Science, 10(2), 134-138. doi: 10.4081/ijas.2011.e26.

Haldar, S., Ghosh, T. K., Toshiwati, & Bedford, M. R. (2011). Effects of yeast (saccharomyces cerevisiae) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with salmonella enteritidis. Animal Feed Science and Technology, 168(1-2), 61-71. doi: DOI 10.1016/j.anifeedsci.2011.03.007.

Halliwell, B., and J. M. C. Gutteridge. 1999. Free Radicals in Biology and Medicine. 3rd ed. Oxford University Press, New York،NYHarbarth S, Harris AD, Carmeli Y, Samore MH. Parallel analysis of individual and aggregated data on antibiotic exposure and resistance in gram-negative bacilli. Clin Infect Dis 2001; 33: 1462–1468.

Hartman, S. 1998. Calcium and selenium enrichment during cultivation improves the postharvest quality and shelf life of mushrooms)Agaricus bisporus). MS Thesis. The Pennsylvania State University University Park.

Hoelzer, K., Wong, N., Thomas, J., Talkington, K., Jungman, E., & Coukell, A. (2017). Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence?. BMC veterinary research, 13(1), 211.

Hossain, M. M., Begum, M., & Kim, I. H. (2015). Effect of Bacillus subtilis, Clostridium butyricum and Lactobacillus acidophilus endospores on growth performance, nutrient digestibility, meat quality, relative organ weight, microbial shedding and excreta noxious gas emission in broilers. Veterinarni Medicina, 60(2), 77-86.

Hashimoto, M., Hossain, M. S., Shimada, T., Yamasaki, H., Fujii، Y. and Shido, O. 2001. Effects of docosahexacnoic acid on annular lipid fluidity of the rat bile canalicular plasma membrane. J. Lipid Res. 42:1160-1168.

Hashimoto, M., Shinozuka, K. and Gamoh, S. 1999a. The hypotensive effect of docosahexaenoic acid is associated with the enhanced released of ATP from the caudal artery of aged rats. J. Nutr. 129:70-76.

Hossain, M. F., Rashid, M., Sidhu, R., Mullins, R., & Mayhew, S. L. (2019). A Simplified, Specific HPLC Method of Assaying Thiamine and Riboflavin in Mushrooms. International journal of food science.

Hashemi, S. R., & Davoodi, H. (2011). Herbal plants and their derivatives as growth and health promoters in animal nutrition. Veterinary research communications, 35(3), 169-180.

Hashemipour, H., Kermanshahi, H., Golian, A., & Raji, A. (2014). Effect of antibiotic alternatives on ileal microflora and intestinal histomorphology of broiler chickens fed wheat based diet. Iranian Journal of Applied Animal Science, 4(1), 135-142.

Hashimoto, M., Shinozuka, K. and Shahdat, H. M. 1998. Antihypertensive effect of all-cis-5, 8, 11, 14, 17-icosapentaenoate of aged rats is associated with an increase in the release of ATP from caudal artery. J. Vasc.

Res. 35:55-62.

Hashimoto, M., Shinozuka, K. and Tanabe, Y. 1999. Hypotension induced by exercise is associated with enhanced release of adenyl purines from aged rat artery. Am. J. Physiol. 276:970-975.

Hassan, H. A., & Ragab, M. S. (2007). Single and combined effects of mannan oligosaccharide (mos) and dietary protein on the performance and immunity response of laying hens. Egyptian Poultry Science 27, 969-987.

Hassan, M., Kjos, M., Nes, I., Diep, D. & Lotfipour, F. 2012. Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. Journal of Applied Microbiology, 113, 723-736.

Hearst, R., Nelson, D., McCollum, G., Millar, B. C., Maeda, Y‹. Goldsmith, C. E., et al. (2009). An examination of antibacterial and antifungal properties of constituents of Shiitake (Lentinula edodes) and Oyster (Pleurotus ostreatus) mushrooms. Complementary Therapies in Clinical Practice, 15, 5e7.

Hofacre, C. L., Beacorn, T., Collett, S., & Mathis, G. (2003a). Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. Journal of Applied Poultry Research, 12(1), 60-64.

Hossain, M. S., Hashimoto, M., Gamoh, S. and Masumura, S1999 .a. Association of age-related decrease in platelet membrane fluidity with platelet lipid peroxide. Life Sci. 64:135-143.

Hung, A. T., Lin, S.-Y., Yang, T.-Y., Chou, C.-K., Liu, H.-C., Lu, J.-J., Wang, B., Chen, S.-Y. & Lien, T.-F. 2012. Effects of Bacillus coagulans ATCC 7050 on growth performance, intestinal morphology, and microflora

composition in broiler chickens. Animal Production Science, 52, 874-879.

Imaeda N. 2000. Influence of the stocking density and rearing reason on incidence of sudden death syndrome in broiler chickens. Poultry Science 79: 201–04.

Jacobs, C. M. (2011). The effects of potential dietary prebiotics and grain particle size on the growth performance and intestinal microfolra of young poultry. (Doctor of Philosophy Dissertation), University of Illinois at Urbana-Champaign, Urbana, Illinois.

Jayakumar, T., Thomas, P. A., & Geraldine, P. (2009). In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom (Pleurotus ostreatus). Innovative Food Science and Emerging Technologies, 10, 228e234.

Jayaraman, S., Thangavel, G., Kurian, H., Mani, R., Mukkalil, R. & Chirakkal, H. 2013. Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with Clostridium perfringens-induced necrotic enteritis. Poultry Science, 92, 370-374.

Jazi, V., Foroozandeh, A. D., Toghyani, M., Dastar, B., & Koochaksaraie, R. R. (2018). Effects of Pediococcus acidilactici, mannan-oligosaccharide, butyric acid and their combination on growth performance and intestinal health in young broiler chickens challenged with Salmonella Typhimurium. Poultry science, 97(6), 2034-2043.

Józefiak, D., Rutkowski, A., & Martin, S. A. (2004). Carbohydrate fermentation in the avian ceca: a review. Animal Feed Science and Technology, 113(1-4), 1-15.

Jeong, S. C., Jeong, Y. T., Yang, B. K., Islam, R., Koyyalamudi, S. R., Pang, G., ... & Song, C. H. (2010). White button mushroom (Agaricus bisporus) lowers blood glucose and cholesterol levels in diabetic and

hypercholesterolemic rats. Nutrition research, 30(1), 49-56.

Kabir, S. M. L. (2009). The Role of Probiotics in the Poultry Industry, Inter. J. Molec. Sci., 10(8): 3531- 3546.

Kabir, S. M. L.; Rahman, M. M.; Rahman, M. B. and Ahmed, S.U. (2004). The dynamic of probiotics on growth performance and immune response in broiler. Inter. J. Poult. Sci., 3(5):361-364.

Kabak, B., Brandon, E. F., Var, I., Blokland, M., & Sips, A. J. (2009). Effects of probiotic bacteria on the bioaccessibility of aflatoxin B1 and ochratoxin A using an in vitro digestion model under fed conditions. Journal of Environmental Science and Health, Part B, 44(5), 472-480.

Kabir, Y., Yamaguchi, M. and Kimura, S. 1987. Effect of shiitake (Lentinus edodes) and maitake (Grifola frondosa) mushrooms on blood pressure and plasma lipids of spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol. 33:341-346.

Kała, K., Krakowska, A., Sułkowska-Ziaja, K., Szewczyk, A., Reczyński, W., Opoka, W‹. et al. (2017). Kinetics of extracted bioactive components from mushrooms in artificial digestive juices. International Journal of Food Properties, 20, 1796–1817.

Kalač, P. (2010a). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chemistry, 113, 9–16.

Kalavathy, R., N. Abdullah, S. Jalaludin and Y.W. Ho‹ .2003Effect of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of brother chickens. Br. Poult. Sci., 44: 139-144.

Kalavathy, R.; Abdullah, N.; Jalaludin, S.; Ho, Y. W. (2003). Effect of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of brother chickens. Br.

Poult. Sci., 44: 139-144.

Kalyoncu F, Oskay M, Salam H, Erdogan TF, Tamer AÜ. Antimicrobial and antioxidant activities of mycelia of 10 wild mushroom species. J Med Food 2010; 13: 415–419

Kalyoncu F, Oskay M. Antimicrobial activities of four wild mushroom species collected from Turkey. Proceeding of the 6th International Conference on Mushroom Biology and Mushroom Products, 2008.

Kalyoncu F, Oskay M. Antimicrobial activities of four wild mushroom species collected from Turkey. Proceeding of the 6th International Conference on Mushroom Biology and Mushroom Products, 2008.

Kassab, A.K.; Elaiwi, H.H.; Al-Murrani, W.K. (2000). Heteropil/ Lymphocyte ration under higher and low temperature against Newcastle antigen in chicken. IPA Agre. Res, 10: 184-192.

Kasuga, A., Y. Aoyagi, and T. Sugahara. 1995. Antioxidant activity of fungus Suihs bovinus (L: Fr.) O. Kuntze. J. Food Sci.1115–60:1113

Kempf M, Rolain JM. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. Int J Antimicrobiol Agents 2012; 39: 105–114

Khan, S. H., Mukhtar, N., & Iqbal, J. (2019). Role of mushroom as dietary supplement on performance of poultry. Journal of dietary supplements, 16(5), 611-624.

Khoobani, M., Hasheminezhad, S. H., Javandel, F., Nosrati, M., Seidavi, A., Kadim, I. T., ... & Tufarelli, V. (2020). Effects of Dietary Chicory (Chicorium intybus L.) and Probiotic Blend as Natural Feed Additives on Performance Traits, Blood Biochemistry, and Gut Microbiota of Broiler Chickens. Antibiotics, 9(1), 5.

Kim, C. H., Shin, K. S., Woo, K. C., & Paik, I. K. (2009). Effect of dietary oligosaccharides on the performance, intestinal microflora and serum

immunoglobulin contents in laying hens. Korean Journal of Poultry Science, 36(2), 125-131.

Kim, G. B., Seo, Y. M., Kim, C. H., & Paik, I. K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers (Version 2010/12/24). Retrieved from .

Kim, H. S., Kacew, S., & Lee, B. M. (1999). In-vitro chemopreventive effects of plant polysaccharides (Aloe barbandensis Miller, Ganoderma lucidum and Coriolus versicolor). Carcinogenesis, 20(81637e1640.

Kim, M.; Shin, H. K. (1998). The water soluble extract of chicory influences serum and liver lipid concentrations, cecal short chain fatty acid concentrations and fecal lipid excretion in rats. J. Nutr., 128: 1731–1736.

Knarreborg, A., Miquel, N., Granli, T., Jensen, B.B., 2002. Establishment anapplication of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. Animal Feed Science and Technology 99, 131–140.

Koch AL. Bacterial wall as target for attack: past, present, and future research. Clin Microbiol Rev 2003; 16: 673–687

Kok, N.; Roberfroid, M.; Delzenne, N. (1996). Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. Bri. J. of Nutr., 76: 881–890.

Kolida, S., & Gibson, G. R. (2011). Synbiotics in health and disease. Annual review of food science and technology, 2, 373-393.

Korakli, M., M. G. Gaenzle, and R. F. Vogel. 2002. Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by Lactobacillus sanfranciscensis. J.

Appl. Microbiol. 92:958–965.

Kues, U., & Liu, Y. (2000). Fruiting body production in basidiomycetes. Application Microbiology and Biotechnology, 54, 141e152.

Lam, Y. S., & Okello, E. J. (2015). Determination of Lovastatin, β-glucan, total polyphenols، and antioxidant activity in raw and processed Oyster culinary-medicinal mushroom, Pleurotus ostreatus (Higher Basidiomycetes). International Journal of Medicinal Mushrooms, 17, 117–128.

Le Marrec, C., Hyronimus, B., Bressollier, P., Verneuil, B. & Urdaci, M. C. 2000. Biochemical and genetic characterization of coagulin, a new antilisterial bacteriocin in the pediocin family of bacteriocins, produced by Bacillus coagulans I4. Applied and Environmental Microbiology, 66, 5213-5220.

Lee, Y. K., & Salminen, S. (2009). Handbook of probiotics and prebiotics. John Wiley & Sons.

Lei, X., Piao, X., Ru, Y., Zhang, H., Péron, A. & Zhang, H. 2015. Effect of Bacillus amyloliquefaciens-based Direct-fed Microbial on Performance, Nutrient Utilization, Intestinal Morphology and Cecal Microflora in Broiler Chickens. Asian-Australasian Journal of Animal Sciences (AJAS), 28, 239-246.

Li, C., Li, P., Tan, Y. M., Lam, S. H., Chan, E. C., & Gong, Z. (2016). Metabolomic characterizations of liver injury caused by acute arsenic toxicity in zebrafish. PLoS One, 11(3), e0151225.

Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez-Miyakawa, M. E., Chi, F., Cravens, R. L., ... & Gay, C. G. (2018). Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Veterinary research, 49(1), 76.

Lim, K. T., C. Hu, and D. D. Kitts. 2001. Antioxidant activity of a Rhus

verniciflua Stokes ethanol extract. Food Chem. Toxicol. .237–39:229

Liong, M. T. and Shah, N. P. (2006). “Effects of a Lactobacillus casei synbiotic on serum lipoprotein, intestinal microflora, and organic acids in rats,” J. of Dairy Sci., vol. 89, no. 5, pp. 1390–1399.

Liu XT,Winkler AL, SchwanWR, Volk TJ, Rott MA, Monte A. Antibacterial

Liu, L., Ni, X., Zeng, D., Wang, H., Jing, B., Yin, Z., & Pan, K. (2017). Effect of a dietary probiotic, Lactobacillus johnsonii BS15, on growth performance, quality traits, antioxidant ability, and nutritional and flavour substances of chicken meat. Animal Production Science, 57(5), 920-926.

Liu, X. Y.; Ma, J.; Park, C. M.; Chang, H. K.; Song, Y. S. (2008). Protective Effect of Dandelion Extracts on Ethanol-Induced Acute Hepatotoxicity in C57BL/6 Mice. J. of Food Sci. & Nutr., 13:269-75.

Liu, X., Yuan, J. P., Chung, C. K., & Chen, X. J. (2002). Antitumor activity of the sporederm-broken germinating spores of Ganoderma lucidum. Cancer Letters, 182, 155e161.

Lodemann, U. 2010. Effects of Probiotics on Intestinal Transport and Epithelial Barrier Function. Bioactive Foods in Promoting Health: Probiotics and Prebiotics. Waltham, USA: Academic Press .

Lolliger, J. 1991. The use of antioxidants in foods. Pages 121–150 in Free Radicals and Food Additives. O. I. Aruoma and B. Halliwell، ed. Taylor & Francis, London, UK.

Macfarlance, G. T.; Cummings, J. H. (1999). Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? Bri. Med. J. 318: 999–1003.

Macfarlane, G. T., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. Journal of applied microbiology, 104(2), 305-344.

Macfarlane, G. T., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. Journal of Applied Microbiology, 104, 305e..344

Macfarlane, S., Macfarlane, G. T., & Cumming, J. H. (2006). Review article: prebiotics in the gastrointestinal tract. Alimentary Pharmacology and Therapeutics, 24, 701e714.

Mahajna, J., Dotan, N., Zaidman, B. Z., Petrova, R. D., & Wasser, S. P. (2009).Pharmacological values of medicinal mushrooms for prostate cancer therapy: the case of Ganoderma lucidum. Nutrition and Cancer, 61(1), 16e26.

Mahfuz, S., He, T., Liu, S., Wu, D., Long, S., & Piao, X. (2019). Dietary inclusion of mushroom (Flammulina velutipes) stem waste on growth performance, antibody response, immune status, and serum cholesterol in broiler chickens. Animals, 9(9), 692.

Manning, T. S., & Gibson, G. R. (2004). Prebiotics. Best Practice and Research Clinical Gastroenterology, 18, 287e298.

Mansoub, N. H. (2010). Effect of probiotic bacteria utilization on serum cholesterol and triglycerides contents and performance of broiler chickens. Global Vet, 5: 184-186.

Manzoor, M., Asrar, M., Leghari, S. U. K., & Ahmed, Z. (2019). Nutritional Value Of Wild Mushrooms From Kharan District Of Balochistan, Pakistan. Fuuast Journal Of Biology, 9(2), 209-214.

Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut pathogens, 10(1), 21.

Marshall, B. M. and Levy S. B. (2011). Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev., 24:718–733.

Mattsson, A., Sjöberg, S., Kärrman, A., & Brunström, B. (2019). Developmental exposure to a mixture of perfluoroalkyl acids (PFAAs) affects the

thyroid hormone system and the bursa of Fabricius in the chicken. Scientific reports, 9(1), 1-14.

McReynolds, J. L., Byrd, J. A., Genovese, K. J., Poole, T. L., Duke, S. E., Farnell, M. B., & Nisbet, D. J. (2007). Dietary lactose and its effect on the disease condition of necrotic enteritis. Poult Sci, 86(8), 1656-1661.

Mehr, M.A.; Shargh, M.S.; Dastar, B.; Hassani, S. and Akbari, M.R. (2007). Effect of different levels of protein and Protexin on broiler performance. Int J Poultry Sci, 6: 573-577.

Midilli, M. & Tuncer, Ş. D. (2001). The effects of enzyme and probiotic supplementation to diets on broiler performance. Turk. J. Vet. Anim. Sci., 25: 895-903. (Turkish with English summary).

Mohan, B., R. Kadirvel, A. Natarajan and M. Bhaskaran، .1996 Effect of probiotic supplementation on growth، nitrogen utilization and serum cholesterol in broilers. Br. Poult. Sci., 37: 395-401.

Muszyńska, B., Kała, K., Firlej, A., & Sułkowska-Ziaja, K. (2016). Cantharellus cibarius culinary-medicinal mushroom content and biological activity. Acta Poloniae Pharmaceutica –. Drug Research, 73, 589–598.

Muszyńska, B., Sułkowska-Ziaja, K., & Wójcik, A. (2013). Levels of physiologically active indole derivatives in the fruiting bodies of some edible mushrooms (Basidiomycota) before and after thermal processing. Mycoscience, 54, 321–326.

Nabizadeh, A. (2012a). The effect of inulin inclusion in low phosphorus diets on some hematological, immunological parameters and broiler chickens performance. Res. J. of Animal Sci., 6: 60–66.

Nakalembe, I., Kabasa, J. D., & Olila, D. (2015). Comparative nutrient composition of selected wild edible mushrooms from two agro-ecological zones, Uganda. Vol. 4SpringerPlus .433

Nworu, C. S., Ihim, S. A., Ugwu, L. E., Laiyemo, K. A., & Akah, P. A. (2014).

Hepato-and nephroprotective activities of a Nigerian local king tuber oyster mushroom, Pleurotus tuberregium (higher Basidiomycetes), in chemically induced organ toxicities in rats. International journal of medicinal mushrooms, 16(4). 319-322.

Niba, A. T., Beal, J. D., Kudi, A. C., & Brooks, P. H. (2009). Bacterial fermentation in the gastrointestinal tract of non-ruminants: Influence of fermented feeds and fermentable carbohydrates. Tropical Animal Health and Production, 41(7), 1393-1407. doi: 10.1007/s11250-009-9327-6.

Okin, D., & Medzhitov, R. (2012). Evolution of inflammatory diseases. Current Biology CB, 22, 733–740.

Orban, J. I., Patterson, J. A., Sutton, A. L., & Richards, G. N. (1997). Effect of sucrose thermal oligosaccharide caramel, dietary vitamin-mineral level, and brooding temperature on growth and intestinal bacterial populations of broiler chickens. [Research Support, Non-U.S. Gov't]. Poult Sci, 76(3), 482-490.

Ozen T, Darcan C, Aktop O, Turkekul I. Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey. Comb Chem High Throughput Screen 2011; 14: 72–84.

Ozyurt B, Iraz M, Koca K, Ozyurt H and Sahin S. 2006. Protective effects of caffeic acid phenethyl ester on skeletal muscle ischemia-reperfusion injury in rats. Molecular Cell Biochemistry 292: 197–203.

Palframan, R., Gibson, G. R., & Rastall, R. A. (2003). Development of a quantitative tool for comparison of the prebiotic effect of dietary oligosaccharides. Letters in Applied Microbiology, 37, 281e284.

Panda A K, Reddy M R and Chawak M M. 2000. Dietary supplementation of probiotic on serum cholesterol,Gut microflora and performance of

broilers. Abstract Poultry Science Association.

Panda, A. K.; Ramarao, S. V.; Raju, M. V. L. N. & Sharma, S. R. (2006). Dietary supplementation of probiotic Lactobacillus sporogenes on performance and serum biochemico-lipid profile of broiler chickens. J. of Poult. Sci., 43: 235_240.

Pang, F. H., M. Q. Xie, and H. H. Ling. 2000. The investigation of immunodulators tested for the results on the control of a coccidial infection. Chin. J. of Vet. Parasitol. (in Chinese) .3–8:1

Park, B. S. (2008). Bifidogenic effects of inuloprebiotics in broiler chickens. J. of Life Sci., 18: 1693–1699.

Park, S. O.; Park, B. S. (2011). Effect of dietary microencapsulated-inulin on carcass characteristics and growth performance in broiler chickens. J. of Animal and Vet. Adv., 10: 1342–1349.

Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. Poultry science, 82(4), 627-631.

Peuranen, S., Tiihonen, K., Apajalahti, J., Kettunen, A., Saarinen, M., Rautonen, N., .2004Combination of polydextrose and lactitol affects microbial ecosystem and immune responses in rat gastrointestinal tract. British Journal of Nutrition 91,.914–905.

Pietras, M.; Barowicz, T. and Ocon, E. (2006). Polish J. of Natural Sci. Suppl., (3): 483-490.

Piray, A. H.; Kermanshahi, H.; Tahmasbi, A. M. & Bahrampour, J. (2007). Effects of cecal cultures and aspergillus meal prebiotic (fermacto) on growth performance and organ weights of broiler chickens. Int. J. Poult. Sci., 6: 340-344.

Pittet D. Infection control and quality health care in the new millennium Am J Infect Control 2005; 33: 258–267.

Piva. (1998). Non-conventional feed additives. J. Anim. Feed Sci., 7: 143–154.

Qureshi, S.; Banday, M. T.; Sheikh, A.; Shakee, I. and Munshi, Z. H. (2015). Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu and Kashmir190 006 India, Ind. J. of Animal Sci., 85 (11): 1248–1254,

Radu-Rusu, C. G., Pop, I. M., & Simeanu, D. (2010). Effect of a synbiotic feed additive supplementation on laying hens performance and eggs quality. Lucrări Ştiinţifice, Seria Zootehnie, 53, 89-93.

Rahman, A. U.; Khan, S.; Khan, D.; Hussain, M.; Ahmad, S.; Sohali, S. M.; Ahmed, I.; Haq, I. U. and Shah, Z. (2009). Use of probiotic in broiler feed at starter phase. arhad. J. Agric., 25(3):469-473.

Rana, K. L., Kour, D., Sheikh, I., Dhiman, A., Yadav, N., Yadav, A. N., ... & Saxena, A. K. (2019). Endophytic fungi: biodiversity, ecological significance, and potential industrial applications. In Recent advancement in white biotechnology through fungi (pp. 1-62). Springer, Cham.

Rea, M. C., Clayton, E., O'connor, P. M., Shanahan, F., Kiely, B., Ross, R. P. & Hill, C. 2007. Antimicrobial activity of lacticin 3147 against clinical Clostridium difficile strains. Journal of Medical Microbiology, 56, 940-946.

Reis, F. S., Martins, A., Vasconcelos, M. H., Morales, P., & Ferreira, I. C. (2017). Functional foods based on extracts or compounds derived from mushrooms. Trends in Food Science & Technology, 66, 48-62.

Reis, M. P., Fassani, E. J., Junior, A. G., Rodrigues, P. B., Bertechini, A. G., Barrett, N., ... & Schmidt, C. J. (2017). Effect of Bacillus subtilis (DSM 17299) on performance, digestibility, intestine morphology, and pH in broiler chickens. Journal of Applied Poultry Research, 26(4), 573-583.

Rycroft, C. E., Jones, M. R., Gibson, G. R., & Rastall, R. A. (2001). A

comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. Journal of Applied Microbiology, 91، 878e887.

Reisinger, N., Ganner, A., Masching, S., Schatzmayr, G., & Applegate, T. J. (2012). Efficacy of a yeast derivative on broiler performance, intestinal morphology and blood profile. Livestock Science, 143(2-3), 195-200. doi:

Ripon, M. M. R., Rashid, M. H., Rahman, M. M., Ferdous, M. F., Arefin, M. S., Sani, A. A., ... & Rafiq, K. (2019). Dose-dependent response to phytobiotic supplementation in feed on growth, hematology, intestinal pH, and gut bacterial load in broiler chicken. Journal of Advanced Veterinary and Animal Research, 6(2), 253.

Roberfroid, M. B. (2000). Prebiotics and probiotics: Are they functional foods? Am J Clin Nutr, 71(suppl), 1682S-1687S.

Rosa LH, Machado KMG, Jacob CC, Capelari M, Rosa CA, Zani CL. Screening of Brazilian Basidiomycetes for antimicrobial activity.Mem Inst Oswaldo Cruz 2003; 98: 967–974

Rossi, M.; Corradini, C.; Amaretti, A.; Nicolini, M.; Pompei, A.; Zanoni, S.; Matteuzzi, D. (2005). Fermentation of Fructooligosaccharides and Inulin by Bifidobacteria: A Comparative Study of Pure and Fecal Cultures. Appl. Environ. Microbiol. 71: 6150-6158.

Salanitro, J.P., Blake, I.G., Muirhead, P.A., Magliom, M., Goodman, J.R., 1978. Bacteria isolated from the duodenum, ileum and cecum of young chicks. Applied and Environmental Microbiology 35, 782–790.

Samanta, A. K.; Jayapal, N.; Senani, S.; Kolte, A. P.; Sridhar, M. (2013). Prebiotic inulin: useful dietary adjuncts to manipulate the livestock gut microflora. Brazil. J. Microbiol., 44: 1–14.

Samanta, A. K.; Senani, S.; Kolte, A. P.; Sridhar, M.; Bhatta, R.; Jayapal, N.

(2012). Effect of prebiotic on digestibility of total mixed ration. Indian Vet. J., 89: 41–42.

Santoso, U., Tanaka, K., & Ohtani, S. (1995). Effect of dried Bacillus subtilis culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. British Journal of Nutrition, 74(4), 523-529.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the united states—major pathogens. Emerging Infectious Diseases, 17(1), 7-15. doi: 10.3201/eid1701.P11101.

Schmidt, E. B., Christensen, J. H. and Ardestrup, L. 2001. Marine ω-3 fatty acid. Basic features and background. Lipids 36:65-68.

Segal-Maurer S, Urban C, Rahal Jr. JJ. Current perspectives on multidrugresistant bacteria. Epidemiology and control. Infect Dis Clin North Am ؛1996 957–939 :10

Silveira G, Nome F, Gesser JC, Sá MM, Terenzi H. Estratégias utilizadas no combate a resistência bacteriana. Quím Nova 2006; 29: 3–7

Singhal, S., Rasane, P., Kaur, S., Singh, J., & Gupta, N. (2020). Thermal degradation kinetics of bioactive compounds in button mushroom (Agaricus bisporus) during tray drying process. Journal of Food Process Engineering, e13555.

Savoie, J. M., & Largeteau, M. L. (2011). Production of edible mushrooms in forests: trends in development of a mycosilviculture. Applied microbiology and biotechnology, 89(4), 971-979.

Salmerón, I., Thomas, K., & Pandiella, S. S. (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. Journal of Functional Foods, 15, 106-115.

Salim, H. M., Kang, H. K., Akter, N., Kim, D. W., Kim, J. H., Kim, M. J., ... & Kim, W. K. (2013). Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poultry science, 92(8), 2084-2090.

Sadeghi, A. A., Shawrang, P., & Shakorzadeh, S. (2015). Immune response of Salmonella challenged broiler chickens fed diets containing Gallipro®, a Bacillus subtilis probiotic. Probiotics and antimicrobial proteins, 7(1), 24-30.

Simmering, R., & Blaut, M. (2001). Pro-and prebiotics–the tasty guardian angels?. Applied Microbiology and Biotechnology, 55(1), 19-28.

Smits, C.H.M., Veldman, A., Verkade, H.J., Beynen, A.C., 1998. The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. Poultry Science 77, 1534–1539.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., Shabbir, M. Z., & Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci, 91(9), 2235-2240. doi: 10.3382/ps.2012-02182.

Solak MH, Kalmis E, Saglam H, Kalyoncu F. Antimicrobial activity of two wild mushrooms Clitocybe alexandri (Gill.) Konr. and Rhizopogon roseolus.)Corda) T.M. Fries collected from Turkey. Phytother Res 2006; 201087–1085:

Soldin S. J.; Brugnara, C.; Wong, E. C. (2003). Pediatric reference Ganges, 4th ed, AACC Press, 10-11.

Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. (2000). The effects of

dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poultry science, 79(2), 205-211.

Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. (2000). The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poult Sci, 79(2), 205-211.

Stanley, D., Hughes, R. J., & Moore, R. J. (2014). Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Applied microbiology and biotechnology, 98(10), 4301-4310.

Stringfellow, K., McReynolds, J., Lee, J., Byrd, J., Nisbet, D., & Farnell, M. (2009). Effect of bismuth citrate, lactose, and organic acid on necrotic enteritis in broilers. Poult Sci, 88(11), 2280-2284. doi: 10.3382/ps.2008-00456.

Swanson, K. S., Grieshop, C. M., Flickinger, E. A., Bauer, L. L., Healy, H.-P., Dawson, K. A., Merchen, N. R., & George C. Fahey, J. (2002). Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. The Journal of Nutrition, 132, 980 - 989.

Synytsya, A., Mickova, K., Synytsya, A., Jablonsky, I., Spevacek, J‹.Erban, V., et al. (2008). Glucans from fruit bodies of cultivated mushrooms Pleurotus ostreatus and Pleurotus eryngii: structure and potential prebiotic activity. Carbohydrate Polymers. doi:10.1016/j.carbpol.2008.11.021.

Tako, E., & Glahn, R. P. (2012). Intra-amniotic administration and dietary inulin affect the iron status and intestinal functionality of iron-deficient broiler chickens. [Clinical Trial]. Poult Sci, 91(6), 1361-1370. doi:

10.3382/ps.2011-01864.

Tam, N. K., Uyen, N. Q., Hong, H. A., Duc, L. H., Hoa, T. T., Serra, C. R., Henriques, A. O. & Cutting, S. M. 2006. The intestinal life cycle of Bacillus subtilis and close relatives. Journal of bacteriology, 188, 2692-2700.

Tan, X.; Sun, Z.; Chen, S.; Huang, Z.; Zhou, C.; Zou, C.; Liu, Q.; Ye, H.; Ye, C.; Wang, A. (2017): Effects of dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile golden pompano Trachinotus ovatus. Fish and Shellfish Immun., 4648(17): 30268-1.

Tao, Y., Zhang, L., & Cheung, P. C. K. (2006). Physicochemical properties and antitummor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. Carbohydrate Research, 341, 2261e2269.

Teo, A. Y.-L. & Tan, H.-M. 2005. Inhibition of Clostridium perfringens by a novel strain of Bacillus subtilis isolated from the gastrointestinal tracts of healthy chickens. Applied and Environmental Microbiology, 71, 4185-4190.

Tietz, N.W. (1995). ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: W.B. Saunders, 76.

Tong, H., Xia, F., Feng, K., Sun, G., Gao, X., Sun, L., et al. (2009). Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of Pleurotus ostreatus. Bioresource Technology, 100, 1682e1686.

Tsai, S. Y., Huang, S. J., Lo, S. H., Wu, T. P., Lian, P. Y& ‹. Mau, J. L. (2009). Flavour components and antioxidant properties of several cultivated mushrooms. Food Chemistry, 113, 578e 584

Tsirtsikos, P.; Fegeros, K.; Balaskas, C.; Kominakis, A. and Mountzouris, K.C.

(2012). Dietary probiotic inclusion level modulates intestinal mucin composition and mucosal morphology in broilers. Poultry Science, 91: 1860–1868.

Vamanu, E., & Vamanu, A. (2010). The influence of prebiotics on bacteriocin synthesis using the strain Lactobacillus paracasei CMGB16. African Journal of Microbiology Research, 4(7), 534-537.

Van Loo, J. (2004). The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. Nutr. Res. Rev., 17: 89-98.

Van Loo, J. (2006). Inulin-type fructans as prebiotics. In G. R. Gibson & R. A. Rastall (Eds.), Prebiotics: Development and application)pp. 59). Hoboken, NJ: John Wiley & Sons.

Vetter, J. (2007). Chitin content of cultivated mushrooms Agaricus bisporus, Pleurotus ostreatus and Lentinula edodes. Food Chemistry6 ،102 e9

Vetter, J., and J. Lelley. 2004. Selenium level of the cultivated mushroom Agaricus bisporus. Acta Aliment. 33:297–301 Falandysz, G. 2008. Selenium in edible mushrooms. J. Environ. Sci.Health C 26:256–299.

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Frontiers in microbiology, 10, 57.

Wagner, J. R., C. C. Hu, and B. N. Ames. 1992. Endogenous oxidative vitro antioxidant, anticholinesterase and antimicrobial activity studies on three Agaricus species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms. Food Chem Toxicol 2011; 49: 1353–1360

Wang R. J., D. F. Li, and S. Bourne. 1998. Can 2000 years of herbal medicine history help us solve problems on the year ؟2000Pages 273–291 in

Biotechnology in the Feed industry. Proceedings of Alltech's 14th Annual Symposium. University Press, Nottingham, UK.

Wang, D., & DuBois, R. N. (2015). Immunosuppression associated with chronic inflammation in the tumor microenvironment. Carcinogenesis, 36, 1085–1093.

Wang, H. X., Ooi, V. E., Ng, T. B., Chiu, K. W. and Cang, S. T. 1996. Hypotensive and vasorelaxing activities of a lectin from the edible mushroom Tricholoma magnivelare. Pharmacol. Toxicol. 79:318-323.

Wang, J., & Zhang, L. (2009). Structure and chain conformation of five water-soluble derivatives of a b-D-glucan isolated from Ganoderma lucidum. Carbohydrate Research, 344, 105e112.

Wang, Y.; Chen, Y. J.; Cho, J. H.; Yoo, J. S.; Wang, Q.; Huang, Y.; Kim, H. J.; Kim, I. H. (2007): The effects of dietary herbs and coral mineral complex on growth performance, nutrient digestibility, blood characteristics and meat quality in finishing pigs. J. Anim. Feed Sci., 16: 397–407.

Warren, J. S.; Ward, P. A. (1986). Oxidative injury to the vascular endothelium. Am. J. Med. Sci., 292: 97–103.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immonomudulating polysaccharides. Applied Microbiology and Biotechnology, 60, 258e274.

Wenk, C. (2003). Herbs and botanicals as feed additives in monogastric animals. Asian-Aust. J. Anim. Sci., 16: 282–289.

WHO. World Health Organization report – Antimicrobial resistance (AMR)./2010amr_20100820/en/index.html. Accessed January 21, 2012

WHO. World Health Organization report on infectious diseases 2000 Overcoming antimicrobial resistance. int/infectious-disease-report. Accessed

January 21, 2012

Wilson, T. A.; Nicolosi, R. J.; Rogers, E. J.; Sacchiero, R. and Goldberg, D. J. (1998). Studies of Cholesterol and bile acid metabolism and early atherogenesis in hamsters fed GT16 239, a novel bile acid sequestrant (BAS), Atherosclerosis, 40: 315-324.

Waneck, C. R. (2011). Preventive Measures to Control Clostridial Outbreaks of Gangrenous Dermatitis in Commercial Broiler Operations (Doctoral dissertation, Texas A & M University.

Wang, Y., Al Farraj, D. A., Vijayaraghavan, P., Hatamleh, A. A., Biji, G. D., & Rady, A. M. (2020). Host associated mixed probiotic bacteria induced digestive enzymes in the gut of tiger shrimp Penaeus monodon. Saudi Journal of Biological Sciences, 27(9), 2479-2484

Willis, W. L., Isikhuemhen, O. S., & Ibrahim, S. A. (2007). Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poultry Science, 86(9), 1856-1860.

Windisch, W.; Schedle, K.; Plitzner, C.; Kroismayer, A. (2008). Use of phytogenetic products as feed additives for swine and poultry. J. Anim. Sci., 86: E140–E148.

Wolever, T.; Spadafora, P.; Cunnane, S.; Pencharz, P. (1995). Propionate inhibits incorporation of colonic [l,2-l3C] acetate into plasma lipids in humans. Americ. J. Clinical Nutr., 61: 1241–1247.

Xiao, R., Power, R. F., Mallonee, D., Routt, K., Spangler, L., Pescatore, A. J., Cantor, A. H., Ao, T., Pierce, J. L., & Dawson, K. A. (2012). Effects of yeast cell wall-derived mannan-oligosaccharides on jejunal gene expression in young broiler chickens. Poult Sci, 91(7), 1660-1669. doi: 10.3382/ps.2011-02035

Xie, Z. Q., and S. Q. Niu. 1996. Comprehensive Book of Natural Resources and Chinese Herb Feed Additives (in Chinese. Xueyuan Science and

Technology Press, Beijing, China.

Xu, Z. R., Hu, C. H., Xia, M. S., Zhan, X. A., & Wang, M. Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry science, 82(6), 1030-1036.

Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang، .2003Effects of dietary fructo oligosaccharide on digestive enzyme activities. Intestinal Microflora and morphology of male broilers. J. Anim. Sci., 82: 1030-.1036.

Xue, G. D., Barekatain, R., Wu, S. B., Choct, M., & Swick, R. A. (2018). Dietary L-glutamine supplementation improves growth performance, gut morphology, and serum biochemical indices of broiler chickens during necrotic enteritis challenge. Poultry science, 97(4), 1334-1341.

Xue, M., and X. S. Meng. 1996. Review on research progress and prosperous of immune activities of bio-active polysaccharides.

Yeo, J. & Kim, K. (1997). Effect of feeding diets containing an antibiotic, a probiotic, or Yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci. 76: 381-385.

Yoshioka, Y., Tabeta, R., Saito, H., Uehara, N. and Fukuoka, F 1998.Antitumor polysaccharides from P. ostreatus (Fr.) Quel. isolation and structure of a â-glucan. Carbohydrate Res. 140: 93-100.

Yu, J. G., and L.Y. Zhu. 2000. The use of Astragalus polysaccharide against infectious bursa disease in chickens. J. Trad Chin. Vet. Med. (in Chinese). 6:3–4.

Yuan, Y. L., B. T. Fang, and Y. X. Zhang. 1993. Efficacy of 8301 polysaccharides as an adjuvant against Avian Pasteurellosis in chickens. J. Trad. Chin. Vet. Med. (in Chinese). 3:6–10.

Yusrizal Chen, T. C. (2003). Effect of adding chicory fructans in feed on broiler

growth performance, serum cholesterol and intestinal length. Inter. J. of Poult. Sci., 2: 214–219.

Zakeri, A., & Kashefi, P. (2011). The comparative effects of five growth promoters on broiler chickens humoral immunity and performance. Journal of Animal and Veterinary Advances, 10(9), 1097-1101. doi: 10.3923/javaa.2011.1097.1101.

Zdunczyk, Z., Jankowski, J., Juskiewicz, J., & Slominski, B. A. (2011). Dietary content and gastrointestinal function of soybean oligosaccharides in monogastric animals. In Soybean-Biochemistry, Chemistry and Physiology. IntechOpen.

Zhang, C. (2004). Evaluation of guar meal as a source of preboitic galactomannans for laying hens. (Doctor of Philosophy Dissertation), Texas A&M University, College Station, TX.

Zhang, Z. F.; Cho, J. H. and Kim, I. H. (2013). Effects of Bacillus subtilis, ubt-mo 2, on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Livestock Sci., 155:343–347.

Zhang, Z. F.; Zhou, T. X.; Ao, X. and Kim, I. H. (2012). Effects of β- glucan and Bacillus subtilis, on growth performance, blood profiles, relative organ weiht and meat quality in broilers fed maize–soybean meal based diets. Livestock Sci., 150:419–424.

Appendix

Appendices (1)

3.2.1 Biochemical tests

3.2.1.1 Cholesterol estimation (mg/dl):

Principle:

Easter of cholesterol+H_2O $\xrightarrow{Chol.\ estease}$ Cholesterol + Fatty acids

Cholesterol +O_2 $\xrightarrow{Chol.\ oxidase}$ Cholest-4-en-one+H_2O_2

H2O+4-Aminophenazone + phenol $\xrightarrow{peroxidase}$ Quinonimine

Reagent:

Reagent (1) Buffer solution: pipes PH 6.9 mmol/L, phenol 26 mmol/L

Reagent (2) vial of enzyme: cholesterol oxidase 300 U/L, peroxidase 1250 U/L, cholesterol esterase 300 U/L, 4-aminophenazone 0.4 mmol/L

Reagent (3): cholesterol standard 200 mg/dl

1. **Manual procedure:** Cholesterol concentration in serum samples was measured according to the following

a. Reagent and serum samples were brought to room temperature

b. Serum sample, blank and standard were treated as follow:

c. Tube contents were mixed and left to stand for 5 minutes at 37°C before reading.

d. the absorbance of the standard was measured and sample was read via spectrophotometer at wavelength 505 nm against the blank

Tubes	Blank	Standard	Sample
Cholesterol standard (s)	-	10 ml	-
Sample	-	-	10 ml
Working Reagent	1 ml	1 ml	1 ml

Calculation:

Result were calculated according to the following equation:

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard)× nn = 200 mg/dl

Appendices (2):

3.2.1.2Triglyceride estimation (mg/dl):

Principle:

Triglyceride $\xrightarrow{lipoprotein\ lipase}$ Glycerol + fatty acid

Glycerol + ATP $\xrightarrow{Glycerol\ kinase,Mg++}$ Glycerol-3-phosphate+ADP

Glycerol-3-P+O_2 $\xrightarrow{3-G-P-oxidase}$ Dihydroxyacetone ne-p+H_2O

H2O2+4-Aminophenazone+p+Chlorophenol$\xrightarrow{peroxidase}$Quinonimine+ H_2O

Reagent:

Reagent (1) buffer solution: pipes buffer PH 7.2, 50 mm0l/L, p- chlorophenol 2 mmol/L

Reagent (2) Enzyme: lipoprotein lipase 150 000 U/I, glycerol kinase 800 U/I, glycerol-3-phosphate oxidase 4000 U/I, peroxidase 440 U/I, 4-aminophenazone 0.7 mmol/L, ATP 0.3 mmol/L.

Reagent (3) triglyceride Standard (S): Glycerol 200mg/dl.

Procedure:

Triglyceride concentration in serum samples was measured according to the following:

a. Wave length/filter. 505nm (Hg546nm)/green
b. Temperature 37°C/R. T
c. Light path 1 cm

Pipette into clean dry test tubes labelled as Blank (B), standard (S), and Test (T).

Mix well and incubated at 37° C for 5 min or at R. T (25° C) for 15min. measure the absorbance of the standard

Calculation:

Results were calculated according to the following equation:

Triglyceride concentration mg/dl = (O.D sample) / (O.D standard) × n = 200 mg/dl

Additive sequence	Blank	Standard	Test
Working reagent	1.0	1.0	1.0

Distilled water	0.01	-	-
Triglyceride standard	-	0.01	-
Sample	-	-	0.01

Appendices (3):

3.2.1.3 HDL-Cholesterol estimation (mg/dl):

Principle:

Cholesterol esters + H_2O $\xrightarrow{Chol.esterase}$ Cholesterol + fatty acid

Cholesterol +½O_2 + H_2O $\xrightarrow{Chol.oxidase}$ Cholestenone + H_2O_2

2 H2O2 + 4-Aminoantipyrine + DCFS $\xrightarrow{peroxidase}$ Quinoneimine + 4H_2O

Reagent:

Reagent (1) Good's buffer (pH 6.6)100 mmol/l, cholesterol esterase 1400 U/l, cholesterol oxidase 800 U/l, catalase 600 kU/l, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) 0.6 mmol/l

Reagent (2) Good's buffer (pH 7.0) 100 mmol/l peroxidase 3 kU/l 4–aminoantipyrine (4-AA) 4 mmol/L

Procedure:

HDL-Cholesterol concentration in serum sample was measured according to the following steps: serum sample 40 – 60 mg/dl 1.04 1.55mmol/l, wavelength 600 nm, temperature 37°C CORMAY HDL DIRECT is intended for automated analysers.

a. Reagent (A, B) and serum sample were brought to room temperature.
b. Serum sample, blank and standard were treated as followed:
c. 0.2 ml of sample was mixed with 0.5 ml of reagent (A) in centrifuge tube and let stand for 10 minute at room temperature.

d. Centrifuged at a minimum of 4000 r.p.m. for 10 minutes.
e. The temperature was collected carefully.
f. Sample supernatant, blank, standard and reagent (B)were treated as follows:
g. Tubes contents were mixed thoroughly and incubated for 10 minute at 37°C.
h. the absorbance (A) of the standard was measured and sample was read via spectrophotometerat wave length 500 nm against the blank.

Calculation: results were calculated according to the following equation:

HDL-cholesterol concentration in the sample (mg/dl) = (Absorbance of the sample/Absorbance of standard) × concentration of standard × sample dilution factor (1.7).

Appendices (4):

3.2.1.4 LDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol ester $\xrightarrow{chol.esterase}$ chol. + fatty acid

Cholesterol + $O_2 \xrightarrow{Chol.oxidase}$ chol. H_2O_2

$2H_2O_2 \xrightarrow{catalase} H_2O + O_2$

Reagent:

Reagent (1) Good's buffer (pH 7,0) 50 mmol/l, cholesterol esterase 600 U/l, cholesterol oxidase 500 U/l, catalase 1200 kU/l, ascorbate oxidase 3 kU/l, TOOS [N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline] 2.0 mmol/l

Reagent (2) Good's buffer (pH 7,0) 50 mmol/l, peroxidase 5 kU/l, 4–aminoantipyrine (4-AA) 4 mmol/l.

Procedure:

wavelength 600 nm, temperature 37°C, CORMAY LDL DIRECT is intended for automated analysers. serum/plasma < 100 mg/dl < 2.59 mmol/l.

As LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should

establish its own reference ranges for local population.

Calculation:

A comparison between LDL cholesterol values determined at Biolis

24i Premium (y) and at COBAS INTEGRA 400 (x) using 52 samples
gave following results:
y = 0.9642 x - 0.8968 mg/dl;
R = 0.9762 (R – correlation coefficient)

Appendices (5):

3.2.2 Liver enzymes concentration:
3.2.2.1Alkaline phosphatase (ALP) activity determination:
Principle:
Kinetic method recommended by International Federation of Clinical Chemistry (IFCC).

2-amino-2-methyl-1-propanol + p-nitrophenylophosphate + H2O $\xrightarrow{ALP}$
4-nitrophenol + 2-amino-2-methyl-1-propanol phosphate

The rate of 4-nitrophenol formation is directly proportional to the ALP activity.

REAGENTS:
Reagent (1) 2-amino-2 methyl-1-propeunol (AMP) 350 mmol/l
Reagent (2) Mg2+ 2.0 mmol/l

	Liquick cor-ALP 500	Liquick cor-ALP "bulk"
1. ALP	3×400 ml	--
2.ALP	1×300 ml	--

Reagent (3) Zn2+ 1.0 mmol/l
Reagent (4) HEDTA 20 mmol/l
Reagent (5) p-nitrophenylphosphate 16.o mmol/l

PROCEDURE
These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers. Applications for them are available on request.
Manual procedure:

Wavelength 410 nm (405/412nm)
Temperature 37°C
cuvette 1 cm

Sample start method:

pipette into cuvette, working reagent 1000µl, bring up the temperature of determination. Then add sample 18µl. Mix and

incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly 1, 2 and 3 minutes. Calculate the mean absorbance change per minute (□A/min.).

Reagent start method:

the determined can be also performed with use of separate 1-ALP and 2-ALP reagents. Pipette into the cuvette 1-ALP 1000µl, bring up to the temperature of determined. Then add sample 17µl. mix well, incubate for 1min. then add 2- ALP 25µl, mix well perform measurement as described for sample start method.

Calculation:

ALP activity [U/I] =
□A/min. x F
Sample start method F=3038
Reagent start method F=3442

Appendices (6):

3.2.2.2 Aspartate aminotransferase (AST) activity determination:

Aspartate aminotransferase activity is (ASAT, AST, GOT) Measured by cormay GOT kit produced by PZ CORMAY S.A. company (Tietz, 1995).

Principle:

L-aspartate + 2-oxoglutarate <ASAT> oxalacetate + L-glutamate
oxalacetate + NADH + H+ <MDH > malate + NAD+

The rate of absorbance changing at λ=340 nm is directly proportional to aspartate aminotransferase activity.

	(24-TRAY)	(36-TRAY)
1-Reagent	6 x 40 ml	8 x 23 ml
2- -Reagent	6 x 12.5 ml	8 x 7.5 ml

Reagent:
Tris (pH 7.8) 80 mmol/l, L-aspartate 240 mmol/l, MDH > 10 µkat/l, LDH > 20 µkat/l, 2-oxoglutarate 15 mmol/l, NADH 0.18 mmol/l

ROCEDURE
These reagents may be used in automatic analysers Prestige 24i, Biolis 24i and Sapphire 400.
1-REAGENT and 2-REAGENT are ready to use.
1-Reagent put on basic position in reagent tray.
2-Reagent put on start position in reagent tray. For reagent blank deionized water is recommended. Reagent blank is required each day

Parameter	Liquick Cor-ALAT 500	Liquick Cor-ALAT "bulk"
1-ALAT	3 x 400 ml	--
2-ALAT	1 x 300 ml	--

Calculation:
A comparison between ASAT values for samples obtained on
Prestige 24i (y) and obtained on COBAS INTEGRA 400 (x) using
100 samples gave following results:
y = 1.1501 x - 2.8845 U/l;
R= 0.9972 (R - correlation coefficient)

Appendices (7):

3.2.2.3 Serum Alanine aminotransferase (ALT) activity determination:

Principle:
L-alanine + 2-oxoglutarate $\xrightarrow{ALAT}$ pyruvate + L-glutamate

pyruvate + NADH + H+ $\xrightarrow{LDH}$ lactate + NAD+

The rate of absorbance changing at l=340 nm is directly proportional
to alanine aminotransferase activity.

Reagent:
Tris (pH 7.5) 100 mmol/l, L-alanine 500 mmol/l, LDH > 36.7 µkat/l 2-oxoglutarate 15 mmol/l, NADH 0.18 mmol/l

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers.
Applications for them are available on request.

Simple start method:

Pipette into the cuvette. working reagent 1000µl, bring up to the temperature of determination. Then add simple 100µl, Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly1, 2 and 3 minutes. Calculate the mean absorbance change per minute (DA/min.).

Calculation:

ALAT activity [U/l] = DA/min. x F

Appendices (8):

3.3 Immunological tests:

3.3.1Serological test: Enzyme Linked Immunosorbent Assay (ELISA)

Principle:

Serum obtained from chickens exposed to Newcastle disease virus contain specific anti-NDV antibodies. Serum, diluted in dilution buffer, is added to an NDV antigen coated plate. Specific NDV antibody in the serum forms an antibody-antigen complex with the NDV antigen bound to the plate. After washing the plate, an infinity purified goat anti-chicken IgG (H+L) peroxidase conjugate is added to each well. The antibody-antigen complex remaining from the previous step binds with the conjugate. After a brief incubation period, the unbound conjugate is removed by a second wash step. Substrate, which contain a chromagen (ABTS), is added to each well. Chromagen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. The relative intensity of color developed in 15 minute (compared to control) is directly proportional to the level of NDV antibody in the serum. After the substrate has incubated, stop

solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at 405-410 nm (Alexander, 2008). Determined by ProFLOK Kit produced by SYNBIOTIC USA/Canada company

Reagent:

a. 1 NDV antigen coated plate
b. 10 µl NDV positive control serum
c. 10 µl Normal control serum (NCS)
d. 100 µl Goat Anti-Chicken IgG (H+L) Peroxidase conjugate solution
e. 40 ml dilution Buffer
f. 10 ml ABTS-Hydrogen Peroxidase Substrate solution
g. 2.5 ml 5X Stop solution (dilute [1:5] with laboratory grade water)
h. 20 ml 20X Wash Solution (dilute [1:20] with laboratory grade water)

Procedure:

Preparation the test plate

a. Remove an NDV antigen test plate from the productive bag and label according to dilution plate identification
b. Add 50 µl dilution buffer to all wells on the test plate.
c. Add 50 µl diluted NDV position control serum to wells A1, A3 and H11. Discard pipette tip.
d. Using an 8 or 12 channel pipette transfer 50 µl/well of each of the diluted serum samples and normal control serum samples from the dilution plate to the corresponding wells of the NDV coated test plate. Discard pipette tips after each row of sample is transferred, transfer of samples to the ELISA plate should be done as quickly as possible.
e. Incubate plate for 30 minute at room temperature.

Wash procedure:

a. Tap out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.

b. Using an 8 or 12 channel pipette (or comparable automatic washing device), fill each well with approximately 300 µl wash solution. Allow to soak in wells for 3 minute: then discard contents into appropriate waste container (waste container should contain bleach solution), tap inverted plate to ensure that all residual liquid is removed.

Calculation:

Calculate a sample to positive (Sp) ratio by equation format:

$$Sp = \frac{(SAMPLE\ ABSORBANCE) - (AVG\ NORMAL\ CONTROL\ ABSORBANCE)}{CORRECTED\ POSITIVE\ CONTROL\ ABSORBANCE}$$

An NDV ELISA titer can be calculated by the following suggested equation:

LOG_{10} TOTER = (1.464×LOG_{10} Sp) + 3.740 TITER = ANTILOG OF LOG_{10} TITER.

الخلاصة

قد تتأثر الاستجابة المناعية وأداء الدجاج اللحم بالصحة البدنية ومستويات التحدي ، وإنتاج دجاج التسمين صناعة تتطور بشكل ديناميكي ، وتعتمد معظم الأعلاف على العزز الحيوي والسابق الحيوي كمصدر للمكملات الغذائية. لذلك تهدف هذه الدراسة إلى استكشاف تأثير المعزز الحيوي (Bacillus subtilis) والسابق الحيوي مثل الفطر (Agaricus bisporus) أو الجمع بينهما في تحسين المناعة الخلطيه والخلوية ، بالإضافة إلى صحة وأداء دجاج التسمين. تم تقسيم إجمالي 160 فروج لحم عمر واحد يوم ،سلالة (روس 308) ، بشكل عشوائي إلى أربع مجموعات وتم تصميمها لتكون أربع مكررات. تم تغذية المجموعة القياسيه (Con) بنظام غذائي أساسي بدون أي إضافات. تم تغذية مجموعة الفطر (Mush) بالنظام الغذائي الأساسي مع (1٪ مسحوق عيش الغراب المجفف). تم تغذية مجموعة Bacillus subtilis (BS) بنظام غذائي أساسي مع 4 × 10^9 cfu/gm ككمعزز حيوي . تم تغذية المجموعة المدمجة (Mush-BS) على نظام غذائي أساسي بنسبة 1 ٪ من مسحوق الفطر المجفف مع نظام غذائي أساسي يحتوي على 4 × 10^9 cfu / gm Bacillus. بدأت الفترة التجريبية من 2020/1/4 إلى 2020/2/7 ، واستمرت لمدة 5 أسابيع. تم جمع عينات الدم في 9 و 18 و 35 يومًا من الدراسة للفحص المناعي والدموي. أظهرت النتائج أن تراكيز إنزيم الكبد AST و ALT و ALP لمجموعات (Mush-BS) و (Mush) و (BS) كانت أقل معنوياً ($P\leq0.05$) مقارنة بمجموعة القياسية (CON). خلاف ذلك ، كانت تركيزات الدهون الثلاثية TG والكوليسترول والبروتين الدهني منخفض الكثافة (LDL، vLDL) للمجموعات المعينة (Mush-BS) و (Mush) و (BS) أقل معنوبة ($P\leq0.05$). ومع ذلك ، بالمقارنة مع (CON) ، زاد تركيز البروتين الدهني عالي الكثافة (HDL) لمجموعات (Mush-BS) و (Mush) و (BS) بشكل ملحوظ ($P\leq0.05$). تم تحسين الامينوكلوبنين IgG المناعي العضدي ضد مرض نيوكاسل ND والتهاب الشعب الهوائية المعدي بشكل ملحوظ ($P\leq0.05$) في مجموعات (Mush-BS) و (Mushroom) و (BS) على التوالي مقارنة مع (Control) في العمر التاسع و الثامن عشر يوما.

اختلف أداء نمو الفراريج بشكل كبير ($P\leq0.05$) بين (Mush-BS) و (Mush) و (BS) في وزن الجسم ، وزيادة الوزنيه ، وتناول العلف ، ونسبة تحويل العلف اختلافات كبيرة ($P\leq 0.05$) مقارنة مع السيطره (CON) من ناحية أخرى ، أظهرت المعلمات المورفولوجية لعمق خبايا الأمعاء الدقيقة وارتفاع الزغابة اختلافًا كبيرًا ($P\leq0.05$) في مجموعات Mush-BS مقارنة بمجموعات السيطرة . أظهرت النتائج وجود فروق غير معنوية ($P\geq0.05$) في المجموعة السيطرة لمؤشر الطحال والجراب مقارنة بمجموعتي (Msh) و (BS) والمجموعات المركبة (Mush + BS) بشكل عام ، وأظهرت النتائج فروق معنوية ($P\leq 0.05$) في المجموعة السيطرة لقياس الأس الهيدروجيني بدلاً من مجموعات (MSH) و (BS) والمجموعات المركبة (Mush + BS).

كما تم العثور على فروق ذات دلالة إحصائية ($P\leq0.05$) مقارنة مع مجموعة التحكم في نسبة H / L بين مجموعات (MSH) و (BS) والمجموعات المركبة (Mush + BS).

في الختام ، فإن إضافة مزيج من المعزز الحيوي والسابق الحيوي إلى نظام الغذائي الأساسي في فروج اللحم يمكن أن يحسن الاستجابة المناعية عن طريق تعزيز المناعة الخلوية والخطية. بالإضافة إلى ذلك ، فهو يحسن توزيع الدهون ، وإنزيمات الكبد ، وأداء دجاج التسمين.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب البيطري

تأثير مسحوق الفطر المجفف (Agaricus bisporus)‘ (Bacillus subtilis) ومزيجها على الأداءالانتاجي وبعض الصفات الفسيولوجية في الدجاج اللاحم

رسالة

مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في علوم الفسلجة والأدوية البيطرية

من قبل

أمير حميد كاظم الشيخ

بإشراف

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2020 ميلادي