THE IMPACTS ON BROILER PEFORMANCE AND YIELD BY REMOVING ANTIBIOTIC GROWTH PROMOTERS AND AN EVALUATION OF POTENTIAL ALTERNATIVES

A Dissertation

by

JOEY L. BRAY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2008

Major Subject: Poultry Science

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ABSTRACT

The Impacts on Broiler Performance and Yield by Removing Antibiotic Growth Promoters and an Evaluation of Potential Alternatives. (December 2008)

Joey L. Bray, B.S., Stephen F. Austin State University;

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Three experiments were conducted to evaluate the impacts of removing antibiotic growth promoters (AGP) on broiler performance and yield and to evaluate alternative products as potential replacements. In experiment one, approximately 552,000 broilers were reared in four solid-wall, tunnel ventilated houses that were divided into two paired-house facilities, each assigned one of two dietary treatments. The treated group received basal diets containing salinomycin (SAL), roxarsone (ROX) and AGP, while the control group received the same diets without ROX and AGP. Removal of ROX and AGP had no affect on average body weight and feed efficiency, while livability was significantly affected negatively by the removal of ROX and AGP. Tender, wing, drum and percentage of total white meat showed significant improvements in yield during the study, while all other parts were not affected by removal of ROX and AGP.

In experiment two, an investigation was conducted to evaluate the effects on performance from feeding *Bacillus subtilis* spores (Gallipro®, Chr Hansen A/S, Denmark), as a direct-fed microbial additive, to commercial broiler chickens. Birds were

divided among two paired-house facilities. The treatment group received basal diets supplemented with *B. subtilis* spores, while the control group was fed the same basal diets containing an AGP. Feed conversion ratio was significantly lower for the treatment group, while average body weight, coccidiosis lesion scores, and footpad scores were not affected by the treatments.

In experiment three, 6,000 broiler chickens were equally divided among four treatment groups and reared to 49 d to determine the effectiveness mannan oligosaccharides (MOS, Bio-Mos®, Alltech, Nicholasville, Kentucky, USA) as an alternative for an AGP program and MOS plus Natustat™ (NAT, Alltech, Nicholasville, Kentucky, USA) as an alternative to an enteric health program (AGP+anticoccidial drug). Average body weight for the control (CON) and antibiotic (ANT) groups was significantly different from the MOS+NAT group, but not the MOS group. Carcass front half, carcass hind half, frame and skin yields were improved for all treatments when compared to the MOS+NAT group. Conversely, percent total white meat yield was improved with the inclusion of MOS when compared to the ANT group.

The findings of this research suggest that the removal of AGP from the diets of commercial broiler chickens does not affect the performance and yield of the birds over a one year production period. Furthermore, *B. subtilis* spores and mannan oligosaccharides provide acceptable alternatives to an AGP program.

DEDICATION

This dissertation is dedicated to my family, wife Cathryn, daughter Madilyn and son Blake. Your love, support, and patience have helped make this possible and your dedication to my success was an inspiration. Also, to my mother, Joye, who always stood beside me and encouraged me to always reach my full potential. Thank you for all of your love and support throughout this process.

ACKNOWLEDGEMENTS

I would like to thank Dr. John B. Carey, my major professor, for his continuous guidance and wisdom throughout the course of my studies, for without your help this would not have been possible. Your assistance during my studies, research projects, and preparation of my dissertation were vital and I thank you.

I would like to thank my committee members, Dr. David Caldwell, Dr. Michael Davis, and Dr. Morgan Farnell, for their guidance and support in my education and research. You all have made a lasting impression on my career and my life, and for that I thank you.

Deep and heartfelt thanks to Dr. Tim Cherry, at Stephen F. Austin State University, for your encouragement to continue my education. Your guidance and support was a tremendous help. Your wisdom and devotion to my success is greatly appreciated.

Thanks also go to my friends and colleagues that helped make this research possible: Crystal Taylor, Danny Rossow, Robert Kavanaugh, Jason Shipp, Pieter Post, and all of the students that helped with the numerous yield studies.

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CHAPTER I

INTRODUCTION

Background

Recently, consumers of animal protein products have taken an interest in the animal agriculture practices used to meet the demands of producing these products. Particular attention has been placed on the health issues of the animals and the intense nature of the production systems. Commercial poultry production has been under tremendous scrutiny due to its dependence on chemotherapy to improve weight gain and feed efficiency, while avoiding enteric health problems common to confined animal feeding operations.

Enteric Disease

Disease can be defined as any deviation or interruption of the normal structure or function of any part, organ or system within the host [1]. Enteric diseases are detrimental to poultry production due to the loss of productivity, increase in mortality, the potential for human health risks associated with food borne illness, and the increase in cost associated with disease prevention and treatment [2]. Pathogenic microorganisms have to elude many natural defenses of the host to cause disease. Low gastric pH, rapid transit through portions of the gastrointestinal tract, competitive intestinal microbiota, and immune defenses are in place to deny pathogenic microorganisms from colonizing the digestive tract and establishing disease. Factors such as stress, nutrition, and injury can leave the host more susceptible to disease. The severity and duration of stress factors,

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This dissertation follows the style of Journal of Applied Poultry Research.

such as suboptimal temperatures, poor environmental conditions, and improper handling can influence the host's vulnerability to disease. Disease may also result from deficiency of vital nutrients or the ingestion of toxic substances from the feed. Nutritional deficiencies can lead to improper function of some natural defenses providing optimal conditions for the establishment of disease. While some nutritional deficiencies may be reversible with supplementation of adequate nutrients, others are irreversible and leave the animal permanently disease prone. Injury, whether temporary or permanent, can lead to primary and secondary infections that result in disease [3].

Disease Prevention Factors

Many management factors have an influence on disease control and prevention. Biosecurity practices are designed to prevent the spread of disease. Most poultry producers have adopted the practice of removing entire flocks from farms before any new replacements are added. This "all-in, all-out" concept decreases the spread of disease from one flock to the next. Furthermore, young poultry that is more disease-sensitive is reared in isolation from older poultry that is more disease-resistant. Poultry housing and environmental factors can influence the instance of disease. Modern poultry facilities have been constructed to minimize conditions conducive for harboring disease organisms. Advancements in ventilation have lead to a reduction of stressful conditions such as excess dust, high levels of ammonia, damp litter, and excessive draft over the birds. Advancements in poultry housing equipment has allowed for more favorable conditions. One example is the creation and adoption of the nipple drinking system. These drinking systems, when properly used, can decrease the amount of moisture being

placed into the litter. Sanitation, mortality disposal, vaccination programs, and rodent control are other critical practices that must be addressed for proper biosecurity. While biosecurity practices are important in disease prevention, it cannot provide total protection against infection. To eliminate the threat of enteric disease and to promote the growth of the birds, producers have relied on the use of antibiotics at subtherapeutic levels.

Brief History of Antibiotic Growth Promoters (AGP)

During the 1940's, advancements made in poultry genetics, nutrition, housing, and marketing fueled the expansion of the US poultry industry [4]. Poultry production became more of an intensive and confined production system in order to efficiently produce more birds in a shorter period of time. Along with increased production, came an increase in the occurrence of disease. During this time period, it was observed that animals fed dried mycelia of Streptomyces aureofaciens showed an increase in growth [5]. It was later discovered that the dried mycelia contained residues of chlorotetracycline, an effective broad spectrum antibiotic. Chlorotetracycline was the first of the tetracycline antibiotics. Moore and colleagues [6] were the first to demonstrate the beneficial effects of feeding antibiotics at subtherapeutic levels to improve performance in poultry. Streptothricin, streptomycin, sulfasuxidine or a combination of streptomycin and sulfasuxidine did not sterilize the intestinal tract of chickens, but showed a reduction of coliform bacteria in the ceca, while the combination of streptomycin and sulfasuxidine increased growth rates of the birds. In 1951, the United States Food and Drug Administration (FDA) approved the use of antibiotics as an

animal feed additive without the prescription of a veterinarian [4]. The industry quickly adopted the use of antibiotic growth promoters and made it the industry standard for production.

Biological Aspects of AGP

Antibiotics fed at sub-therapeutic levels promote growth and feed efficiency in poultry and other animals [7]. The mechanism of action for AGP has been explained as an interaction between the antibiotics and the intestinal microbial population [5]. Four major mechanisms of action for AGP have been reviewed to explain their beneficial effects on performance [7, 8, 9] These mechanism consist of the following: 1) AGP inhibit endemic subclinical infection, thus reducing the metabolic cost of the innate immune system; 2) AGP reduce the growth-depressing metabolites produced by microbes, such as ammonia and bile degradation products; 3) AGP reduce microbial use of nutrients; and 4) AGP enhance the uptake and use of nutrients, due to the thinning of the intestinal wall in AGP-fed animals [10]. These mechanisms suggest that, either directly or indirectly, the intestinal microflora depresses the growth of the animal. The reduction of intestinal microbial population could be the underlying beneficial action of AGP.

AGP-associated Problems and Concerns

For over 60 years, the US poultry industry has relied on the use of chemoprophylaxis with antibiotics, for the control and prevention of enteric diseases. Potential problems and concerns have resulted in global changes for the use of AGP, with the most drastic changes occurring in the European Union. One potential problem is

the increased resistance of pathogenic bacteria to the approved antibiotics used for growth promotion [11]. An early sign of resistance to streptomycin in turkeys was reported shortly after the approval of AGP use by the FDA [12]. Tetracycline resistance was associated with feeding growth-promoting levels to chickens in the late 1950's [13, 14]. By the 1980's, resistance to numerous antimicrobial agents by pathogenic bacteria was reported worldwide [15]. The occurrence of AGP-associated resistant bacteria has lead to concerns of human health risks. The possibility of zoonotic bacteria with antibiotic resistance linked to animal use of AGP that could be contracted by human recipients has stimulated change [11]. The major driving force of these changes has been the pressure placed on the poultry industry by consumers of animal meat proteins that perceive AGP-associated human health risks to be serious [16]. This consumer pressure has lead to major retailers and restaurant chains compelling poultry producers to voluntarily reduce the use of AGP.

Banning of AGP use in the European Union (EU)

In 1986, Sweden was the first country to ban the use of antimicrobials for growth-promoting purposes [15]. In 1995, Denmark banned the use of Avoparcin (a vancomycin-like compound) from use as an AGP in food animals due to reports of resistance in isolates from conventional and organic poultry farms. In 1997, the Commission of the EU banned Avoparcin in all EU member states [7]. After the banning of Avoparcin, the EU Commission launched an investigation into the use of all AGP approved for use in their member states. It was determined that the use of AGP could increase the instance of microbes with resistant genes and pose a potential for humans if

they are transferred to persons. Therefore, the World Health Organization and the Economic and Social Commission of the EU concluded that the use of antimicrobials in food animals is a public health concern. From this conclusion, a plan was set forth to withdraw the use of all remaining approved AGP from all EU member states by January 1, 2006 [5].

AGP Usage in the United States

Little regulatory activity has taken place in the US with the use of AGP [7]. In 2005, fluoroquinolones were banned from use in poultry production in the US [16]. The similarity of fluoroquinolones to drugs used in human medicine has lead to their removal for therapeutic use. The use of AGP is under tremendous scrutiny in the US, the pressure applied by the consumers of animal protein products remains as the major influence of voluntary AGP removal.

Search for AGP Alternatives

The mandatory removal of AGP in the EU and the voluntary removal of these drugs in the US have resulted in a worldwide search for suitable alternatives to counter the expected decline in poultry performance. Some potential alternatives that have been tested include probiotics, prebiotics, competitive exclusion cultures, organic acids, and plant extracts. Probiotics are defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance [17]. These products can be comprised of a single bacteria strain or a mixture of strains. Prebiotics are defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of intestinal

bacteria [18]. Both probiotics and prebiotics have ideal characteristics which are shown in Table 1. Several microbial strains have been tested for use as probiotics in food animals, including species of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *E. coli*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, and varieties of yeast species. Each has unique characteristics that make them suitable for use as probiotics. Prebiotics that have been tested for animal use include the oligosaccharides (fructo-, trans-galacto-, gluco-, glyco-, malto-, xylo-, and sucrose thermal), lactulose, and lactitol [2]. These nondigestible ingredients can be fermented by the intestinal microflora and utilized as nutrients, decreasing the competition for nutrients between the microbial population and the intestinal tract. Competitive exclusion (CE) cultures are defined as mixtures of adult intestinal microflora exhibiting disease resistance. Newly-hatched chicks are inoculated with this disease resistant adult intestinal microflora to provide protection. Also known as the Nurmi-concept, this practice can provide immediate protection to young chicks that are prone to disease without an established intestinal microflora [19]. Mannan oligosaccharides (MOS) are nondigestible carbohydrates that are the main component in the outer cell wall of yeast cells (*Saccharomyces* spp.). While similar to prebiotics, MOS is not thought to selectively enrich intestinal bacteria, instead they are thought to bind to pathogenic bacteria and remove them from the intestinal tract [20]. Mannose is the main component of MOS and is a unique sugar. Many bacteria have mannose-sensitive receptors, called Type 1 fimbriae, which allow the bacteria to attach to the sugar rich epithelial lining of the intestinal tract. MOS can attach to this receptor and denying the bacteria from attaching to the intestinal tract. The MOS-bound bacteria can then be

Table 1. Ideal characteristics of probiotics and prebiotics[1]

Probiotics
Be of host origin
Non-pathogenic
Withstand processing and storage
Resist gastric acid and bile
Adhere to epithelium or mucus
Persist in the intestinal tract
Produce inhibitory compounds
Modulate immune response
Alter microbial activities
Prebiotics
Be neither hydrolyzed or absorbed by mammalian enzymes of tissues
Selectively enrich for one or a limited number of beneficial bacteria
Beneficially alter the intestinal microbiota and their activities
Beneficially alter luminal or systemic aspects of the host defense system

[1]Adapted from Simmering and Blaut [21]; Patterson and Burkholder [2].

flushed from the intestinal tract, decreasing the instance of pathogenic bacterial colonization [22]. This property makes MOS more of a CE product than a prebiotic. Organic acids are organic compounds that have acidic properties that can penetrate the bacterial cell wall and disrupt cell physiology [23]. Formic and propionic acids are examples that typically affect pH-sensitive bacteria, such as spp. of *E. coli*, *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, and *Clostridium perfringens*. Bacteria that are pH-sensitive cannot deal with drastic changes in the pH gradient between intracellular and extracellular environments. Various plant extracts have shown to have antimicrobial abilities. Essential oils of various plants have shown the most potential for antimicrobial action. These products are typically used as a blend of different extracts, such as thyme, clove, black pepper, oregano, and yucca [22].

Objectives of the Study

The objectives of this body of research was to evaluate the impacts of removing in-feed, sub-therapeutic antibiotics, fed for growth promotion purposes, from the diets of commercial broiler chickens over a one year production period. Further studies were conducted to examine the potential for a probiotic (*B. subtilis* spores), competitive exclusion product (mannan oligosaccharide), and plant extract blend (plant extracts + mannan oligosaccharide + organic minerals) as potential alternatives to AGP, while determining their effects on broiler performance parameters.

CHAPTER II

REVIEW OF LITERATURE

Consequences of AGP Removal

The main expected consequence from the ban of AGP in the EU was a reduction in the use of antibiotics in animal production, eliminating the risk of transferring microbes with resistant genes to humans [5]. There has been conflicting data about the true consequences of removing antibiotics for growth promotion in the EU. One report explains that the broiler industry in Denmark has seen little affect from the banning of AGP from 1995 to 2002. Productivity and livability were not affected, but an increase in feed conversion has been seen over this time period. Mortality due to necrotic enteritis did not increase after the ban of AGP in Denmark. However, the use of the ionophore (ION), salinomycin, which has activity against *Clostridium perfringens* did increase steadily after the AGP ban. This may reflect the attempt of producers to control necrotic enteritis with the use of salinomycin [7]. On the other hand, some reports suggest that although the removal of AGP resulted in lower overall use of antimicrobials, the result has been an increase in the use of therapeutic antibiotics to combat infections [11, 24].

In the US, a report by Chapman and Johnson [25] indicates that in 1995 an ANT was used in the starter, grower, and withdrawal diets by 94.3, 98.2, and 75.1% of broiler production units, but by 2000, there use had declined to 64.8, 66.9, and 48.1%, respectively. It was determined that between the years of 1995 and 2000, BMD was most frequently used in the starter and grower diets, whereas, VIR was most frequently found in the withdrawal diet. Broiler production units decreased their use of an ION +

ROX + antibiotic (ANT) combination from 1995 to 2000. This decline was likely due to the fact that ANT has a higher added cost to the feed as compared to ION and ROX, and coupled with producers belief that the growth-promoting effects once observed from these drugs was no longer evident. Furthermore, this data reflects the intense use of the same ANT by a majority of the broiler producers in the United States over an extended period of time, greatly increasing the chance of ANT-resistant populations.

In 2002, Engster et al. [26] conducted a comprehensive study to examine the effects of withdrawing AGP from the diets of commercial broiler chickens reared on the same farms, in two different geographic locations (Delmarva Peninsula and North Carolina), over a 3 year period of time. The control treatment received commercial diets containing a coccidiostat, ROX, and AGP program that was currently being used by the broiler integrator, and was compared to the trial treatment which received the same diets with no AGP. Results of the study showed that the removal of the AGP program reduced the average livability of the broilers by 0.2% on the Delmarva Peninsula and 0.14% in North Carolina. Furthermore, average body weight was decreased on the Delmarva Peninsula and in North Carolina by 0.03 and 0.04 lb, respectively. Feed conversion ratio increased in both locations by 0.016 and 0.012, respectively. This study indicates that removing the AGP program from the diets of broilers can have a negative impact on performance, but the information is dependent upon the length of the study and geographic locations evaluated. Further data is need to determine if the length of time can be adjusted to decrease the negative impacts seen, as well as, to determine if the geographic location and type of drugs has an effect on overall performance.

Probiotics

Fritts and colleagues [27] studied the effects of *Bacillus subtilis* spores C-3102 (Calsporin), at an inclusion rate of 30 g/ton of completed feed, on broiler performance and carcass microbiological contamination in two trials. At 42 d, the combined results showed Calsporin significantly increase body weight and improved feed conversion at 21 and 42 d. Aerobic plate counts, coliforms (non *E. coli*) and *Campylobacter* were significantly reduced in both studies on carcasses fed Calsporin when compared to those fed the control diet. In 2004, four trials were conducted to evaluate the effects of Calsporin (*Bacillus subtilis*) spores, used as a direct-fed microbial probiotic, for improving broiler performance as an alternative to AGP [28]. Broiler chicks were reared for an average of 41 d for trials 1-3 and 49 d for trial 4. Trial diets included a basal diet with 0.05% Calsporin (contributing 0.003% as spores) with no AGP, while the control diets included the same basal diets with no Calsporin and no AGP for all trials. Hooge et al. [28] showed that Calsporin significantly increased body weight in all experiments with an average of 2.90% (+0.113 lb) increase, while decreasing feed conversion in 2 of the first 3 trials (average -1.46%). For trial 4, two additional treatments were added to the pervious treatments, which included the basal diet plus BMD at 55 ppm, and basal diet with Calsporin plus BMD. Results showed that the Calsporin diets produces higher body weights and lower feed conversion than the control diets, but not significantly better that the BMD and Calsporin/BMD supplemented diets.

Further research has demonstrated that probiotic species need not be active to be beneficial to the host. Canadian researchers tested two strains of disrupted, cobalt-

enriched, lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus casei*) and a disrupted fungal mycelium (*Scytalidium acidphilum*) as in-feed probiotic additives at two levels, on broiler performance and immune response [29]. Low level *L. casei*, high level *L. acidophilus*, and high level fungal mycelium showed improved performance over the negative control treatment, but did not enhance the immune response in the birds. However, high level *L. casei* and low level *L. acidophilus* significantly stimulated a higher immune response of IgA compared to the negative control, 10 d post-immunization. Similar research demonstrates that the 10 "generally recognized as safe" (GRAS) probiotic isolates used in poultry production are capable of increasing heterophil activity (oxidative burst and degranulation) *in vitro* [30]. *Bacillus subtilis*, *Lactococcus lactis lactis*, and *Lactobacillus acidophilus* isolates stimulated the greatest heterophil activity *in vitro*. These probiotic isolates were fed to broiler chicks and heterophil response was evaluated 24 h post-treatment. All three isolates showed a significant increase in oxidative burst and degranulation as compared to chicks receiving no probiotic isolate. The innate immune system (i.e., heterophils) responds much quicker to pathogens compared to the humoral immune system, indicating that probiotics can provide protection to newly-hatched chicks sooner.

Prebiotics

Studies that have examined the effects of prebiotics have shown conflicting results making it difficult to assess these additives. Fructooligosaccharide (FOS) has been the dominant prebiotic studied for poultry production. FOS fed to broiler chickens at a concentration of 0.375% yielded consistent improvement in growth rate and feed

efficiency [31]. However, FOS did not significantly reduce *Salmonella* contamination when the birds were processed. Fukata et al. [32], demonstrated the reduction of *Salmonella enteritidis* by feeding FOS. Two trials were conducted in which broiler chicks were fed diets containing 0.1% FOS and were challenged with *S. enteritidis* at 7 d. No significant reduction of *Salmonella* was seen in the first trial. In the second trial, the FOS treatment showed a significant reduction of *S. enteritidis* in the ceca when compared to the control, unchallenged birds at 1 and 7 d postchallenge. By 14 d, no significant reduction was seen among the FOS treatment in both studies. In a similar study, the effect of FOS fed at different levels on *Salmonella* colonization was examined [33]. FOS fed at a level of 0.375% had little effect on colonization, although 0.75% FOS reduced the colonization of *Salmonella* by 12% compared to the control group. Lactose cannot be enzymatically digested by the chicken, therefore making it a prebiotic ingredient. Chickens fed diets containing lactose exhibited reduced colonization of *Salmonella typhimurium* in the ceca [34]. Fernandez et al. [35] concluded that reduction of Salmonella colonization by feeding prebiotics, increases the prevalence of intestinal bacteria spp. of *Bifidobacterium* and *Lactobacillus.*

Competitive Exclusion Concept

Several factors can reduce the efficacy of conventional CE products in chickens, such as the use of antibiotics, stress factors, disease, feed withdrawal, and contamination from the hatchery [36]. Zhang et al. [37] conducted studies to select isolates that could reduce *Salmonella* in chickens. Isolates were taken from 9 adult *Salmonella* and *Campulobacter jejuni* negative chickens. To ensure the isolates resistance to

Salmonella/*C. jejuni* plating assays were conducted using 6 strain of *Salmonella* and *C. jejuni*. Isolates that showed inhibitory zones were typed as *Lactobacillus salivarius* (6 strains), *Streptococcus cristatus*, and *Streptococcus mitis* (2 strains). These isolates were then tested *in vivo* by feeding them to broiler chicks on d of hatch and the following d. On d 3, the birds were challenged with nalidixic acid-resistant *Salmonella*. At d 10, *Streptococcus* isolates were reduced *S. typhimurium* by 16%, while the *L. salivarius* isolates decreased *S. typhimurium* by 21% compared to the control group.

Mannan oligosaccharides fit the definitions of both prebiotics and the CE concept. Due to its inability to selectively enrich intestinal bacteria and its potential to bind to bacterial receptors, MOS better fits into the category of CE. Spring et al. [20] tested the ability of different enteric pathogens and coliforms to trigger agglutination of yeast cells and MOS preparations. They determined that 5 species of E. coli and 7 species of Salmonella agglutinated to yeast cells and MOS products. Broiler chicks were challenged at 3 d with *Salmonella typhimurium* 29E and fed a diet supplemented with 4,000 ppm of MOS (Bio-MOS®) or an unsupplemented control diet in three trials. Results of all three trials showed a significant reduction in cecal *S. typhimurium* 29E at 10 d. A second series of three consecutive trials were conducted using *S. dublin* as the challenge organism and again MOS diets significantly reduced the cecal concentations of *S. dublin* at 10 d. Finally, a trial was conducted with *S. typhimurium* 27A, which does not express type-1 fimbriae, to evaluate the effects of MOS on cecal concentrations. Cecal concentrations were not significantly lowered for *S. typhimurium* 27A, but were

numerically lower. Further research has shown that when MOS is used in combination with antibiotics a synergistic effect is seen to improve broiler performance [38, 39].

Organic Acids

The antimicrobial mechanisms of organic acids are not fully understood, but they are capable of exhibiting bacteriostatic and bacteriocidial properties depending on the physiology of the organism and the physiochemical characteristics of the external environment [40]. Feeding different levels of a combination of formic and propionic acids (0.5 to 0.68%), reduced the incidence of *Salmonella* colonization compared to the untreated control treatment [41]. Cox et al. [42] tested the effects of butyric and lactic acids at a level of 0.5%, in diets fed to two groups of newly hatched chicks. On d 2, the two groups fed organic acids were challenged with *S. typhimurium*. Ceca were collected from 6 chicks at 7, 14, and 21 d to determine the prevalence of *S. typhimurium*. No significant reduction was experienced at d 7, but lactic acid reduced the colonization by 1.6 logs. By d 21, both acids had significantly reduced intestinal colonization.

Plant Extracts

The antimicrobial properties of various plant extracts have been tested in recent years. The essential oils (EO) of certain plant varieties have been shown to have antimicrobial effects in many *in vitro* studies. Studies using EO of thyme, clove, oregano, black pepper, and cinnamon have shown to have inhibitory effects on bacteria species *in vitro*; including S. typhimurium, E. coli, S. pullorum, and Clostridium sporogenes [43, 44, 45, 46, 47]. Mitsch et al. [48] tested the effects of two different blends of EO on *Clostridium perfringens* in broiler chickens. The two blends contained

different concentrations of EO from thyme (thymol) and oregano (carvacrol), with consistent levels of clove (eugenol), turmeric (curcumin), black pepper (piperin), and were fed at 100 ppm throughout the study. The first blend reduced the prevalence of *C. prefringens* in the feces at d 14, 21, and 30; in the jejunum and cecum on d 14 and 21; and in the cloaca on d 14. The second blend reduced *C. perfringens* in the jejunum on d 14 and 30 and in the cloaca on d 30. Compounds found in plant extracts have been shown to have anticoccidial properties as well. *Yucca schidigera* extract was shown to have a synergistic effect when supplemented through the diet of broilers vaccinated against coccidiosis [49]. Improvements to average daily gain and feed conversion were significant at d 42 and the intestinal villus length was higher at 6 d. Studies testing a blend of oregano and yucca extracts, along with organic minerals, improved average body weights, feed conversion and mortality (%) of broilers challenged with *H. meleagridis* [50]. Another study using the same blend supplemented in turkey diets, showed a reduction in the severity of intestinal lesions caused by dual infection of *Cochlosoma anatis* and coccidiosis (*Eimeria* spp.) compared to nonsupplemented, challenged birds [51].

CHAPTER III

PERFORMANCE COMPARISON BETWEEN THE USE AND NON-USE OF AN ENTERIC HEALTH MEDICATION PROGRAM OVER FIVE CONSECUTIVE COMMERCIAL BROILER FLOCKS

Introduction

Over the past 60 years, antibiotics and anticoccidial drugs have been used to improve performance in agricultural animal production by reducing the burden of pathogens in the gastrointestinal tract [7, 25, 52]. The polyether ionophorous coccidiostats have been used extensively in broiler production for the control of coccidiosis [53]. Monensin (MON) and Salinomycin (SAL) were approved for use in broiler feeds by the FDA in 1971 and 1983, respectively, and since have become two drugs of choice for the prevention of coccidiosis to date [54]. These drugs achieve control by altering the permeability of protozoan cell membranes for alkaline metal cations thereby upsetting osmotic balance [55]. Antibiotics can be used therapeutically for treatment of poultry diseases, but are more commonly used in a prophylactic manner. Bacitracin Methylene Disalicylate (BMD) and Virginiamycin (VIR) have been included in poultry diets at sub-therapeutic levels since their approval for increased rate of gain and improved feed efficiency [54]. These antibiotics may also prevent the occurrence of the bacterial infection, necrotic enteritis, caused by *Clostridium spp* [56]. Roxarsone (ROX), another feed additive commonly used in broiler diets is an arsenical drug used for improving weight gain, feed efficiency and skin pigmentation [54]. ROX is also approved to aid anticoccidials in the control of *Eimeria tenella* oocysts [57]. Further

field experience has demonstrated the ROX may be effective at suppressing *Salmonella* and possibly other enteric organisms that can lead to food safety hazards in meat products [58].

While antibiotics and anticoccidials are effective in their own respect, overall intestinal health shows greatest improvement when these products are used in combination in broiler diets. The combination of an ionophore, roxarsone and an antibiotic in the starter and grower diets and an antibiotic alone in the withdrawal diet has become the industry standard [52]. While MON and SAL are only approved for control of coccidiosis, field experience has revealed that these drugs have an effect on controlling gram positive bacteria, combining them with an antibiotic and ROX takes advantage of synergism between the drugs [59].

Consumer pressure has forced the poultry industry worldwide to examine pathogen resistance from using feed additives on a continuous basis for prophylactic prevention of disease and improved performance [11,55]. Concerns arise from antimicrobial resistance to antibiotics used in animal feeds possibly resulting in microbial resistance in human medicine. This has lead to the ban of antibiotic growth promoters by the European Union in 2006, and the continuous decrease of using antibiotics at sub-therapeutic levels in the United States [7]. The objective of this study was to evaluate the effects on performance and yield by withdrawing antibiotic growth promoters from rations fed to commercial broiler over five consecutive flocks.

Materials and Methods

Animals and Housing

This study was conducted in four solid-wall, tunnel ventilated commercial broiler houses, with dimensions of 43 ft wide and 500 ft long located on the Stephen F. Austin State University Broiler Research Center. Each house was identical in feeding, water and ventilation equipment. The four houses were divided into two paired-house facilities; with each paired-house facility received one of two treatments (treated or control) consistently throughout the five consecutive flocks. For each flock, 27,600 straight-run broiler chicks were placed in each house at a stocking density of 0.78 ft^2 per bird. Multiple breeds of birds were placed throughout the trial with the majority being Ross 708. At the hatchery, an equal number of chicks from the respective breeder flocks were randomly divided prior to placement in the paired-house facilities. Birds were reared to an average of 49 d under standard commercial industry practices. The same environmental and lighting regimes were used consistently from flock to flock. Birds received light for 23 hr at an intensity of 3.0 fc for seven days. From d 8 to 21, the photoperiod was reduced to 12 hr/day and the intensity was lowered to 0.10 fc. The photoperiod was increased 2 hr each week for the remainder of the flock while the light intensity remained the same. Birds were placed on built-up litter from five previous flocks and no clean pine shavings were added between flocks.

Feeding and Dietary Treatments

Birds were fed standard commercial corn-soy based diets formulated to meet the requirements of broilers chickens. Feeding phases consisted of a starter, grower,

withdrawal I (WDI) and withdrawal 2 (WDII) diets with feed changes approximately occurring at 18 d, 35 d, and 42 d, respectively. Feed and water was provided *ad libitum* via an automated feeding system and nipple drinkers. The treated group was fed a basal diet that included SAL (60.0 g/ton starter and grower), ROX (45.4 g/ton starter and 34.0 g/ton grower), BMD (50.0 g/ton starter and 25.0 g/ton grower), and VIR (10.0 g/ton WDI); while the control group was fed the same diets containing SAL (60.0 g/ton starter and grower) but without ROX, BMD and/or VIR. Each flock received the same treatment over the course of the study. Samples were taken from each batch of feed and analyzed to ensure diets contained proper levels of coccidiostat, ROX, antibiotics and/or the absence of ROX and antibiotics.

Yield Study and Data

A yield study was conducted at the completion of each flock using a total of 280 birds from the four houses. At 48 d of age, 70 birds per house (35 males and 35 females) were randomly selected from each house. Each house was divided into five, 100 ft sections where 14 birds (7 males and 7 females) were selected to ensure a uniform representation of the house. A numbered wing-tag was placed in the wing of each bird and the birds were individually weighed and recorded. The birds were then removed from the house and placed in an isolation pen where feed was removed 12 hr prior to processing. Water was not removed from the birds until immediately prior to processing. Each yield study was conducted at approximately 49 d of age. At the Stephen F. Austin State University Pilot Processing Facility, the birds were stunned then bled using a knife to sever the jugular vein, scalded, defeathered, and manually eviscerated. The head,

neck, and paws were removed and discarded. Carcasses were then cut into front and hind halves and were weighed along with the abdominal fat. The front halves were skinned, wings were removed, breast filet and tenders (pectoralis major and pectoralis minor muscles) were deboned, leaving the frame (spine and rib cage). The hind halves were dissected to remove the drums and thighs, leaving the back. All parts were weighed individually and yields were calculated relative to final live bodyweight. Total white meat (breast filet + tenders) and percent total white meat ((total white meat / live bodyweight) * 100) were later calculated.

The remaining broilers in the houses were taken to a processing plant and slaughtered under a commercial setting. Each paired house treatment group was removed, processed and tracked through the plant separately.

Data Collected

For each flock of the trial, 200 randomly-selected birds (100 males and 100 females) were individually weighed for each treatment group on d 18, 35 and 48. Birds were selected equally (10 males and 10 females) from five, 100 ft sections within each house to ensure uniform distribution. Using the commercial processing data of the remaining chickens, average body weights, feed conversion, adjusted feed conversion, livability and condemnation were calculated for each paired house treatment group. Coccidiosis lesion scores [60] of the duodenum, ileum and ceca were recorded from 5 randomly-selected birds per house at 14, 21, 28, 35 and 42 d of age. Performance and yield data were analyzed by using the general linear models procedure of SAS software

[61]. When significance between the treatments was observed ($P < 0.05$) means were separated using the least squares means test with the PDIFF option of this procedure.

Results and Discussion

Growth Performance

Table 2 shows the means of the treated and control groups for the 200 chickens that were weighed per treatment group at d 18, 35 and 48. At 18 d of age, the control group had an equal or higher average body weight for each of the five flocks, with the difference for flock 2 being significant. The overall five flock cumulative average between the treatments was 0.02 kg, with no significant difference. On d 35, the control group had a higher average body weight for each flock and a cumulative average differential of 0.02 kg, but the difference was not significant. At d 48, the control group had an equal or higher numerical average body weight for flocks 1, 2, and 3, but for flocks 4 and 5, the treated group had a numerically higher average body weight.

TABLE 2. The effect of removing growth-promoting antibiotics (GPA) and roxarsone (ROX) on average bodyweight of broilers at 18, 35 and 48 d of age

	Average Bodyweight (kg) [2]					
	18 d		35 d		48 d	
Flock [1]	Treated [3]	Control [4]	Treated	Control	Treated	Control
1 (06/08/06 – 07/27/06)	0.60 [a]	0.62 [a]	1.75 [a]	1.78 [a]	2.65 [a]	2.67 [a]
2 (08/10/06 – 09/28/06)	0.48 [b]	0.50 [a]	1.74 [a]	1.76 [a]	2.74 [a]	2.74 [a]
3 (10/12/06 – 11/30/06)	0.48 [a]	0.49 [a]	1.84 [a]	1.85 [a]	2.73 [a]	2.76 [a]
4 (12/22/06 – 02/08/07)	0.53 [a]	0.56 [a]	1.80 [a]	1.83 [a]	2.78 [a]	2.76 [a]
5 (03/06/07 – 04/24/07)	0.48 [a]	0.48 [a]	1.80 [a]	1.82 [a]	2.81 [a]	2.80 [a]
Cumulative Average (1-5 flock)	0.51 [a]	0.53 [a]	1.79 [a]	1.81 [a]	2.74 [a]	2.74 [a]

[1]Placement date of flock – ending date of flock.
[2]Average bodyweight of 200 randomly-selected, individual bird weights per treatment group.
[3] Basal diets with coccidiostat, ROX and GPA.
[4] Basal diets with coccidiostat.
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

However, the 5 flock cumulative average body weight between the treatments at 48 d of age was equal and therefore no significance was detected.

Table 3 shows the impact of removing ROX and GPA from the diet of broilers processed in a commercial processing plant. The data reflects an average of 54,000 broilers per treatment taken to market at approximately 49 d of age. The control group had a consistently higher numerical average body weight of 0.05 kg at the completion of flocks 1 and 2. By flock 3, the treated group had a 0.03 kg higher numerical average body weight as compared to the control group. The average body weight between the two groups was virtually equal for flocks 4 and 5. The cumulative average body weights for the five flocks show the control group had an overall 0.01 kg higher average than the treated group which was not significantly different. Most producers would expect the control group to have a higher average body weight for flocks 1 and 2 due to the absence of antibiotics coupled with a low microbial challenge in the house. By flock 3, with the absence of antibiotics they expect the microbial challenge to increase significantly and see the trend change to the advantage of the ROX and GPA treated group, this was not the case for this study. Engster et al. [26] demonstrated that average body weight was not adversely affected by removal of GPA for about one year.

Actual feed conversion seem to follow a similar trend as average body weight, with the control group having an equal or lower numerical feed to gain ratio than the treated group at 49 d of age for flocks 1, 4 and 5. The treated group had a lower numerical feed conversion for flock 2 and 3 with a difference from the control of 0.01

and 0.04, respectively. Cumulative feed conversion over the five flocks of the treated group was 0.01 lower than the control group, which was not statistically significant.

TABLE 3. The effect of removing growth-promoting antibiotics (GPA) and roxarsone (ROX) on average bodyweight, feed conversion and adjusted feed conversion of broilers at 49 d of age

	Average Bodyweight (kg) [2]		Feed Conversion (g:g) [3]		Adjusted Feed Conversion (g:g) [4]	
Flock [1]	Treated [5]	Control [6]	Treated	Control	Treated	Control
1 (06/08/06 – 07/27/06)	2.62	2.67	1.83	1.80	1.62	1.59
2 (08/10/06 – 09/28/06)	2.60	2.65	1.81	1.82	1.61	1.61
3 (10/12/06 – 11/30/06)	2.62	2.59	1.90	1.94	1.69	1.74
4 (12/22/06 – 02/08/07)	2.60	2.60	1.92	1.92	1.71	1.72
5 (03/06/07 – 04/24/07)	2.62	2.63	1.96	1.96	1.75	1.74
Cumulative Average (1-5 flock)	2.61 [a]	2.62 [a]	1.88 [a]	1.89 [a]	1.68 [a]	1.68 [a]

[1]Placement date of flock – ending date of flock.
[2]Average bodyweight of remaining broilers processed under a commercial setting.
[3]Feed Conversion = (lb feed/lb total bodyweight)
[4]Adjusted to a 5 lb bird and 1500 calories with 7 weight/point of feed conversion.
[5] Basal diets with coccidiostat, ROX and GPA.
[6] Basal diets with coccidiostat.
[a] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

Feed conversion was adjusted for a 5 lb bird and a 1500 calorie (ME) diet with 7 weight/point of feed conversion. The control group had a numerically equal or lower adjusted feed conversion for flocks 1, 2, and 5 with an average differential of 0.02 points. Adjusted feed conversion was numerically lower for the treated group for flocks 3 and 4 with an average differential of 0.03 points. When adjusted feed conversion was averaged over the five flocks both groups were equal and were not significantly different.

Table 4 shows the differences between the treatment groups for livability (%) and condemnation (%) of the paired-house groups at 49 d of age. Livability was shown

to be negatively affected over the five flock study by the omission of GPA and ROX from the diets. The treated group had a higher livability percentage for every flock and the cumulative average of 0.37% was statistically different, when compared to the control group. Condemnation at the processing plant varied from flock to flock between the treatments throughout the study. The treated group had a lower cumulative condemnation percentage at 49 days of age, but was not significantly different from the control group.

TABLE 4. The effect of removing growth-promoting antibiotics (GPA) and roxarsone (ROX) on broiler livability (%) and condemnation (%) at 49 d of age

	Livability [2] (%)		Condemnation [3] (%)	
Flock [1]	Treated [4]	Control [5]	Treated	Control
1 (06/08/06 – 07/27/06)	98.29	97.73	0.45	0.86
2 (08/10/06 – 09/28/06)	98.05	97.76	0.41	0.51
3 (10/12/06 – 11/30/06)	98.28	97.82	0.54	0.45
4 (12/22/06 – 02/08/07)	97.70	97.16	0.34	0.44
5 (03/06/07 – 04/24/07)	97.98	97.95	0.41	0.33
Cumulative Average (1-5 flock)	98.05 [a]	97.68 [b]	0.43 [a]	0.52 [a]

[1] Placement date of flock – ending date of flock.
[2] Livability (%) of remaining broilers processed under a commercial setting.
[3] Condemnation (%) of remaining broilers processed under a commercial setting.
[4] Basal diets with coccidiostat, ROX and GPA.
[5] Basal diets with coccidiostat.
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

Coccidiosis Lesion Scores

Coccidiosis lesion scores were examined throughout the study to evaluate the effects of withdrawing ROX and GPA from the diets. Coccidial lesion scores between the treatment groups were significantly correlated with a value, r = 0.9141 (P = 0.0298),

from flock to flock. Lesion scores between the two treatment groups were not affected by the treatments as shown in Figure 1.

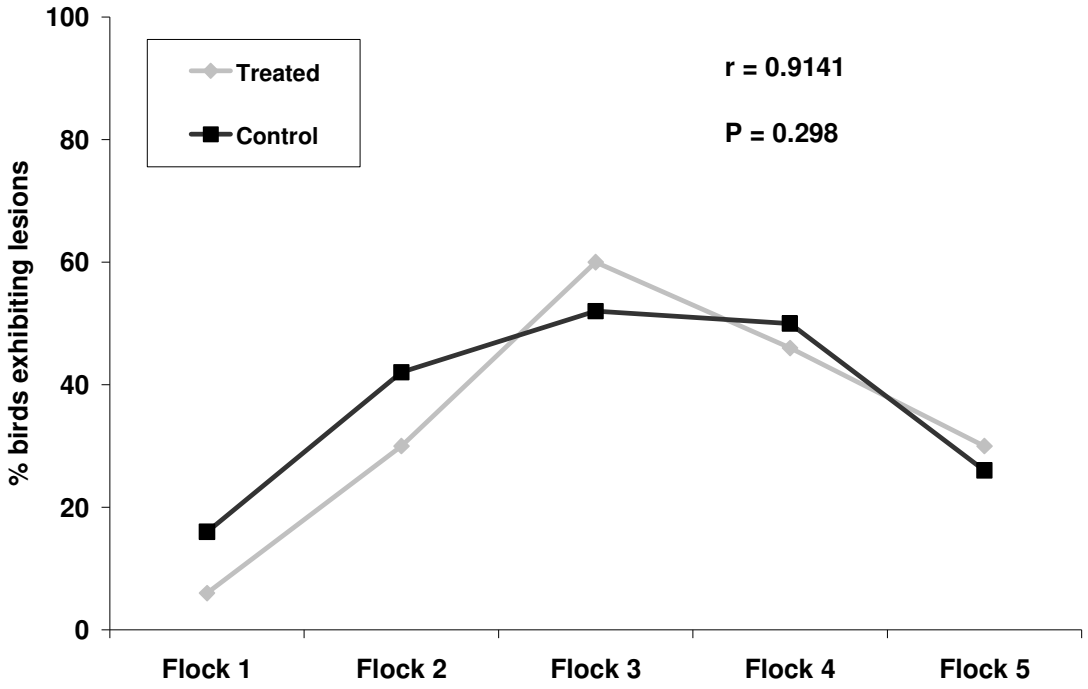

Figure 1. Correlation of coccidiosis lesion scores between treatment groups from flock to flock. (P < 0.05). Treated = basal diets with coccidiostat, roxarsone, and AGP; Control = basal diets with coccidiostat only.

Yield Performance

Average live body weight of the birds selected and tagged for the yield study were not significantly different for any of the flocks or when accumulated for the entire study. The control group had numerically higher average eviscerated carcass weights for flocks 1-3, while the treated group weights were numerically higher for the last two

flocks. The control group had a 1,952 g average carcass weight compared to a 1,933 g average weight for the treated group over the course of the study, this difference was not significant. After the carcasses were divided into front and hind halves and the abdominal fat pad was removed, the control group had a numerically higher cumulative average for each respective product when compared to the treated group.

Table 5 shows the yield of the breast filet, tenders and wings after the front half was dissected into each of the respective cuts. Breast yield for the control group was numerically higher for flock 1-3, but was higher for the treated group for flocks 4 and 5. Cumulative numerical average breast yield for the control group was slightly higher than the treated group with a differential of 4.62 g. The numerical difference was not statistically significant; therefore the removal of ROX and GPA had no affect on breast yield. The control group had a numerically equal or higher tender yield over the course of the study with the exception of flock 4. At the completion of flock 2, the control group had an average tender yield of 108.86 g and was significantly different than the 98.43 g average yield for the treated group. The control treatment had a 2.27 g greater cumulative average for tenders which were significant. Average wing yield was equal or higher for the control group for flocks 1-3, with a flock 2 average of 221.81 g being significantly different than the treated group average of 212.28 g. However, the cumulative average for the study was not statistically different between the two groups with an average of 213 g.

Table 6 shows the sum of breast filet and tender yield as total white meat production for each group. Again, the control group had a numerically higher total white

meat average for the first three flocks, while the treated group average was numerically higher for flock 4 and 5. By the end of the study, the control group had produced 7.35 g more total white meat than the treated group, which was not significantly different.

TABLE 5. The effect of removing growth-promoting antibiotics (GPA) and roxarsone (ROX) on breast fillet, tender, and wing yield of broilers at 49 d of age

	Breast Fillet (g)		Tenders (g)		Wings (g)	
Flock [1]	Treated [2]	Control [3]	Treated	Control	Treated	Control
1 (06/08/06 – 07/27/06)	391.45 [a]	404.60 [a]	94.80 [a]	96.62 [a]	205.48 [a]	206.38 [a]
2 (08/10/06 – 09/28/06)	413.68 [a]	439.53 [a]	98.43 [b]	108.86 [a]	212.28 [b]	221.81 [a]
3 (10/12/06 – 11/30/06)	410.50 [a]	412.32 [a]	99.34 [a]	102.51 [a]	211.83 [a]	211.83 [a]
4 (12/22/06 – 02/08/07)	437.26 [a]	420.03 [a]	107.96 [a]	102.97 [a]	220.45 [a]	214.10 [a]
5 (03/06/07 – 04/24/07)	435.45 [a]	435.00 [a]	106.14 [a]	106.14 [a]	215.46 [a]	210.47 [a]
Cumulative Average (1-5 flock)	417.67 [a]	422.29 [a]	101.15 [b]	103.42 [a]	213.00 [a]	213.00 [a]

[1] Placement date of flock – ending date of flock.
[2] Basal diets with coccidiostat, ROX and GPA.
[3] Basal diets with coccidiostat.
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

TABLE 6. The effect of removing growth-promoting antibiotics (GPA) and roxarsone (ROX) on total white meat and percentage total white meat yield of broilers at 49 d of age

	Total White Meat [2] (g)		Percentage Total White Meat [3] (%)	
Flock [1]	Treated [4]	Control [5]	Treated	Control
1 (06/08/06 – 07/27/06)	485.80 [a]	501.67 [a]	18.59 [a]	19.23 [a]
2 (08/10/06 – 09/28/06)	511.65 [a]	549.30 [a]	19.17 [b]	19.85 [a]
3 (10/12/06 – 11/30/06)	509.84 [a]	515.28 [a]	18.85 [a]	18.88 [a]
4 (12/22/06 – 02/08/07)	545.22 [a]	523.45 [a]	19.62 [a]	19.02 [a]
5 (03/06/07 – 04/24/07)	541.14 [a]	540.68 [a]	19.35 [a]	19.56 [a]
Cumulative Average (1-5 flock)	518.73 [a]	526.08 [a]	19.12 [a]	19.31 [a]

[1] Placement date of flock – ending date of flock.
[2] Total White Meat = (breast filet + tenders)
[3] Percentage Total White Meat = (total white meat / live body weight)*100
[4] Basal diets with coccidiostat, ROX and GPA.
[5] Basal diets with coccidiostat.
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

Table 6 shows the percentage of total white meat produced related to live body weight by group. Percentage of total white meat was numerically higher for the control group for the majority of the study, with the exception of flock 4. The control group (19.85 %) had a significantly higher percentage of total white meat for flock 2, as compared to the treated group (19.17 %). The cumulative average over all flocks shows that the removal of ROX and GPA from the diet had no adverse affect on the percentage of total white meat produced.

The hind half was dissected to evaluate the drums, thighs and back yield of the carcass. Drum yield was significantly higher for the control group for the first flock of the study and remained numerically higher for flocks 2-3. The treated group had a numerically higher drum yield for flock 4, while the groups flock 5 averages were equal. Both treatments had an equal cumulative average yield for drums of 270.34 g. The control group had a numerically higher thigh yield for flocks 1 & 2, while the treated group had heavier thigh yields for flocks 3-5. The treated group had a 1.36 g numerically higher cumulative average over the control group which was not significant. Back yield was numerically higher for the control group for flocks 1-3 while the treated group had numerically higher yields for flocks 4 & 5. The control group had a numerically higher cumulative average back yield of 4.99 g as compared to the treated group, which was not significantly different.

CHAPTER IV

EFFICACY OF *BACILLUS SUBTILIS* SPORES (Gallipro®) ON PERFORMANCE OF COMMERCIAL BROILER CHICKENS

Introduction

Enteric diseases are economically important to the poultry industry due to lost productivity, increased mortality, cost of prevention measures, and the associated contamination of poultry products for human consumption. Changes in the poultry industry, such as an increase in antibiotic resistance, banning sub-therapeutic antibiotic usage in the European Union, declining usage with potential banning of these drugs in the United States, and an overwhelming consumer demand for the removal of these drugs, has lead to an increase in finding alternatives to antibiotics for poultry production [2]. Organic poultry production is a sector of the industry that has gained popularity with consumers. Consumers are willing to pay a premium for organic poultry products that meet governmental regulations prohibiting the use of antibiotics for any reason [22]. Many potential alternatives to antibiotics have been tested, both *in vivo* and *in vitro*, for their ability to improve bird performance and reduce the spread of disease.

Probiotics is one alternative ingredient that has shown potential to improve performance that is comparable to antibiotics. Probiotics are defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance [17]. Probiotics can be comprised of a single or multiple bacterial species, generally containing species of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *E. coli*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, and a variety of yeast species [2]. Probiotics

should have certain characteristics to be beneficial as a direct-fed microbial. For instance, the probiotic should not be hydrolyzed nor absorbed by enzymes or tissues of the host. Probiotics should have the ability to selectively enrich for one or a limited number of intestinal bacteria that are beneficial to the host. Furthermore, probiotics should be able to beneficially alter the activities of the intestinal microbiota. Finally, probiotics should beneficially stimulate the host's immune system [21].

A number of *Bacillus* spp. have been considered safe for use in poultry production. Bacillus spp. can exist as vegetative cells or when placed in a stressful state produce spores that are heat resistant and tolerant to bile salts [62]. These characteristics are advantageous for poultry production, allowing the spores to be included in the feed while withstanding the high temperatures of the pelletizing process and survive the effects of bile salts in the intestinal tract. *Bacillus subtilis* spores have been shown to withstand heat up to 100^{o}C for several minutes and continue to germinate into vegetative cells after exposure to 0.5% bile salts [63]. Strains of *B. subtilis* have been shown to exclude pathogenic bacteria, including spp. of *E. coli*, *Salmonella*, *Clostridium*, *Streptococcus* and *Campylobacter*, from colonizing in the gastrointestinal tract of poultry [62, 64, 65, 66]. Both the humoral and innate immune systems can be potentiated by *B. subtilis* spp. in the gastrointestinal tract of chickens [30, 67, 68, 69]. Improvements in performance parameters, including weight gain and feed efficiency, have been reported when using various *B. subtilis* strains as probiotics in poultry [27, 28, 70, 71]. The objective of this study was to evaluate the effects of in-feed, *B. subtilis* spores

(Gallipro®, Chr Hansen A/S, Denmark) on the performance of commercial broiler chickens.

Materials and Methods

Animals and Housing

This experiment was conducted at the Stephen F. Austin State University Broiler Research Center in four solid-wall, tunnel ventilated commercial broiler houses, with dimensions of 43 ft wide and 500 ft long. Each house was identical in feeding, water and ventilation equipment. The four houses were divided into two paired-house facilities; with each paired-house facility receiving diets either supplemented with in-feed, *B. subtilis* spores (Gallipro®, Chr Hansen A/S, Denmark) or diets containing a standard AGP program. Each broiler house was filled with 27,600 Ross 708, straight-run broiler chicks, with a total placement of 55, 200 broilers per treatment. Birds were placed at a stocking density of 0.78 ft^2 per bird. In order to ensure uniformity between the treatment groups, chicks from different breeder flocks were equally distributed between the two groups at the hatchery prior to arrival at the farm. Under standard commercial industry practices the birds were reared for 48 d. The environmental and lighting regimes were consistent from house to house. Birds received light for 23 hr at an intensity of 3.0 fc for seven days. The length and intensity of light was reduced at d 8 to 12 hr/day and 0.10 fc., respectively. The length of lighting was increased 2 hr each week for the remainder of the flock while the light intensity remained the same. Birds will be placed on built-up litter from eight previous flocks and no clean pine shavings will be added prior to the placement of birds.

Feeding and Dietary Treatments

All diets were commercially made with a corn-soy base and formulated to meet the NRC requirements for broilers chickens. Feeding phases consisting of a starter, grower, withdrawal I (WDI) and withdrawal 2 (WDII) diets were fed with feed changes approximately occurring at 18 d, 35 d, and 42 d, respectively. The treatment group received basal diets at each phase that was supplemented with *B. subtilis* spores (0.25 lb/ton starter – WDII), and were compared to a control group receiving the same basal diets containing Flavomycin (2.0g/ton starter and grower). Both treatment groups received a coccidiostat program comprised of Salinomycin (60.0 g/ton starter), Monensin (100 g/ton grower) and Narasin (72 g/ton WDI); and the growth enhancer, Roxarsone (ROX) (45.4 g/ton starter and 22.7 g/ton grower). Samples were taken from each batch of feed and analyzed to ensure diets contain proper levels of coccidiostat, ROX, antibiotics and *B. subtilis* spores. Feed and water were provided *ad libitum* via an automated feeding system and a nipple drinker system.

Floor Pen Data

Prior to the placement of birds, a total of 8 floor pens, each measuring 6 ft X 6 ft, were evenly divided between the two paired-house facilities. Four replicate floor pens were equally spaced on the brood end of one house per treatment group. In each pen, 50 randomly-selected chicks were placed at a stocking density of 0.72 ft^2 per bird, and reared to 48 d of age. Each pen was fed *ad libitum* via one hanging tube feeder and received water *ad libitum* via a nipple drinker system. All feed was weighed and recorded prior to placement into the pen.

Data Collected

Throughout the experiment each floor pen was collectively weighed for each treatment group on d 18, 35 and 48. Average body weight and feed conversion ratio was calculated for each floor pen and treatment group at d 18, 35 and 48. At 48 d of age, all birds were removed from the houses and transported to a local commercial processing facility to be slaughtered. All birds for each paired-house facility was removed separately and followed through the processing facility, in order to calculate average body weights, feed conversion, adjusted feed conversion, percent mortality, and percent condemnation. On d 14, 21, 28, 35, and 42, five randomly-selected birds were selected from each house, humanely euthanized, and necropised. Coccidiosis lesion scores [60] of the duodenum, ileum and ceca were examined and recorded. The bird's footpads were scored on a 0 to 3 scale (0 = normal; 1 = slight degradation; 2 = moderate degradation; 3 = severe degradation). Performance data was analyzed by using the general linear models procedure of SAS software [61]. Means were separated using the Duncan's Multiple Range Test, if differences between the treatments were significant ($P < 0.05$).

Results and Discussion

Floor Pen Data

The floor pen data for average body weights at d 18, 35 and 48 are shown in Figure 2. The treatment group had a numerically higher average body weight as compared to the control group at 18 and 48 d, with a differential of 0.06 and 0.03 kg, respectively. At 35 d of age, the control group had higher numerical average body weight than the treatment group with a differential of 0.02 kg. No significant differences

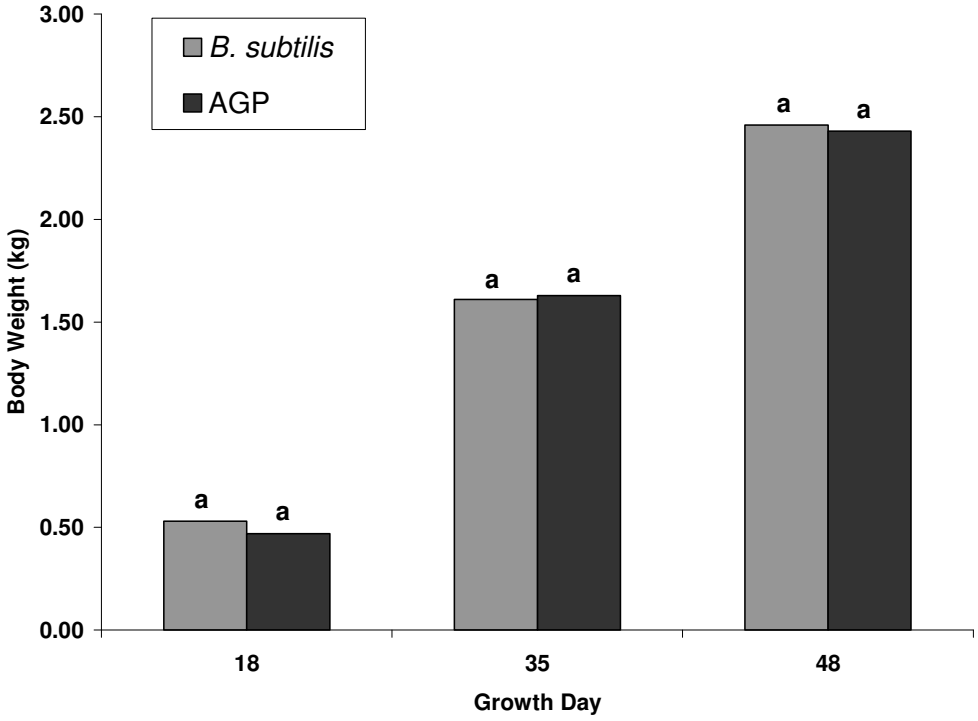

Figure 2. Treatment effect on average body weight of floor pen birds for growth days 18, 35 and 48 ($P < 0.05$). *B. subtilis* = *Bacillus subtilis* spores (0.25 lb/ton starter – WDII); AGP = Flavomycin (2.0g/ton starter and grower). Four replicate pens per treatment containing 50 birds/pen.

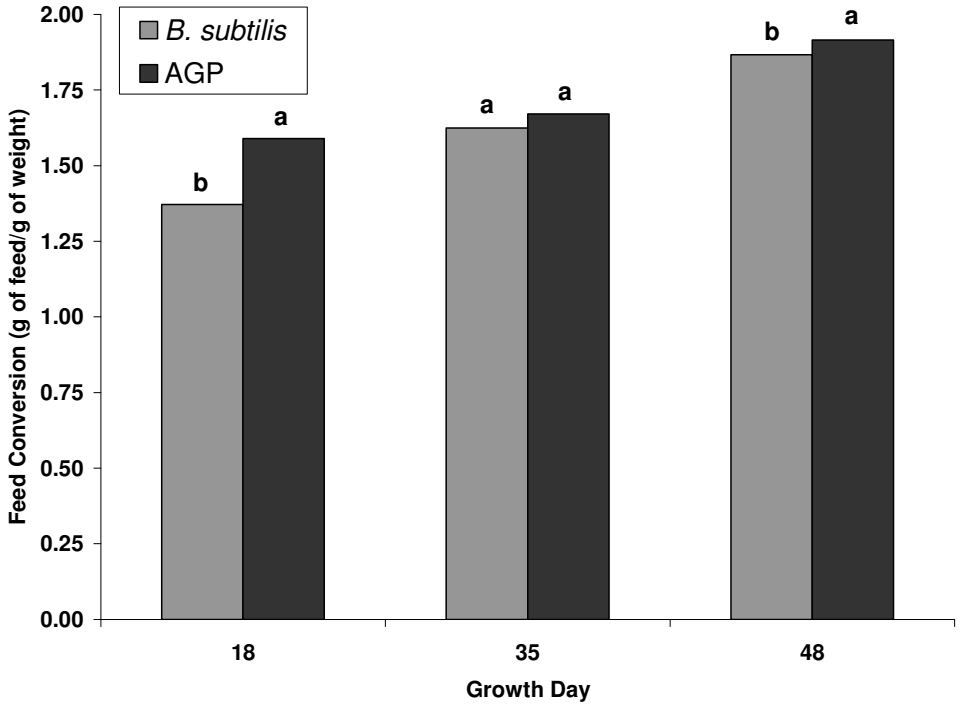

Figure 3. Treatment effect on feed conversion of floor pen birds for growth days 18, 35 and 48 ($P < 0.05$). *B. subtilis* = *Bacillus subtilis* spores (0.25 lb/ton starter – WDII); AGP = Flavomycin (2.0g/ton starter and grower). Four replicate pens per treatment containing 50 birds/pen.

were observed for average body weights between the treatment groups at d 18, 35 and 48. The effect of the treatments on feed conversion ratio for the birds reared in the floor pens are shown in Figure 3. Feed conversion ratio was calculated for the 50 birds/pen at 18, 35 and 48 d of age. There was a significant difference between the treatment and control groups at d 18 and 48. At d 18, the treatment group had a 0.22 lower feed conversion than the control group, while at d 48 the difference was 0.04. The feed conversion ratio at d 35 was not significantly different between the groups.

Commerical Paired-house Facility Data

Table 7 shows the effects of *B. subtilis* spores and an AGP program on the performance of 110,400 broiler chickens evenly distributed between two commercial paired-house facilities reared to 48 d of age. At 48 d of age, the control group fed diets containing Flavomycin had a higher numerical average body weight than the treatment group receiving diets containing *B. subtilis* spores. The treatment group was more feed efficient than the control group at 48 d with a 0.02 point lower feed conversion ratio. The feed conversion ratio was adjusted for a 5.0 lb average bird, fed a 1500 kcal average diet, with 7 weight points of feed conversion. After calculating the adjusted feed conversion ratio, the treatment group still had a numerically lower adjusted feed conversion ratio when compared to the control group, with a differential of 0.02 points. Mortality (%) was consistently lower in the control group when examined at d 7, 14, and 48, with differentials of 0.11, 0.63, and 0.08 %, respectively. Condemnation (%) at the processing plant was numerically lower for the treatment group at 48 d of age. The

majority of the condemnation difference between the treatments was due to septicaemia and toxemia, and airsacculitis, which were both higher for the control group.

Coccidiosis lesion scores were taken at d 14, 21, 28, 35, and 42 to determine the effectiveness of the coccidiosis control program used and to evaluate any potential effects between the treatments. Coccidial lesions were similar between the treatment groups from week to week, with the amount and severity of lesions increasing gradually from d 28 to 42 for both groups. Lesion scores between the two treatment groups were not affected by the treatments (Data not shown). Footpads were scored at d 14, 21, 28, 35, and 42 to examine any ulcerations or degradation of the skin tissue due to litter conditions. Litter conditions were consistent between the houses and were maintained at a relatively low moisture content, therefore all birds necropsied throughout the experiment did not exhibit any signs of ulcerations or degradation and were scored as normal (Data not shown).

TABLE 7. The effect of *B. subtilis* spores and an antibiotic growth promoter program on performance parameters of commercial broiler chickens in paired-house facilities[1] at 48 d of age

Measurement	Treatment	Control
Average Body Weight (kg)	2.42	2.44
Feed Conversion Ratio[2]	1.85	1.87
Adjusted Feed Conversion Ratio[3]	1.70	1.72
Mortality (%)[4]		
7 d	0.80	0.69
14 d	1.60	0.97
48 d	1.78	1.70
Condemnation (%)[5]	0.30	0.34

[1] Paired-house facility contains 55,200 broiler chickens receiving one of two dietary treatments.
[2] Feed conversion ratio = g of feed / g of weight.
[3] Adjusted feed conversion ratio = [(((feed conversion – (avg body weight – 5.0 lb))/ 7 weight points)* 1435 kcal)/1500 kcal].
[4] Mortality (%) = [(total mortality/total bird placement)*100].
[5] Condemnation (%) = [(total gross live weight/total gross condemnation weight)*100].

CHAPTER V

EFFICACY STUDY OF SUPPLEMENTING Bio-Mos® AND Natustat™ FOR ANTIBIOTIC GROWTH PROMOTERS ON BROILER PERFORMANCE AND YIELD

Introduction

There have been global changes in the use of antibiotics for growth promotion in the poultry industry. Antibiotic growth-promoters (AGP) are fed to poultry to increase the rate of gain and improve feed efficiency 54]. Some AGP aid in the prevention of necrotic enteritis, such as bacitracin methylene disalicylate (BMD) and virginiamycin (VIR). Roxarsone (ROX) is an arsenical drug that is often used in combination with an AGP program to improve weight gain and feed efficiency. ROX may be used in conjunction with an ionophore coccidiostat (i.e., salinomycin (SAL)) to aid in the control of coccidiosis, caused by *Eimeria tenella* [72]. There are several concerns related to the use of AGP in poultry production have contributed to these changes. Development of resistance to antibiotics by pathogenic bacteria has been the major concern and was observed as early as the 1950's, shortly after discovering the beneficial effects of AGP on production [7]. Antimicrobial resistance has increased over the years, potentially due to the intensive use of antibiotics at subtherapeutic levels to increase weight gain and improve feed efficiency [25]. Further concern is the human health risks related to antimicrobial resistance by mainly zoonotic bacteria that can be spread from infected animals to humans [11]. The potential for public health risks has lead poultry product consumers to place significant pressure on the poultry industry to change the practice of

feeding antibiotics for disease prevention. This increased pressure by the consumer has resulted in voluntary reduction of AGP use by some major poultry producers in the US and has forced restaurant chains to eliminate the use of products from chickens grown using AGP [16].

The poultry industry has been forced to explore potential alternatives to AGP. Competitive exclusion (CE) cultures have been a potential alternative to AGP that has been tested and shown to improve the health and performance of chickens. Since, newly-hatched chicks lack intestinal microflora, they are especially prone to enteric pathogens until the microbiota are established [73]. In 1971, a severe outbreak of *Salmonella infantis* occurred among broiler flocks in Finland. Antibiotic treatment was unsuccessful at relieving the problem, so E. Nurmi at the National Veterinary Institute, Helsinki, Finland, took a different approach by isolating cultures of cecal microflora from adult chickens and administered them to newly-hatched chicks [19]. This concept of taking adult cecal cultures that were resistant to *Salmonella* and establishing adult-type resistance in the young chicks in now referred to as the competitive exclusion or the Nurmi-concept. One mode of action for CE cultures is to compete with pathogens for attachment sites on the mucosal surface of the intestine, thereby excluding these pathogenic species [74].

Mannan-oligosaccharides (MOS, Bio-Mos®, Alltech, Nicholasville, Kentucky, USA) is a non-digestible, complex carbohydrate that is the main component in the outer cell wall of yeast cells (*Saccharomyces cerevisiae* var. *boulardii*), and is thought to act by binding and removing pathogens from the intestinal tract and stimulates the immune

system [2]. One potential mode of action for MOS is its ability to adsorb pathogenic bacteria that contain type-1 fimbriae with mannose-sensitive lectin proteins [75]. Another probable mode of action that has been reported for MOS is an improvement in the function of the intestinal tract by increasing villi height, uniformity and integrity [76]. MOS products are thought to stimulate gut associated and systemic immunity by acting as non-pathogenic microbial antigens [77]. MOS products are thought of as CE products because of these probable modes of action.

Many researchers have shown that MOS have a positive effect on BW and FCR, while either having no effect or lowering mortality in chickens and turkeys [28, 52, 78, 79]. Research has shown that some strains of pathogenic bacteria can trigger agglutination of MOS and reduce the susceptibility of bacterial colonization in the intestinal tract [20, 35]. Further research has shown that when MOS is used in combination with antibiotics a synergistic effect is seen to improve broiler performance [38, 39].

Natustat™ (NAT, Alltech, Nicholasville, Kentucky, USA), is a proprietary plant derived product, used as an alternative for the control of histomoniasis, caused by *Histomonas meleagridis*, in poultry [50, 80], and dual infection of *Cochlosoma anatis* and coccidiosis (*Eimeria* spp.) in turkeys [51]. NAT is a natural product that is comprised of at least one yeast-derived mannan-oligosaccharide, along with organic mineral nutrients and plant extracts. This product has been shown to provide improvements to BW, FCR and mortality (%) of broilers challenged with *H. meleagridis* [50]. NAT supplemented diets reduces the severity of intestinal lesions caused by dual

infection of *Cochlosoma anatis* and coccidiosis (*Eimeria* spp.) in turkeys when compared to nonsupplemented, challenged birds [51]. The objective of this study was to evaluate the effectiveness of MOS as an alternative to a commercially standard AGP program or a combination of MOS/NAT as an enteric health replacement program. The effects of MOS and NAT on broiler performance and yield were observed.

Materials and Methods

Animals and Housing

A total of 6,000, straight-run Cobb X Cobb broiler chickens were reared at the Stephen F. Austin State University Poultry Research Center. This floor-pen facility contains 48 pens, each measuring 10 ft. X 10 ft., in which 125 birds were randomly assigned to each pen at a stocking density of 0.80 ft^2 per bird. The birds were reared for 49 days to a target average weight of 6.00 lb. Environmental conditions were controlled by negative air exchange (tunnel ventilation) and emulated commercial settings. The floor-pens contained built-up litter comprised of wood shavings from six previous flocks.

Feeding and Dietary Treatments

Corn-soy based basal diets were fed and formulated to meet or exceed the requirements of broilers chickens. Diets were prepared and pelletized at the Stephen F. Austin State University Poultry Research Feed Mill prior to bird placement. Feeding phases consisting of a starter, grower, finisher and withdrawal (WD) diets were fed with feed changes approximately occurring at 14 d, 28 d, and 40 d, respectively. The starter diet was fed as a crumble with the remaining diets feed as pellets. Feed and water were

provided *ad libitum* via 4 hanging tube feeders and 2, 10 ft. nipple drinker systems per pen. Each pen was randomly assigned 1 of 4 dietary treatments, with each treatment having 12 replications setup in a randomized block design. The treatments consisted of the following: control (CON), basal diets containing SAL (50.0 g/ton starter, 60 g/ton grower and finisher), and no AGP or CE products; antibiotic program (ANT), basal diets containing SAL (50.0 g/ton starter, 60 g/ton grower and finisher), BMD (50 g/ton starter, grower, and 25 g/ton finisher), ROX (45.4 g/ton grower and 39 g/ton finisher), and VIR (10 g/ton WD); Bio-Mos® replacement (MOS), basal diets containing SAL (50.0 g/ton starter, 60 g/ton grower and finisher), and Bio-Mos® (4 lb/ton starter, 2 lb/ton grower,1 lb/ton finisher and WD); and Bio-Mos®+Natustat™ replacement (MOS+NAT), basal diets containing Natustat™ (2 lb/ton starter and grower), and Bio-Mos® (1 lb/ton finisher and WD). The MOS/NAT birds were vaccinated prior to placement in the pens with Coccivac®-B coccidiosis vaccine and were not fed a coccidiostat.

Yield Study and Data

A yield study was conducted at the completion of the experiment using a total of 384 birds. At 49 d of age, 8 birds per pen (4 males and 4 females) were randomly-selected from each pen. Males and females were differentiated by visual appearance and sexual characteristics. Each treatment had 12 replications for a total of 96 birds per treatment. Each bird had a numbered wing-tag that was placed in one wing and the birds were individually weighed and recorded. The birds were removed from the pens and placed in an isolation pen with feed until 12 hr prior to processing. Water was provided for the birds until immediately prior to processing. At the Stephen F. Austin State

University Pilot Processing Facility, the birds were stunned and manually eviscerated. The head, neck, and paws were removed and discarded. Carcasses were then split into front and hind halves and weighed along with the abdominal fat. The front halves were skinned, then dissected to obtain the wings, deboned to obtain the breast fillet and tenders (pectoralis major and pectoralis minor muscles), leaving the frame (spine and rib cage). The hind halves were dissected to remove the drums and thighs, leaving the back. All parts were weighed individually and yield data was calculated relative to final live bodyweight. Total white meat (breast fillet + tenders) and percent total white meat ((total white meat / live bodyweight) * 100) will be calculated at a later period.

Data Collected

All feed was weighed and recorded prior to placement in the pens. The birds in each pen were weighed collectively at d 49, along with the remaining feed. Daily mortalities were weighed, necropsied and probable cause of death was recorded. Average body weight, feed conversion ratio, mortality adjusted feed conversion ratio and percent mortality were calculated at 49 d of age. Performance and yield data were analyzed by using the general linear models procedure of SAS software [61]. If significance differenced between the treatments are observed ($P < 0.05$), means will be separated using the Duncan's Multiple Range Test.

Results and Discussion

Performance Data

The treatment effects on average body weight, feed conversion ratio, mortality-adjusted feed conversion ratio, and percent mortality at 49 d of age is shown in Table 8.

Average body weight for the CON and ANT groups were significantly different than the MOS+NAT treatment on d 49 by a differential of 0.11 kg, but was not significantly different than the MOS group. No significant differences were detected between the treatments for feed conversion ratio, mortality-adjusted feed conversion ratio, and percent mortality at 49 d of age. The MOS+NAT treatment was vaccinate with a coccidiosis vaccine prior to placement, which could have had an effect on overall performance.

TABLE 8. Effects of antibiotic growth-promoters, Bio-Mos, and Natustat on BW, FCR, mortality-adjusted FCR, and mortality of broilers at 49 d of age

Treatment [1]	BW (kg)	FCR (kg:kg)	Mortality-adjusted FCR (kg:kg)	Mortality (%)
CON	2.96 [a]	1.94 [a]	1.92 [a]	5.28 [a]
ANT	2.96 [a]	1.94 [a]	1.92 [a]	4.41 [a]
MOS	2.91 [ab]	1.95 [a]	1.93 [a]	4.09 [a]
MOS+NAT	2.85 [b]	1.97 [a]	1.95 [a]	5.68 [a]

[1]Treatment codes: CON = control; ANT = antibiotic growth-promoter; MOS = mannanoligosaccharide (Bio-Mos); MOS+NAT = Bio-Mos/Natustat (plant-derived blend)
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

Yield Data

Yield data was collected and analyzed from 384 randomly-selected birds at 49 d of age. Table 9 shows the effects of the treatments on the parts that comprise the front half of the carcass. The front half consists of the wings, breast, tenders, frame, and skin. There were no significant differences between the treatments for wing, breast, and tender yield. All treatments were significantly different from the MOS+NAT group for frame weight, with the MOS group having the highest frame yield. ANT group had the highest

skin yield of the treatments and along with the CON and MOS groups was significantly different from the MOS+NAT treatment group at 49 d of age.

TABLE 9. Treatment effects of antibiotic growth-promoters, Bio-Mos, and Natustat on wing, breast, tender, frame, and skin yield of broilers at 49 d of age.

Treatment[1]	Wing (g)	Breast (g)	Tender (g)	Frame (g)	Skin (g)
CON	224.07 [a]	449.06 [a]	102.51 [a]	304.36 [a]	106.59 [a]
ANT	230.88 [a]	450.42 [a]	105.69 [a]	303.91 [a]	111.13 [a]
MOS	221.81 [a]	449.96 [a]	103.87 [a]	305.72 [a]	107.50 [a]
MOS/NAT	223.62 [a]	440.44 [a]	104.78 [a]	287.12 [b]	99.34 [b]

[1]Treatment codes: CON = control; ANT = antibiotic growth-promoter; MOS = mannanoligosaccharide (Bio-Mos); MOS+NAT = Bio-Mos/Natustat (plant-derived blend)
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

The d 49 yield data for the parts that comprise the hind half of the carcass are shown in Table 10. The drums, thighs, back, and abdominal fat comprise the hind half of the carcass. Differences between the treatments for drums, thighs, back, and abdominal fat at 49 d of age were not significant when compared.

TABLE 10. Treatment effects of antibiotic growth-promoters, Bio-Mos, and Natustat on drum, thigh, back, and abdominal fat yield of broilers at 49 d of age.

Treatment[1]	Drum (g)	Thigh (g)	Back (g)	Abdominal fat (g)
CON	289.39 [a]	343.37 [a]	235.41 [a]	57.61 [a]
ANT	291.66 [a]	350.63 [a]	237.68 [a]	60.33 [a]
MOS	280.32 [a]	340.19 [a]	230.88 [a]	59.87 [a]
MOS/NAT	283.95 [a]	335.66 [a]	227.25 [a]	54.43 [a]

[1]Treatment codes: CON = control; ANT = antibiotic growth-promoter; MOS = mannanoligosaccharide (Bio-Mos); MOS+NAT = Bio-Mos/Natustat (plant-derived blend)
[a] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

Data in Table 11 demonstrates the effects of the treatments on the whole carcass weight, the collective means of the front and hind halves of the carcass, the total white meat yield and the percentage of total white meat produced. The ANT group, followed by the CON and MOS groups, significantly improved carcass front half yield when compared to the MOS+NAT group. Carcass hind half yield was significantly improved by for the CON group, along with the ANT and MOS groups over the MOS+NAT group at d 49. No significant differences were detected between the treatments for whole carcass weight and total white meat yield. Conversely, when the percent total white meat yield was calculated relative to the live weight of the bird, the MOS group had a significantly higher percentage of white meat as compared to the ANT group, but was not different from the MOS+NAT and CON treatment groups.

TABLE 11. Treatment effects of antibiotic growth-promoters, Bio-Mos, and Natustat on carcass, front half, hind half, total white meat, and percent total white meat yield of broilers at 49 d of age

Treatment[1]	Carcass Weight [2] (kg)	Carcass Front Half [3] (kg)	Carcass Hind Half [4] (kg)	Total White [5] Meat (kg)	Percent Total White Meat [6] (%)
CON	2.11 [a]	1.20 [ab]	0.88 [a]	0.55 [a]	18.48 [ab]
ANT	2.12 [a]	1.22 [a]	0.86 [ab]	0.57 [a]	18.14 [b]
MOS	2.05 [a]	1.19 [ab]	0.85 [ab]	0.55 [a]	18.70 [a]
MOS/NAT	2.00 [a]	1.15 [b]	0.82 [b]	0.55 [a]	18.51 [ab]

[1]Treatment codes: CON = control; ANT = antibiotic growth-promoter; MOS = mannanoligosaccharide (Bio-Mos); MOS+NAT = Bio-Mos/Natustat (plant-derived blend)
[2] Carcass Weight = carcass wt. – (neck + head + paws + giblets)
[3] Carcass Front Half = wings + breast + tenders + frame + skin
[4] Carcass Hind Half = drums + thighs + back
[5] Total White Meat = Total White Meat = (breast + tenders)
[6] Percent Total White Meat = ((breast + tenders) / live body weight)*100
[a,b] Means between treatment groups without a common superscript are significantly different ($P < 0.05$).

CHAPTER VI

SUMMARY AND CONCLUSIONS

The primary aim of this research was to determine the impact of AGP removal from the diets of commercial broiler chickens, over a one year production period. Furthermore, to determine the effectiveness of a probiotic (*B. subtilis* spores), competitive exclusion product (mannan oligosaccharide), and plant extract blend (plant extracts + mannan oligosaccharide + organic minerals) as alternatives to AGP.

In the first study, the removal of ROX and AGP from the diets of commercial broilers over five consecutive flocks showed no negative effects on broiler performance. Feed conversion was not affected by the removal of ROX and AGP from the diets throughout the study. The cumulative average feed conversion and adjusted feed conversion were not significantly different at 49 d. Livability percentage was negatively affected by the removal of ROX and AGP over the five consecutive flocks. The removal of ROX and AGP had no affect on coccidiosis lesion scores throughout the course of the study. Overall carcass yield was not affected by the removal of these drugs from the diets. While the data from this study demonstrated that broilers reared without ROX and AGP in a commercial setting can perform as well as birds receiving prophylactic antimicrobial drugs, these results must be interpreted in context. Research has shown that broiler performance may not be negatively affected until after the first year without prophylactic drug use [26] and the conditions of the environment can have a large role in the success or failure of an enteric health medication program [81].

In study two, feed conversion ratio was improved by the inclusion of *B. subtilis* spores at d 18 and 48, while not affecting average body weight. The inclusion of *B. subtilis* spores had no affect on coccidiosis lesion scores and footpad condition. *B. subtilis* spores (Gallipro®), fed to commercial broiler chickens at an inclusion rate of 0.25 lb/ton, proved to be beneficial as an alternative to antibiotic growth promoters.

In study three, broilers reared to 49 d fed diets supplemented with MOS had average body weights, feed conversion ratio and mortality that was statistically equal to broilers fed diets that either did or did not contain AGP. All treatments had a significantly higher carcass front half, carcass hind half, frame and skin yield when compared to the MOS+NAT treatment. MOS supplemented diets significantly improved the percentage of total white meat yield relative to the live weight of the bird when compared to diets including AGP. MOS can be used as an alternative to AGP that provides equal performance and yield for broilers reared to 49 d under simulated commercial settings. The combination of MOS+NAT was not found to be an acceptable replacement for a standard enteric health program comprised of a coccidiostat, ROX and AGP.

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