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Effects Of Phase Feeding Different Levels Of Fungus Myceliated Grain On Broiler Performance And Health

Joi N. Jackson
North Carolina Agricultural and Technical State University

Effects of Phase Feeding Different Levels of Fungus Myceliated Grain on Broiler Performance and Health

Joi N. Jackson

North Carolina A&T State University

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Joi N. Jackson

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Approved by:

_____	_____
Dr. Willie Willis Major Professor	Dr. Mulumebet Worku Committee Member
_____	_____
Dr. Radiah Minor Committee Member	Dr. Sang Oh Committee Member
_____	_____
Dr. Omoanghe Isikhuemhen Committee Member	Dr. Ralph Noble Department Chairperson

_____
Dr. Sanjiv Sarin
Dean, The Graduate School

Biographical Sketch

Joi N. Jackson was born in Orange, New Jersey on January 4, 1984. She was in the first graduating class of the Appomattox Regional Governors School in Petersburg, Virginia where she graduated with honors. After graduating from high school, she went on to attend Virginia State University to study Animal Science where she graduated *cum laude*. She then went on to attend North Carolina Agricultural and Technical State University to work on her master's degree. She was accepted on the Graduate Merit Assistantship Scholarship and remained in excellent academic standing throughout the course of her graduate studies. She has participated in scientific conferences and farm related educational demonstrations. She has assisted undergraduates with laboratory procedures, participated in multiple research projects, conducted class lectures and has co-authored three publications. Her project was supported by the Evans-Allen USDA grant funding. She is a candidate for the Master of Science degree in Animal Health Science.

Dedication

This thesis is dedicated to my mother, grandmother, brother and loving family. I could not love you more. I give a special recognition to my father who through sickness has shown me exceptional strength. Their love, support, patience and encouragement have helped me through many obstacles. Your dedication to my success has been an inspiration in which I am truly thankful.

I shall be telling this with a sigh

Somewhere ages and ages hence:

Two roads diverged in the woods, and I - -

I took the one less traveled by,

And that has made all the difference.

- *Robert Frost*

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List of Symbols and Abbreviations

AA	Amino Acids
ANOVA	Analysis of Variance
AMGP	Antimicrobial Growth Promoter
AMR	Antimicrobial Resistance
β	Beta
BW	Body Weight
d	Day
C°	Degrees of Celsius
EPG	Eggs Per Gram
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FE	Feed Efficiency
FMG	Fungus Myceliated Grain
GI	Gastrointestinal
GIT	Gastrointestinal Tract
GLM	General Linear Model
g	Grams
GALT	Gut Associated Lymphoid Tissue
HRP	Horseradish Peroxidase
hr	Hour
IgA	Immunoglobulin A
IgG	Immunoglobulin G

IACUC	Institutional Animal Care and Use Committee
kg	Kilograms
LSM	Least Square Means
µg	Microgram
µl	Microliter
ml	Milliliter
NRC	National Research Council
NCSU	North Carolina State University
PF	Phase Feeding
$NANO_3$	Sodium Nitrate
spp.	Species
Trt	Treatment
USDA	United States Department of Agriculture
wk	Week
WBC	White Blood Cell

Abstract

A study was conducted to evaluate the effect of phase feeding a combination of 3 different medicinal mushrooms (*Lentinus edodes*, *Ganoderma lucidum*, *Pleurotus ostreatus)* via Fungus Myceliated Grain (FMG) on broiler performance and health after natural and experimental exposure to field strains of *Eimeria*. A total of 300, day-of-hatch, female broiler chickens were weighed and randomly assigned into the following dietary treatment conditions: 1) 1 % starter, 2) 1 % grower, 3) 1 % starter-grower-finisher, 4) 5% starter, 5) 5% grower, 6) 5% starter-grower-finisher, 7) 10% starter, 8) 10% grower and 9) 10% starter-grower-finisher. Each treatment was replicated 3 times with 10 chickens per replicate. Half the broilers in each treatment were challenged with a mixture of three pathogenic *Eimeria* spp. (*E. acervelina, E. maxima* and *E. tenella*) via oral gavage at 4 weeks of age. The body weights of broilers given FMG at the 1% inclusion level were significantly lower compared to broilers in the 5% and 10% starter phase. *Eimeria* counts for challenged broilers in the 5% grower phase were significantly higher than that of broilers given the 1% and 10% inclusion. Elevations in IgA and IgG levels were observed in the grower phase at the 1% level as well as the starter-grower-finisher phase at the 10% level.

CHAPTER 1

Introduction

Poultry production in the U.S. had increased significantly after the Second World War as the supply of beef and pork became limited (Food and Agriculture Organization (FAO), 2010). Prior to the war, the majority of poultry flocks were raised within the backyards of local farmers as a source of food for family consumption. Today, the commercial broiler industry has evolved greatly from backyard flocks into large-scale, high-density, mass production operations. Since the 1960's, the consumption of chicken per capita has increased greatly from approximately 30 lbs in 1965 to 80 lbs in 2012 (The National Chicken Council, 2012).The increase in broiler productivity reflects consumer demand based on preference for high-quality products at relatively low costs. At present, poultry is the number one meat consumed in the United States (The National Chicken Council, 2012). Internationally, the popularity of poultry meat is second only to that of pork (Best, 2011).

Broiler meat is a healthy alternative to red meat, and is rated as one of the most important sources of animal protein in the world (Pan et al., 2012). Nutritionally, people consume broiler meat because it is high in protein, low in fat and it possesses an abundant source of almost all of the essential amino acids. Aside from their incredible nutritional value, broilers have one of the best feed to food conversion ratios of any other livestock species grown for meat (Aiello, 1998; Scanes, Brant, & Ensminger, 2004). Genetic selection for large meat producing birds along with the proper nutritional support provides producers with broilers that are able to reach market weight in excess of 4 pounds (1.8 kg) in as little as 7 weeks (49 days). The ability for broilers to grow quickly allows for shorter rearing periods and faster marketing production.

Feed is considered to be the most expensive aspect of poultry production. Therefore, it is essential to discover ways of reducing the cost of feed without compromising feed quality. In 1997, Emmert and Baker reported a method of nutrient delivery referred to as phase feeding (PF). Phase feeding is a feeding regimen based off the nutritional requirements of broilers defined by the National Research Council (NRC) (1994). The nutritional needs of broilers decrease as the bird ages, therefore it is not necessary to maintain costly feed additives throughout the entire grow-out period. Since feed cost per meat yield is a main concern for broiler producers, it is essential that costs associated with broiler rearing remains relatively low. The PF nutritional system was designed as a precision feeding method for broilers that has the potential to decrease dietary costs by aiding in the improvement of feed efficiency (FE), growth potential, health and weight maintenance.

Growth and productivity have always been important factors in the broiler industry. Most practical broiler diets are formulated with a mixture of the necessary nutrients to ensure maximum performance and good health. Proper nutrition aids in growth promotion, viability and the proper development of the immune system. Formulating diets for its effect on broiler intestinal health is becoming inevitable in the poultry industry. Many scientific studies suggest that nutrition and gut health are intricately correlated in broilers (Choct, 2009; Yang, Iji, & Choct, 2009). Therefore, diets can have a marked effect upon gut health and proper gut health greatly influences the nutrient utilization of feed thus improving weight gain.

Any deterrent to broiler health can cause a decrease in profitability. The poultry industry is challenged frequently with the threat of infectious diseases that can alter productivity and diminish broiler well-being. Enteric diseases can be damaging to poultry productions due to increases in mortality, loss of productivity, decreases in efficient feed utilization and the

potential for human health risks that are linked to food borne illnesses. In addition, costs related to disease prevention and treatment can cause hefty reductions in profits. Coccidiosis is a common parasitic disease of poultry that causes considerable economic loss, especially in the production of broilers. The global economic impact of coccidiosis infections in poultry flocks is estimated to be about $3 billion annually (Bal, 2009). The incidence of coccidiosis in commercial poultry has increased considerably since the incorporation of intensive confined rearing conditions. The high density confinement methods of poultry rearing have favored the spread of this disease in commercial flocks worldwide. Intense focus has been placed on coccidiosis because of its significance in the poultry industry.

Considerable steps have been taken in the field of poultry nutritional science within the last century in regards to improving both broiler feed and overall health. Antimicrobial growth promoters (AMGPs) have played an important role in the development of modern commercial poultry production. The addition of AMGPs in broiler diets at sub-therapeutic levels had, at one time, been the principal means of disease control in large commercial production flocks. However, antimicrobial feed additives can modulate some of the beneficial effects of the natural micro-flora found in the gut. In addition, exposing pathogens continuously to a low-dose of antibiotics can increase their resistance to these products. For these reasons, consumer demands have prompted new market niches for the poultry industry to deliver products that have a reduced risk of pathogens and are free of antibiotics and other drugs.

Induced pressure over the concern of drug resistant organisms in poultry products has prompted investigations into the development of non-chemotherapeutic alternative feed additives (Apata, 2009). There are a plethora of studies that have been carried out in recent years on a variety of potentially health enhancing ingredients that could be incorporated into the diets of

poultry flocks for improvement of production and reduction of disease (Griggs & Jacob, 2005). One approach that has gained increased attention is the use of medicinal mushrooms as immune-modulators. Mushrooms have been shown to possess medicinal properties that work to enhance the normal functions of the gut (Aida, Shuhaimi, Yazid, & Maaruf, 2009). The incorporation of medicinal mushrooms as natural feed additives in the basal diets of broilers may provide immune-enhancing properties against coccidiosis infection and other diseases, thus increasing growth and improving feed efficiency, intestinal integrity and overall performance (Hashemi & Davoodi, 2010). Medicinal mushrooms contain polysaccharides and other physiochemical properties that have been documented in broiler associated gut studies to improve the quality and quantity of the beneficial microbial flora that inhabit the gut (Guo et al., 2004b; Willis, King, Iskhuemhen, & Ibrahim, 2009). Enrichment of microbial activity has been directly associated with the enhancement of immunity, feed efficacy and general health in an organism (Isolauri, Sutas, Kankaanpaa, Arvilommi, & Salminen, 2001).

The specific objectives of this study were to evaluate the overall influence of FMG placed into phase feed rations at different inclusion levels on the performance and health of broilers after natural and experimental exposure to field strains of *Eimeria.*

CHAPTER 2

Literature Review

2.1 The Importance of Nutrition on Poultry Performance and Health

Proper nutrition is critical for both the health and well-being of broilers. Increasing awareness has placed emphasis on the link between nutrition, good health and proper immune function. This has prompted many commercial poultry producers to make health maintenance and disease prevention through diet a main priority in husbandry practices. To maintain health and productivity, broilers must be given a diet that completes their nutritional needs. This means supplying feed that incorporates all the basic nutrition in which a broiler will need to thrive throughout production. Nutrient efficiency in broilers is often obtained by using precision animal nutrition (PAN) (Sifri, 1997).

Precision animal nutrition is a method of providing an animal with a diet that specifically meets its nutritional requirements (Sifri, 1997). Providing diets based on PAN to broilers is necessary for optimal growth and development. However, PAN can be challenging to deliver as it is often difficult to target the precise nutritional requirements of an animal at specific stages of its life (Ferket, Van Heugten, Van Kempen, & Angel, 2002; Oviedo-Rondón & Waldroup, 2002). For instance, nutritional needs for amino acids (AA) are reported to be the highest for broilers around 0-3 weeks of age (Emmert & Baker, 1997). Therefore, providing a singularly formulated, complete diet throughout the entire growth period can lead to nutritional excesses or deficiencies. The basic aim of precision nutrition is to alleviate the over and under feeding of AA in animal diets by supplying only what is necessary to meet the animals' nutritional needs (Emmert & Baker, 1997). Since AA requirements can be influenced by a variety of dietary, genetic and environmental factors (Taherkhani, Shivazad, Zaghari, & Shahneh, 2008), many

different methods of precision feed delivery programs for broilers have been investigated (Makinde, 2013; Shariatmadari, 2009).

2.1.1 Phase feeding broiler diets. In 1994, the NRC established a set of recommendations specific for meat producing poultry that involved feeding broilers nutrients according to their age and actively level. This proposal united the feeding regimen of both pullets and cockerels into three fixed periods of development: starter (0-3 weeks of age), grower (3-6 weeks of age) and finisher (6-8 weeks of age) according to AA dietary needs (National Research Council, 1994; Warren & Emmert, 2000). This feeding regimen works on the principle of supplying growing broilers with a diet that is initially high in protein content which subsequently decreases as the bird matures and requires less protein and more energy for weight maintenance (Ewing, Pesti, & Bakalli, 2001).

The body weights (BW) of commercial meat-type chickens increase rapidly within 6-8 weeks post hatch (Toudic, 2006). Large amounts of AA are needed during this crucial period of growth to support the development of healthy muscle tissue (National Research Council, 1994). Proficient conversions of AA by broilers into breast meat during the grow-out period can boost carcass yields and decrease costs associated with feed. Since protein requirements for broilers decrease as the bird ages, it is not economically feasible to feed a diet that is fortified with high amounts of AA continuously throughout each stage of development (Dudley-Cash, 2004; Pope, Loupe, Pillai, & Emmert, 2004).

Continuous improvements in the genetic potential of meat birds coupled with innovations in dietary formulations in addition to the increased demand of varied broiler markets (Costa, Houston, Gunter, & Pesti, 2002; Swaggerty et al., 2009), have made the recommendations of the NRC for broiler diets nearly obsolete (Pope & Emmert, 2001, 2002;

Pope et al., 2004). The proposed regimen, set up nearly 20 years ago, no longer related to the grow-out periods of current commercial broiler operations (Warren & Emmert, 2000). It became necessary for researchers to consider revisions within the suggested feeding program of the NRC almost immediately after its proposal. In 1997, Emmert and Baker formally introduced phase feeding (PF) as a method of feed delivery that incorporated the NRC (1994) recommendations of precision nutritional requirements for broilers, yet allowed for variations in production times. Multiple studies involving modifications in PF programs have been conducted to fit the flexible amino acid requirements associated with modern broiler production systems (Emmert & Baker, 1997; Pope et al., 2004). Phase feeding in broilers has also been utilized as a way to incorporate a variety of supplements and additives into feed for observation of their effects on overall health, performance and to reduce cost (Benites, Gilharry, Gernat, & Murillo, 2008; Driver, Pesti, Bakalli, & Edwards, 2006; Payne & Southern, 2005; Willis, Isikhuemhen, Hurley, & Ohimain, 2011). At present, variations in PF methods have been universally applied to broiler production operations.

2.2 Antimicrobial Drug Usage

Antimicrobials have been used as additives in poultry feeds to reduce the occurrence of illness and to improve the utilization of feed for many years. These substances are either produced by microorganisms or synthetically manufactured and are used to kill or inhibit the growth of microbes such as bacteria, fungi, and protozoan (Schwarz, Kehrenberg, & Walsh, 2001). Antimicrobials are generally differentiated into groups according to the microorganisms in which they specifically target. For instance, coccidiostats are a group of antimicrobial agents generally used to arrest the development of *Eimeria*, the causative agent of coccidiosis in poultry (McEwen & Fedorka-Cray, 2002); while bactericidal compounds commonly termed antibiotics,

destroy potentially pathogenic bacterium that typically contaminate poultry such as S*almonella* and *Escherichia coli* (Kohanski, Dwyer, Hayete, Lawrence, & Collins, 2007).

Antimicrobials used in livestock and poultry act primarily on the animal's gastrointestinal (GI) micro-flora by modifying bacterial populations found within the gut (Yang et al., 2009). Detailed knowledge on the selective actions of antimicrobials on the intestinal bacteria of broilers are often absent within literature. However, it has been generally suggested that antimicrobials may aid in the integrity of the intestines by: protecting nutrients from bacterial degradation; reducing the amount of bacteria that naturally colonize the gut allowing for better digestibility and nutrient absorption; decreasing the amount of destructive toxins that these natural bacterium release and reducing the occurrence of intestinal infections (Butaye, Devriese, & Haesebrouck, 2003; Doyle, Bill, & Coffee, 2001).

The use of antimicrobial agents as feed additives has played a major role in the successful growth and prevalence of the poultry industry. Commercial broiler industries adopted the use of antimicrobials in poultry feed as growth promoters shortly after their approval for use in agricultural animal feeds by the Food and Drug Administration (FDA) in the early 1950's without the need of a prescription from a veterinarian (Jones & Ricke, 2003). Antimicrobial growth promoters describe compounds that are used at continuously low levels to improve animal growth and feed conversions while reducing the incidence of morbidity and mortalities (Butaye et al., 2003). They are generally administered to either prevent infections (sub-therapeutic) or treat outbreaks of disease (therapeutic) (Schwarz et al., 2001). According to authors Piva and Rossi (1999), the sub-therapeutic use of antibiotics has the following aims: to serve as a prophylactic in young animals to help reduce the occurrence of enteric diseases and to prevent the onset of feed-induced pathogens in adults. Young broilers chicks (0-7 days of age)

are more susceptible to enteric pathogens since their immune systems are underdeveloped and naïve (Swaggerty et al., 2009). Older broilers have more established immune systems but are still vulnerable to a variety of enteric pathogens that can compromise the integrity of their health.

The spread of infective pathogens is encouraged by the congested and stressful conditions under which most production animals are reared (Butaye et al., 2003). It had become necessary for commercial producers to incorporate ways of reducing the chance of disease outbreaks occurring in broiler flocks since infectious agents have the ability to reduce the carcass yields of production food animals resulting in economic losses. Consequently it had become, at one time, common practice for production animals such as broilers to be fed antimicrobials in their diets for the prevention of disease (McEwen & Fedorka-Cray, 2002).

2.2.1 Concerns over antimicrobial growth promoters. AMGPs are not as celebrated today as they once were 60 years ago (Cook, 2004). The continuous use of antimicrobial drugs has promoted the emergence of multi-drug resistant strains of microbes (Apata, 2009). As a result, they are not considered as effective now as when they were initially introduced. The incidence of antimicrobial resistance (AMR) has led to a considerable amount of public distrust for food safety regarding broilers. High incidences of AMR among the pathogenic and commensal bacteria that populate poultry and frequency of resistant bacterial strains signify a great public health hazard (Apata, 2009). There are many retrospective and prospective studies that document the occurrence of resistant pathogens in broilers (Luangtongkum et al., 2006; Van den Bogaard et al., 2001; Van den Bogaard et al., 2002).

An initial sign of resistance to AMGPs began with the usage of streptomycin in turkeys (Starr & Reynolds, 1951). This occurred shortly after antibiotics were approved for use as additives in production animals without the prescription of a veterinarian in the early 1950s.

Shortly thereafter, streptococci resistance to tetracycline had been reported in AMGP fed poultry (Barnes, 1958; Dibner & Richards, 2005). A couple years later, in 1969, the incidence of antimicrobial resistance had been reported by the Swann Committee, a division of the British government. The agency issued a report requesting to restrict the use of AMGPs that were used in both animal and human medicine, since the discovery of *Salmonella enterica* serovar Typhimurium resistance to oxytetracycline in food animals (Cogliani, Goossens, & Greko, 2011). This event was one of the first to confirm the presence of resistant microbes observed globally. Since then, many regulatory changes have taken place to reduce the use of in feed animal AMGP additives by incorporating limitations and bans on the use of sub-therapeutic levels of antibiotics in production animal rations (Buchanan et al., 2008). For instance, the use of penicillin and tetracycline as AMGPs were banned in the United Kingdom in the 1970's (Hughes & Heritage, 2004). Sweden and Denmark banned the use of antimicrobials as AMGPs in production animal feed entirely in 1986 and 1999 (Aarestrup et al., 2001; Wierup, 2001). And the United States followed suit by banning the use of enrofloacin in 2005 (Buchanan et al., 2008; Revington, 2003).

2.2.2 Alternatives to antimicrobial growth promoters. The overuse of antimicrobials can lead to the disruption of the normal micro-flora of the gut which allows pathogens to breach and overwhelm the hosts' immune system (Cervantes, 2008). In addition, the rapid development of multi-drug resistant pathogens, increasing consumer pressure for a more natural and less chemically enhanced food, as well as regulatory change has prompted researchers to search for alternative solutions (Noirot, 2010). According to literature, there are three basic strategies that can be used to cope with the loss of AMGPs, they are: 1) pathogen reduction; 2) augmentation of the immune response and; 3) nutritional strategies and/or additives that either improve

performance, or aid in the production of beneficial gut microbial flora (Ferket, 2003; Ferket, 2004; Revington, 2003).

Several natural feed additives have been investigated as sources of potential immune-modulatory dietary additives in poultry (Allen & Fetterer, 2002). For example essential oils, organic acids, and phytogenic compounds have been shown to enhance production of gastric secretions, stimulate blood circulation, and reduce levels of pathogenic bacteria (Buchanan et al., 2008). Findings by investigators Buchanan et al. (2008) suggest that formic, acetic, and propionic acids reduce the prevalence of *Salmonella* and *Campylobacter* bacteria found in the intestines of broilers. In recent years, an innovative approach to broiler health enhancement and immune system enrichment has involved the use of medicinal mushrooms incorporated into broiler diets via feedstuffs or extracts (Giannenas et al., 2010; Guo et al., 2004a; Willis et al., 2008; Willis, Isikhuemhen, & Ibrahim, 2007).

2.3 Medicinal Mushrooms

Mushrooms have been used as a food source by many cultures worldwide since antiquity (Chang & Miles, 2004). Nutritionally, mushrooms are a nearly perfect food. Studies have indicated that several edible forms of fungi are rich in carbohydrates, vegetable proteins, essential amino acids, dietary fibers, vital vitamins and minerals (Garcha, Khanna, & Soni, 1993; Lakhanpal & Rana, 2005; Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999). In addition to their nutritional importance, several mushrooms have long been recognized for having medicinal properties. One such genre of fungi, Basidiomycetes, are described as a class of higher fungi whose members have fleshy, macro-fungi, are both edible (*Agaricus*) and non-edible (*Amanita*), and are rich in bioactive compounds (Hobbs, 1995; Madigan, Martinko, & Brock, 2006).

The majority of basidiomycetes produce visible fruiting bodies in addition to a mass of hyphae generally referred to as mycelium, which constitutes the vegetative part of the fungus. The fruiting bodies of basidiomycetes are reproductive structures that produce spores and are generally viewed growing above ground (Cheung, 2008; Hobbs, 1995). The mycelia are complex networks of filamentous hyphae that grow within a medium such as wood, feed grains or ground soil (Madigan et al., 2006). The mycelia secrete strong enzymes into the environment in which it colonizes and breaks down complex carbohydrates into simpler sugars in which it uses to nourish itself (Hobbs, 1995; Marley, 2009). Both the fruiting bodies and mycelium of basidiomycetes have been used as a functional food source (Cheung, 2008). Basidiomycetes have an extensive history in folk medicine, specifically Traditional Chinese Medicine and have been incorporated into modern medicines and medical practices as a way to promote health (Wasser & Weis,1999).

2.3.1 Medicinal mushrooms and poultry health. In recent years, advances in scientific and medical research have prevailed in the identification, isolation, purification and effective use of several bioactive molecules found in medicinal mushrooms (Smith, Rowan, & Sullivan, 2002). Basidiomycetes contain biologically active polysaccharides such as beta-glucans, lectins, and terpenoids in the cell walls of their fruiting bodies and mycelium (Wasser, 2011). The bioactive polysaccharides, particularly β-glucans, found in these fungi vary in chemical composition and have been shown to possess strong immunomodulation behavior (Horowitz, 2011; Wasser, 2002). Constituents of basidiomycetes are reputed to possess; antitumor, anti-cholesterol, antibacterial, antiviral, anti-diabetic, anti-allergic and anti-parasitic properties (Dalloul, Lillehoj, Lee, Lee, & Chung, 2006; Wasser & Weis, 1999).

There is increasing evidence that mushrooms may play a major role in the immune-enhancement of poultry. Information regarding the use of mushrooms as a way to promote

performance and health in chickens, however, is limited. However, there are some scientific reports that do attest to the administration of fungi to enhance the overall productivity and health of poultry (Dalloul et al., 2006; Guo, Savelkoul, Kwakkel, Williams, & Verstegen, 2003). Mushrooms have been known to have a natural prebiotic effect on the gastrointestinal environment of many organisms including poultry. The oral consumption of fungi can encourage the stimulation of the microbiological ecosystem within in gut, resulting in immunity enhancement.

An in vivo study conducted by Guo et al. (2004b) evaluated the potential effects of the shiitake mushroom extract along with other natural products on chicken growth and the integrity of the cecal ecosystem. In this study, the shiitake mushroom extract was compared against that of a well-known anticoccidicial agent, apramycin. Chickens were naturally infected with avian *Mycoplsma gallisepticum*, an economically significant pathogen in poultry, prior to the study. Results concluded that both the shiitake extract and apramycin significantly stimulated the growth of chickens although the body weights of the birds given extracts were significantly lower than those given apramycin. However, birds who received the shiitake extract were found to have a higher population of beneficial bacteria within the ceca compared to those given apramycin (Guo et al., 2004b). This suggests that the bioactive compounds found within the mushroom extract may have the potential to positively influence the growth of beneficial intestinal micro-flora in diseased birds. A study conducted by Willis et al. (2009) also suggested the advantageous effects of mushroom extracts given to chickens on the positive influential growth of beneficial microbial populations within the intestines. In this study, the effects of administrating shiitake (*L. edodes*) mushroom extract on broiler intestinal bifidobacteria and *Salmonella* bacteria populations were evaluated. Findings from this study found that the

supplementation of mushroom extracts in broiler diets aided in increasing populations of bifidobacteria while suppressing the growth of potentially pathogenic *Salmonella* populations. In another study by Willis et al. (2007) the continual shedding of beneficial bacteria in broiler feces was reported after the administration of shiitake mushroom extract. This may indicate that the bioactive components of the shiitake mushroom extract enhanced conditions within the intestines allowing for greater bifidobacteria proliferation. Dietary extracts from the shiitake mushroom have also been found to improve egg production in layer hens while reducing the *Salmonella* bacterial populations during molt (Willis et al., 2008).

Dalloul et al. (2006) reported a study incorporating the use of fungi in poultry feed. In this experiment, a lectin extracted from the *F. fraxinea* mushroom was given to chickens orally challenged with *Eimeria*. The goal of this study was to evaluate the effect of the *F. fraxinea* mushroom extract on poultry immunity against coccidiosis. The investigators found that the *F. fraxinea* extract produced a strong proliferative response in lymphocytes and significantly protected chickens against weight loss associated with coccidiosis. Scientific studies regarding the use of mushrooms incorporated into chicken fed via FMG utilized for their prebiotic effects on chicken performance and immunological health have been reported by Willis et al. (2009, 2010a, 2010b, 2012 & 2013). These studies collectively demonstrated benefits from the inclusion of mushrooms into the diets of broilers and laying hens by preferentially improving body weights, increasing beneficial bacterial populations, reducing the fecal shedding of *Eimeria* oocysts and decreasing the population densities of potentially pathogenic *Salmonella* bacteria. The relationship between intestinal health and overall immunity in chickens has been reported by several authors. For example, Guo et al. (2004c) demonstrated the immune enhancement of *E. tenella* infected chickens fed diets supplemented with mushrooms and herb extracts. Selegean et

al. (2009) investigated the use of extracellular fractions containing polysaccharides from the *P. ostreatus* mushroom on its ability to stimulate the immune systems of broilers and its potentially synergistic relationship with conventional poultry vaccines against the Infectious Bursal Disease virus, during the first two weeks post hatching. The use of mushroom components as immunomodulators in poultry water and feed have shown promising results suggesting that the use of medicinal mushrooms may be one way of creating immune-competence in poultry with an approaching post-antibiotic era.

2.3.1.1 Lentinus edodes (shiitake). Shiitake is an amber colored mushroom that has long been a symbol of prosperity and longevity in traditional Asian culture. This species of edible fungus is indigenous to Eastern Asia and can be found growing on the wood logs of a variety of native deciduous trees to include that of the shii tree (*Castanopsis cuspidate)*, in which the shiitake mushroom is partially named (Wasser, 2005). Shiitake is one of the 5 most cultivated edible fungi worldwide, being second only to *Agaricus bisporus*, the button mushroom (Chang & Miles, 2004; Jones, 1998; Wasser, 2005). Shiitake, nicknamed the "monarch of mushrooms", is well-known for its high nutritional value and health benefits. It is a nutritionally sound, flavorful food containing a high percentage of water, proteins, lipids, enzymes, carbohydrates, vitamins and minerals (Hobbs, 1995; Wasser, 2005). Extracts and dried whole products of the mushroom are often used in herbal remedies (Stengler, 2005). Shiitake gained its popularity particularly for its medicinal properties after extensive scientific investigational studies revealed its ability to regulate chronic health conditions such as hypertension, cardiovascular disease and diabetes (Broadhurst, Polansky, & Anderson, 2000; Chen & Raymond, 2008; Houston, 2005). Components of the shiitake mushroom have been used in the treatment of ulcers, allergies and parasitic infections (Bilay et al., 2011; Jones, 1995). Additional studies using shiitake have

publicized the mushroom's antitumor, immunostimulatory, and antiproliferative properties (Borchers, Keen, & Gershwin, 2004; Kaneno et al., 2004; Wasser, 2005).

Eritadenine, found in the mycelia and fruiting body of shiitake, have been found to dramatically reduce the blood cholesterol of rats that had received diets high in cholesterol (Shimada, Morita, & Sugiyama, 2003). A polysaccharide called lentinan, also isolated from the shiitake mushroom, has been shown to regress tumors in mice experimentally infected with aggressive cancers (Vetvicka & Vetvickova, 2012). Shiitake has also been found to contain a substance called cortinelin, a broad spectrum antibacterial agent (Carvalho, Van Der Sand, Rosa, Germani, & Ishikawa, 2008). The administration of shiitake extract to broilers has been shown to reduce pathogenic *Salmonella* infections (Willis et al., 2009). The shiitake mushroom has a relatively low toxicity and has been reported to be tolerated well by both humans and animals in health related studies (Hobbs, 1995).

2.3.1.2 Ganoderma lucidum (reishi). *Ganoderma lucidum*, commonly known as Reishi, is a woody mushroom that is commonly known for parasitizing the wood of trees and causing them to decay. The mushrooms' cap is distinctively kidney-shaped and reddish in color. For centuries, the Reishi mushroom has been highly regarded as one of the best known and well characterized medicinal mushrooms due to its wide variety of biological activities. Reishi is regarded as the "plant of immortality" in Asian culture because of its many health benefits and extended usage in Chinese, Japanese and Korean medicine (Meschino, 2002). Beneficial compounds of the Reishi mushroom include β-glucans, triterpenes, sterols, lactones, vitamins and minerals (Hobbs, 1995). These compounds work by stimulating the immune system and increasing the amount of active cells and thus the potential for their effects. It has been reputed to possess antitumor, immunomodulatory, cardiovascular, respiratory, hepatoprotective, antitumor

and anti-inflammatory properties (Chang & Miles, 2004; Zhu, Chang, Wong, Chong, & Li, 1999).

2.3.1.3 Pleurotus ostreatus (pleurotus). Pleurotus is commonly called the oyster mushroom because its fleshy pileus is generally white and semi-circular resembling that of an oyster shell (Hobbs, 1995). Pleurotus grows in clusters on the wood of deciduous trees. It can be found growing in parts of Asia, Europe and North American and is cultivated in many parts of the world. Pleurotus has a high nutritional content and contains many essential amino acids and vitamins (Hobbs, 1995). The active compounds of the oyster mushroom include polysaccahrides, lectins, peptides, lovastatin-type compounds and ergosterols.

2.4 Poultry Immunity

Intense poultry production systems with densely populated flocks, under which most commercial broilers are raised, can cause birds to endure a great deal of undue stress (Kabir, Rahman, Rahman, & Ahmed, 2004). Stress, generally defined as a physical, chemical, or emotional factor that causes bodily or mental strain, may be a causative factor of disease (Heritage, 2005; Pease, 2006). Health issues in the poultry industry can be induced by a combination of stressful factors including: stress induced by improper management, nutritional stress, environmental stress, physiological stress and pathological stress (Downing & Bryden, 2002; Hill, 1983; Rosales, 1994; Siegel, 1980; Tankson, Vizzier-Thaxton, Thaxton, May, & Cameron, 2001). Confinement rearing coupled with higher bird densities have increased flock risks of exposure to various protozoal, bacterial, viral and fungal pathogenic agents via feed, water, air, litter and direct contact with other birds (Kogut, 2009; Yegani & Korver, 2008).

For many pathogens, the avian body is a nearly ideal habitat as it offers protection from outside elements, a ready source of nutrients, and is a means for transmission to new hosts and

environments (Campbell & Reece, 2008). Chickens have developed a sophisticated immune system similar to that of mammals that is capable of defending the bird against a broad spectrum of disease causing pathogens (Korver, 2006). The two primary functions of the avian immune system are recognition and response (Kindt, Goldsby, & Osborne, 2006). Defense mechanisms in chickens include both nonspecific (innate) and specific (acquired or adaptive) immune responses. These systems work together to combat potential threats from bacteria, viruses, parasites and other foreign antigenic material. Innate and adaptive immunity are considered essential factors for the survival and health of the host.

2.4.1 Innate immunity. In the chicken, innate immunity is composed of both barrier defenses (feathers, skin, and mucosal linings), as well as cellular defenses that are activated immediately following exposure to pathogens. Leukocytes or white blood cells (WBC) provide a non-specific immunological defense to the host (Qureshi, Hussain, & Heggen, 1998). Granulocytes, macrophages and other innate immune cells all play a major role in pathogenic recognition and elimination. These cells inactivate foreign biological agents directly by lysis, agglutination, or phagocytosis (Hub, 2011). Avian innate immunity is found to be functional at hatch and development continues to occur throughout the first week of a birds life (Dibner et al., 1998; Korver, 2006).

2.4.1.1 Innate immune cells. Granulocytes are polymorphonuclear luekocytes (PMN) that have secretory granules in their cytoplasm. Avian granulocytes include heterophils, eosinophils and basophils (Thrall, Weiser, Campbell, & Allison, 2004). Heterophils are one of the most abundant granulated leukocytes in the avian body and are documented to be highly efficient in phagocytosis and bacterial inhibition (Weiss & Wardrop, 2010). Avian heterophils are functionally similar to mammalian neutrophils although they differ morphologically and

biochemically (Swaggerty et al., 2009). Heterophils have been shown to increase in number at sites of acute inflammation and during long periods of induced stress (Davis, Maney, & Maerz, 2008; Swaggerty et al., 2009). Using artificial irritants, some investigators have been able to illustrate the influx of heterophils during the acute phase of inflammation in broilers. Brickford (2007) suggests that heterophil numbers may increase due to inflammation, stress, infection and tissue damage.

Not much is known about the general functions of both the avian eosinophil and basophil (Thrall et al., 2004). The mammalian eosinophil, on the other hand, is well documented and has been described to have the ability to form protection against helminthic infestations, and is frequently associated with allergic responses (Machnicka-Rowińska & Dziemian, 2003). Studies using parasitic antigens and other stimulants in an attempt to induce peripheral eosinophilia in birds have proven rather unsuccessful (Maxwell, 1980; Maxwell & Burns, 1981; Maxwell & Burns, 1986) although eosinophilia had been reported in studies involving gastrointestinal nematode infestations (Thrall et al., 2004).

Avian basophils contain histamine, as do their mammalian counterparts, which suggest that they may play an important role in inflammation and hypersensitivity reactions (Chand & Eyre, 1978; Maxwell & Robertson, 1995; Mitchell & Johns, 2008). According to information reviewed by Weiss and Wardrop (2010), both clinical and experimental evidence suggests that the avian basophil may have some involvement in host response to internal and external parasites. Though basophilia in birds is said to be rare it has been reported in respiratory disease, clamydiosis infections and in trauma to tissue (Doneley, 2010). Additionally it is assumed that these leukocytes participate in the initial phase of acute inflammation and may be associated with a stress response in birds (Maxwell, Robertson, Mitchell, & Carlisle, 1992; Thrall et al., 2004).

Avian monocytes are the largest leukocyte in avian peripheral blood and they are functionally similar to mammalian monocytes. Monocytes in general, perform phagocytic activities and transform into macrophages after migration into various body tissues. Monocytes are often associated with acute infectious or chronic inflammatory responses (Mitchell & Johns, 2008). An influx of monocytes is often observed in both infectious and inflammatory diseases (Qureshi, 2003). After monocytes develop into tissue macrophages, they become capable of degrading and presenting antigenic peptides to immune lymphocytes thus activating the acquired immune system (Mitchell & Johns, 2008; Qureshi, 2003).

When the physical barriers of the innate immune system are breached, these immune cells work both directly and indirectly on disabling the invading microbe. If infection persists, the bird's innate immune responses utilize antigen presentation and cellular signaling to activate the acquired immune response.

2.4.1.2 Acquired immunity. Humoral immunity is the acquired function of the immune system through which antibodies are produced in response to an antigenic challenge (Qureshi et al., 1998). These antibodies are produced in response to foreign antigenic stimuli by modified B lymphoctyes (B-cells). B-cells originate in the bursa of Fabricus, a primary lymphoid organ that is unique to the avian species. The Bursa of Fabricius is a small, hollow, sac like organ that is connected to the cloaca, an excretory organ in birds (Glick, 1979). The bursa is the site of B cell differentiation and maturation. After B-cells have fully matured, they are released from the bursa to circulate within the blood and lymphatic fluids. B-cells that are exposed to and stimulated by foreign antigen within the body will either develop into memory cells or differentiate into specialized lymphocytes called plasma cells (Brand, Gilmour, & Goldstein, 1976). Memory B-cells have a prolonged life span and are capable of "remembering" specific pathogens. Plasma

cells produce and secrete cell surface soluble proteins known as immunoglobulin (Ig) or antibodies (Tizard, 2002).

2.4.1.3 Antibodies. Antibodies are glycoproteins that are used by the immune system to identify and neutralize foreign microbes. Two major antibody isotypes found in poultry include; immunoglobulin G (IgG), and immunoglobulin A (IgA) (Tirziu & Ere, 2010; Tizard, 2002). Avian antibodies differ slightly from that of mammalian antibodies by way of structure and function although they are predominantly homologues.

Avian IgG, sometimes termed IgY, are similar to both mammalian IgG and IgE functionally. Avian IgG is often referred to as IgY because specific studies have shown that it differs both structurally and biochemically from that of mammals. Therefore, IgG and IgY are generally used interchangeably in literature. Avian IgG is the predominant antibody produced against established and systemic infections (Perry, 2006; Scanes et al., 2004).

The avian IgA function is similar to mammalian IgA. It is the predominant antibody found in bodily secretions and is the major antibody found within the intestinal tract. Antibodies are an important component of the acquired immune system as they provide antigen-specific immunity to extracellular pathogens. An increase in circulating antibodies within the blood or lymphatic fluids can suggest infection within the body.

2.4.2 Gastrointestinal tract and immunity. Gastrointestinal immunity is perhaps the most important aspect of the avian immune system. The primary role of the gastrointestinal tract (GIT) in any animal is to digest and absorb nutrients in order to meet the demands for normal growth and development (Isolauri et al., 2001). The GIT also functions as a barrier against pathogens from micro-organisms. The intestinal mucosa lining of the GIT provides a protective layer of defense against the constant presence of antigens from food and micro-organisms in the

gut lumen. Isolauri et al. (2001) reported that the protection against potentially harmful agents is ensured by many factors, this includes saliva, gastric acid, peristalsis, mucus, intestinal proteolysis, intestinal flora, and epithelial cell membranes. The micro-flora of the GIT is a mixture of beneficial bacteria, fungi and other protozoa that work symbiotically with the host in creating homeostasis in the intestinal tract. Intestinal micro-flora has been shown to have these beneficial effects on the body: vitamin production, stimulation of the immune system through nonpathogenic mechanisms, and inhibition of the growth and establishment of harmful micro-organisms (Yegani & Korver, 2008). The mucosal surface of the gut is one of the primary pathogen portals of entry to the bird (Korver, 2006). For example, avian enteric diseases such *Eimeria* gain entry to a bird through the alimentary tract where they proliferate and cause severe tissue damage resulting in host morbidity or mortality. Therefore, the immune responses of the GIT must be able to combat such infectious agents without disturbing the homeostasis and normal physiological activities of the gut.

The GIT is composed of the gut associated lymphoid tissue (GALT), one of the largest secondary lymphoid organs in the body. The GALT is composed of several types of immune cells and tissue and is essential for protection of the host against invasion from pathogenic organisms. Pathogens entering the GIT become trapped in the mucosa where they are inactivated by secreted GALT components such as IgA (Kindt et al., 2006 ; Korver, 2006). As a result the inactivated microbes are unable to colonize the gut and are therefore passed out of the digestive tract. In this way the GIT is able to control the presence and absorption of antigens within the intestine. Many reports have suggested that the key to keeping chickens immuno-competent is by the proper management of the GIT (Kidd, 2004).

2.5 Coccidiosis in Poultry

Adverse effects on the GI tract can cause major economic losses. Such is the case with avian coccidiosis, a prevalent and severe enteric infection of poultry. Coccidiosis affects many different species of animals and is of great economic significance in livestock and poultry. Coccidiosis is one of the most prevailing diseases affecting the U.S. broiler industry with estimated annual total cost resulting in millions dollars (Allen & Fetterer, 2002; David Chapman, 2003; Chapman, 2009; Dalloul & Lillehoj, 2005; Noirot, 2010). Contribution to losses are attributed by the costs of in-feed chemotherapeutic prophylactics (growth promoters), therapeutic treatments if the application of growth promoters prove unsuccessful and losses due to morbidity, mortality, inefficient feed utilization, poor feed conversion and impaired growth rates (Allen & Fetterer, 2002; Badran & Lukesová, 2006; Dalloul & Lillehoj, 2005).

2.5.1 Etiology and epidemiology. Coccidiosis is one of the most prevalent parasitic diseases of broilers. It is generally described as an acute, intracellular parasitic disease that primarily affects the intestinal tract of the host often resulting in morbidity, impaired growth and poor feed utilization. Avian coccidiosis in poultry is caused by single-celled parasitic protozoan of the genera *Eimeria* (Boughton, 1937; Cervantes, 2008; Fanatico, 2006). These parasites, belonging to the sub-kingdom Protozoa and to the phylum Apicomplexa (Aiello, 1998), are predominantly host specific and are generally characterized by their replication in host cells as well as their ability to cause extensive damage to the intestinal lining (Aiello, 1998; Conway & McKenzie, 2007).

Coccidia are ever present in poultry-rearing operations and are generally found in the intestines of mostly young birds that are 3 weeks of age or older (Damerow, 1994). Coccidia are transmitted to healthy chickens that ingest oocysts that have been shed in the feces of infected

chickens. Gradual exposures to small amounts of *Eimeria* in the environment allow young chicks to gain a natural resistance to these pathogens over a period of time. In such cases flocks may only have mild or subclinical infections where they exhibit little to no clinical signs, this is referred to as coccidiasis (Aiello, 1998; Conway & McKenzie, 2007; Damerow, 1994). Clinical disease generally manifests when birds are exposed to excessive amounts of *Eimeria* within their environment (Damerow, 1994).

In a recent review, Conway and McKenzie (2007) suggest that exposure to active *Eimeria* oocysts usually begins shortly after day old chicks are placed onto poultry house litter. Litter oocysts counts are generally low during the first 2 to 3 weeks of placement but tend to increase rapidly at 4 to 6 weeks and decrease again to low levels by 7 and 8 weeks. This pattern of infection indicates that coccidiosis infections are more likely to occur in broiler chicks at 3 to 6 weeks of age (Chapman, 1984; Conway & McKenzie, 2007). Birds raised in high density flocks are at greater risk for developing coccidiosis as they are more likely to become stressed (Damerow, 1994). As stated previously, flocks are vulnerable to the rapid spread of infectious agents and disease outbreaks under intense rearing conditions. Scientific reports by both Badran et al. (2006) and Noirot (2010) suggested that the pathogenicity of *Eimeria* in poultry flocks is influenced by and often a result of an imbalance between: 1) the parasites in relation to their numbers and infectivity, 2) the host in relation to its susceptibility, natural and acquired resistance and ability to recover from infection and 3) the rearing environment in relation to proper management techniques.

2.5.2 *Eimeria* spp. affecting poultry. In broiler chickens there are seven (Schnitzler, Thebo, Mattsson, Tomley, & Shirley, 1998) species of *Eimeria* that have been described in literature; *Eimeria acervulina, Eimeria praecox, Eimeria maxima, Eimeria mitis, Eimeria*

necatrix, Eimeria tenella, and *Eimeria brunette* (Aiello, 1998; Cervantes, 2008; Chapman, 1978; David Chapman, 2003; Schnitzler et al., 1998). Of these species, however, only three; *E. acervulina, E. maxima* and *E. tenella* have been widely recognized as being the most pathogenic (Aiello, 1998; Cervantes, 2008; Fanatico, 2006).

2.5.3 Clinical signs and symptoms. Most chickens are exposed to and parasitized by more than one species of *Eimeria*, with mixed infections found frequently in the field (Cervantes, 2008). Collectively, all species of avian *Eimeria* are capable of producing morbidity in broilers with their pathogenicity varying from mild to moderate and very severe. Altogether signs and symptoms can include; reduced pigmentation, severe bloody diarrhea, dehydration, impaired growth, lethargy, depression, drooping and suppressed appetite. Other recognizable signs include poor feed utilization, lowered feed intake, impaired weight gain, weight loss and mortality in severe cases (Fanatico, 2006; Noirot, 2010). Birds that are able to recover from infection may never regain their productivity and therefore may have to be culled. Birds that recover and regain productivity and those that harbor sub-clinical infections may serve as permanent reservoirs of infection.

CHAPTER 3

Methodology

3.1 Animal Husbandry and Experimental Design

This study was performed to evaluate the effects of dietary medicinal mushrooms on the immunity, coccidiosis control, general health, product safety and overall performance of broiler chickens utilizing three different inclusion levels at specific grow-out phases. This trial was performed at the Poultry Research Farm at North Carolina Agricultural and Technical State University located in Greensboro, NC. The treatment, housing, handling, management and humane euthanasia of subjects confirmed to all rules and regulations set by the Institutional Animal Care and Use Committee (IACUC).

A total of three hundred, day-of-hatch, female (Ross x Ross) broiler chicks were obtained from a local commercial hatchery (Siler City, NC). All chicks were vaccinated against Newcastle disease, infectious bronchitis and Mareks's disease prior to release from the hatchery. No form of coccidiosis protection was provided. The chicks were divided into groups, weighed and randomly assigned to one of the following dietary treatment conditions: 1) 1 % starter, 2) 1 % grower, 3) 1 % starter-grower-finisher, 4) 5% starter, 5) 5% grower, 6) 5% starter-grower-finisher, 7) 10% starter, 8) 10% grower and 9) 10% starter-grower-finisher. Each treatment condition was replicated a total of 3 times with 10 chicks per replicate.

Birds were housed in floor pens, one for each replicate treatment group, on unused litter with one hanging tube feeder and one drinker per pen. The lighting program was set to be continuous throughout the trial. The temperature was maintained at 35° C at the start of the trial and reduced weekly thereafter until reaching 24°C. Tunnel ventilation was set on an automatic periodic cycle timer and provided adequate air flow conducive for poultry rearing.

At 28 d of age, half the birds in each treatment replicate group challenged with a mixture of three pathogenic *Eimeria* spp. strains*; E. acervulina, E. maxima* and *E. tenella.* via oral gavage. Each experimental subject was challenged via oral gavage with 1 ml of a 49,000 oocysts/ml suspension solution. Mortalities were evaluated and recorded daily. Birds were weighed in groups according to their corresponding treatments using a standard industrial scale. Blood samples were collected from broilers for WBC differential count analysis. Fecal sampling for oocysts counts were taken on d 49. The IgG and IgA antibody concentrations, carcass and bursa organ weights for each treatment group were recorded at the conclusion of the trial (d 49).

3.2 Fungus Myceliated Grain Preparation

Shiitake, Reishi and Pleurotus mushrooms were cultivated at the Mushroom Biology and Fungal Biotechnology Laboratory at North Carolina Agricultural and Technical State University Farm (Greensboro, NC). At the laboratory, sorghum (white milo), a common cereal grain used in the poultry industry, was soaked in water overnight and drained the following day. Five kilograms of the sorghum grain was loaded into individual micropore fitted bags (Unicorn Mfg Co. Texas, USA). The bags were sterilized at 121°C for 3 h. Afterwards the sterilized grain was individually inoculated with *L. edodes*, *G. lucidum* and *P. ostreatus* mycelium and incubated at 25°C for 2 wk before use. The individual medicinal mushroom and grain biomass conjugant were transported to the Poultry Research Center at NCAT where they were dried. The compound grain was then ground into a powder that was used as a supplement in the basal meal dietary ration in each treatment.

3.3 Diet

Broilers in all treatment groups were fed a basal starter (1-14 d), grower (15-28 d) and finisher (29-49 d) diet in correspondence with modified grow-out phases. The basal diets were

supplemented with a 1%, 5% and 10% FMG medicinal mushroom mixture consisting of a combination of Shiitake, Reishi and Pleurotus. The supplemented diets were then administered to the individual treatment groups during their respective stages of development in correspondence with the treatment design. Feed and water, both free of drugs and medications, were provided to all treatment groups *ab libitum*.

Table 1

Composition of Basal Meal Diets Provided by NCSU

Ingredients		**Amount**	
	Starter	**Grower**	**Finisher**
Corn	1167	1324	1410
Soybean meal	716	563	478
Corn micro-flush	19.94	20.73	20.30
Limestone fine	19.42	20.40	21.37
Dicalcium phosphate (18.5%)	41.77	36.92	31.47
Lysine (78.5%)	0.01	1.26	4.27
Methionine (99%)	3.80	2.67	2.01
Threonine	1.06	0.02	1.58
Salt	10.00	10.00	10.00
PX NCSU Br Mineral (TM90)	4.00	4.00	4.00
Choline chloride (60)	4.00	4.00	4.00
PX NCSU Br Vitamin (NCSU90)	1.00	1.00	1.00
Selenium Premix NCSU (0.02%)	2.00	2.00	2.00
Poultry fat (Miter)	10.00	10.00	10.00
Total batch weight	2000	2000	2000

3.4 White Blood Cell Differential Counts

Blood samples were collected from one bird per replicate treatment group randomly using the brachial/ulna wing vena-puncture method of sampling. The blood was collected in heparinized micro-hematocrit tubes to avoid blood coagulation and immediately placed unto pre-cleaned microscope slides using the two slide wedge method. All slides were marked for identification using an industrial permanent fade and water resistant marker. Prepared slides were then transported to a laboratory located on the campus of NCAT to be air dried overnight.

Subsequently, each slide was fixed with methanol and stained according to the HEMA 3 manual staining system protocol. The slides were allowed to air dry completely before examination. For a detailed analysis of avian WBC, all slides were examined using an oil immersion lens under 100 x magnification by means of a compound light microscope. A total of 100 avian heterophils, lymphocytes, monocytes, eosinophils and basophils were identified and enumerated using manual counters with results expressed as percentages.

3.5 Enzyme-Linked Immunosorbent Assay

Whole blood samples were obtained from 3 experimentally challenged and 3 unchallenged broilers per treatment group at the conclusion of the trial (d 49) via the jugular vein at the time of euthanasia. The blood was collected in tiger top collection tubes, packed on ice, placed into a cooler and transported to a laboratory at NCAT for processing. Serum was separated from whole blood using centrifugation. The sera was decanted into another set of tubes and stored in a minus 80°C, ultra-low temperature laboratory freezer until analyzed.

3.5.1 Detection of chicken IgA and IgG concentrations. Chicken ELISA kits were used to quantify the amount of antibody levels present within the collected serum samples. Both standards and samples were analyzed using the Chicken (Cat. No. E33-103) kits manufactured by Bethyl Laboratories. All standards were ran in duplicates and all samples were ran in triplicates. A micropipette was used to coat a 96-well microtiter plate with the 50 ul of primary antigen diluted in ELISA Coating Buffer (Cat. No. E107). The microtiter plate was covered and incubated for 1 h at room temperature (20-25°C). The antigen was then decanted and the plate was washed five times with an ELISA Wash Solution buffer (Cat. No. E106) using a Nunc automatic plate washer. The wells where then filled with 100 ul of a blocking solution Buffer (Cat. No. E104) and incubated for 30 min at room temperature. The wash steps were repeated

before adding 50 ul of both standards and samples serially diluted in Sample/Conjugate Diluent 10% Tween 20 (Cat. No. E108). After a 1-h incubation at room temperature, the plates were washed again and a second antibody conjugated with horseradish peroxidase (HRP) was added to the wells, followed by a 1-h incubation at room temperature. The wash steps were repeated followed by adding tetramethylbenzidine substrate (TMB) Enzyme Substrate, TMB (Cat. No. E102) to each well. The plate was then developed under low light conditions for 15 minutes. The reaction was stopped by adding 50 ul of stop solution ELISA Stop Solution (Cat. No. E115) to each occupied well. Absorbency was read at 450 nm and the total antibody titer average was recorded.

3.6 *Eimeria* Oocyst Counts

Pooled fecal samples were taken from broilers in each treatment group on d 49. A total of four birds from each group were placed into battery brooder cages where they were held overnight. Fresh fecal samples were collected the next day. The samples were collected in labeled 50 ml conical tubes, placed in a cooler with ice and transported to the Laboratory Animal Resource Unit at NCAT for processing. The samples were processed within 24 hours to prevent further oocycts development. Oocysts counts were performed using a modified version of the standard McMasters oocysts chamber counting technique. Two grams of fresh feces from each sample were weighed out using an electronic laboratory balance. Measured samples were placed in sterile containers and mixed thoroughly with 30 ml of sodium nitrate. The fecal suspension was filtered through a double layer of cheesecloth into a new container. The filtrate was mixed again before being transferred into both chambers of the McMaster slide. The loaded slides were allowed to sit for 5 minutes before analysis. Slide samples were read at a 10 x 10 magnification

using a compound microscope. The number of oocysts within each chamber grid were counted, multiplied by 50 and recorded as eggs per gram (EPG).

3.7 Carcass and Bursa Weights

Carcass and bursa weight percentages were determined on d 49 by selecting four birds per treatment group and by pooling from replicates. They were then humanly killed, de-feathered and eviscerated. The bursa's were separated from the rest of the internal organs and weighed.

3.8 Statistical Analysis

All data except mortality percentages were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). The Least Square Means (LSM) procedure was used to separate treatment means. Differences among treatment groups were determined using the Duncan's multiple-range test. Mean values within the starter, grower or starter-grower-finisher phases having the same alphabets are not significantly different at $P \leq 0.05$ or $P \leq 0.1$.

CHAPTER 4

Results and Discussion

4.1 Body Weights

4.1.1 Unchallenged and challenged broiler body weight gain at 7 weeks (d 49). The mean body weights of unchallenged broilers on d 49 are shown in (Figure 1). Overall, there were no significant effects or interactions ($p>0.05$) on the body weights of unchallenged broilers in regards to dietary phase or inclusion level. In addition, there were no statistical differences found among the treatment levels and dietary phases of individual treatment groups. Although not statistically significant, there was a numerical trend improvement in the body weights of unchallenged broilers in treatment group 3 (1% starter-grower-finisher).

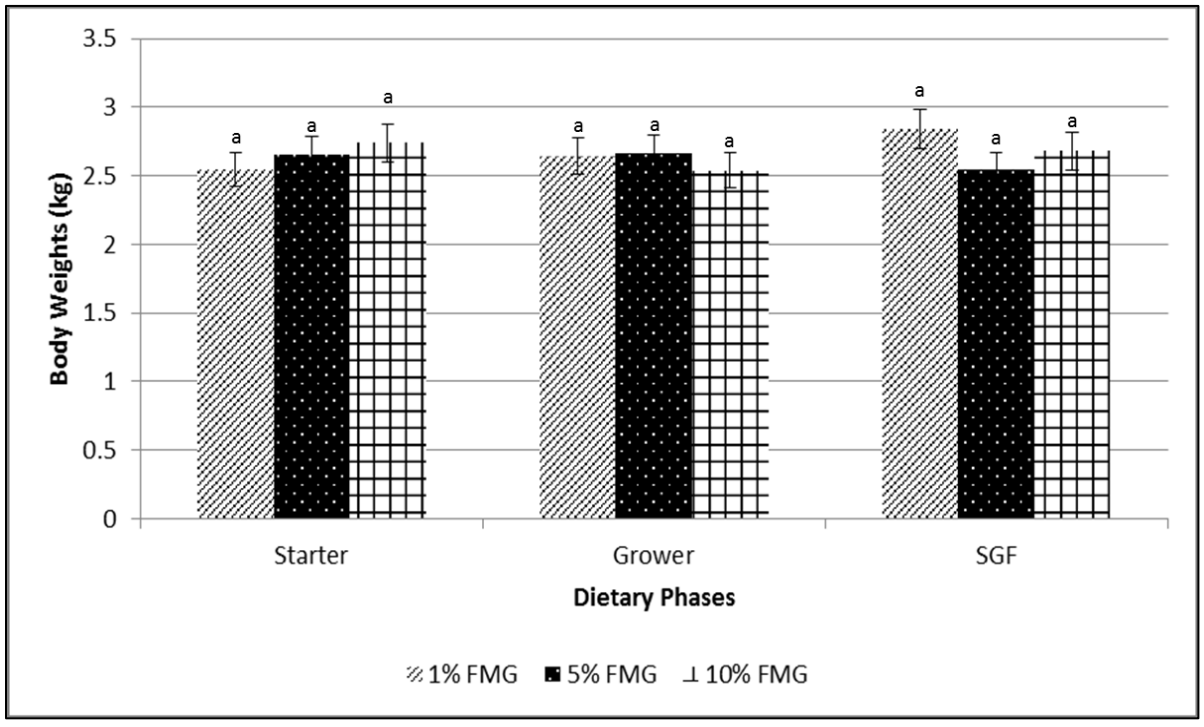

Figure 1 Effects of FMG Diet on Unchallenged Broiler Body Weights d 49

The mean body weight gains of challenged broilers are presented in (Figure 2). There were no statistically significant ($p>0.05$) effects observed among dietary phases or inclusion

levels on the body weight gains of challenged broilers. However, there were significant differences ($p<0.05$) observed on d 49 for broilers who were experimentally challenged with *Eimeria*. Challenged broilers belonging to treatment groups 4 (5% starter) and 7 (10% starter) had statistically higher ($p<0.05$) body weights compared to challenged broilers in treatment group 1 (1% starter), which had a statistically lower body weight value. While citing a numerical trend found among unchallenged broilers, body weight improvements were also observed for challenged broilers in treatment group 3 (1% starter-grower-finisher).

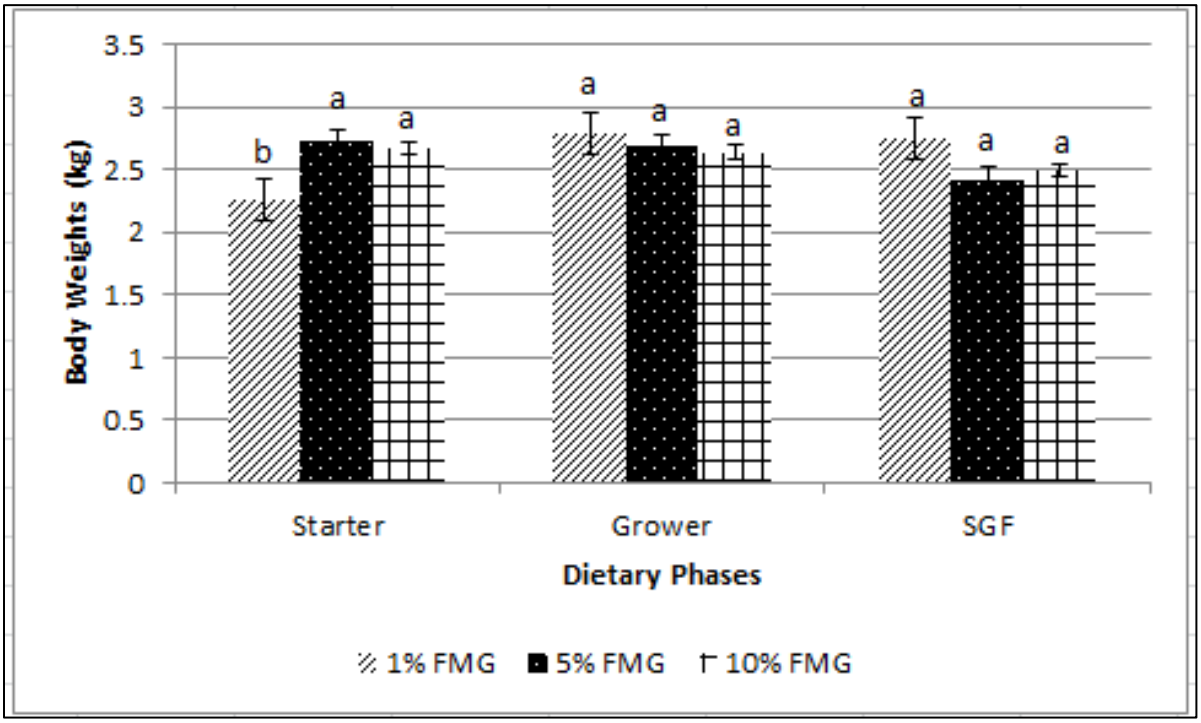

Figure 2 Effects of FMG Diet on Challenged Broiler Body Weights d 49

Overall, there were no statistical differences among the body weights of unchallenged broilers receiving FMG supplementation in the starter, grower or starter-grower-finisher phases at any dietary level on d 49. This may indicate that certain medicinal mushrooms fed in combination to chickens via FMG at different levels may offer some immune protection against natural *Eimeria* infections and may aid in growth performance. It appears that administrating

FMG at the 1% level to challenged broilers influenced body weights. It may be notable to mention the numerical improvements to body weights of both unchallenged and challenged broilers in treatment group 3 (1% starter-grower-finisher).

Natural *Eimeria* infections in broilers are caused by prolonged exposure to active oocyst found ubiquitous within their rearing environment. Shedding of oocysts by birds that are infected with high parasitic loads of *Eimeria* spp. increases the exposure and infection risks of clinically healthy broilers. In this study, unchallenged and experimentally challenged broilers were housed together within each treatment group. It was perceived that the challenged birds would serve as a reservoir of infection for the sentinel broilers with whom they cohabited. Reduced body weights in broilers are often a sign of disease. Avian coccidiosis, specifically, is a particularly detrimental disease of poultry and infestations can lead to intestinal hemorrhaging, impaired nutrient absorption and depressed growth rates (Conway & McKenzie, 2007).

Feeding broilers a diet of 5% shiitake FMG has been shown to provide some level of immunity against *Eimeria* (Willis et al., 2011). It cannot be assumed that variations in body weights of unchallenged birds or challenged birds were a direct result of coccidiosis infection. However, a correlation between coccidiosis and induced weight depression has been documented in previous literature (Willis et al., 2012). Differences in body weights may also be influence by other factors such as the different physiochemical properties of polysaccharides and their biological effects on the broiler GIT (Guo, Savelkoul, Kwakkel, Williams, & Verstegen, 2003).

4.2 Carcass Yield Percentages

Carcass yields for unchallenged broilers on d 49 post slaughter are shown in (Figure 3). Dietary treatment effects and interactions on broiler carcass yields were non-significant ($p>0.05$).

In addition there were no statistical differences ($p>0.05$) observed in carcass yields among treatment groups. No carcass yields for challenged broilers were obtained from this study.

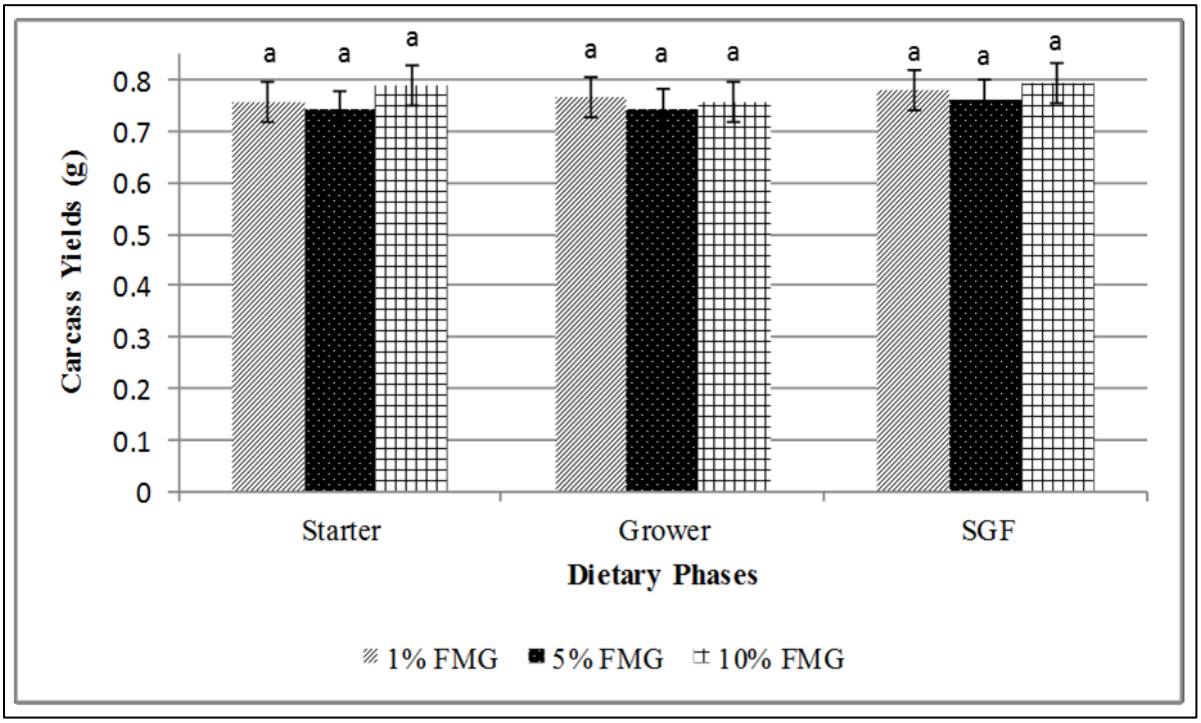

Figure 3 Effects of FMG Diet on Unchallenged Broiler Carcass Yields d 49

4.3 Mortality Percentages

The mortality rates of broilers from placement to slaughter during a 7-week rearing period are represented in (Table 2). Numerically, the mortality rate percentages for broilers belonging to treatment groups 3 (1% starter-grower-finisher (6.6%), 4 (5% starter (10%), 5 (5% FMG grower (3.3%), 6 (5% starter-grower-finisher (10%), 7 (10% starter (10%), 8 (10% grower (6.6%) and 9 (10% starter-grower-finisher (6.6%) were relatively low. No mortalities were observed in treatment groups with broilers receiving 1% starter and 1% grower diets. Mortalities in broilers can be a result of a number of external factors. Throughout this study, signs of morbidity in broilers were infrequent. Mortalities observed in this study did not seem to be significantly influenced by treatments. In previous investigations conducted by researchers at

this site, FMG supplements aided in the performance enhancement of both challenged and naturally infected broilers by reducing the percentage of disease related mortalities.

Table 2

Mortality Percentages of Broilers on d 49

Treatment Name	Group #	# of Birds/Trt on d 49	Mortality Percentage (# of Deceased Birds)
1 % FMG-Starter	**1**	30	0%
1 % FMG-Grower	**2**	30	0%
1 % FMG- S/G/F* **	**3**	28	6.6% (2)
5 % FMG-Starter	**4**	27	10% (3)
5 % FMG-Grower	**5**	29	3.3% (1)
5 % FMG- S/G/F	**6**	27	10% (3)
10 % FMG-Starter	**7**	27	10% (3)
10 % FMG-Grower	**8**	28	6.6% (2)
10 % FMG- S/G/F	**9**	28	6.6% (2)

* Trt= Treatment; ** FMG = Fungus Myceliated Grain; ***S/G/F= Starter, Grower and Finisher

4.4 *Eimeria* Oocyst Counts

Eimeria oocysts counts performed on unchallenged broilers were statistically significant ($p>0.1$) between treatment groups. The oocysts counts of unchallenged broilers are shown in (Figure 4). However, a significant ($p<0.1$) effect of dietary levels on broiler fecal oocysts shedding was observed at the 5% level in the starter, grower and starter-grower-finisher phases. A numerical trend was observed for fecal oocyst elevations in the 5% inclusion levels of unchallenged broilers belonging to treatment groups 4 (5% starter), 5 (5% grower) and 6 (10%

starter-grower-finisher). Broilers in treatment group 3 (1% starter-grower-finisher) had the highest overall fecal oocyst count numerically, compared to all other treatment groups.

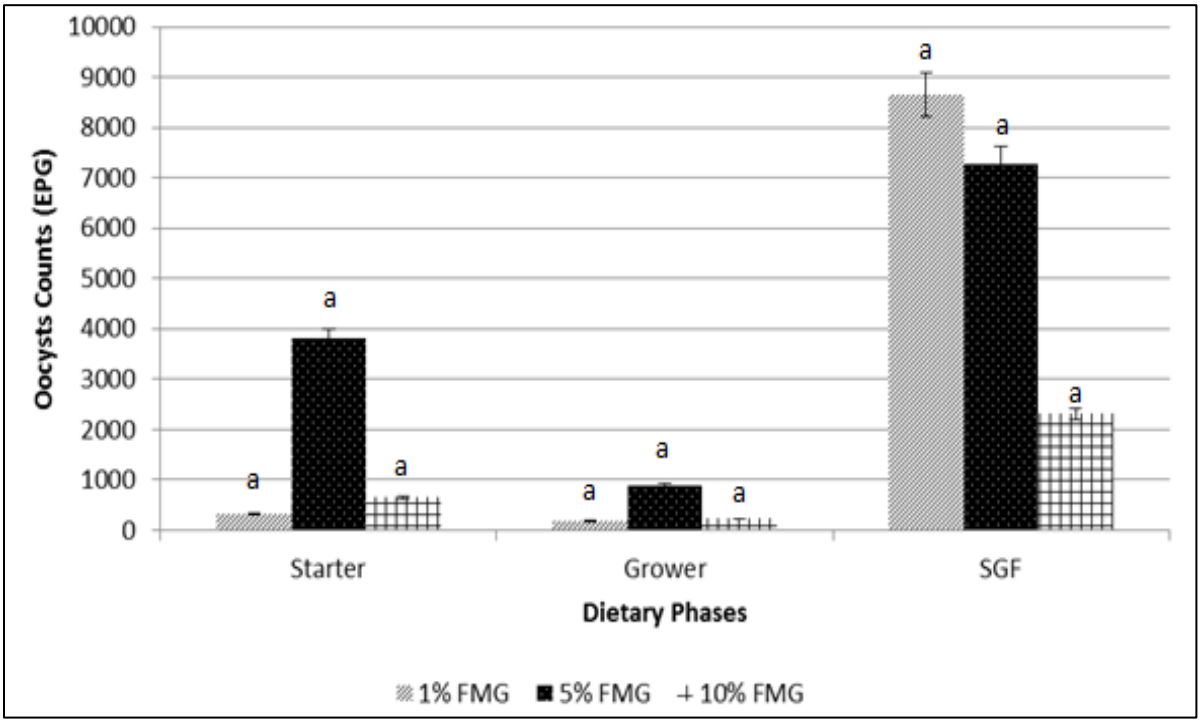

Figure 4 Effects of FMG on *Eimeria* oocysts Counts in Unchallenged Broilers d 49

There were significant ($p<0.05$) differences in the oocysts fecal shedding of experimentally challenged broilers as presented in (Figure 5) on d 49. Additionally, there was a significant ($p<0.05$) interaction between dietary level and grow-out phase influenced the oocyst shedding in birds. Treatment group 5 (5% grower) had the highest numerical difference compared to that of treatment groups 2 (1% grower) and 8 (10% grower). There were no statistically significant ($p>0.05$) differences in the dietary levels in neither the starter nor the starter-grower-finisher phases. *Eimeria* oocysts counts did not appear to negatively affect the body weights of broiler chickens. For instance, a high oocysts count in the grower phase at the 5% level did not cause depression in the body weights of boilers within the same treatment

group. In addition, the increased oocysts counts in treatment group 5 (5% grower) did not reflect negatively on the body weight gain of broilers within the same treatment group.

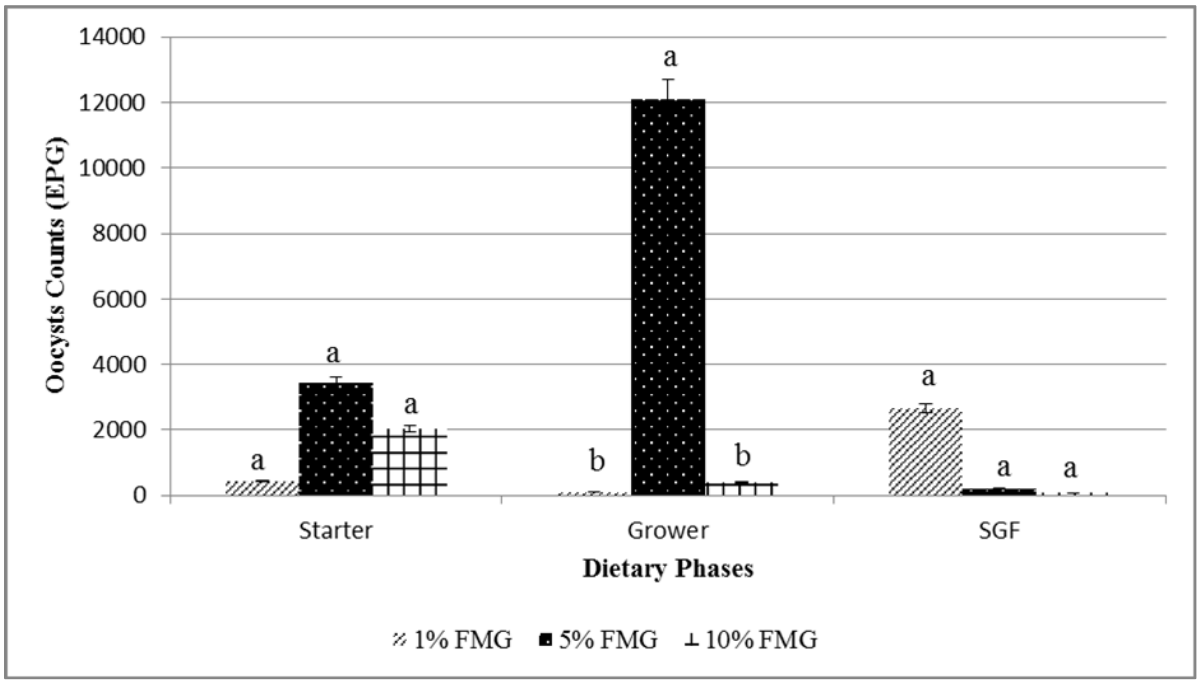

Figure 5 Effects of FMG on *Eimeria* oocysts Counts in Challenged Broilers d 49

4.5 White Blood Cell Differential Counts

4.5.1 Heterophil counts. Heterophil values for unchallenged broilers are represented in (Figure 6). A significantly higher ($p<0.05$) heterophil differential percent value was observed in treatment groups 2 (1% grower) and 5 (5% grower) as compared to unchallenged broilers in treatment group 8 (10% starter-grower-finisher). A significantly higher ($p<0.05$) heterophil cell count was also observed in broilers belonging to treatment group number 9 (10% starter-grower-finisher). Broilers in treatment group 6 (5% starter-grower-finisher) had a significantly lower heterophil value as compared to treatment group 9 (10% starter-grower-finisher). The heterophil values for broilers belonging to treatment group 3 (1% starter-grower-finisher) did not differ statistically from other treatment groups within the same experimental phase.

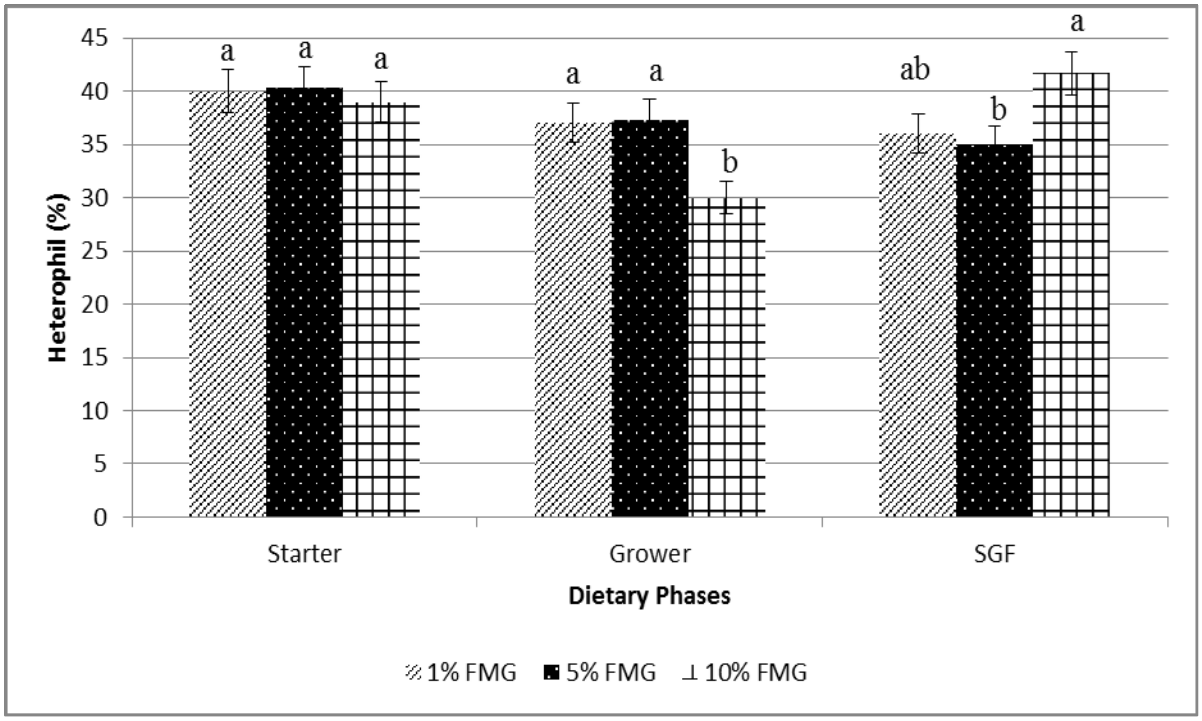

Figure 6 Effects of FMG Diet on Heterophil % of Unchallenged Broiler d 49

A significantly higher ($p<0.05$) heterophil differential value was observed for challenged broilers in treatment group 5 (5% grower) as compared to treatment group 2 (1% grower) and 8 (10% grower), which had significantly lower values (Figure 7). In addition, there was a significantly higher ($p<0.05$) heterophil value observed in broilers belonging to treatment group 6 (5% starter-grower-finisher) as compared to broilers in treatment group 9 (10% starter-grower-finisher) whose values were statistically lower. Heterophil values for broilers in treatment group 3 (1% starter-grower-finisher) had comparable values to both treatment groups 6 (5% starter-grower-finisher) and 9 (10% starter-grower-finisher) and therefore did not differ from them significantly. There were no statistical differences observed among dietary levels in the starter phase. Overall, heterophil values for challenged broilers in all treatment groups were slightly elevated.

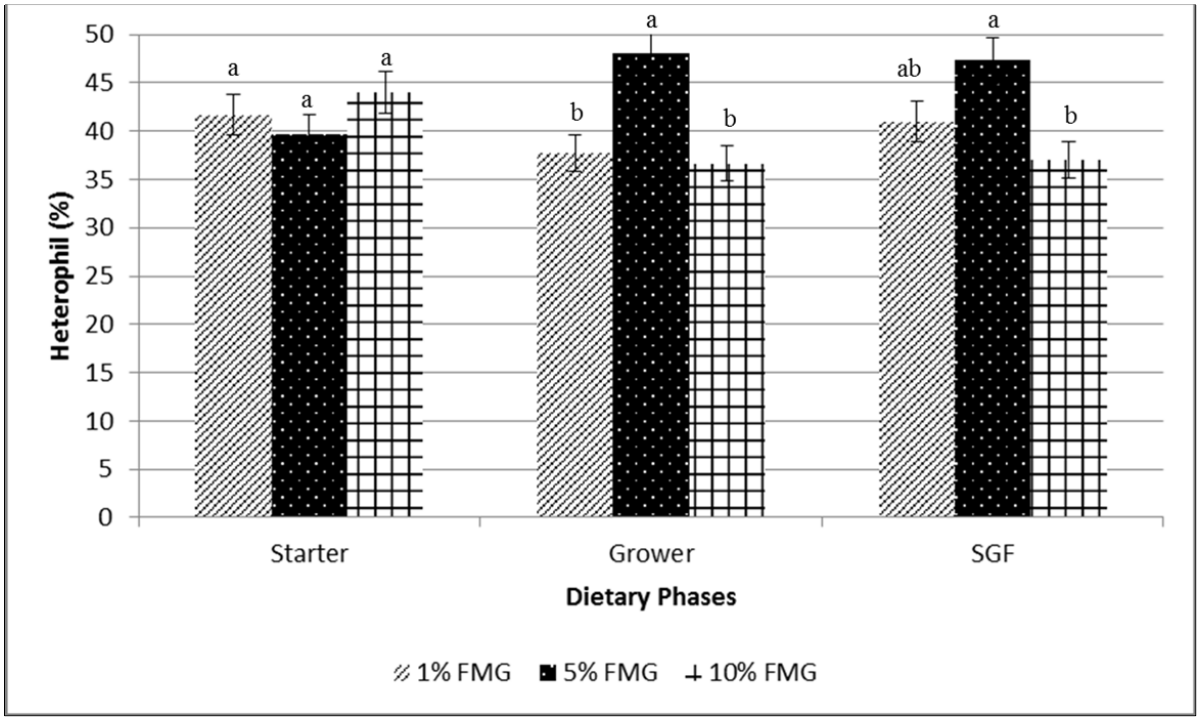

Figure 7 Effects of FMG Diet on Heterophil % of Challenged Broilers d 49

Heterophils are the second most abundant granulocyte in chickens and are proficient modulators of the avian immune system. Heterophils are highly phagocytic and are capable of generating an early primary innate response to pathogenic microbes (Harmon, 1998). The heterophil counts for both unchallenged and challenged chicks, in this study, fell within the normal avian hematological range (15-50%) (Campbell & Ellis, 2007). However, there were statistically significant elevations in heterophil counts among treatment groups for both unchallenged and challenged birds. It has been reported that heterophil numbers increase when birds are placed under stressful conditions (Maxwell & Robertson, 1998). Therefore, even slight elevations in heterophil counts may be indicative of an immunological challenge.

4.5.2 Lymphocyte counts. Lymphocyte values for unchallenged broilers on d 49 are represented in (Figure 8). A statistically lower lymphocyte value was seen in broilers belonging to treatment group 7 (10% starter) as opposed to treatment groups 1 (1% starter) and 4 (5%

starter) whose values were significantly higher ($p<0.05$). The lymphocyte differential value for unchallenged broilers belonging to treatment group 3 (1% starter-grower-finisher) was significantly higher ($p<0.05$) than that of broilers belonging to treatment group 6 (5% starter-grower-finisher) which was significantly lower. The lymphocyte differential values for broilers in treatment group 9 (10% starter-grower-finisher) did not significantly differ from treatment groups 3 (1% starter-grower-finisher) and 6 (5% starter-grower-finisher). Statistically, there were no significant differences ($p>0.05$) among the lymphocyte differential values of challenged broilers.

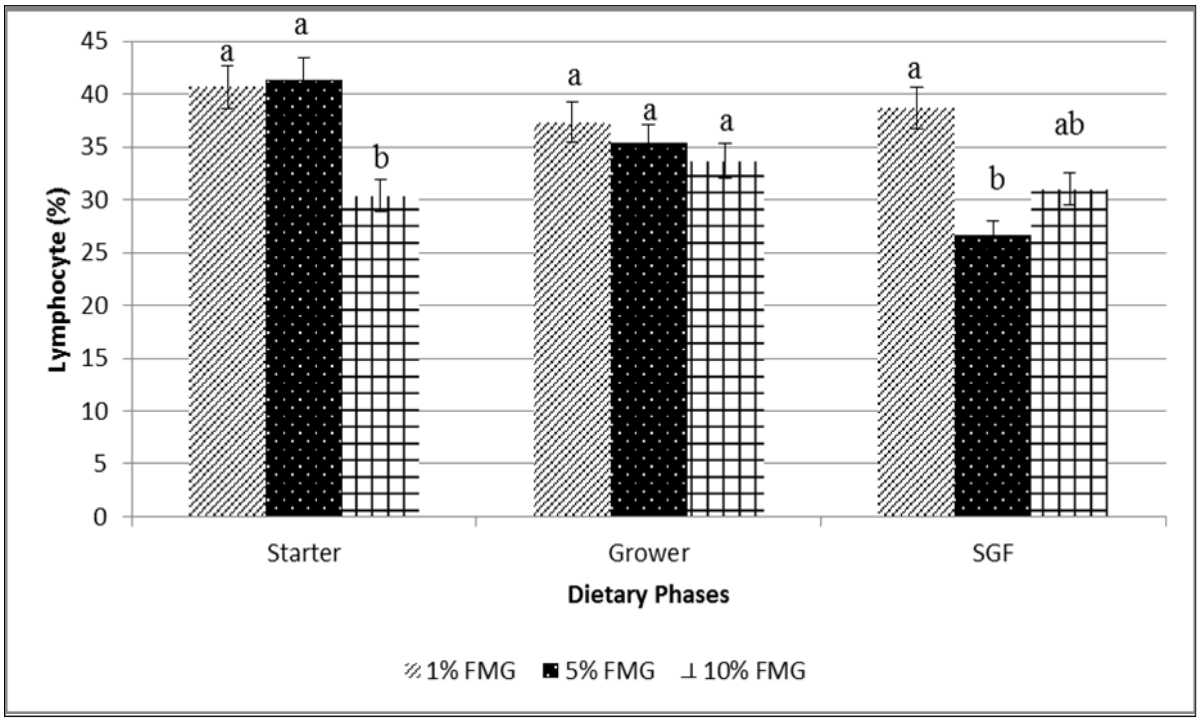

Figure 8 Effects of FMG Diet on Lymphocyte % of Unchallenged Broilers d 49

Lymphocytes values for challenged broilers are shown in (Figure 9). Lymphocytes cell values were also all within normal range according to literature (29-84%) (Campbell & Ellis, 2007). A fluctuation in lymphocyte values may be linked to a later *Eimeria* challenge (d 28) than would normally occur naturally within a broilers environment in production. In another study,

broilers given a 5% FMG diet consisting of shiitake and challenged with *Eimeria* spp. at 28 d had elevated lymphocyte values when compared to other treatment groups (Willis, Isikhuemhen, Minor, Hurley, & Ohimain, 2010b). Generally, lymphocytes are the most numerous WBC in chickens and are activated upon antigenic stimulation. However, in this study, heterophil counts were more numerous than lymphocytes on d 49. Campbell et al., (2007) noted that immunologically healthy chicks should have more circulating lymphocytes than heterophils. It has been reported that *Eimeria* infections in young broilers can prompt the activation and proliferation of local lymphocytes (Swinkels et al., 2006). No conclusive interpretation can be drawn from this data.

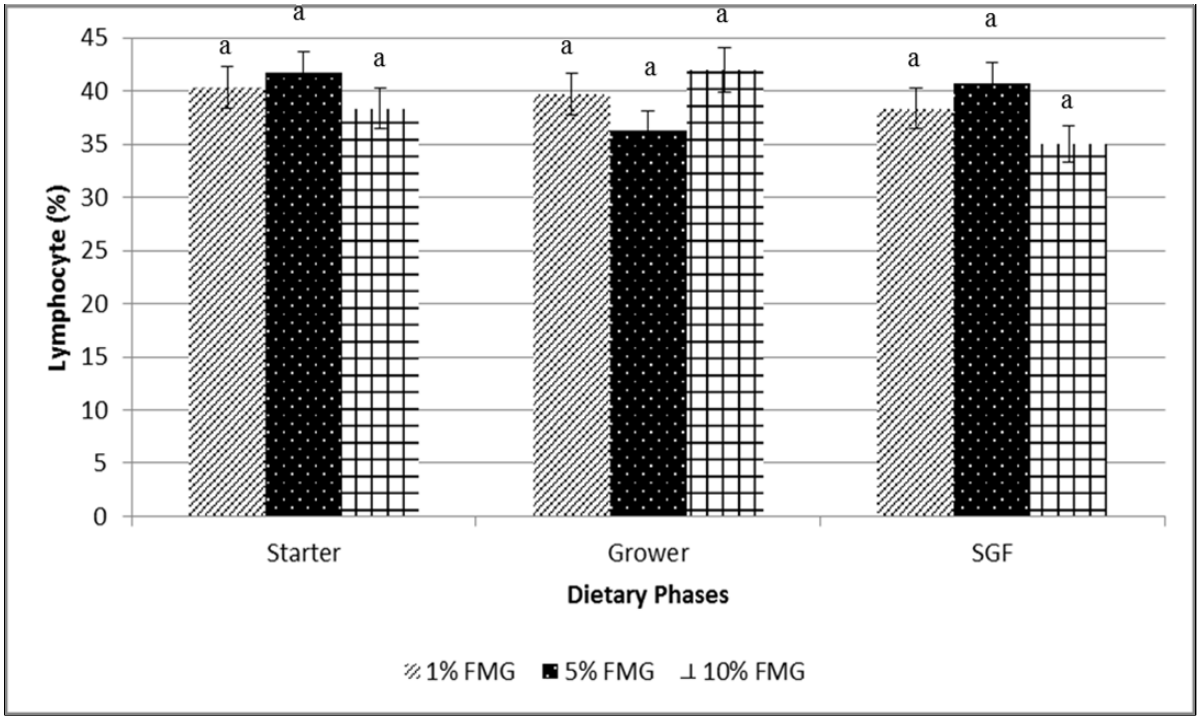

Figure 9 Effects of FMG Diet on Lymphocyte % of Challenged Broilers d 49

4.5.3 Monocyte counts. Unchallenged boilers in treatment group 5 (5% grower) were significantly lower ($p<0.05$) than those monocyte values for broilers in treatment groups 2 (1% grower) and 8 (10% grower) (Figure 10). There were also statistical differences found in broilers

fed FMG supplementation throughout duration of the study. In treatment group 9 (10 % starter-grower-finisher), the monocyte values for unchallenged broilers was statistically higher ($p<0.05$) than that of treatment group 3 (1% starter-grower-finisher), but did not differ statistically from the values of broilers in treatment group 6 (5% starter-grower-finisher).

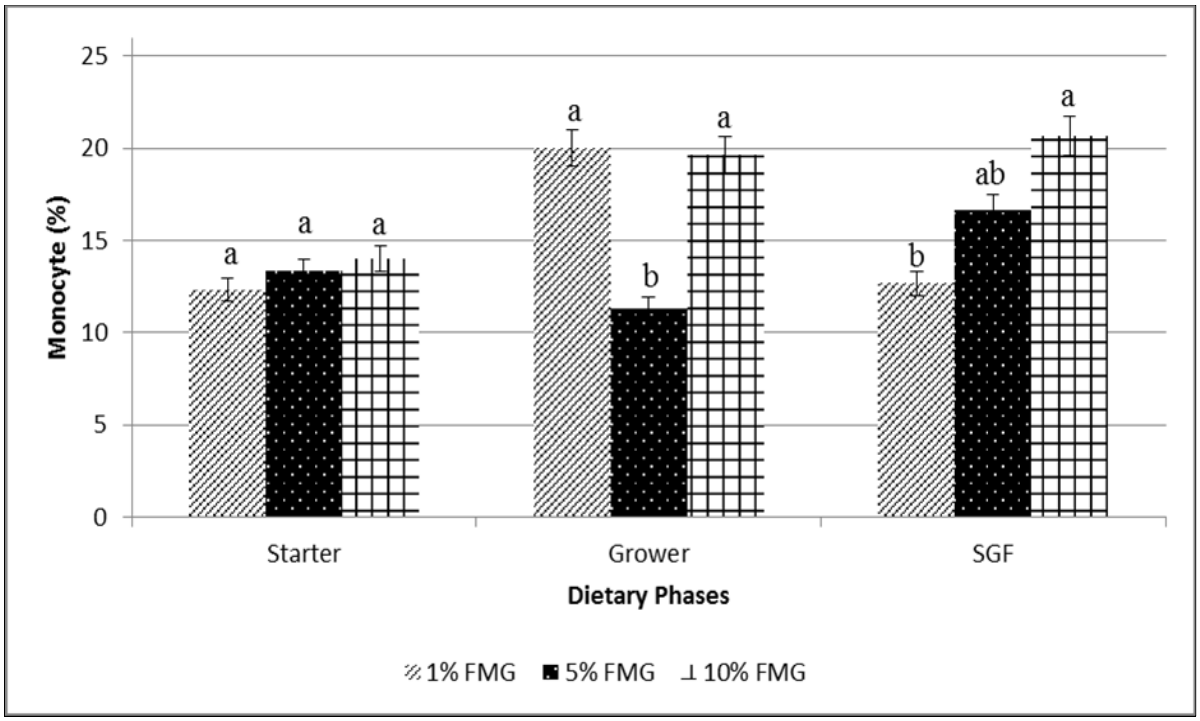

Figure 10 Effects of FMG Diet on Monocyte % of Unchallenged Broilers d 49

The monocyte values of challenged broilers (Figure 11) were statistically higher ($p<0.05$) in treatment group 7 (10% starter) than in treatment groups 1 (1% starter) and 4 (5% starter). Statistical differences were not found among challenged broilers in other treatment groups. Monocyte cell counts had the greatest variance among hematological cells. According to literature, monocyte values observed within this study were not within the normal range of 0-7% (Campbell & Ellis, 2007). However, in a previous study involving the administration of FMG to broilers, monocyte values ranged from 19 and 36% (Willis et al., 2010b). A scientific report

suggests that the activity of monocytes can be influenced by a variety of factors including; diet, environment, genetics and antigenic stimuli (Qureshi, 1998).

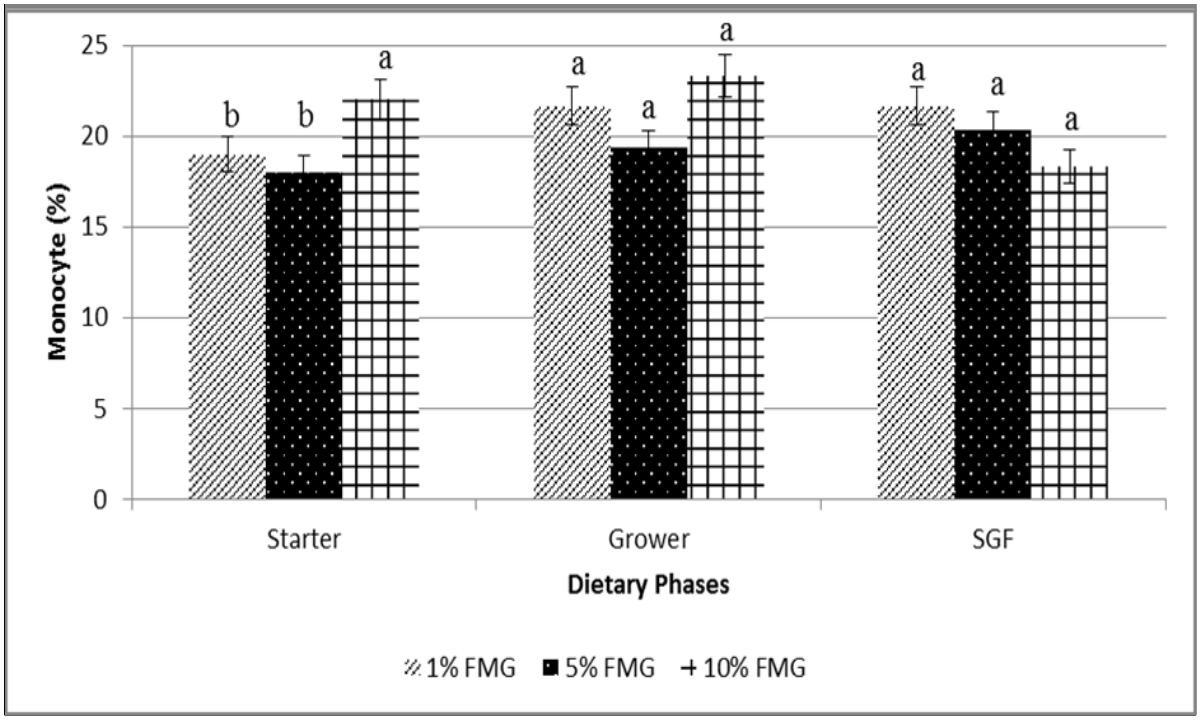

Figure 11 Effect of FMG Diet on Monocyte % of Challenged Broilers d 49

4.6 Chicken IgA Concentrations

The concentrations of unchallenged broilers IgA are presented in (Figure 12). The IgA concentrations of unchallenged broilers in treatment group 2 (1% grower) were significantly higher ($p<0.05$) when compared to treatment group 8 (10% grower) which had a significantly lower concentration. Broilers in treatment group 5 had an IgA serum concentration that was comparable to those of both treatment groups 2 (1% grower) and 8 (10% grower). There were also statistically significant ($p<0.05$) elevations in IgA serum concentrations in broilers belonging to treatment group 9 (10% starter-grower-finisher). Significantly decreased ($p<0.05$) levels of chicken serum IgA concentrations were observed in treatment group 3 (1% starter-grower-finisher). The IgA levels in broilers belonging to treatment group 6 (5% starter-grower-

finisher) did not differ significantly from neither treatment group 3 (1% starter-grower-finisher) or 6 (5% starter-grower-finisher).

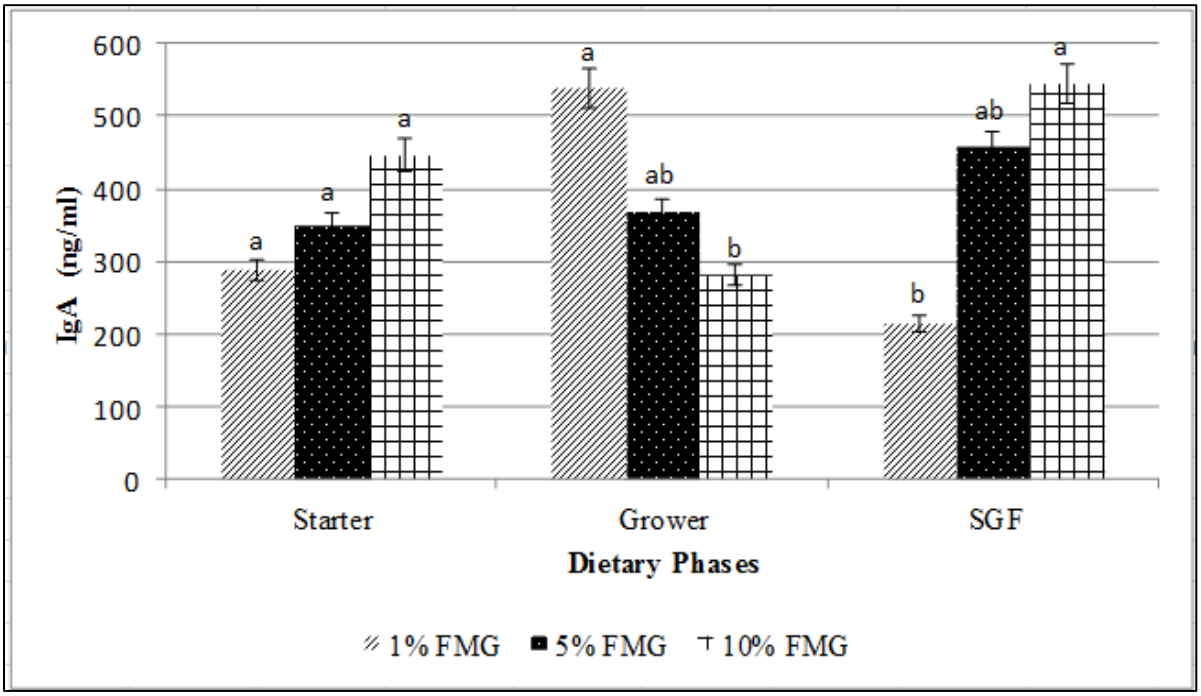

Figure 12 Effects of FMG Diet on IgA Concentrations in Unchallenged Broilers d 49

The IgA concentrations of challenged broilers are presented in (Figure 13). In respect to the IgA levels of broiler chickens experimentally challenged with common field strains of *Eimeria*, there were no statistically significant ($p>0.05$) differences between the dietary phase ($p>0.05$) and FMG inclusion level main effects. In addition, there were also no statistically significant interactions ($p>0.05$). However, there was a statistically significant ($p<0.05$) difference among the FMG supplemented treatment groups. Treatment groups 1 (1% starter) and 4 (5% starter) had statistically higher IgA concentration values when compared to treatment group 7 (10% starter). Concentration levels of IgA were elevated in treatment group 2 (1% grower) which differed significantly from treatment group 8 (10% grower) but not from treatment group 5 (5% grower). In addition, broilers in treatment group 3 (1% starter-grower-

finisher) had a significantly higher IgA concentration compared to broilers in treatment group 6 (5% starter-grower-finisher).

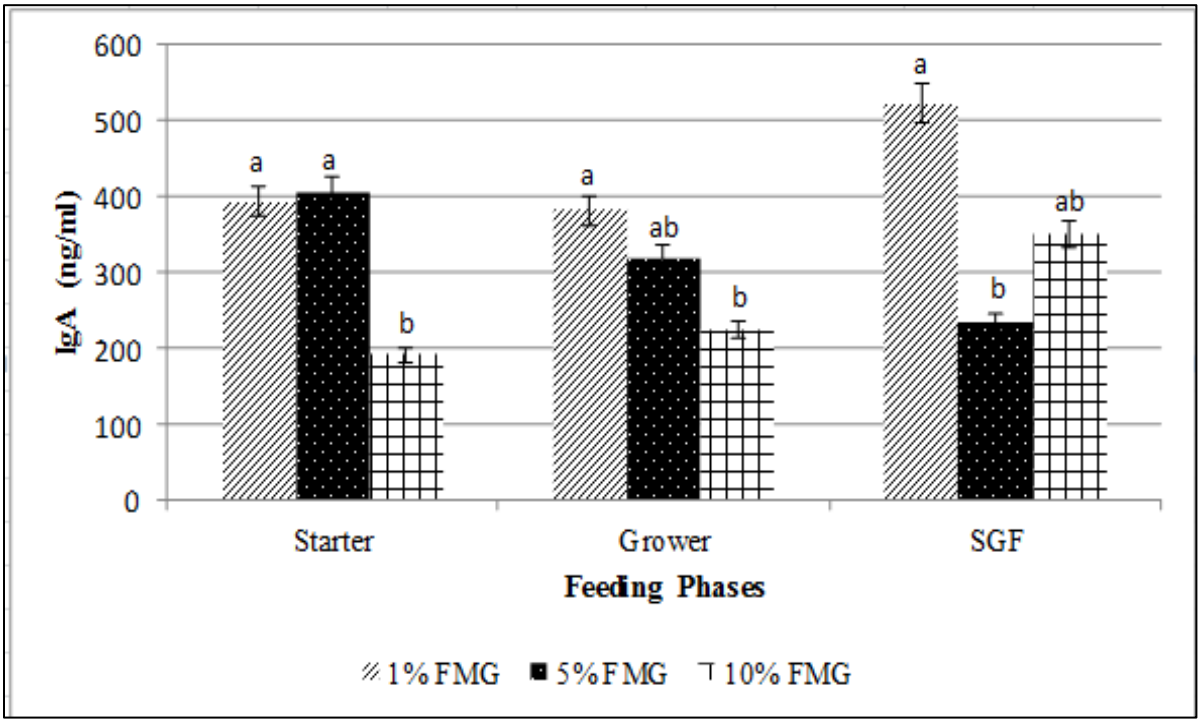

Figure 13 Effects of FMG Diet on IgA Concentrations in Challenged Broilers d 49

Chicken IgA is the primary immunological defense against pathogens which invade the body of their host through mucosal surfaces, especially those lining the GIT. IgA is normally found in body secretions and aids in the prevention of adherence and invasion of pathogens in the gut (Perry, 2006). Elevations in serum IgA may be an immunological indicator of gut infections in chickens. In an unpublished research conducted at the at this site, it has been noted that elevated IgA levels in chickens were a result of feeding chicks a diet consisting of medicinal mushrooms which caused stimulation of the immune system.

4.7 Chicken IgG Concentrations

Statistically there were significant effects between the dietary phase ($p<0.05$) and FMG inclusion level main effects on the means of IgG concentrations in unchallenged broilers (Figure

14). There was a significant influence ($p<0.05$) within the dietary treatment phase on broiler IgG concentration levels in treatment group 4 (5% starter) which had the highest serum IgG values. Broilers in treatment group 1 (1% starter) had significantly lower ($p<0.05$) serum IgG concentration values.

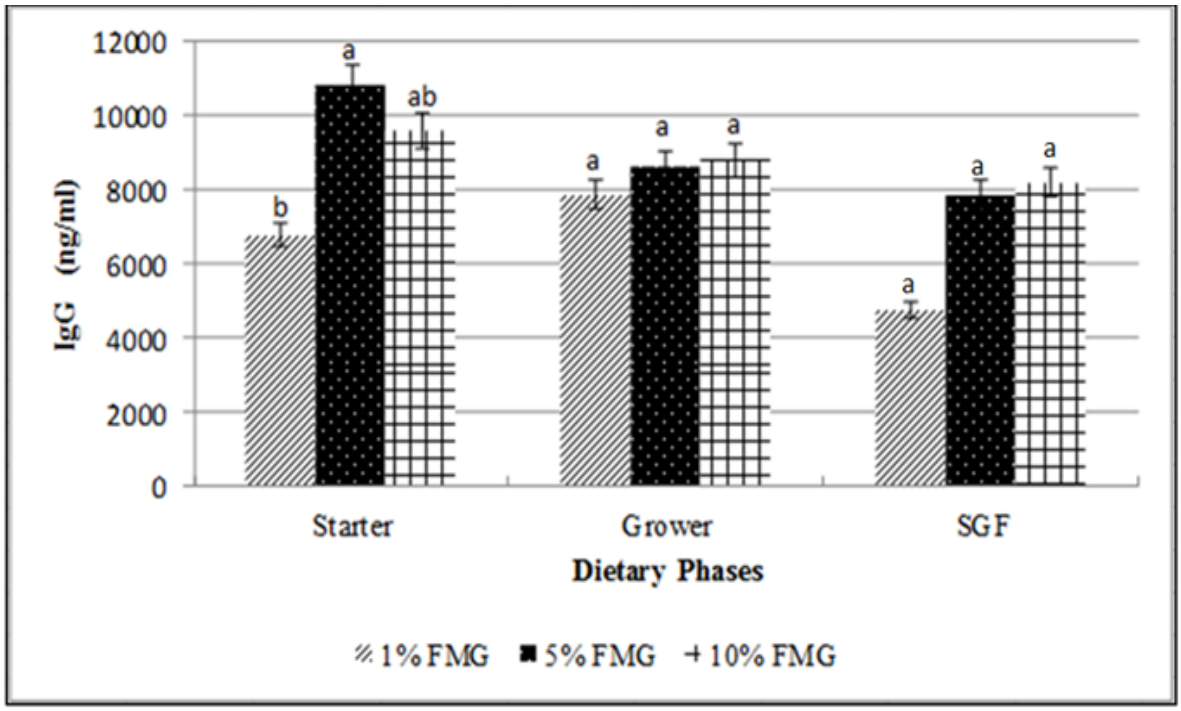

Figure 14 Effects FMG Diet on IgG Concentrations in Unchallenged Broilers d 49

There were no statistically significant ($p>0.05$) dietary phase and level effects on the IgG concentrations of challenged broilers (Figure 15). In addition, there were no statistically significant observations for the IgG concentration levels among the treatment groups of challenged broilers.

Avian IgG is the most abundant antibody found within the blood (Perry, 2006; Scanes et al., 2004). It can be found nearly anywhere in the body and is responsible for neutralizing antigens. IgG production is generally stimulated by infection and inflammation. The average concentration of IgG in the blood serum of chickens is approximately 600-1300 µg/ml (Bethyl

Laboratories, Inc, USA). Although serum IgG concentration values fell within normal range for chickens, most IgG values were elevated.

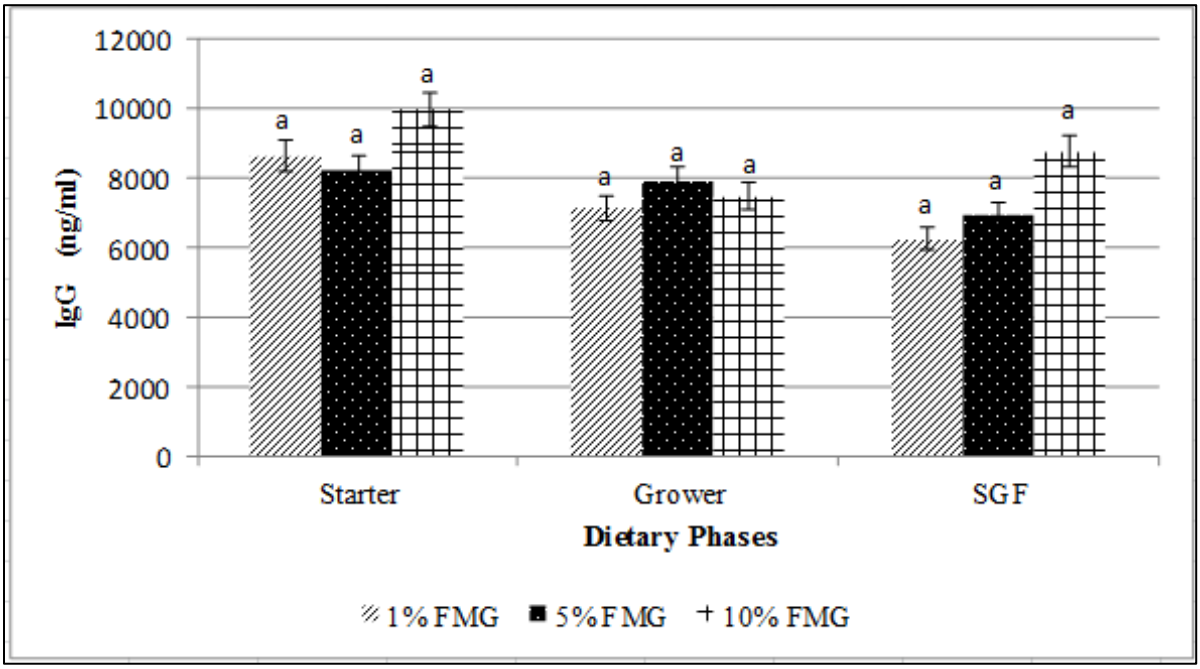

Figure 15 Effects of FMG Diet on IgG Concentrations in Challenged Broilers d 49

Elevated levels of serum IgG may serve as an indicator for chronic infections. In addition, as mentioned earlier, the IgG antibody in broilers is functionally similar to both mammalian IgG and IgA. Therefore, the elevated levels of IgG in both unchallenged and challenged broilers may be an indicator of a chronic parasitic infection (coccidiosis) which would coincide with the elevated levels of IgA which can be indicators of intestinal gut infections.

4.8 Bursa Weights

There were no statistical ($p>0.05$) differences found regarding the influence of dietary phase and level effects nor interactions on unchallenged broiler bursa weights (Figure 16). However, the bursa weights of broiler chicks did vary significantly ($p<0.05$) among treatments. Broilers belonging to treatment group 7 (10% starter), had significantly higher bursa weights

when compared to unchallenged broilers in treatment group 4 (5% starter). The bursa weights for broilers in treatment group 1 (1% starter) were comparable to the values observed in treatment groups 4 (5% starter) and 7 (10% starter) and therefore did not differ from either group statistically. Unchallenged broilers in treatment group 9 (10% starter-grower-finisher) had the highest overall bursa weight values for broilers being fed a supplemental diet of FMG in all dietary phases. Broilers in treatment group 3 (1 % starter-grower-finisher) had significantly lower ($p<0.05$) bursa weight values when compared to treatment group 9 (10% starter-grower-finisher). Overall, broilers supplemented with dietary FMG in the grower phase had numerically lower bursa values as compared to broilers receiving FMG in the starter and starter-grower-finisher phases.

The Bursa of Fabricius is an essential lymphoid organ in chickens that plays an important role in adaptive immunity. Bursa size can reflect the immunological health status of birds (Glick, 1979). Healthy broilers produce larger bursas than broilers whose immune systems have been compromised (Glick, Chang, & Jaap, 1956). Glick et al., (1956), also reported that the Bursa of Fabricius plays an important role in antibody production. Therefore, it may be perceived that unchallenged broilers in treatment groups with higher bursa have superior immune systems compared to broilers whose bursa weights were smaller. Although, there is no apparent evidence that bursa size had any significant influence on the lymphocyte values or antibody titers of unchallenged broilers in this study, there has been a noticeable immunological response to *Eimeria* infections in both challenged and unchallenged broilers. In addition, there have been reports that have indicated positive effects on the integrity of the immune system of chickens given medicinal mushrooms in their diets (Guo et al., 2004a; Willis et al., 2012; Willis et al., 2013). Therefore, it may be suggested that broiler diets supplemented with certain mushrooms

can exert positive health attributes via bursa performance. There are no data from challenged broiler bursa weights presented for comparison.

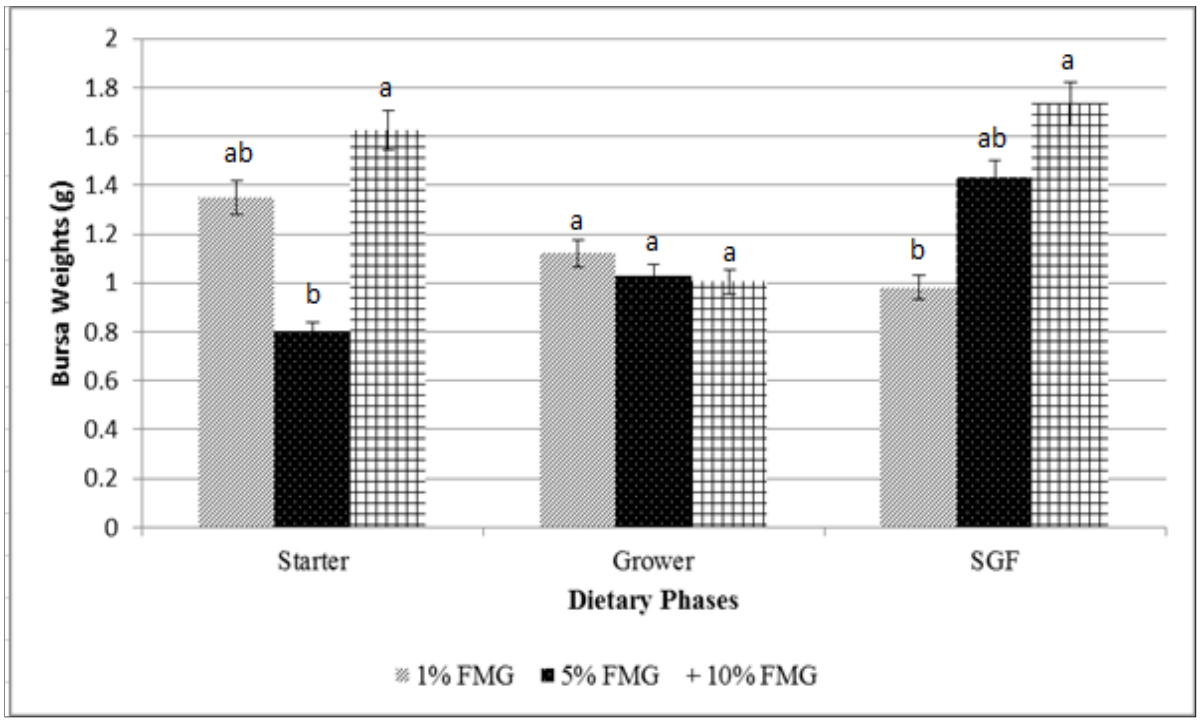

Figure 16 Effects of FMG Diet on Unchallenged Broiler Bursa Weights d 49

CHAPTER 5

Summary and Conclusion

The present study was designed to evaluate the effects of dietary FMG supplements administered to broiler chicks at different dietary phases and at different stages of development on broiler performance and health. A positive impact from the medicinal mushrooms utilized in this study was observed in the performance enhancement protection of broilers having been exposed to *Eimeria* spp. The final body weights of both experimentally challenged and naturally exposed broilers were primarily uniform and did not reflect the loss in weight normally associated with chickens clinically infected with coccidiosis. Chickens that were directly challenged with *Eimeria* spp. and administered a diet supplemented with FMG at the 1% inclusion level within the starter phase showed a significant depression in body weight compared to all other treatment groups. Although depressed body weights can be a result of many different factors, it is quite possible that supplying chicks with a relatively low inclusion of FMG at a young age does not provide enough protection against avian coccidiosis. On the contrary, based on numerical trends, broilers in both the unchallenged and challenged treatment groups had better growth performance when supplemented with a 1% inclusion of FMG in the grower and starter-grower-finisher phases.

At the end of the trial, chickens who were directly challenged with *Eimeria* spp., that had been given diets supplemented with FMG at all inclusion levels and in all grow-out phases had relatively low fecal oocyst counts with the exception of broilers who received a 5% FMG inclusion within the grower phase. In general, the parasitic load of both unchallenged and challenged broilers did not negatively impact their final body weights. Relatively low mortality rates support the positive effect that FMG supplementation had on broilers' performance within

all treatment groups. Therefore, it cannot be concluded as to whether or not it may be reasonable to avoid administering a combination of medicinal mushrooms to broilers at the 5% inclusion level in the grower phase based upon observations in oocyst output and shedding. However, a relatively low amount of fecal oocysts shedding in the starter and grower phases of unchallenged broilers, and in the starter and starter-grower-finisher phases of challenged broilers can be linked to improved performance via FMG supplementation at the 1%, 5% and 10% inclusion levels.

Fluctuations in avian hematological values have been reported as being a normal occurrence. Percent values for heterophils and lymphocytes in both challenged and unchallenged broilers were within the normal hematological range for chickens. Monocyte values, however, where notably elevated and could be indicative of infection. Although, based on the observations of relatively low oocysts counts in both unchallenged and challenged broilers, it cannot be proposed that the increase in monocyte values were in direct relation to *Eimeria* spp. infection. Observed elevations in the IgA and IgG concentration levels of both unchallenged and challenged broilers did not conclusively indicate whether or not FMG supplementation had any influence on the immunological response of broilers. However, it appears that higher levels of IgA at the 1% inclusion level in the grower phase of both unchallenged and challenged broilers were superior to that of any other treatment group and may be indicative of nutrition based immune enhancement.

From the data obtained from this study, it can be concluded that that use of FMG in the diets of chickens exposed to *Eimeria* has an effect on performance and health. However, due to the variability in the results found between each measured parameter, it is difficult to interpret the exact influence that FMG had on the performance and health of broilers in one particular diet.

5.1 Recommendations

Future research is required to determine which level of dietary FMG and at which stage of growth is optimal for broiler performance and health. Broilers in this study were challenged with *Eimeria* spp. later than what is normally observed with natural infections; therefore the potential impact of parasitic burden may not have been reflected in broiler performance and health parameters. Future investigations should be conducted in which all broilers are challenged with *Eimeria* spp. within a period of time that natural *Eimeria* infections would normally occur. Further studies should compare the performance and health parameters of broilers that are experimentally challenged with *Eimeria* separately from birds with natural infections. A trial set up in this way may generate more conclusive observations on performance and health parameters. A larger sample size of broilers may result in improved data analysis and reduce variability in parameter measurements.

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