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Evaluation of Different Probiotic Strains Supplemented in Commercial Broiler Rations and their Influences on Performance, Yield, and Intestinal Microbiota.

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EVALUATION OF DIFFERENT PROBIOTIC STRAINS SUPPLEMENTED IN COMMERCIAL BROILER RATIONS AND THEIR INFLUENCES ON PERFORMANCE, YIELD, AND INTESTINAL MICROBIOTA.

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ABSTRACT

The objective of this study was to evaluate *Bacillus megaterium*, *Bacillus licheniformis*, and *Bacillus subtilis*, as probiotic strains compared to Bacitracin Methylene Disalicylate (BMD) 50 as the antibiotic growth promotor, supplemented in commercial broiler rations and their influences on performance, yield, and intestinal microbiota. This trial was completed as a randomized-block design with 4,800 birds split into 96, 5'x10' pens, and randomly assigned to one of eight treatment groups. The birds were placed at a stocking density of 1.00 ft^2/bird, (50 birds/pen), and reared on used pine shaving for 55 days. Throughout the study, bird performance, and intestine samples were measured. A yield study was completed at the end of the study to determine meat yield for all retail cuts. Results show treatment 3, (LS + AGP), which consisted of the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus subtilis*, and BMD with Maxiban in the starter and grower diets as the coccidiostat, performed better overall compared to the negative and positive control treatments.

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CHAPTER I

Introduction

The poultry industry is one of the leading sectors of the animal industry with an increase in demand of poultry products around the world. Global production is forecasted to increase one percent to a record 90.4 million tons of poultry products by 2017 (USDA, 2016). Therefore, poultry integrators must meet demands placed on the industry by increasing both performance and yield. Since the beginning of commercialized broiler production, the final goal for the producers has been to keep cost as low as possible. One of the largest issues broiler producers face is the health of the birds. Diseases are an important concern to poultry producers because of lost productivity, increased mortality, and the health related issues with consumers eating the meat. Medicating poultry has been performed by the industry for many years with the treatment of antibiotics (Saif, 2003). Consumers are driving the market toward replacing antibiotics. Probiotics could be the next choice for integrators. Unlike antibiotics, probiotics are living organisms and rely on survival and replication in the gastro-intestinal tract (Patterson & Burkholder, 2003). Probiotics are aimed at promoting the growth of beneficial gut microbes. Some more recently introduced probiotics

are competitive exclusion products which help to eliminate other microbes. During this study we evaluated different probiotic strains used as supplements in commercial broiler rations and evaluated their influences on performance, yield, and intestinal microbiota.

Statement of Problem

Antibiotic free chicken is currently trending within the United States of America. The European Union has banned the use of human antibiotics as growth promotors in animal feed since 2006 (Wang et al., 2016). The main expected consequence of the ban is a reduction in the amount of antibiotics used in animal production, and therefore the risk of transferring resistant genes from microbes to the human population (Castanon, 2007). With such urgency of banning antibiotics in the U.S., probiotics and prebiotics may become the next viable option for substitution. *Bacillus* is an established bacteria within the intestinal tract of the bird, therefore using this bacteria as a probiotic could possibly help support the overall health.

Research Objectives

The objective for this study was to supplement three different probiotic strains of bacteria, at various combinations, within commercial broiler chicken diets and evaluate the effects on bird performance, meat yield and intestinal microbial ecology. Performance evaluation was achieved by comparing average body weights per pen across all treatments. Data was collected to calculate feed conversion ratio (FCR), and adjusted feed conversion ratio. Intestinal samples were collected on day 25, d35, & d55 and analyzed for 16 rRNA genomic

sequencing. A yield study was conducted to determine meat yield weights among the treatment.

CHAPTER II

Literature Review

Probiotics in Poultry

The key to successfully rearing poultry to the desired body weight for market without antibiotic growth promoters (AGP) is to control and maintain a healthy and diverse gut microflora (Barug et al., 2006). Specific carbohydrates, prebiotics, probiotics, and beneficial microorganisms have been identified to eliminate potential pathogens and alter intestinal microflora (Barug et al., 2006). Essentially, prebiotics and probiotics both have the same mode of action: to increase resistance of infection and reduce the risk of increasing potential pathogens. Combinations of prebiotics and probiotics are known as synbiotics (Patterson & Burkholder, 2003). According to the currently adopted definition by Food and Agricultural Organization/ World Health Organization (FAO/WHO, 2001) probiotics are “living microorganisms which when administered in adequate amounts confer a health benefit on the host”. Probiotic bacteria have the ability to bind to intestinal mucus, and it has been suggested that adhesion may be a key for applying their protective effect (Bernet et al., 1994).

Increased resistance to antibiotics in humans has attracted attention in governmental and public interest to eliminate the usage of antibiotics in

animals. Recent changes in legislation have driven the need for different variety of feed requirements on the use of antimicrobials. The gastrointestinal (GI) tract is densely populated with microorganisms which interact closely and intensely with the host and ingested feed. Sub-therapeutic use of probiotic microorganisms, prebiotic substrates that enrich certain bacterial populations, or a combination of the two have been an alternative method to antibiotics in livestock (Patterson & Burkholder, 2003). Sub-therapeutic is defined as, a drug at a lower dosage than required for a therapeutic effect. Probiotic, which means “for life” in Greek (Gibson & Fuller 2000), has been defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Fuller, 1989). Pathogens need to overcome many obstacles to colonize and create an infection in the gut. Probiotics help fight infections with beneficial bacteria, unlike antibiotics where a bactericidal drug kills the bacteria; however a bacteriostatic drug inhibits the replication of the bacteria and requires a functional immune system to eliminate the bacteria from the body (Saif, 2003). Competitive exclusion (CE) is a term that has been used to describe the protective effect of the natural or native bacterial flora of the intestine in limiting the colonization of some bacterial pathogens (Jeffrey, 1999). CE may provide products for the poultry industry in combating the occurrence of intestinal disease and reduction of pathogens (Jeffery, 1999). Carbohydrates are at the center of cell to cell functions. Specific surface carbohydrates permit viruses and bacteria to attach to

the cell surface, to colonize, and in the case of a pathogen, cause disease (Barug et al., 2006). Different carbohydrate structures can have different biological activities. Monosaccharides, sugars, are known for directing the movement of cells and proteins throughout the body, organizing embryonic development, regulating hormones, and regulating the immune system (Benz & Schmidt, 2001).

Probiotics can be administered to the chicken through the diet, water, or by liquid spray. Probiotics currently are viewed as production enhancers to affect microflora positively to promote performance and protect against colonization of harmful bacteria (Hume, 2011). The concept of probiotics is not entirely new, the distribution of how, when, and where to use them is the key factor. The most common additives include *Bacillus*, lactic acid bacteria and yeast, out of which *Aspergillus*, *Bacillus*, *Bifidiobacterium*, *Candida*, *Lactobacillus* and *Sterptomyces* are widely used in the broiler industries (Islam et. al., 2004; Gil et al., 2005; Willis et al., 2007; Apata, 2008). The ultimate goal of commercial application of probiotics is to increase economic profitability modulated by 3 hopeful results: 1) a demonstrable increase in animal performance; 2) reduction in morbidity and mortality in the animals; and 3) reduction in human pathogenic bacterial populations (Flint & Garner 2009). These goals are not mutually exclusive and beneficial outcomes are, in fact, tightly interwoven with one another.

Bacillus Bacteria

Bacillus licheniformis, Bacillus subtilis, and *Bacillus megaterium* were the probiotics used in this trial. *Bacillus* is a genus of bacteria that are gram-positive, rod-shaped and found widespread in the environment. They are usually called “soil bacteria”, even though they can be found in soil, water, dust, air and feces (*Bacillus* Bacteria, 2012). Bacterial spores are particularly well suited for use as live microbial products as they are metabolically dormant and highly resilient to environmental stresses, indicated by Cartman et al., (2008). These essential properties are highly desirable from a commercial perspective and spore-based products have a long shelf life and retain their sustainability during distribution and storage. Saif (2003) stated that *Bacillus spp.* occasionally has been associated with embryo mortality and yolk sac infections in chickens and turkeys. Certain strains of *Bacillus* interfere with intestinal colonization of enteric pathogens and have value as probiotics (Saif, 2003). Knarreborg and colleagues (2008) showed that the addition of *Bacillus* spores in broiler chicken feed increased the microbial diversity in the ileum and increased the growth of lactic acid bacteria in the birds fed *Bacillus* organisms compared to the control birds.

Bacillus licheniformis produces keratinases (subtilisins) and have the ability to degrade feathers. Feather degradation is associated with focal ulcerative dermatitis of turkey breast skin but a correlation between keratinase

exposure and lesion formation has not been investigated (Saif, 2003). Knap and colleagues (2011) have shown that studies with *B. licheniformis* spores as a probiotic has the ability to prevent necrotic enteritis (NE) and could be an alternative to prophylactic use of antibiotics to overcome NE under commercial conditions. *Bacillus licheniformis* could therefore be of direct use in preventing antibiotic-resistant pathogens in chickens (Knap et al., 2011).

It has been suggested that *Bacillus subtilis* will associate with the gut wall and favor the balance of beneficial intestinal microflora (Jiraphocakul, et al., 1990). Research by Tactacan and colleagues (2013) showed that an adequate level of dietary *B. subtilis* spores supplemented was equally as effective as Bacitracin Methylene Disalicylate (BMD) in mitigating the subclinical effects of NE in broiler chickens. Replacing BMD with *B. subtilis* would be not only reasonable but profitable in the commercial industry.

In 1884, De Bary named *Bacillus megaterium* “big beast” because of its large size with a volume approximately 100 times that of *Escherichia coli* (De Bary, 1884). *B. megaterium* is a gram-positive, mainly aerobic spore-forming bacterium found in widely diverse habitats from soil to seawater, sediment, rice paddies, honey, fish, and dried food (Vary et al., 2007). The poultry industry has used feather wastes as an ingredient in animal feed stuffs because feathers are almost pure keratin protein. Generally, they become feather meal used as animal

feed after undergoing physical and chemical treatments. These processes require significant energy and also destroy certain amino acids (Papadoulos et al., 1986). Therefore, biodegradation of feather keratin by microorganisms represents an alternative method to improve the nutritional value of feather remains and to prevent environment pollution. *B. megaterium* has the capability of keratin degradation from research shown by Park and colleagues (2007) and degraded whole chicken feathers completely within seven days.

Gut Health in Poultry

Gut health is critical when discussing performance of broilers in the commercial industry. A well balanced ration sufficient in energy and nutrients is exceptionally important in maintaining a healthy gut. It is not surprising considering the gut holds more than 640 known different species of bacteria, contains over 20 different hormones, digests and absorbs the vast majority of nutrients, and accounts for 20% of energy the body uses (Choct, 2009). The balance of the microflora in the gut reflects the performance of the bird itself. Nutrient uptake from the diet will affect the probiotic, prebiotic, or antibiotic needed to perform ideally. Not only is the gut a major organ for digestion and absorption, it is also the first protective mechanism against pathogens which can enter host cells and tissues (Choct, 2009).

The conflicting side to a healthy gut would be one infected by bacteria such as, *Clostridium, Salmonella*, and *Campylobacter*. Necrotic enteritis is defined as, an acute chronic enterotoxaemia caused by *Clostridium perfringens* and characterized by fibrino-necrotic enteritis, usually of the small intestine (McMullin, 2004). This disease occurs sporadically, but mortality can be very high in untreated flocks. Infection of NE mainly occurs by fecal to oral transmission (McMullin, 2004). Clinical signs are depression, ruffled feathers, immobility, and dark colored diarrhea. Illness is caused by the proliferation of *C. perfringes* (type C) often occurs in association with outbreaks of coccidiosis or any other situation which causes damage to the lining of the intestine (Pattison, 1993). Coccidiosis is extremely difficult to control. A coccidiostat is included in the diet in an attempt to control the disease, without totally eliminating the coccidia, described by Pattison (1993). The idea is to allow the coccidia to survive and reproduce in the gut of the bird in sufficient numbers to stimulate immunity. There are few strategies and tools available for control and prevention of *C. perfringens*. The most cost-effective control will most likely be obtained by balancing the composition of the diet as stated by Van Immerseel and colleagues (2004).

The genus *Salmonella* contains many species of bacteria, all of which may cause problems, though some more than others. Two species, *S. pullorum* and *S. gallinarum* are generally restricted to poultry (Pattison, 1993). Techniques at commercial processing facilities can result in carcass or meat contamination of

products. If the product is mishandled then low numbers of salmonella organisms can multiply quickly up to a level at which they are capable of causing food poisoning in humans. Efforts are being made by the USDA Food Safety and Inspection Services to reduce *Salmonella* in the processing facilities (USDA, 2017). There are a few preventative measures taken to reduce the amount of pathogenic bacteria. A few examples are biosecurity measures, competitive exclusion, vaccination, host genetic selection, and the use of antimicrobial alternatives (Lin, 2009).

Campylobacter, primarily *Campylobacter jejuni* and *Campylobacter coli*, are well adapted to the avian host and reside in the intestinal tract of birds (Saif, 2003). Studies have shown that despite extensive colonization, *Campylobacter* infections produce little or no clinical diseases in poultry. Saif also states that although thermophilic campylobacters are not significant pathogens for poultry, they are of importance to food safety and public health with *C. jejuni* being responsible for the majority of campylobacteriosis. *Campylobacter* has now emerged as a leading bacterial cause of foodborne gastroenteritis in humans around the world (Saif, 2003).

Intestinal Microbiota

Key reasons for maintaining a healthy gut is because the gut is responsible for digestion and absorption of nutrients. If the gut is impaired in any

way, digestion and absorption of feed will be altered, as well as, performance and overall health of the bird. Factors such as injury, stress, and nutrition can leave the host more susceptible to disease. Age of the bird is critical for developing a resilient immune system. Within the first few hours of a newly hatched chick, the normal gut bacteria (microflora) that inhabit the intestine become established (Jeffrey, 1999). Functions of the microflora are to breakdown ingested food, produce some vitamins and mostly provide a natural barrier of protection to harmful bacteria that enter the host cell (Jeffrey, 1999). The overall microbiota varies from bird to bird, and broilers can contain many different bacteria species in the gastrointestinal community. Environmental settings can also affect the performance of the microbial communities based on management practices and litter control. According to Apajalahti & Bedford (2000), these factors affect birds both directly and indirectly by recycling microbes and weakening immunity. Poultry litter is another main source of infection to the birds and meat contamination. Litter is consisted of a mixture between poultry wastes with bedding materials that cover the floor in commercial poultry houses (Grimes et al., 2006). Rice hulls, processed paper pellets, sand, peanut hulls, crushed corn cobs, chopped straw and wood shavings are most commonly used due to feasibility and convenience of resources in specific regions of the world (Ritz et al., 2009). Viability of these bedding materials is due to their absorption and storage properties of poultry wastes during the rearing of broilers. Tewolde and

colleagues (2005) presented in studies that litter is high in phosphorus and nitrogen content, which is being used as a fertilizer for crops. Broiler litter is also shown to be a better soil conditioner than synthetic fertilizers by Tewolde and colleagues (2005). At the opposite end of the spectrum, Brake (1992) has stated that continuous growth in the poultry industry has led to a problem with litter disposal, which has resulted in farmers recycling used bedding material inside chicken houses. Nevertheless, recycling of litter can lead to increased levels of parasitic and bacterial infections of the birds. Poultry litter is considered an environmental ecosystem with an extensive range of biotic properties. Barker (1996) stated that the physiochemical properties within the litter environment favor the establishment of a large microbial and parasitic community that are mostly of intestinal origin. According to the USDA, 12.3 million metric tons of poultry litter is produced annually for every 8.5 million broilers (Chamblee, 2002), causing a high number of bacteria which would be a problem for their disposal.

Bacteria, such as lactic acid producing bacteria, are at the historical core of the discussion when the use of probiotic heath supplements and therapeutics are considered (Hume, 2011). Lactic acid bacteria make up a group of bacteria that degrade carbohydrates with the production of lactic acid. A few examples of bacteria containing lactic acid are *Streptococcus, Lactobacillus, Lactococcus*, and *Leuconostoc*. These bacteria are gram-positive that do not form spores and are able to grow both in the presence and absence of oxygen. Lactic acid

bacteria can also manufacture compounds needed to survive and grow in many types of environments.

A number of known microorganisms, mainly the lactic acid producing bacterial species *enterococci, bifidobacteria*, and *lactobacilli,* as well as a smaller number of unidentified microbial cultures are normally used as probiotics (Barug et al., 2006).

Stress Related Bacteria

Stresses in poultry production are a harsh reality. The development of the poultry industry has been due in part, to the ability of the chicken to accommodate many of the stresses imposed on them by modern production techniques. Such stressors include genetic selection for increased growth rate and egg numbers, environmental and management changes, increased diseases challenges and exposure to a wide array of pharmaceuticals and vaccines needed to maintain a healthy flock. It is important to understand what bacteria are related to stress and how bacteria can affect the health of the bird. When conditions are perfect, such as, feed quality and diet, temperature, water pH, and ventilation, there is a low amount of stress on the chicken. As conditions vary, the intestinal microbiota will fluctuate. *Lactobacilli* and *Bifidobacterial* species seem to be stress sensitive, and these populations tend to decrease when a bird is under stress (Patterson & Burkholder 2003). *Lactobacillus* is a bacteria producing

lactic acid from the fermentation of carbohydrates. *Bifidobacteria* is a gram-positive anaerobic bacterium, usually found in the GI-tract of the bird (Patterson & Burkholder 2003). The reason these microbes are so unusual in the beneficial bacteria world, is that the bacteria form endospores under stressful conditions (*Bacillus* Bacteria, 2012). The endospores have a tough coating in order to protect the dormant bacteria within. This coating is able to last for years and can resist extreme heat, radiation, extreme freezing, drying, and chemical disinfectants (*Bacillus* Bacteria, 2012).

Antibiotics as Growth Promotors

Studies by Castanon (2007) stated the growth promoter effect of antibiotics was discovered in the 1940s, when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved their growth. The United States Food and Drug Administration approved the use of antibiotics as animal feed additives without veterinary prescription in 1951 (Jones & Ricke, 2003). Antibiotics are the chemical products obtained from certain strains of micro-organisms at low concentrations that can inhibit the growth of other micro-organisms, and may even cause their death. In the past, the use of antibiotics in food, as treatment and either at a lower level of care (as growth promoters) was widespread (Visek, 1978 & Shane, 2005), but the use of antibiotics in livestock and poultry may increase bacterial resistance.

Concerns from consumers have shifted the market of the broiler industry by substituting antibiotics with probiotics, prebiotics or other alternatives. Consumers are worried the antibiotic given to the animal could be transferred to the human upon digestion of the meat. The European Union has banned the use of human antibiotics as growth promotors in animal feed since 2006 (Wang et al., 2016). The main expected consequence of the ban is a reduction of the amount of antibiotics used in animal production, and therefore the risk of transferring to persons of microbial with resistant genes to antibiotics (Castanon, 2007).

In this research, the antibiotic growth promotor of choice was Bacitracin Methylene Disalicylate (BMD). BMD is used for the prevention and control of necrotic enteritis, increased rate of weight gain and improved feed efficiency. It preserves the integrity of the gut wall, helping absorb the nutrients needed from the feed within the diet (Miller et al., 2017). This antibiotic also reduces subclinical and clinical disease, resulting in greater productivity and decreased mortality. Much of the work with antibiotic growth promoters continues to be from the standpoint of studying the effects on easily cultured bacterial populations such as *Lactobacilli* and *Clostridium perfringes* and poultry health rather than resulting physical changes to the gastrointestinal tract (Engberg et al., 2000).

Pyrosequencing of DNA

A variety of methods are available for sequencing DNA, but Sanger and pyrosequencing are two of the most commonly used today. The Sanger method is also known as terminator sequencing because DNA fragments of varying lengths are synthesized by incorporating both nucleotides and dideoxyterminators (deoxyribonucleotide triphosphates [dNTPs] and dideoxynucleotide triphosphates [ddNTPs], respectively) (Harrington, 2013).

Pyrosequencing is designated as a sequence-by-synthesis technique because DNA synthesis is monitored in real time. It is based on the pioneering and sophisticated, basic science work of Pal Nyren, PhD, who first demonstrated in 1987 that DNA polymerization can be monitored by measuring pyrophosphate production, which can be detected by light (Nyren, 1987). Small change in bacterial population could be easily analyzed with the advancement in high-throughput techniques like pyrosequencing. Pyrosequencing is one of the next generation sequencing methods that has revolutionized the field of biology. It provides an ample amount of volume of information from a small amount of sample collected. Pyrosequencing employs sequencing by enzymatic approach and is based on detecting the activity of DNA polymerase with another chemiluminescent enzyme (Manoharan, 2013). The pyrophosphates (ppi) released during DNA syntheses (as the dNTPs are added to the new strand by DNA polymerase) are proportionally converted in to a chemiluminescent signal by a cascade of enzymatic reactions and quantified (Ronaghi, 1998; Ronaghi,

2001). At present, the widely used method for pyrosequencing is the bacterial 16S rRNA Tag-Encoded FLX Amplicon Pyrosequencing (bTEFAP) approach.

Studies by Van Kley, (2013) has shown that overall, pyrosequencing has helped to further understand the diversity of cecal microbial communities, demonstrate a method to determine the dynamics of microbial communities and also establish the functionality of the microbial community with broiler performance.

Research conducted by Nonnenmann and colleagues (2010) studying the bacteria and fungi present in organic dust from commercial poultry houses have been largely limited to culture-based techniques (Clark, 1983 & Lee, 2006). The low cost and the fast processing speed of this pyrosequencing technology may revolutionize the ability to identify the distribution and concentration of bioaerosols, stated by Nonnenmann and colleagues (2010).

Another method, so the data obtained is more reliable, has been developed by one of the Roche companies, 454 life sciences, is a high-throughput DNA sequencing method which employs massive parallel sequencing-by-synthesis approach (Manoharan, 2013). With this method, we could sequence any double-stranded DNA which enables a variety of applications including de novo whole genome sequencing, re-sequencing of

whole genomes and target DNA regions and metagenomics (454 Life Sciences, 2011).

Veterinary Feed Directive 2017

The Food and Drug Administration (FDA) has adjusted the animal drug regulations to implement the veterinary feed directive (VFD) drugs section of the Animal Drug Availability Act of 1996 (ADAA) (FDA, 2017). This amendment is intended to improve the efficiency of FDA's VFD program while protecting human and animal health.

VFD drugs are a list of animal drugs intended for use in or on animal feed which are limited to use under the professional supervision of a licensed veterinarian. Any animal feed containing a VFD drug can only be fed to animals based upon an order, called a veterinary feed directive (VFD), issued by a licensed veterinarian in the course of the veterinarian's professional practice (FDA, 2017).

Disease Prevention

Understanding interactions between animals and humans is critical in preventing outbreaks of zoonotic disease. Biosecurity practices are designed to prevent the spread of disease. Rodent control, vaccination programs, disposal of

mortality and sanitation practices are other critical actions important to disease prevention, although it cannot provide total protection against infection.

Many management factors have an influence on disease control and prevention. People are considered to be one of the largest vectors as to carrying poultry diseases onto poultry farms, particularly on their footwear, clothing and hands. Contaminated vehicles, equipment, flies and wild birds are also factors that could potentially carry diseases into a poultry farm. Bahrndorff and colleagues (2013) stated in his research that by using fly screens to prevent flies from entering broiler chicken houses, it was possible to reduce the prevalence of *Campylobacter spp*. positive flocks from 41.4% to 10.3%.

The clean-out period between flocks in the poultry houses is critical to the health of the forthcoming flock. Young chicks are more disease-sensitive than older birds. Poultry housing and environmental factors can influence the occurrence of disease. Poor chicken litter conditions may include wet or poor bedding quality, inadequate litter depth, poor site drainage, house condensation problems, improper management of the drinkers, cooling and ventilation systems, and not maintaining uniform bird density in the houses (Malone, 2004).

CHAPTER III

Materials and Methods

Animals and Housing

This study began on March 11, 2016 and was completed on May 5, 2016. This study was conducted using one day old, co-mingled, mixture of male and female, Cobb chickens supplied by Pilgrim's Pride in Nacogdoches, Texas. A total of 4,800 birds, 600 birds per treatment group, were placed within 96, 5'X10' floor pens in a randomized-block design at the SFASU Poultry Research Center. Birds were randomly divided within the pens at a stocking density of 1.00 ft^2/bird (50 birds/pen). Birds were reared on used pine shavings for 55 days. Food and water were administered *ad libitum* basis throughout the study to the birds. Water was provided via Lubing FeatherSoft® nipple drinkers, while feed was provided via two, 30lb. hanging tube feeders. All 4,800 broilers were reared in the same house under standard commercial industry practices. This house is split into two equal halves with each half containing 48 individual pens. These pens were then separated into twelve different blocks with each block containing one pen for each of the eight treatment groups. The experiment was arranged specifically to minimize environmental variation created by the bird's location within the house.

The LED light bulbs of choice in this research were Overdrive A19 bulbs. The lighting program used followed an industry standard lighting program. The birds received 24 hours of light at 100% intensity for the first 6 days. On day 7, the intensity level was reduced to 80%. By day 8, the intensity level was reduced to 60%. Day 9, the intensity level was reduced to 40%. Days 10-14, the intensity level was reduced to 20% and remained at this intensity for the remainder of the study. Total hours of light were reduced from 24 hours to 16 hours for days 7-14. Reducing hours of light during the growing period allows the birds to develop their skeletal system prior to putting on large amounts of muscle. At day 15-48, the total amount of hours was increased to 18. From day 49 to the end of the study, total hours of light were increased again to reach 20 hours. The reasoning for reduction of lighting intensity as the birds grow is because of behavioral developments. The birds will tend to fight to show dominance and cause scratch marks on the skin. Once the lights have been reduced to a dimmer intensity, the fighting will be reduced causing the birds to relax and become calm.

Table 1. Lighting Treatment

Lighting Treatment					
Growth Day	**Intensity**	**Transition Period (min)**	**Total Hours of Light**	**Cycle 1**	**Cycle 2**
0 to 6	**100%**	**0**	**24**	**00:00-00:00**	
7	**80%**	**30**	**16**	**06:00-22:00**	
8	**60%**	**30**	**16**	**06:00-22:00**	
9	**40%**	**30**	**16**	**06:00-22:00**	
10 to 14	**20%**	**30**	**16**	**06:00-22:00**	
15 to 48	**20%**	**30**	**18**	**06:00-24:00**	
49 to END	**20%**	**30**	**20**	**06:00-02:00**	

Experimental Treatment and Groups

This study had a total of 8 different treatment groups (600 birds with 12 replications/TX). The individual treatments associated with probiotics, antibiotic growth promotor, coccidiostat and vaccines are listed in Table 2. Probiotics were supplemented at 2.0 x 10^6 CFU/gram of feed. CFU stands for Colony Forming Units.

Treatment 1 was the negative control (NC). Negative control is a treatment without any products or additives mixed within the diet with the exception of Maxiban in the starter and grower diets as the coccidiostat. Treatment 2 was the positive control (PC), which is the standard diet with an antibiotic growth promotor, BMD. BMD was added in the starter and grower diets at 50 grams per ton, and Maxiban was also added in the starter and grower diets as the coccidiostat. Treatment 3, (LS + AGP), had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus subtilis,* and BMD with Maxiban in the starter and grower diets as the coccidiostat. Treatment 4, (LMS + AGP), had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus megaterium, Bacillus subtilis*, and BMD with Maxiban in the starter and grower diets as the coccidiostat. Treatment 5, (LS + NO AGP), had the following probiotics and no antibiotic growth promotors: *Bacillus licheniformus* and *Bacillus subtilis*, with Maxiban in the starter and grower diets

as the coccidiostat. Treatment 6, (LMS + NO AGP), had the following probiotics and no antibiotic growth promotors: *Bacillus licheniformus, Bacillus megaterium*, and *Bacillus subtilis*, with Maxiban in the starter and grower diets as the coccidiostat. Bioshuttle is the application of a vaccine on day of hatch, and then followed up by a coccidiostat within the grower to help clean up after the vaccine. Treatment 7, (LS + Bioshuttle), had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus subtilis*, with BMD in the starter and grower along with Salinomycin as the coccidiostat at a rate of 45 grams per ton in the grower diet only. Advent coccidiosis vaccine was also administered to treatment 7 (LS + Bioshuttle), at day of hatch. Treatment 8 (LMS + Bioshuttle) had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus megaterium, Bacillus subtilis,* with BMD in the starter and grower along with Salinomycin in the grower diets only as the coccidiostat. Advent coccidiosis vaccine was also administered to treatment 8 at day of hatch.

Table 2. Dietary Treatment Groups

Treatment #		Test Articles				
		Diet	Probiotic*	Antibiotic Growth Promotor	Coccidiostat	Coccidiosis Vaccine
1	Negative Control	Standard Diet			Maxiban in Starter & Grower	
2	Positive Control	Standard Diet		BMD 50 g/ton in Starter & Grower	Maxiban in Starter & Grower	
3	LS + AGP	Standard Diet	*B. licheniformis, B. subtilis*	BMD 50 g/ton in Starter & Grower	Maxiban in Starter & Grower	
4	LMS + AGP	Standard Diet	*B. licheniformis, B. megaterium, B. subtilis*	BMD 50 g/ton in Starter & Grower	Maxiban in Starter & Grower	
5	LS + NO AGP	Standard Diet	*B. licheniformis, B. subtilis*		Maxiban in Starter & Grower	
6	LMS + NO AGP	Standard Diet	*B. licheniformis, B. megaterium, B. subtilis*		Maxiban in Starter & Grower	
7	LS + Bioshuttle	Standard Diet	*B. licheniformis, B. subtilis*	BMD 50 g/ton in Starter & Grower	Salinomycin 45 g/ton in Grower Only	Advent Vaccine at day of hatch
8	LMS + Bioshuttle	Standard Diet	*B. licheniformis, B. megaterium, B. subtilis*	BMD 50 g/ton in Starter & Grower	Salinomycin 45 g/ton in Grower Only	Advent Vaccine at day of hatch

*Probiotics were supplemented at 2.0 x 10^6 CFU/gram of feed.

Feed

All basal diets were supplied by Pilgrim's Pride. The basal diets were formulated to be complete for all nutrients required by the birds. Feeding phases consisted of a starter (S), grower (G), withdrawal (W) diets with feed changes occurring at approximately at day 15, day 33, and day 55, respectively. Diets were back formulated to meet the negative control treatments needs prior to arrival to the SFASU Research Feed Mill. Diets were formulated according to the treatments, mixed, pelletized, weighed and recorded. Feed samples were retained for analysis. The feed was provided *ad libitum* throughout the study via tube feeders.

Performance Data

All birds in each pen were counted and weighed collectively on days (d) 1, d15, d33, and d55. These days represented approximate times for feed change, d15 was the end of the starter phase, d33 was the end of the grower phase, and d55 was the end of the withdrawal phase. All feed was weighed and recorded prior to delivery to each pen. The chickens were delivered by Pilgrim's Pride on their delivery truck in chick trays. On day one, the farm crew, along with help from Dr. Joey Bray, counted and weighed 50 chicks on a Doron 8000XL table top scale before placement into their pen. While in the procedure of weighing the chicks, any deformities, open navels, or cull birds were euthanized properly via cervical dislocation. Weights were analyzed to determine average body weight per treatment group. In order to collect the data, each bird was placed in an aluminum cage with five separate levels, with load cells on the bottom to capture the collective weight. All feed remaining in each pen on assigned weigh days were placed on the scale, weighed, and recorded. Data was used to calculate feed conversion ratio, and adjusted feed conversion ratio for each treatment. Mortality was checked daily. All mortality was collected, weighed, necropsied, and recorded. Probable cause of death was noted.

Intestinal Sampling for 16S rRNA Sequencing

Intestinal samples were collected from 8 randomly selected birds per treatment group on d25, d35, and d55 then analyzed for total microbiome by Omega Bio-Tek, Inc. in Norcross, GA.

16S rRNA sequencing is designated as a sequence-by-synthesis technique because DNA synthesis is monitored in real time. It employs sequencing by enzymatic approach and is based on detecting the activity of DNA polymerase with another chemiluminescent enzyme. Pyrosequencing also provides an ample amount of volume of information from a small amount of sample collected. Because of its fast processing speed, pyrosequencing technology may revolutionize the ability to identify the distribution and concentration of bioaerosols (Nonnenmann et al., 2010).

Yield Study

At the completion of the study, four randomly-selected birds/pen (two males & two females identified by sexual characteristics), for a total of 384 birds were individually weighed, recorded, and wing tagged. A numbered wing tag was placed in the wing web of each bird for further individual identification throughout the yield process. Birds from each treatment group remained together and were placed in individual isolation pens until the time of processing. Birds were provided feed and water until 10 hours prior to processing, when the feed only was removed until transit time.

The processing stage begins at the euthanasia station where the birds are electrically stunned before severing the carotid artery and jugular vein in the neck. Once the birds have been exsanguinated, they were transferred to the scalder. The scalder is filled with water and the temperature is set to 140°F while turning for 90 seconds. Inside the scalder is a rotating plate which tumbles the chickens causing the feather follicles to open and feathers to loosen from the skin. The next step in the processing phase is to transport them into the plucker. The plucker has rubber finger-like studs along the bottom and wall of the machine, when added with water, the inside of the machine rotates clockwise for 90 seconds plucking the feathers from the skin of the bird. Once completed, the hocks are removed from the lower portion of the bird and discarded

appropriately. The birds are hanged upside down on a rotating shackle line for removal of the neck and tail. After the neck and tail have been removed, the birds are then eviscerated, removing all organs and intestines. At this point, the front-half and hind-half are separated. The front-half remains whole until later. The hind-half is then cut into sections, such as drums, thighs, back and fat pad. All parts of the carcass are then placed within a colander where the hind-half parts are recorded through a scale connected to a computer. Once the hind-half has been removed and placed into the chiller, the front-half is cut up into breast, tenders, wings, frame and skin. The skin weights recorded were only from the breast of the carcass. The same procedure of weighing the front-half of the carcass was repeated like the hind-half.

Birds were processed for yield analysis and the following weights were recorded: without giblets (WOG), front-half carcass, hind-half carcass, breasts, tenders, wings, drums, thighs, frame, back, abdominal fat pad, and skin. All remaining birds were processed by Pilgrim's Pride for commercial distribution. The yield analysis helped determine if the probiotics had any effect on intestinal microbiota, and performance.

Data Interpretations

The data was collected at the Stephen F. Austin State University Poultry Research Center and was statistically analyzed using Statistical Analysis System (SAS 9.2) (Cary, NC). The data was interpreted using a one-way, analysis of variance (ANOVA) using the PROC GLM procedure. When significance between the treatments was observed, at the alpha level of $P < 0.05$, means were separated using the least squares means test with the PDIFF option of this procedure. Means were separated using Duncan's Multiple Range Test. Duncan's Multiple Range Test allowed the results to be analyzed by testing all treatment groups against themselves. Duncan's Multiple Range Test was only performed to determine the differences between performance and yield data. Dunnet's Multiple Range Test allowed the results to be tested individually against the control group. Dunnet's test was only used to determine the difference in treatments compared to the control group for intestinal pyrosequencing data.

CHAPTER IV

Results and Discussion

At the completion of the study, all of the performance parameters and yield data were collected and evaluated by the researcher and poultry thesis research director. The following is a compilation of the results determined from this research trial. Treatment 1 was the negative control (NC). Negative control is a treatment without any products or additives mixed within the diet with the exception of Maxiban in the starter and grower diets as the coccidiostat. Treatment 2 was the positive control (PC), which is the standard diet with an antibiotic growth promotor, BMD. BMD was added in the starter and grower diets at 50 grams per ton, and Maxiban was also added in the starter and grower diets as the coccidiostat. Treatment 3, (LS + AGP), had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus*, *Bacillus subtilis*, and BMD with Maxiban in the starter and grower diets as the coccidiostat. Treatment 4, (LMS + AGP), had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus megaterium*, *Bacillus subtilis*, and BMD with Maxiban in the starter and grower diets as the coccidiostat. Treatment 5, (LS + NO AGP), had the following probiotics and no antibiotic growth promotors: *Bacillus licheniformus* and *Bacillus subtilis,* with Maxiban in the starter and

grower diets as the coccidiostat. Treatment 6, (LMS + NO AGP), had the following probiotics and no antibiotic growth promotors: *Bacillus licheniformus, Bacillus megaterium*, *and Bacillus subtilis*, with Maxiban in the starter and grower diets as the coccidiostat. Bioshuttle is the application of a vaccine on day of hatch, and then followed up by a coccidiostat within the diet to help stimulate the vaccine. Treatment 7, (LS + Bioshuttle), had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus*, *Bacillus subtilis*, with BMD in the starter and grower along with Salinomycin as the coccidiostat at a rate of 45 grams per ton in the grower diet only. Advent coccidiosis vaccine was also administered to treatment 7 (LS + Bioshuttle), at day of hatch. Treatment 8 (LMS + Bioshuttle) had the following probiotics and antibiotic growth promotors: *Bacillus licheniformus, Bacillus megaterium*, *Bacillus subtilis*, with BMD in the starter and grower along with Salinomycin in the grower diets only as the coccidiostat. Advent coccidiosis vaccine was also administered to treatment 8 at day of hatch.

Growth Performance

Average body weights and feed conversion ratios were measured on multiple occasions throughout the study. Feed conversion ratio is a measurement of feed efficiency. Feed conversion ratio is a mathematical measure to calculate the animal's ability to use the nutrients given in the diet (Shike, 2011). Since feed makes up the primary cost for broiler producers, feed efficiency is very important for profit yields. The broiler industry attempts to reduce the amount of feed required to grow birds to a constant weight in a given period of time. For this study, d15, d33, and d55 were chosen as they were the days that the broilers switched diets. At d15, the chickens had completed eating the starter diets and were switched to a grower diet. At d33, they switched from grower to withdrawal. At d55, all feed was removed as the birds were prepared for processing. For this study, the average body weight, feed conversion ratio and adjusted feed conversion ratio were determined. Treatment means for average body weight and feed conversion ratio are shown in Table 3. Table 4 displays the analysis of variance for the response variable of the average body weight and feed conversion ratio.

Table 3. Average Body Weight & Feed Conversion Ratio for Treatments 1-8, Day 15, & 33

TX	Treatment	Day 15		Day 33	
		Avg. Body Weight (lbs.)	Feed Conversion (lb:lb)	Avg. Body Weight (lbs.)	Feed Conversion (lb:lb)
1	NC	0.688^{abc}	1.24^{ab}	3.525^{ab}	1.71^{a}
2	PC	0.678^{abc}	1.26^{a}	3.563^{a}	1.67^{ab}
3	LS + AGP	0.696^{ab}	1.24^{ab}	3.591^{a}	1.67^{b}
4	LMS + AGP	0.706^{a}	1.21^{b}	3.574^{a}	1.66^{b}
5	LS + NO AGP	0.695^{ab}	1.22^{ab}	3.514^{ab}	1.67^{ab}
6	LMS + NO AGP	0.675^{bc}	1.23^{ab}	3.519^{ab}	1.67^{ab}
7	LS + Bioshuttle	0.660^{c}	1.24^{ab}	3.449^{b}	1.68^{ab}
8	LMS + Bioshuttle	0.667^{bc}	1.24^{ab}	3.421^{b}	1.68^{ab}

*Means with the same letters are not significantly different ($p<0.05$).

Table 3 shows the average body weight and feed conversion ratios for days 15 & 33. On day 15, there was a significant difference for average body weight when the researcher compared treatment 4 and treatment 6, 7, & 8 but not significant when compared to the remaining treatments. Treatment 4 showed the largest body weight and treatment 7 showed the smallest body weight. Feed conversion ratio for day 15 also showed treatment 4 was significantly different from treatment 2 but was not significant when compared to the remaining treatments. Treatment 4 had the lowest feed conversion, while treatment 2 had the highest feed conversion.

On day 33, average body weight for treatments 2, 3, & 4 were significantly different from treatments 7 & 8, but were not significantly different from treatments 1, 5, & 6. Treatment 3 had the largest body with the lowest feed conversion ratio, following closely behind was treatment 4. Treatment 3 had an increase in body weight and feed conversion compared to the other treatments. The results also showed treatments 3 & 4 were significantly lower than treatment for feed conversion ratio, but not significantly different when compared to the remaining treatments.

Table 4. ANOVA of Body Weight, Treatments 1-8, Day 15 & 33

Source	DF	Mean Square	
		D15	D33
Block	11	0.00411553	0.03313741
Treatment	7	0.00297321	0.04234866
Error	77	0.00101445	0.01500450
Total	95	0.14419583	1.81629896
Pr>F		0.0090	0.0113

Table 5. ANOVA of Feed Conversion Ratio for Treatments 1-8, Day 15 & 33

Source	DF	Mean Square	
		D15	D33
Block	11	0.00211847	0.00825786
Treatment	7	0.00292842	0.00338557
Error	77	0.00236479	0.00259141
Total	95	0.22589063	0.31407396
Pr>F		0.2925	0.2587

Figure 1 compares the average body weight and feed conversion ratio for day 15. The bar graph represents the relationship of the average body weight and feed conversion ratio. It supports the claim that Table 1 showed statistical differences between treatments treatment 4 and treatment 6, 7, & 8 for average body weight. Feed conversion ratio for day 15 also showed a significant difference between treatment 2 and treatment 4.

Figure 1. Average Body Weight and Feed Conversion Ratio for Day 15

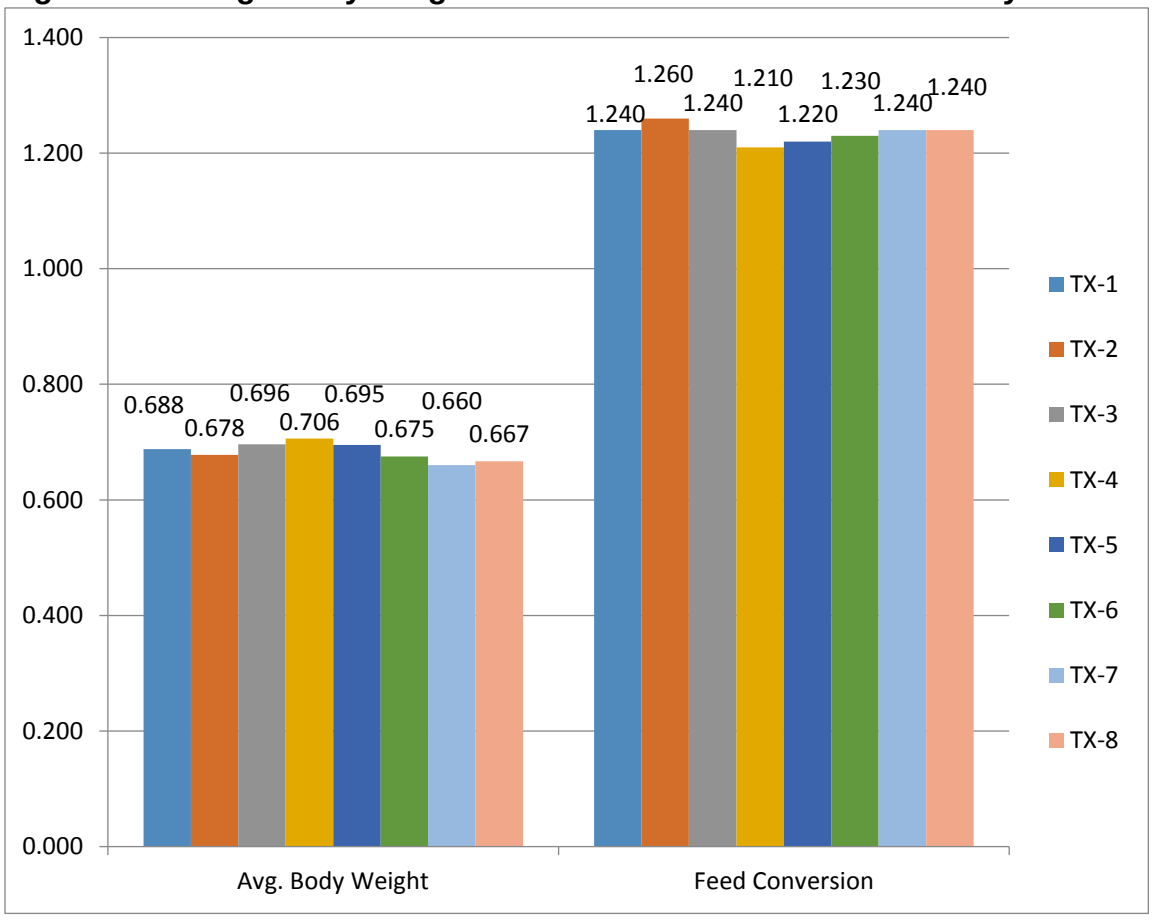


Figure 2 compares the average body weight and feed conversion ratio for day 33. The bar graph represents the relationship of the average body weight and feed conversion ratio. It supports the claim from Table 1 showing treatments 2, 3, & 4 were significantly different from treatments 7 & 8 for average body weight. Figure 2 also shows a significant difference between treatment 1, compared to treatment 3 & 4 for feed conversion ratio.

Figure 2. Average Body Weight and Feed Conversion Ratio for Day 33

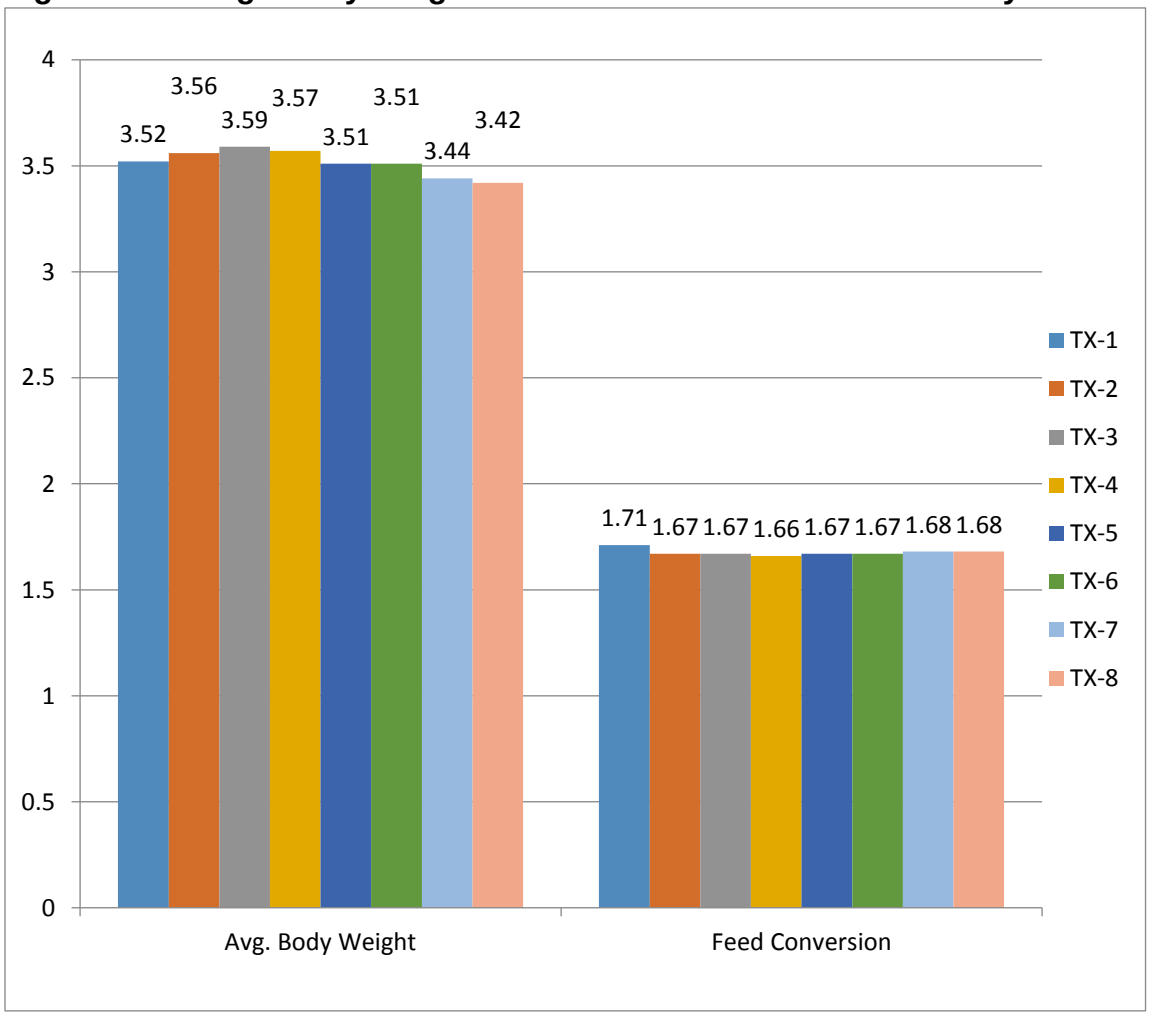


Table 6 shows the average body weight, feed conversion ratio and adjusted feed conversion ratio for treatments 1-8 at day 55. There was no significant difference at day 55 for average body weight or feed conversion ratio. Although there was no significant differences, treatment 3, produced the largest average body weight but the feed conversion ratio was only 0.03 lbs. higher than treatment 6 & 8, which had the lowest feed conversion ratio. Treatment 1 & treatment 8 showed to have the smallest average body weight. Treatment 1 reported the highest feed conversion at a 1.94 while treatment 8 produced one of the lowest feed conversion ratios at a 1.90.

Table 6. Average Body Weight, Feed Conversion Ratio, and Adjusted Feed Conversion Ratio without Mortality, Treatments 1-8, Day 55

Day 55			
Treatment	Avg. Body Weight (lbs.)	Feed Conversion (lbs:lbs)	Adj. Feed Conversion (lbs:lbs)
1	7.605[a]	1.942[a]	1.794[a]
2	7.623[a]	1.942[a]	1.789[a]
3	7.700[a]	1.937[a]	1.776[a]
4	7.652[a]	1.922[a]	1.766[a]
5	7.666[a]	1.915[a]	1.760[a]
6	7.653[a]	1.903[a]	1.749[a]
7	7.653[a]	1.933[a]	1.779[a]
8	7.607[a]	1.908[a]	1.760[a]

*Means with the same letters are not significantly different ($p<0.05$).

Feed conversion ratio was adjusted for a 7 lb. bird on a 1450 calorie diet with a 7 point weight/point of feed conversion ratio. The following formula was used to calculate adjusted feed conversion ratio.

Step 1: (Actual Avg. Body Weight – 7.00) / 7 = X1

(Actual Feed Conversion Ratio – X1) = X2

Step 2: (X2 * 1450) kcal / 1500 std kcal = Adjusted Feed Conversion ratio for Avg. Body Weight

Table 7. ANOVA of Average Body Weight, Day 55

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Block	11	0.75387812	0.06853437	1.62	0.1097
Treatment	7	0.08518229	0.01216890	0.29	0.9569
Error	77	3.25733021	0.04230299		
Total	95	4.09639063			

Table 8. ANOVA of Feed Conversion Ratio, Day 55

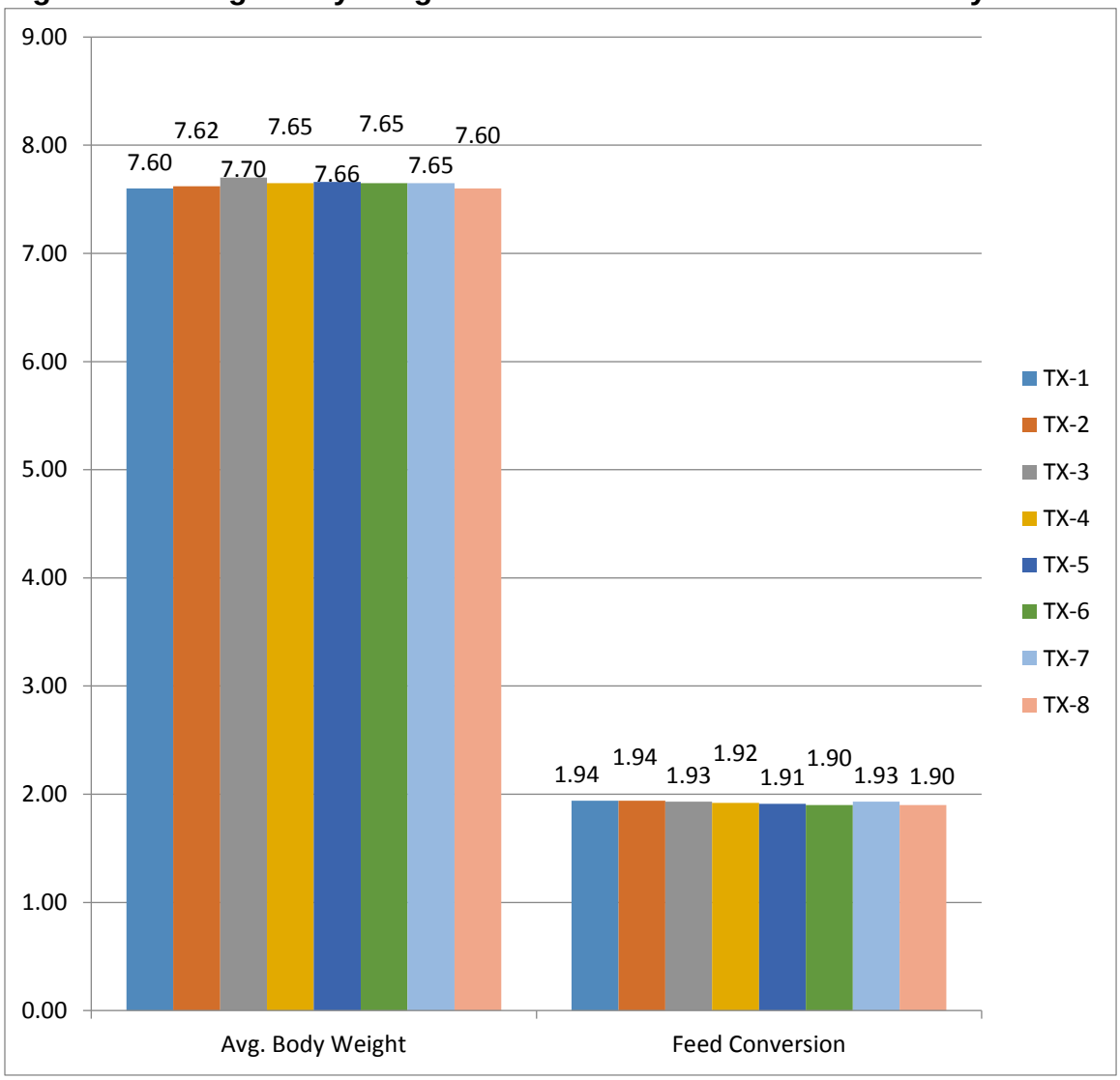
Source	DF	Type I SS	Mean Square	F Value	Pr>F
Block	11	0.03951146	0.00359195	1.16	0.3320
Treatment	7	0.02005729	0.00286533	0.92	0.4946
Error	77	0.23938021	0.00310883		
Total	95	0.29894896			

Table 9. ANOVA of Adjusted Feed Conversion Ratio without Mortality, Day 55

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Block	11	0.07783750	0.00707614	1.31	0.2337
Treatment	7	0.02036250	0.00290893	0.54	0.8018
Error	77	0.41506250	0.00539042		
Total	95	0.51326250			

Figure 3 compares the average body weight and feed conversion ratio for day 55. The bar graph represents the relationship of the average body weight and feed conversion ratio. It supports the claim of Table 1 showed there was no significant difference between treatments 1-8.

Figure 3. Average Body Weight and Feed Conversion Ratio for Day 55



Mortality

Mortality was measured on the same days as both average body weight and feed intake. A significant difference was not only seen in percent livability, but also in mortality percent in treatments 6 & 7 compared to treatment 5 but not the remaining treatments. It is also noted that, average mortality body weight showed a significant difference in treatment 7 compared to treatment 2; the remaining treatments were not significantly different. Treatment 5 had the lowest average mortality bodyweight and lowest percent mortality but an average mortality body weight of 7.833, which indicates broilers died near the end of the study.

Table 10. Percent Mortality (%), Treatments 1-8, Day 1-55

Percent Mortality			
Treatment	Day 1-55		
	Percent Livability (%)	**Percent Mortality (%)**	**Average Mortality Bodyweight**
1	94.167^{ab}	5.833^{ab}	6.728^{ab}
2	94.500^{ab}	5.500^{ab}	6.274^{b}
3	94.167^{ab}	5.833^{ab}	7.552^{ab}
4	94.167^{ab}	5.833^{ab}	7.128^{ab}
5	95.333^{b}	4.667^{b}	7.833^{ab}
6	92.167^{a}	7.833^{a}	7.768^{ab}
7	91.833^{a}	8.167^{a}	9.688^{a}
8	93.667^{ab}	8.167^{ab}	7.659^{ab}

*Means with the same letters are not significantly different ($p<0.05$).

Table 11. ANOVA of Mortality, Treatments 1-8, Day 1-55

Source	DF	Total Mortality			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	52.00000000	4.72727273	2.03	0.0363
Treatment	7	29.33333333	4.19047619	1.80	0.0991
Error	77	179.1666667	2.3268398		
Total	95	260.5000000			

Table 12. ANOVA of Total Mortality Body Weight, Treatments 1-8, Day 1-55

Source	DF	Total Mortality Body Weight			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	228.4544365	20.7685851	1.73	0.0833
Treatment	7	86.1932490	12.3133213	1.02	0.4221
Error	77	926.836639	12.036839		
Total	95	1241.484324			

Table 13. ANOVA of Total Mortality Percent (%), Treatments 1-8, Day 1-55

Source	DF	Total Mortality Percent (%)			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	208.0000000	18.9090909	2.03	0.0363
Treatment	7	117.3333333	16.7619048	1.80	0.0991
Error	77	716.666667	9.307359		
Total	95	1042.000000			

Pyrosequencing

Lactobacillus is a genus of gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria, normally found in the GI-tract (Makarova et al., 2006). *Lactobacillus* creates lactic acid from the utilization of carbohydrates. *Enterococcus* is a large genus of lactic acid bacteria of the phylum *Firmicutes*. *Enterococci* are gram-positive cocci that often occur in pairs or short chains, and are difficult to distinguish from *Streptococci* on physical characteristics alone and are usually found in the intestinal tract (Gilmore, 2002). *Brachybacterium* is a coryneform bacterium from poultry deep litter (Collins et al., 1988). *Bacillus* is a genus of gram-positive, rod-shaped bacteria and a member of the phylum *Firmicutes*. Under stressful environmental conditions, the bacteria can produce oval endospores that are not true 'spores', but to which the bacteria can reduce themselves and remain in a dormant state for very long periods (Turnbull et al., 2002). *B. subtilis* has proved a valuable model for research. Other species of *Bacillus* are important pathogens, causing anthrax and food poisoning. *Virgibacillus* is a genus of gram-positive, rod-shaped bacteria and a member of the phylum *Firmicutes. Virgibacillus* species can be obligate aerobes, or facultative anaerobes and catalase enzyme positive. Under stressful environmental conditions, the bacteria can produce oval or ellipsoidal endospores in terminal, or sometimes subterminal, swollen sporangia (Kampfer et al., 2010). *Paenibacillus* is a genus of facultative anaerobic, endospore-

forming bacteria, originally included within the genus *Bacillus* and then reclassified as a separate genus in 1993 (Ash et al., 1993). Bacteria belonging to this genus have been detected in a variety of environments, such as: soil, water, rhizosphere, vegetable matter, forage and insect larvae, as well as clinical samples.

Escherichia is a genus of gram-negative, nonspore forming, facultatively anaerobic, rod-shaped bacteria from the family *Enterobacteriaceae* (Madigan & Martinko, 2005). In those species which are inhabitants of the gastrointestinal tracts of warm-blooded animals*, Escherichia* species provide a portion of the microbially derived vitamin K for their host. *Streptococcus* is a genus of coccus (spherical) gram-positive bacteria belonging to the phylum *Firmicutes* and the order *Lactobacillales* (Ryan & Ray, 2004). Cell division in this genus occurs along a single axis in these bacteria; thus they grow in chains or pairs, meaning easily bent or twisted, like a chain. *Clostridium* is a genus of gram-positive, anaerobic bacteria, which includes several significant human pathogens, including the causative agent of botulism and an important cause of diarrhea, *Clostridium difficile* (Maczulak, 2011). *Clostridium* endospores have a distinct bowling pin or bottle shape, distinguishing them from other bacterial endospores, which are usually ovoid in shape. *Clostridium* species inhabit soils and the intestinal tract of animals, including humans (Maczulak, 2011). *Staphylococcus* is a genus of gram-positive bacteria. Under the microscope, they appear round, and form in

grape-like clusters. Found worldwide, they are a small component of soil microbial flora.

Pyrosequencing data is shown below in tables 14, 15, & 16. There were no significant differences between any treatments 1, 3, 5, & 7 for day 25, 35, or 55. The study consisted of 4 replications of each treatment. The researcher only selected to test the samples for treatment 1, 3, 5 & 7 because treatments without *Bacillus megaterium* proved to perform better.

Pyrosequencing Data

Table 14. LSMEANS – Dunnett for Treatments 1, 3, 5, & 7, Day 25

Day 25					
Genus	Treatment				
	1 (NC)	3 (LS+AGP)	5 (LS + NO AGP)	7 (LS + Bioshuttle)	Pr > F
Lactobacillus	90975.00	98951.25	96167.00	88175.25	0.0905
Enterococcus	14663.25	9922.75	11459.00	9697.00	0.7593
Escherichia	370.75	948.50	209.50	32.75	0.5489
Streptococcus	20949.50	6759.50	33153.75	4601.00	0.1811
Clostridium	7074.25	4500.50	4436.00	3898.50	0.6983
Brachybacterium	2175.50	5268.25	3566.25	2708.75	0.5977
Staphylococcus	2932.00	6003.50	2054.50	3024.75	0.4509
Bacillus subtilis	839.50	1244.00	687.25	700.75	0.5010
Virgibacillus	641.75	579.50	739.00	682.75	0.4596
Paenibacillus	522.25	183.75	111.50	163.25	0.3286

Table 15. LSMEANS – Dunnett for Treatments 1, 3, 5, & 7, Day 35

Day 35					
Genus	Treatment				
	1 (NC)	3 (LS+AGP)	5 (LS + NO AGP)	7 (LS + Bioshuttle)	Pr > F
Lactobacillus	22259.75	20565.00	27420.50	36500.75	0.2388
Enterococcus	47981.25	47985.25	60271.00	37140.25	0.4273
Escherichia	32292.75	27625.50	32379.25	19538.25	0.2745
Streptococcus	5262.25	12865.50	8335.25	7661.75	0.2337
Clostridium	1941.25	4692.00	4963.25	4132.50	0.5169
Brachybacterium	180.75	229.50	404.00	237.25	0.5114
Staphylococcus	676.50	542.50	438.50	820.75	0.0876
Bacillus subtilits	1506.50	1608.75	645.75	751.00	0.8113
Virgibacillus	124.75	161.50	29.25	66.00	0.6298
Paenibacillus	189.00	202.50	116.50	183.75	0.3587

Table 16. LSMEANS – Dunnett for Treatments 1, 3, 5, & 7, Day 55

Day 55					
Genus	Treatment				
	1 (NC)	3 (LS+AGP)	5 (LS + NO AGP)	7 (LS + Bioshuttle)	Pr > F
Lactobacillus	126203.25	111335.75	141655.25	122360.50	0.8487
Enterococcus	19006.25	18872.50	5846.00	21146.25	0.8864
Escherichia	132.75	7792.00	1948.75	4678.25	0.6869
Streptococcus	3101.75	2599.75	1156.75	2535.75	0.7961
Clostridium	267.50	2266.75	996.00	3086.75	0.4651
Brachybacterium	3643.00	3078.25	1653.00	3173.75	0.2651
Staphylococcus	2040.25	485.25	362.00	617.25	0.4592
Bacillus subtilis	1170.75	1162.00	225.00	361.25	0.0805
Virgibacillus	1448.00	422.50	117.00	93.00	0.4109
Paenibacillus	195.75	126.75	41.50	83.25	0.1676

Carcass Yield

Table 17. Yield Data Results by Treatment, Day 55

Day 55								
Weight of Parts (lbs.)	Treatment							
	1 (NC)	2 (PC)	3 (LS+ AGP)	4 (LMS+ AGP)	5 (LS+NO AGP)	6 (LMS+ NO AGP)	7 (LS+ Bioshuttle)	8 (LMS+ Bioshuttle)
Average Live Weight	7.78[a]	7.78[a]	7.83[a]	7.77[a]	7.79[a]	7.67[a]	7.80[a]	7.72[a]
WOG	5.73[a]	5.67[a]	5.77[a]	5.72[a]	5.75[a]	5.68[a]	5.72[a]	5.66[a]
Carcass – Front Half	3.40[a]	3.38[a]	3.42[a]	3.38[a]	3.41[a]	3.39[a]	3.41[a]	3.31[a]
Carcass – Hind Half	2.23[a]	2.17[ab]	2.23[a]	2.23[a]	2.22[a]	2.17[ab]	2.20[a]	2.07[b]
Breast	1.44[a]	1.44[a]	1.50[a]	1.45[a]	1.46[a]	1.43[a]	1.48[a]	1.44[a]
Tenders	0.33[a]	0.32[a]	0.34[a]	0.31[a]	0.35[a]	0.33[a]	0.32[a]	0.31[a]
Wings	0.59[a]	0.57[ab]	0.58[ab]	0.59[a]	0.58[ab]	0.58[ab]	0.59[a]	0.56[b]
Drums	0.75[a]	0.71[a]	0.73[a]	0.73[a]	0.73[a]	0.72[a]	0.72[a]	0.72[a]
Thighs	0.94[ab]	0.93[ab]	0.97[ab]	0.98[ab]	0.99[a]	0.94[ab]	0.96[ab]	0.92[b]
Skin	0.21[ab]	0.21[ab]	0.22[a]	0.22[a]	0.22[a]	0.19[b]	0.22[a]	0.20[ab]
Fat Pad	0.12[a]	0.11[ab]	0.11[ab]	0.10[ab]	0.10[ab]	0.12[ab]	0.10[b]	0.12[a]
Frame	0.78[a]	0.78[a]	0.76[a]	0.79[a]	0.78[a]	0.79[a]	0.78[a]	0.78[a]
Back	0.54[a]	0.52[ab]	0.51[ab]	0.52[ab]	0.53[ab]	0.50[b]	0.51[ab]	0.50[ab]

*Means with the same letters are not significantly different ($p<0.05$).

The yield study was conducted by randomly selecting 4 birds, 2 females and 2 males identified by sexual characteristics, from each treatment pen within each pen. This allowed for a representative sample to be used for the entire flock.

Table 18. ANOVA of Hind Half for Treatment 1-8, Day 55

Source	DF	Hind Half			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.55562676	0.05051152	0.68	0.7544
Treatment	7	1.05810566	0.15115795	2.05	0.0488
Error	364	26.90084661	0.07390342		
Total	383	52.03945410			

A significant difference was recorded in the hind half carcass yield ANOVA Table 18 (p=0.0488). Treatment 8 produced the lowest average hind half carcass weight with 2.07 lbs. when compared to the heaviest weights at 2.24 lbs. shown by treatments 1, 3, 4, 5, & 7.

Table 19. ANOVA of Skin for Treatment 1-8, Day 55

Source	DF	Skin			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.00904192	0.00082199	0.41	0.9517
Treatment	7	0.03328808	0.00475544	2.37	0.0223
Error	362	0.72680692	0.00200775		
Total	381	0.79192230			

The ANOVA Table 19 showed a significant difference (p=0.0223) in skin weights. Treatment 6 produced the lightest skin weights at 0.19 lbs. while treatments 3, 4, 5, & 7 weighed in at 0.22 lbs.

Table 20. ANOVA of Fat Pad for Treatment 1-8, Day 55

Source	DF	Fat Pad			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.02720909	0.00247355	1.04	0.4139
Treatment	7	0.03153354	0.00450479	1.89	0.0707
Error	358	0.85495607	0.00238815		
Total	377	0.91479762			

The results from ANOVA Table 20, shows no significant difference among treatments. Treatment 1 & treatment 8 were heavier with fat pad yields of 0.127 lbs. and 0.126 lbs. Treatment 7 was also noted to be lighter at 0.100 lbs.

Table 21. ANOVA of Back for Treatment 1-8, Day 55

Source	DF	Back			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.03897412	0.00354310	0.67	0.7711
Treatment	7	0.05283879	0.00754840	1.42	0.1967
Error	361	1.92167152	0.00532319		
Total	380	3.37478228			

No significant differences were detected for back yield as seen in ANOVA Table 21. Treatment 1 was the heaviest with a yield weight of 0.542 lbs. Treatment 6 was the lightest yield weight with 0.502 lbs.

There were no significant differences shown in the ANOVA tables 22-30 live weights, without giblets, front-half, breast, tenders, wings, drums, thighs, and frame.

Table 22. ANOVA of Live Weight for Treatment 1-8, Day 55

Source	DF	Live Weight			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	6.787499	0.617045	1.56	0.1089
Treatment	7	0.901969	0.1288528	0.33	0.9422
Error	364	144.0570760	0.395761		
Total	383	321.8270490			

Table 23. ANOVA of Without Giblets for Treatment 1-8, Day 55

Source	DF	WOG			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	2.95463250	0.26860295	1.00	0.4436
Treatment	7	0.52830919	0.07547274	0.28	0.9610
Error	363	97.2697298	0.2679607		
Total	382	195.7984387			

Table 24. ANOVA of Front Half for Treatment 1-8, Day 55

Source	DF	Front Half			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	2.20517572	0.2004705	1.46	0.1429
Treatment	7	0.3795218	0.0542174	0.40	0.9046
Error	364	49.83723516	0.1369154		
Total	383	74.19370150			

Table 25. ANOVA of Breast for Treatment 1-8, Day 55

Source	DF	Breast			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.90027681	0.08184335	2.26	0.0114
Treatment	7	0.17936120	0.02562303	0.71	0.6658
Error	362	13.11108152	0.03621846		
Total	381	18.03109247			

Table 26. ANOVA of Tenders for Treatment 1-8, Day 55

Source	DF	Tenders			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.09770325	0.00888211	0.82	0.6190
Treatment	7	0.06469097	0.00924157	0.85	0.5430
Error	362	3.91589478	0.01081739		
Total	381	4.21327522			

Table 27. ANOVA of Wings for Treatment 1-8, Day 55

Source	DF	Wings			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.03487429	0.00317039	0.72	0.7179
Treatment	7	0.04275196	0.00610742	1.39	0.2082
Error	362	1.59060247	0.00439393		
Total	381	2.55895611			

Table 28. ANOVA of Drums for Treatment 1-8, Day 55

Source	DF	Drums			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.11296376	0.01026943	1.20	0.2884
Treatment	7	0.04088500	0.00584071	0.68	0.6892
Error	362	3.11005130	0.00859130		
Total	381	6.30928357			

Table 29. ANOVA of Thighs for Treatment 1-8, Day 55

Source	DF	Thighs			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.12673154	0.01152105	0.68	0.7563
Treatment	7	0.19412819	0.02773260	1.64	0.1228
Error	362	6.11982573	0.01690560		
Total	381	9.87675216			

Table 30. ANOVA of Frame for Treatment 1-8, Day 55

Source	DF	Frame			
		Type 1 SS	Mean Square	F Value	Pr>F
Block	11	0.29767364	0.02706124	3.56	<.0001
Treatment	7	0.02245445	0.00320778	0.42	0.8882
Error	362	2.74887481	0.00759358		
Total	381	5.15784165			

CHAPTER V

Summary and Conclusion

From this study, it was determined probiotics *Bacillus licheniformus, Bacillus megaterium*, and *Bacillus subtilis* could all potentially be substituted for antibiotic growth promotors with no negative impact on average body weight, and feed conversion ratio. When compared to the negative control, treatment 1, treatment 3 produced the highest average yield weights. The results shown in Table 3 indicate the information near the beginning of the study, treatments 3 & 4 have a slightly heavier bird than treatments 5 & 6 at day 15, and again on day 33. This trend could be due to the fact that the probiotic might not have had enough time to establish itself within the microbiota of the intestinal tract. The bioshuttle programs in treatments 7 & 8 were lighter on average body weights on days 15 and 33. This is expected because of the vaccine given at the beginning of the study causes a mild infection in order to stimulate immunity. While treatments 7 & 8 were behind the other treatments at d15, & d33, they exhibited compensatory gain to be similar to the rest by d55. Looking at the data in Table 3, no significant differences were shown on day 55 for average body weight or average feed conversion ratio. Yet, treatment 5 did not include an antibiotic growth promotor and proved to be within 0.04 lbs. of the average body weight and a 0.02 lbs. lower feed conversion than treatment 3. Treatment 6 had the same average body weight as treatment 4 at 7.65 lbs. but had a lower feed conversion with a

difference of 0.02 lbs. at 1.90 lbs. Even though main cuts from the yield weights did not show a significant difference, it is noted that the treatments with probiotics included were similar to the control groups. Studies by Apata (2008) indicated that *Lactobacillus* in addition to broiler chick diets significantly improved growth performance, increased nutrient digestibility and stimulated humoral immune response. Although we did not see any statistical difference in treatments with probiotics, the weights proved to be equal to or heavier compared to the control groups. Further studies should be conducted to determine more significant differences between probiotics and antibiotic growth promotors. The intestine samples that were analyzed throughout this study did not show a significant difference between any of the treatments. The samples taken from the intestines proved to be clean and did not show any evidence of challenge within the bird. Again, further studies should be conducted to see the possibility for probiotics to be substituted for antibiotic growth promotors.

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