USING A DIRECT-FED MICROBIAL IN BROILER BREEDERS TO REDUCE BROILER PROGENY LAMENESS

by

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ABSTRACT

Bacterial chondronecrosis with osteomyelitis is a substantial problem in the poultry industry today. Many studies have been undertaken to determine the cause of BCO and how to reduce it in broiler chickens. Some studies have suggested the original bacterial contamination that causes BCO comes from the broiler breeder flock and unsanitary hatcheries. Figuring out a way to reduce this bacterial contamination is crucial in the fight to reduce BCO in broilers. This thesis focuses on the feeding of probiotics in broiler breeders to reduce bacterial contamination on the egg shell, therefore passing less harmful bacteria to the offspring. Four treatment groups were used. Chicks from control fed hens were assigned to a control diet (CT-CT) or a *Bacillus subtilis* (BSS) diet (CT-BSS). While chicks from probiotic fed hens were assigned to a control diet (BSS-CT) or a BSS diet (BSS-BSS). During the study, at 28 days, a wire-floor ramp was placed in all pens in between feed and water to force broilers to traverse in order to eat and drink. This ramp system has been used in previous studies to induce BCO in order to study its pathogenesis.

During the course of the study, any visually lame and dead broilers were removed, leg lesions scored and bacteria swabs taken. At the end of the study, 56 days, 30 remaining healthy broilers from every pen were evaluated to determine if BCO lesions were present and the severity of lesions. Broilers from BSS-CT treatment group had significantly lower FCR and higher average body weight gain. Broilers from the same treatment also had significantly lower BCO lesion incidence and the severity of these lesions were also significantly lower than that of the control treatment. This data suggests that feeding broiler breeders a probiotic will have a positive effect in reducing bacterial contamination of broiler egg shells, therefore reducing broiler progeny lameness.

INDEX WORDS: Probiotic, *Bacillus subtilis*, wire-floor panel, Bacterial chondronecrosis with osteomyelitis

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DEDICATION

This Thesis is dedicated to the memory of my father, Judson Lamar Owen. Your wisdom, love, guidance and humor are sorely missed. And to my pup, Sally the Pug.

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CHAPTER 1

INTRODUCTION

Broiler chicken lameness is a serious issue in the broiler industry. Previous studies suggest that BCO is caused by *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus* spp. and other bacteria. The broilers develop bacteremia and these bacteria travel through the blood and adhere to cartilage. BCO is most commonly linked to necrosis due to contamination in the tibia head and the femoral head, especially in the growth plate. Knowing where the bacteria comes from plays an important role in the prevention of BCO. One previous study showed that the same strain of *S. aureus* present in chick fluff at a hatchery was found in the necrotic lesions of those same broilers. This same bacteria has also been linked to the breeder environment. These findings lead us to believe that the likely source of infection comes from the hatchery, and before that the breeder flock (McNamee and Smyth, 2000). Another study showed that when chicks were inoculated 1-2 days post hatch with a *S. agnetis*, they had an 8 fold higher incidence of BCO than the control broilers. This once again shows that pre or post hatch exposure is the most likely source of this bacterial contamination (Al-Rubaye et al., 2014). Hygiene and management in both the hatchery and breeder environments is necessary in the fight to help overcome broiler lameness (Wideman, 2016; McNamee and Smyth, 2000). A different study was performed on the broiler side where probiotics instead of antibiotics were used as a prophylactic treatment for reducing BCO in broilers that were raised on wire-flooring. This study showed that feeding broilers a probiotic from 1 day of age had a significant affect in reducing BCO in those

broilers. This study also showed that raising broilers on a wire-floor structure allows us to research the etiology, pathogenesis and treatments for BCO (Wideman et al., 2012).

Prior to the proposed study a smaller, pilot study was conducted to determine if feeding broiler breeders a probiotic would have an effect on the incidence and severity of BCO in the broiler offspring. In this study, broiler breeders were fed a probiotic from 26 weeks of age. Their eggs, and those collected from a control set of breeders, were collected and hatched for the broiler study. Eggs were also collected from both groups and cultured to determine if a difference could be found in the number of bacteria on the blunt end of the eggs between the two groups. The broiler offspring were then hatched and separated into 3 groups: 2 groups from hens fed a probiotic and 1 group from the control hens. The groups from the probiotic fed breeder were further divided into probiotic-fed broilers and control-fed broilers. Each treatment was separated into 2 pens, 6 pens total. Broilers were monitored for lameness throughout the study. Any found to be lame were euthanized and necropsied and any lesions found were scored and swabbed for bacteria culture. On day 28, the wire-floor ramp was placed between the feed and the water to force the broilers the walk over in order to eat and drink. On day 56, the end of the study, 30 broilers from each pen were gait scored to determine if any difference in the visual lameness of each group. In addition to gait scoring, 30 birds were also euthanized and necropsied and both the femoral head and tibia head were scored for femoral head necrosis and tibia head necrosis. The results from this study showed that there was a higher portion of birds from the hen fed probiotic, control fed broiler group that had less severe femoral head lesions. This led us to conclude from this small study that feeding hens a probiotic does have an effect on the severity

of lameness in the offspring. This study needs to be performed on a much larger scale to obtain more significant results (Owen, unpublished).

CHAPTER 2

LITERATURE REVIEW

Introduction

Broiler chickens have many challenges. A major challenge for them is bacterial contamination. Unfortunately, this is an unstoppable force. The way broilers are raised today in the U.S. does not allow for a sterile environment, nor could it be practiced. There are many means of suspected contamination. These include the broiler breeder parent flock via vertical transmission, poor hatchery conditions, the facilities in which broiler are raised, the feed mill, through the water and drinker line and the intestinal microbiome of the broiler from very early on in life. In the U.S., as in many other countries, broilers are grown in confinement housing at high stocking density, on reused litter and low lighting to promote a sedentary lifestyle (Wideman, 2016). It is impossible to remove all bacterial contamination, but reducing it may prove vital for the fast growing broilers of today. Over the past 2 decades the level of lameness in broilers has increased substantially. It is estimated that close to 1% of fast, heavy growing broilers have some form of lameness (Smith, 1954; Carnaghan, 1966; Nairn and Watson, 1972; McCaskey et al., 1982; Kibenge et al., 1983; Mutalib et al., 1983a,b; Griffiths et al., 1984; Duff, 1990a; Pattison, 1992; Riddell, 1992; Thorp et al., 1993; Thorp, 1994; Thorp and Waddington, 1997; McNamee et al., 1998; Butterworth, 1999; McNamee and Smyth, 2000; Bradshaw et al., 2002; Dinev, 2009; Stalker et al., 2010; Wideman et al., 2012; Wideman and Pevzner, 2012; Wideman and Prisby, 2013). This is thought to be related to their fast growing nature and bacterial

contamination. Bacterial chondronecrosis with osteomyelitis is the most common cause of lameness in broilers. Reducing BCO in broilers could have major impacts on the welfare of these birds and prevent large economic losses to the poultry industry (Riddell et al., 1992).

Bacterial Chondronecrosis with Osteomyelitis

Bacterial chondronecrosis with osteomyelitis (BCO) is the most common cause of lameness in broilers. It consists of bacterial infection and necrosis forming in the proximal femur head, proximal tibia head and sometimes the flexible thoracic vertebrae in heavy, fast growing broiler chickens. This disease is caused by many different aspects all working together. The main cause is bacterial contamination in the aforementioned infected areas (Wideman, 2016). While it has been shown that inoculating broilers with high doses of bacteria with the goal of forming BCO, does in fact cause BCO like symptoms to form within 1-3 days post inoculation (Al-Rubaye et al., 2014), it typically takes more than just bacteria to present symptoms of lameness. Most broilers with the disease present symptoms around 5 weeks of age. Typically it is a combination of things that cause BCO in particular. Heavy, fast growing broilers' bones and growth plates elongate at a very fast rate. By the time they have reached 8 weeks they will have grown to over 4kg. Putting on that much weight so fast has an effect on the bone integrity. Broilers this heavy are subjected to extra torque and stress on the proximal tibia head and proximal femur head. Osteochondrotic clefts or micro fractures form in these areas. This occurs because the proximal end of the bone elongates faster than the distal end (Wideman, 2016). These clefts and micro fractures are just the beginning of the process, necrosis has not yet set in. In addition to the bacteria and clefts and micro fractures, blood flow to the affected areas plays a role. Today's broilers do not live a very active life. They are grown in conditions where light is

restricted in order to restrict their movement to promote fast growth. Feed and water are readily available to these birds so they do not need to move around much at all. They spend the majority of their life in a sitting position. This restricts blood flow to various areas of the body, most specifically to the proximal tibia and femur head. There are a complex array of vasculature in these growth plates. Once opportunistic bacteria invade these areas, blood flow becomes even more compromised. Bacteria create emboli in the vast array of capillaries and vessels, then blood ceases to flow to some areas of the growth plates. Due to lack of blood flow, necrosis sets in. Once necrosis has developed, antibiotics cannot reach the sites for treatment and the lameness often becomes terminal (Wideman, 2016).

Bacteria contamination

BCO is caused by opportunistic bacteria being released into the blood stream and proliferating in the proximal tibia head and proximal femur head of heavy, fast growing broilers. As mentioned before, clefts and micro fractures occur in these joints due to the fast growing nature of these broilers. These bacteria include *Staphylococcus aureus*, *Streptococcus spp*., *Escherichia coli*, and *Enterococcus cecorum* are commonly found in BCO related lesions. The pathogenesis of BCO is varied. While it is a bacterial contamination, the source of bacteria can come from several avenues. The intestinal microflora are a main source of contamination. Other channels of translocation include the respiratory tract and the integument.

It has been theorized that the harmful opportunistic bacteria that can cause lameness comes from a pre or post-hatch exposure to the bacteria (Al-Rubaye et al., 2014; Wideman et al., 2015). These researchers suggest that the bacteria originate from one or more source including breeder flocks and in poorly cleaned hatcheries. These bacteria such as *Staphylococcus aureus, E. coli,*

Enterococcus cecorum. and *Streptococcus spp*. have all been found to inhabit the infected joints associated with broiler lameness including the proximal tibia head and proximal femur head. Broiler breeders live in very stressful environments. This stress starts shortly after hatch with vent sexing and vaccination. This stressful environment continues in the pullet rearing facility with feed restriction to control body weight and promote uniformity and continue to have rigorous vaccination programs (Enting, et al., 2007; Katanbaf et al., 1989; Hocking et al., 1993). These stressors can have an impact on the integrity of the GI tract that prohibits broilers from being able to digest all nutrients properly (Chichlowski, et al., 2007; Enting et al., 2007). Once pullets are sexually mature around 21 weeks, they are mixed with roosters in the breeding house and begin mating. Broiler breeders go through the rigors associated with laying eggs and mating. They are still feed restricted though they are fed on a daily basis in lay. Breeders have to deal with a partially raised slatted flooring, and feeding equipment they must access and maneuver around (Rosales, 1994; Cobb Broiler Breeder Management Guide, 2016). One of the biggest stressors is the mating itself and oviposition. It takes a tremendous amount of effort and energy on the behalf of the broiler breeder hen to produce an egg from start to finish. Just like in broilers, any stressors these birds encounter has an effect on their body. While feather quality and pigmentation of the comb and wattle are visual indicators of stress, internal signs of stress are harder to see. A broiler breeder that is stressed or hurt will go out of egg lay, or become "broody." Breeder hens can also go through a period known as molting in which they lose their feathers and the reproductive tract regresses. This molting process gives the birds a break from egg production and time to regenerate the reproductive tract (Rosales, 1994). Stress also affects broiler breeder hens the same way that broilers are affected. A stressful environment can lead to the lumen in the GI tract sloughing off to reveal the tight junctions in the gut. At this time, any

harmful bacteria in the GI tract can escape through these tight junctions and enter the blood stream. Once these bacteria enter the blood stream they travel all throughout the body. Due to the slat flooring and heavy body weights of broiler breeders, leg lameness is a common problem. Clefts and micro fractures can form in the proximal femoral head and proximal tibia head. These clefts and micro fractures are perfect areas for the bacteria to colonize. Once the bacteria proliferates, blood flow to the area slows down and eventually cannot reach these areas. Due to restricted blood flow, necrosis forms and can start to cause pain. Hens that are in pain like this are not very likely to move around and get food and water. Eventually, due to over mating and an inability to gain access to the slats to eat and drink, these lame hens either need to be removed or they die. This creates not only welfare concerns for broiler breeders and economic concerns for declines in egg production, but also in the microflora in the gut of these birds. Breeders translocate bacteria to their offspring via the egg shell (Wideman, 2016).

It is theorized that the harmful bacteria such as *Staphylococcus spp*. and *E. coli* penetrate the egg shell to contaminate the developing embryo. This bacteria becomes part of their early gut flora. Bacterial contamination occurs through the egg shell due to its porous structure. Few bacteria can make it through, and even fewer make it past the membrane and the albumen. The albumen has antimicrobial properties that make it very difficult for bacteria to survive once they have entered the egg shell. Egg whites in fertile eggs have a complex protein structure that allow them to kill certain harmful bacteria from reaching the developing chick. These proteins are available during the first half of incubation before the albumen is drawn into the amniotic fluid, which the chick then consumes (Guyot et al., 2016). In a study done by Guyot, 2016, antimicrobial properties of egg were analyzed and several bacteria species were tested for survival in the egg white. These species include *Staphylococcus aureus*, *Listeria monocytogenes*,

Streptococcus uberis, *Escherichia coli*, and *Salmonella enterica.* In this study no significant reductions in the amount of *S. aureus*, *E. coli* and *S. enterica* were found for these bacteria during the first half of incubation.

Probiotics

A probiotic is defined as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" (Heyman and Ménard, 2001). Many probiotics are used in poultry production today due to a decline in antibiotic use. Consumers are starting to lean more towards using probiotics instead of antibiotics due to concern by the consumer of increased bacterial resistance to antibiotics used in human medicine, so many poultry companies have made the switch to No Antibiotics Ever (NAE). There are a wide range of products on the market available that use different types of organisms such as bacteria, yeast and fungi to create a positive gut flora (Fox, 1988). Through creating a positive environment in the gastrointestinal tract of broilers, probiotics provide several benefits including promoting growth and immune function, protecting against pathogens, enhancing bone strength and fighting against parasites (Khan, 2013). Because there are different types of probiotics, they work in various ways and their success depends on how they interact with the host organism. Several factors influence their ability to survive in the host. They must be of the correct strain to establish themselves within the gut. Probiotics must have certain amount of colony forming units or CFU's in order to be viable against other bacteria and remain competitive. One study shows that a higher amount of CFU's has an impact on microflora found in the ceca, body weight and digestibility (Mountzouris et al., 2010). Probiotics should be able to withstand acid and bile. It should be a strain that is specific and has a high survival rate within the gastrointestinal tract of

poultry. The specific strain chosen should not cause any harm to the bird, or more specifically the GI tract. It must be able to adhere to the wall of the GI tract and handle the stresses of nutrition and production. And most importantly, the probiotic should be able to help reduce the number of pathogenic bacteria located within the GI tract (Choudhari et al., 2008). Age and genetic disposition play a role as well. One of the most important factors to consider when choosing a probiotic is the stress level of the birds when administered. Some probiotics may not be able to handle the stress put on the GI tract by very active birds, such as breeders (Chichlowski, 2007).

Young broilers are also subjected to the stresses of incubation, hatch, vaccination and placement within their first few days of life, so choosing a probiotic that works towards keeping a healthy GI tract is very important. Nutrition also plays an important role in whether or not a probiotic can be successful. Early nutrition is critical. Newly hatched chicks do not have a stabilized microflora in their GI tract which makes it very easy for pathogens to gain access and colonize. Chicks are first exposed to microorganisms present on the egg shell, chick dust, the hatchery environment and after that the production process (Coates and Fuller, 1977). Species in use as probiotics include *Lactobacillus bulgaricus, L. plantarum, L.acidophilus, L. helveticus, L. lactis, L. salivarius, L. casei, Bacillus subtilis, Enterococcus faecium, Streptococcus thermophilus, Enterococcus faecalis, Aspergillus oryzae, Saccharomyces cerevisiae, BiJidobacterium spp.* and *E. coli* (Starvic, 1987; Fuller, 1989; Mohan et al., 1996; Yoruk et al., 2004; O'Dea et al., 2006; Choudhari et al., 2008; Hassanein and Soliman, 2010). For this paper, *Bacillus subtilis* will be reviewed as a potential probiotic for use in broiler breeders to reduce egg shell bacterial contamination that is believed to cause broiler progeny lameness. In broilers, a study was performed to determine if feeding a *Bacillus subtilis* C-3102

probiotic from day 1 to day 42 had an effect on broiler performance and to determine if microbiological contamination of the carcass was reduced (Fritts, et al, 2000). In this study, probiotic treated broilers were kept separate from control group to prevent any cross contamination. At the end of the study, birds were processed and cultured for *Salmonella* incidence (positive/negative) and *E. coli*, and *Campylobacter*. In regards to broiler performance, broilers given a probiotic had higher body weights and better feed conversion ratio than broilers fed a control diet. Testing of pre-chilled carcasses from both groups also showed that all birds in the control group tested positive for *Salmonella* while other birds had less than half positive, and probiotic fed broilers had significantly lower levels of *Campylobacter* and coliforms present. Fritts *et al* (2000) showed that feeding a *Bacillus subtilis* probiotic has positive impacts on not only broiler performance, but gastrointestinal microflora as well. These findings are important from a broiler production and food safety points of view. Other studies have shown similar results in regards to positive impacts on broiler performance. Murugesan, 2014 also performed a study on broilers using a *Bacillus subtilis* direct-fed microbial. In that study, broilers were given a very high dose of coccidia vaccine and fed a probiotic from day 1 to day 28 and raised on reused litter. At the end of this experiment, it was found that by giving the broilers a higher dose of coccidial vaccine it reduced broiler performance. The probiotic was not able to make up for that, but did increase integrity of the epithelium in the intestines by positively impacting the tight junctions which can control the passage of pathogens to the blood stream (Murugesan et al, 2014.)

Probiotics are also used to help strengthen the gastrointestinal tract by prevent leakage of pathogenic bacteria the blood stream that can cause infections in bones. Multiple modes of action make this possible. These include lowering the gut pH making it impossible for some pathogenic

bacteria to survive, competing with pathogenic bacteria for adhesion to the gut epithelium, nutrient competition, stimulating the immune system, creating a toxic environment for bacteria growth and some produce antimicrobial properties (Khan et al., 2013). Not all probiotics can perform these functions, and probiotics are specific for attacking certain pathogenic bacteria making it important to know which to use based on function, though there is no consensus on which probiotics to use in what combinations and doses. Further research is required in this area.

Broiler breeders live in very stressful environments and this can have an impact on gut integrity and breeder performance. Previous studies have shown that using probiotics can have a positive impact on egg production and egg quality and can even decrease bacterial contamination of the egg shell (Khan et al., 2013). Connections have been made between pre and post hatch exposure to pathogenic bacteria (Wideman, 2016). Factors such as egg shell weight, breaking strength and thickness have all been improved by the use of different probiotics. Other probiotics have shown that they have no positive effects on egg production, egg weights and specific gravity (Khan et al., 2013). As mentioned earlier, in order for a probiotic to be successful, the right parameters must be met. A *Lactobacillus* strain of probiotic was used in a study with Single-Comb White-Leghorn commercial egg layers. This study showed that using a probiotic increased egg shell thickness due to more calcium being absorbed in the intestines due the positive environment of the gut (Nahashon, 1994). Being able to reduce the amount of pathogens being spread to offspring via the use of probiotics has not been studied, but it is an area that should be explored.

Importance of *Bacillus subtilis*

Several studies have been performed to understand how *Bacillus subtilis* probiotics interact with broiler gut flora. *Bacillus* are used as probiotics because they are able to withstand

high acid content and bile salts found in the gut of broiler chickens. The *Bacillus* are also heat resistant and activate innate immune function in broilers (Latorre et al., 2014; Rhee et al., 2004). In addition, they produce enzymes that help improve nutrient absorption and prevent the growth of pathogenic bacteria by decreasing substrates they need to grow. These enzymes include protease, lipase, cellulase, xylanase, phytase, and keratinase (Latorre, et al., 2014, Hendricks et al., 1995; Monisha et al., 2009; Mazotto et al., 2011; Mittal et al., 2011; Shah and Bhatt, 2011; Jani et al., 2012). They also increase the growth of *Lactobacillus*, which is a beneficial bacteria in the gut, by producing subtilisin and catalase while decreasing the pH of the intestine (Latorre et al., 2014, Hosoi et al., 2000). It has also been found that some isolates can release antimicrobial substances against such bacteria as *Staphylococcus aureus*, *Enterococcus faecium*, and *Clostridium* (Latorre, et al., 2014, Hoa et al., 2000; Hong et al., 2008). Most probiotics work best when continuously fed, *B. subtilis* being one of these probiotics. However, studies have shown in mice that even after a single gavage dose of the probiotic *B. subtilis* spores, these spores were in the feces 7 days after the initial gavage (Hoa et al. 2001). However, the mode of action of *Bacillus* is not completely known, but it is thought that environmental factors, such as temperature, pH and humidity, play a role in the success of this probiotic (Latorre et al., 2014). In one study, several strains of *Bacillus subtilis* were isolated from broiler gut flora and tested against each other for changes made on the gut flora. *Bacillus subtilis* KD1 had the overall best performance. It not only increased *Lactobacillus* levels in the gut, but also significantly decreased *E. coli*. *Lactobacillus* levels were also 10 fold higher than the control groups and the antibiotic supplemented groups (Wu, et al., 2011). In another study, Manafi et al., 2016 showed that even when inoculating broilers with *E. coli*, they showed a decrease in *E. coli* in the intestine and increased nutrient digestibility. Multiple studies have shown that the use of *B. subtilis* spore

forming probiotics reduce harmful gram-positive (*Staphylococcus aureus*, *Enterococcus faecium*, and *Clostridium*) and Gram negative bacteria (*E. coli* and *Salmonella)* in the intestine (Manafi et al., 2016, Hoa et al., 2000; Hong et al., 2008). These studies prove that the use of spore forming probiotics such as *B. subtilis* are able to with stand harsh environments and are beneficial to poultry as a host.

Lameness-causing bacteria such as *Staphylococcus aureus, E. coli,* and *Enterococcus* can be found in the intestine of healthy broilers. *Bacillus subtilis* probiotics have shown they are capable of reducing these bacteria in the GI tract of broilers.

Brooding techniques in young broilers

Brooding is often an overlooked area when it comes to raising broilers. The GI tract in these young broilers is still developing. As chicks grow, villi in the intestine continue to develop creating more surface area. The most critical time for villi growth is 2-5 days post hatch (Collett, 2017). Providing the optimum environment for these broilers is critical. Cold stress can cause chicks to succumb to vaccine. Heat stress in broilers can lead to dehydration. This breaks down the lining of the GI tract allowing for tight junctions in the gut to open, releasing bacteria into the blood stream resulting in septicemia, thus leading to BCO. Not introducing chicks to feed by 1 day post hatch can have detrimental effects on the still developing microflora of the gastrointestinal tract of these young chicks. Feeding too much protein early on can also have long lasting consequences. At this stage, young chicks have difficulty absorbing proteins. Too high a level of protein can cause breakup of tight junctions as well (Collett, 2017). Feeding digestible feed is crucial for the development of villi and microvilli in the gut to form and help create a successful environment for probiotics to work.

Wire-flooring panels

Wire-flooring has been in use as a research tool with poultry to study lameness in commercial egg layers, but only recently has been used to study lameness in broilers. Wire-flooring panels create instability of the footing allowing for additional torque and stress on the proximal femur head and tibia head (Gilley et al., 2014). Wideman at The University of Arkansas, have successfully triggered lameness on multiple occasions using wire-flooring panels (Wideman et al., 2010, 2012, 2013). Several types of flooring panels have been used as models for these experiments; a flat panel with wire floor and panels that are sloped on either side to create a speed bump affect. In one experiment, multiple flooring models were studied to determine the best model for inducing lameness in a laboratory setting. These included litter flooring, sloped wire-flooring panels (varying degrees of slope from 33-66˚) and flat wire-flooring panels (Gilley et al., 2014). Sloped panels were also implemented with limbo bars forcing the broilers to crouch when reaching the apex creating additional torque and stress. A pagoda top speed bump was also used to force birds into a crouched position going up and down both sides of the ramp. Another scenario had a ramp placed beneath a water line so that the broilers had to climb the ramp. Ramps were placed at 14, 28 or 42 days of age. No differences were found in the onset of lameness in regards to the day of placement. It was also found that raising broilers on flat wire-flooring triggered lameness more often than the sloped ramps which allowed birds to have access to litter (Gilley et al., 2014).

Gait Scoring

Gait scoring is a tool that can be used to assess lameness in broilers. Assessing lameness in broilers is a good way to monitor flock condition. Poultry companies today are held to high standards in regards to animal welfare. Monitoring broilers for general activity based on gait

score is an excellent way to identify emerging diseases within a flock. Poultry producers need a fast and easy way to identify any lameness or diseases in a flock. Due to the heavy, fast growing nature of broilers, leg lameness is a common problem with poultry today. There are several different gait scoring methods in use today. The U.S. Gait scoring system (Webster et al., 2008) that is composed of 3 criteria. The Kestin system (Kestin et al., 1992) is a more complex 6 point system. Both systems score birds from mobile, with no signs of lameness, to signs of severe lame and immobile. Webster et al., 2008 conducted a study to evaluate the between-observer agreements from both of the methods on broilers obtained from commercial hatcheries. In this study observers used the 6 point system and the 3 point system. While both appeared to have high between-observer agreement, the 3-point system, due to its lower variability had better agreement among observers (Webster et al., 2008). Other methods of gait scoring include using camera systems to evaluated broilers automatically allowing for observers to not enter the commercial production facilities to conduct gait scores (Silvera, et al., 2017, Aydin et al., 2010). Methods such as this are not currently in commercial poultry use, but could provide an alternative in the future.

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CHAPTER 3

USING A DIRECT-FED MICROBIAL IN BROILER BREEDERS TO REDUCE BROILER PROGENY LAMENESS[1]

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Abstract

Broiler breeders are feed restricted to control body weight and improve reproduction, mobility and natural mating. It is thought that this stressful environment may encourage vertical transmission of bacteria to progeny. In the modern broiler, the incidence of bacterial chondronecrosis with osteomyelitis (BCO) is the most common cause of lameness. The objective of this study was to determine if feeding broiler breeder hens a direct fed microbial would reduce BCO in the progeny. In addition, determine if feeding the same direct-fed microbial to the progeny would have an impact on the incidence of BCO. The chicks in this study were from breeders fed a standard breeder feed (CT) or breeders fed *Bacillus subtilis* (BSS) supplemented feed. The CT chicks were fed an unsupplemented broiler diet control diet (CTCT) or *Bacillus subtilis* diet (CTBSS), while the chicks from the BSS fed hens were fed *Bacillus subtilis* (BSSBSS) or a control diet (BSSCT) (7 pens/treatment; 50 chicks/pen). Lameness was induced by placing a 60 degree angle wire-floor ramp between the feed and water at 28d. It should be noted that study incidences of femoral and tibia lesions are higher than commonly found without the added stress of the speed bumps. At 56d, 30 of the remaining broilers from each pen were individually gait scored (0-2 system), and 30 were necropsied to identify femoral or tibia lesions. Severity of lesions were scored on a scale of 1 to 8. From necropsy the severity of femoral head lesions was higher in the CTCT group than the BSSCT (3.6, 3.3 average score respectively). No differences in severity of tibia lesions were noted among the groups. The incidence of femoral and tibia lesions were similar in all of the groups as well. Hens fed *Bacillus subtilis* produced broilers that had a slower progression of femoral head disease.

Key words: breeders, broilers, *Bacillus subtillis*, lameness, and femoral head

Introduction

Lameness in broiler breeders today is major issue with poultry producers today. Broilers grow heavier and faster than that of their 1950 counterparts. This causes welfare concerns and economic concerns from birds lost due to this disease. Bacterial chondronecrosis with osteomyelitis (BCO) is the main cause of lameness in broilers. It has been suggested in previous studies that the cause of this bacteria can be linked to hatcheries and before that, the broiler breeder parent flock. In this study, a *Bacillus subtilis* direct-fed microbial was fed to a broiler breeder flock in combination with a control diet to determine if it could reduce bacterial contamination to the broiler offspring and reduce BCO. Some broiler offspring were also fed the direct-fed microbial to determine if a combination effort was more effective at reducing this disease. Wire-flooring panels used by Dr. Wideman at the University of Arkansas were implemented to induce BCO in these broilers so that it could be studied. This study showed that by using a *Bacillus subtilis* direct-fed microbial in broiler breeders, we could significantly reduce the incidence and severity of BCO in broiler progeny.

Description of problem

Broiler lameness is a major economic and welfare concern for the poultry industry today. Due the heavy, fast growing nature of broilers, leg lameness can be very problematic. Bacterial chondronecrosis with osteomyelitis (BCO) is the main cause of broiler lameness. BCO is caused by bacterial infections located within the proximal femur head and the proximal tibia head. Clefts and micro fractures form in the growth plates of these joints due to their large body sizes for such young birds. Next, bacteria are released into the blood stream and spread to these clefts. Bacteria can enter the blood stream through several means such as the respiratory system, open

wounds and the digestive tract. Poor nutrition can lead to the lumen of the gut sloughing off and allowing for bacteria to exit into the blood stream. Once the bacteria inhabit the clefts and micro fractures, blood flow to the infected areas becomes compromised and necrosis sets in.

Previous studies have suggested that the same bacteria that are present in the infected BCO lesions are also found in breeder flock environments and in hatcheries. Poorly sanitized and disinfected hatcheries are a breeding ground for bacteria. Chicks are most susceptible to diseases at this stage. Breeder flocks also live in very stressful environments. They must overcome the constant rigors of mating, slat flooring, oviposition and feed restriction. Stress induced bacteria shed onto the egg shell of broiler offspring can have major implications to young chicks. This can be a means of early bacterial contamination of broilers.

Direct-fed microbials have been used in previous studies to improve broiler performance by allowing for a healthier gut by reducing harmful bacteria and allowing for more nutrients to be absorbed. The objective of this study was to determine if feeding broiler breeder a direct-fed microbial with reduce the incidence and severity of BCO in broiler offspring, and if feeding the same offspring a direct-fed microbial helps reduce it as well.

Materials and methods

A flock of 800, Cobb 500 broiler breeder hens were raised at the University of Georgia Poultry Research Center, and randomly assigned to either a control or *Bacillus subtilis* supplemented breeder diet. *Bacillus subtilis* is a direct-fed microbial that has improved egg shell quality in Leghorn flocks and was used in this study to determine if egg shell quality and/or broiler traits could be improved in progeny. Fertile eggs were collected at 60 weeks of age, 3-4 times a day by treatment and incubated for 21 days by treatment. From the 2 treatment groups of

hatched chicks, 910 broiler chicks were randomly selected from the control group and the *Bacillus subtilis* group. The broiler chicks were vaccinated for coccidiosis and taken to Southern Poultry Research Group for broiler grow out. Chicks from control fed hens were assigned to a control diet (CT-CT) or a BSS diet (CT-BSS). While chicks from *Bacillus subtilis* fed hens were assigned to a control diet (BSS-CT) or a BSS diet (BSS-BSS). Each pen started with 65 broilers and there were 7 replicate pens of each treatment.

The broiler chicks were allocated to 28, 2.4 x 3.6 m pens inside an environmentally controlled poultry research house on used litter. Chicks were brooded with 2 radiant heat lamps per pen, along with two round tube feeders and 2 bell drinkers. Feed and water were given *ad libitum* to 56 days of age. During the experiment the broilers were monitored for mortality, temperature, lighting, water, feed, and litter condition. From day 14 to 28 any dead or visibly lame broilers were removed from the pens, humanely euthanized and necropsied to determine cause of lameness. Dead broilers were also necropsied to determine cause of death and results were recorded. On day 28 a wire-flooring panel speed bump ramp (Varying degrees of slope from 33-66˚) (Wideman et al., 2010, 2012, 2013) was placed centrally in each pen between the feed and water. The number of broilers was reduced in each pen to 50 birds. From day 28 to 56 any dead or visibly lame broilers were removed from the pens, humanely euthanized and necropsied to determine cause of lameness. Dead broilers were also necropsied to determine cause of death. On day 56 a random sample of 30 broilers from the remaining broilers of each pen were gait scored on a scale of 0, 1, or 2 (Webster, et al., 2008). A score of 0 indicates that the broiler showed no visible signs of lameness when walking a total of 5m in one direction. A score of 1 indicates that the broiler was able to walk 5m without sitting down, but still showed visible signs of lameness. And a score of 2 indicates that the broiler was unable to walk the entire 5m

without sitting down and showed severe visible signs of lameness. All of the broilers from each pen were then humanely euthanized. Another random sampling of 30 broilers were taken from each pen after being euthanized. These broilers were necropsied and femoral and tibia lesions were assessed and recorded. Histologic samples were taken from the tibias, and placed in formalin.

Approximately 10 samples per pen of tibia lesions were fixed in 10% buffered formalin for at least 48 h. The samples were then trimmed to fit into cassettes using a bone saw (Buehuler IsoMet diamond watering blade). The samples in cassettes soaked in decalcification solution (one half distilled water and formic acid and one half sodium formate and distilled water) for 1-2 days, then rinsed in running tap water for 1-2 h. Decalcified samples were routinely processed overnight (Tissue-Tek VIP6 and Leica ASP 300), embedded in paraffin, and sectioned at 4 microns. Routine staining with hematoxylin and eosin (H&E, Hacker HCM 3030) was performed and glass slides were cover slipped (Hacker HCM 6000). Sections were viewed with a (Leica DM 2500 LED) light microscope. Samples were scored for the occurrence of rickets, tibia dyschondroplasia, micro fractures, chondrosis and osteomyelitis. Micro fractures were scored on 2 severity levels. Level 1 indicated that clefts were present in the growth plate and areas of hemorrhage and fibrin were present as well. A level 2 showed larger areas of these same signs. Signs of chondrosis were scored by severity as well. Level 1 showed mild signs of degeneration of the chondrocytes. Level 2 showed moderate signs of degeneration of the chondrocytes in multiple areas or large areas. A level 3 showed these same signs along with areas of fibrin and edema. Osteomyelitis were also scored based on severity. A level 1 indicated there were signs of necrosis located in the growth plate. A level 2 indicated areas of fibroplasia along with necrosis

and a level 3 indicated chronic osteomyelitis with areas of severe fibroplasia and high numbers of osteoclasts present.

In the breeder study, eggs (n=16/treatment) were sampled at 44-54 weeks of age for bacterial load. MacConkey agar and Phenylethyl Alcohol Blood Agar (PEA) 5% media were used to determine the presence of *E. coli* and *Staphylococcus spp*. These samples were taken by collecting 2 nest clean eggs from each of 8 control pens and *Bacillus subtilis* pens, (n=16/treatment). The egg sampling was completed every 2 weeks, for a total of 6 sample periods. These nest clean eggs were collected wearing gloves to ensure no human bacteria flora contamination of egg samples. The blunt end of each egg was pressed onto both selective agar plates. Care was taken to not use the same spot on the egg for each plating (egg testing method, personal communication with Dr. Gregorio Rosales). At the end of the study, all remaining feed and broilers were weighed from each treatment.

Results

Broiler Performance

Feed intake was similar among treatments (Table 1), while FCR and average body weight were significantly different. Control broilers fed no direct-fed microbial were less efficient than broilers that came from hens consuming direct-fed microbial or when broiler feed was supplemented with direct-fed microbials or both. The CT-CT broiler had the lightest body weights when compared to the BSS-CT broilers that were the heaviest. The other treatments were intermediate in body weight. Broilers that consumed direct-fed microbial supplement or came from hens that consumed the microbial supplement had lower FCR and heavier average body weight in comparison to those fed standard breeder or broiler diets.

Broilers were gait scored at 56d, there were no significant differences among the treatments for ability to walk 1.5m (Figure 3).

28-56d Mortality

There were no significant differences in mortality before the wire flooring panels were installed at 28d. Mortality was not different from 5 to 7 weeks of age (data not shown). There was an increase in mortality between weeks 5 and 6 that declined off during week 7 and increased again at week 8 (Table 2). Differences can also be seen at week 8 with the highest mortality average occuring in the BSS-BSS group at 5.31%, and was significantly different from the other treatments which range from 0.92% to 2.21%.

This overall mortality was further divided between femur lesion and tibia lesion related mortality. Similar to the overal mortality, femur related mortality follows the same trend. There is a spike in mortality at week 6, 2 weeks after the wire-flooring panels were implemented, it drops off again in week 7 and significant differences were observed in week 8. Once again, the BSS-BSS group had the highest spike in mortality at 1.98% and the lowest mortality occurred in the CT-CT group at 0.32% (Table 3).

Tibia related mortality also follows the same trend as the overall mortality and the femur related mortaltiy, spiking at week 6 and again at week 8. Significant differences were seen in both of these spikes. At week 6, CT-CT had the highest at 4.11% and the lowest belonged to the BSS-CT group at 1.19%. At week 8, the lowest once again belonged to the BSS-CT group 0.32%, but the highest belonged to BSS-BSS, 3.37%. (Table 3).

56d lesion incidence

Incidence was very high in both femur lesions and tibia lesions, reaching 90% or above for tibias in all treatment groups. Differences were seen in regards to left femur lesions and total

incidence of femur lesions. The highest amount of left femur lesions belongs to BSS-BSS and CT-CT groups at 85.71% and 87.14% respectively. This is also the case for the overall femur lesions, the lowest amount of femur lesions are the CT-BSS and BSS-CT treatment groups both at 80.95%, while the highest is CT-CT once again at 87.38% (Table 4).

56d Lesion Severity

Like the lesion incidence, we see the differences in the severity in the left femur head and the severity of all the femurs combined (Table 5). Severity follows the same pattern as the lesion incidence in that the less severe scores fall in the CT-BSS and BSS-CT treatments with an average score of 3.15 and 3.20. The overall severity of femur lesions is the same with the CT-BSS and BSS-CT treatments having the lowest at 3.34 and 3.28 and the highest belonging to the CT-CT group at 3.55. No differences were seen among the tibia lesions in regards to severity (Table 5).

56d Lesion Cultures

Swabs were taken from any lesions grossly identified in 56 day old broilers. *Staphylococcus spp*., *Streptococcus spp*., and *E. coli* were present but no treatment had a significant effect on either the number or species of bacteria present (data not shown).

56d Histology

At 56 days, approximately 10 histologic samples of the proximal tibia head were taken from each pen. Samples were scored for the occurrence of rickets, tibia dyschondroplasia, micro fractures, chondrosis and osteomyelitis. The presence of early rickets was found in all treatments groups, the highest percentage in the CT-BSS broilers with 53% of the samples having lesions. The BSS-BSS, BSS-CT and CT-CT groups were 37.6%, 38% and 33.8% respectively. The incidence of tibia dyschondroplasia was low for the probiotic treated groups with BSS-BSS, CT-

BSS and BSS-CT at 1.4, 4.4 and 5.6% respectively for tested necropsied birds. The CT-CT group was higher at 14.1%.

Micro fractures, lesions of chondrosis and osteomyelitis were scored for both incidence and severity. Intermediate levels of micro fractures were seen in all treatment groups. Incidences by treatment BSS-BSS, CT-BSS, BSS-CT and CT-CT were 15.9, 11.7, 14.1 and 18.3%, respectively (Table 6). Severity of micro fractures was found to be low in all treatments with BSS-CT being the only group with severity in the level 2 range, at 20%. Chondrosis incidence had a wider range with the highest incidence level of 27.5% occurring in the BSS-BSS broilers and lowest in the control broilers with 12.7%. The severity of chondrosis also varied in respect to the treatment groups (Table 8). The majority fell in the level 1 category with highest at 92.9% belonging to the BSS-CT group and lowest at 66.7% in the control group. Level 2 severity also varied with the lowest incidence in treatment BSS-CT at 6.7% and highest in CT-CT at 22.2%. Level 3 severity for chondrosis was only present in the control group with 11.1% of the samples having a score of 3. Incidence of osteomyelitis in the samples was relatively low with the highest incidence belonging to the BSS-BSS group at 5.8% and lowest in CT-BSS group at 2.9%. Severity varied greatly in regards to osteomyelitis (Table 9). BSS-CT had 100% of samples scored as a level 1, and CT-CT had 33.3%. Level 2 severity was only seen in the BSS-BSS group at 25%. Level 3 severity had the greatest variance with CT-BSS have 100% of samples scored a 3, BSS-BSS at 75% and CT-CT at 66.7%. All treatments had similar amounts of samples considered normal, containing no lesions, ranging from 11.6% to 12.7% (Table 6).

Discussion

Bacterial chondronecrosis with osteomyelitis is a significant in the poultry industry, especially for ABF (antibiotic free) and NAE (no antibiotics ever) production. Targeting the bacterial contamination that causes BCO is paramount in the fight to reduce it in broiler chickens. Several studies have shown that likely sources of the bacteria come from both a pre or post hatch exposure to these harmful bacteria (Al-rubaye et al., 2014). These exposures include the broiler breeder parent flock and poorly cleaned hatcheries (Wideman, 2016). This study examined the breeder parent flock to the broiler in regard to reducing bacterial contamination. The theory was the *Bacillus subtilis* direct-fed-microbial would promote a more positive gut environment in the broiler breeder flock thus reducing the presence of bacteria such as *Staphylococcus aureus, Staphylococcus spp., Escherichia coli,* and *Enterococcus cecorum* that can cause BCO by reducing the shed of these bacteria onto the egg shell, therefore reducing bacterial contamination of the broiler progeny.

In this experiment, significant differences were seen in broilers that were treated with probiotics and in broilers that were the offspring of broiler breeders that were given the same *Bacillus subtilis* probiotic. Feeding broiler breeders a probiotic appeared to have the most positive affect on broilers that were not fed a probiotic. This treatment group was significantly different from the control group both lesion severity and lesion incidence. This shows us that even though the BSS-CT group had higher overall body weights than the control group, which would typically lead to more micro fractures and clefts in the growth plates of the tibia and femur head (Ytrehus et el., 2007). It should also be noted that bird weight may play a role in the overall mortality over the course of the experiment. Broilers from treatments with higher mortality earlier on in the experiment typically came from groups with the highest body weights,

while the lower amounts of mortality came from birds with lower body weights throughout the experiment. Previous studies have shown a positive influence on FCR, BW and BW gain from the use of probiotics (Cavazzoni et al., 1998; Jin et al., 1998; Zulkifli et al., 2000; Kabir et al., 2004; Mountzouris et al., 2007; Samli et al., 2007; Awad et al., 2009). This is thought to be due to the probiotic resulting in more villi growth in the gastro intestinal tract allowing for more surface area for more nutrient absorption from the feed (Caspary, 1992). While having better nutrient absorption capabilities can have a positive impact on growth and performance it can also positively affect the bone integrity of broiler chickens (Ytrues, et al., 2007). More uptake of calcium leads to more calcification of the matrix of the growth cartilage (Ytrues, et al., 2007). The results from this study suggest that using a direct-fed microbial in the breeder flock can aid in the reduction BCO in the progeny.

Conclusions and applications

1. Using a *Bacillus subtilis* direct-fed microbial in a broiler breeder flock can reduce the severity and incidence in the broiler progeny flock. 2. Only one type of direct-fed microbial was used in this study, but others have shown their worth in previous studies at bettering broiler performance and enhancing gut health. Using another direct-fed microbial, such as *Lactobacillus spp.*, alone or in combination with a *Bacillus subtilis* in broiler breeders could prove even more benficial to broiler flocks in the future. 3. With many companies switching to No Antiobiotics Ever, the use of direct-fed microbials is an alternative in breeder and broiler flocks to reduce the amount of pathogens. Therefore, using this *Bacillus subtilis* direct-fed microbial has more than one benefit to poultry companies by reducing bacteria that causes BCO in broilers.

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Table 1. Performance of 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment Diets[1]	Feed Intake	FCR[3]	Body Weight (kg)
BSS-BSS	296.5_{a}[2]	1.946_{b}	2.936_{ab}
CT-BSS	293.94_{a}	1.934_{b}	2.955_{ab}
BSS-CT	307.58_{a}	1.902_{b}	3.086_{a}
CT-CT	291.98_{a}	1.999_{a}	2.850_{b}

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]Means within a column with different letters are significantly different at $P \leq .05$.
[3]Feed Conversion Ratio.

Table 2. Overall Mortality (%) at 5 to 8 weeks in broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	Wk 5	Wk 6	Wk 7	Wk 8
BSS-BSS	3.14_{a2}	3.86_{a}	1.49_{a}	5.31_{a}
CT-BSS	4.00_{a}	4.10_{a}	1.53_{a}	2.21_{b}
BSS-CT	4.57_{a}	2.39_{a}	1.84_{a}	0.92_{b}
CT-CT	2.57_{a}	5.88_{a}	0.63_{a}	2.16_{b}

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.

[2]Means within a column with different letters are significantly different at $P \leq .05$.

Table 3. Femur and tibia related mortality (%) at 5 to 8 weeks in broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Femur related mortality				
Treatments diets[1]	Wk 5	Wk 6	Wk 7	Wk 8
BSS-BSS	1.43_{a2}	1.50_{a}	0.00_{a}	1.98_{a}
CT-BSS	1.43_{a}	0.92_{a}	1.54_{a}	0.95_{ab}
BSS-CT	0.86_{a}	3.02_{a}	0.00a	1.24_{ab}
CT-CT	0.86_{a}	2.66_{a}	0.31a	0.32_{b}
Tibia Related Mortality				
Treatments	Wk 5	Wk 6	Wk 7	Wk 8
BSS-BSS	0.29_{a}	2.71_{ab}	0.57_{a}	3.37_{a}
CT-BSS	1.14_{a}	2.36_{ab}	0.00_{a}	1.92_{ab}
BSS-CT	1.43a	1.19_{ab}	0.62_{a}	0.32_{b}
CT-CT	0.86_{a}	4.11_{a}	0.32a	0.63_{b}

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.

[2]Means within a column with different letters are significantly different at $P \leq .05$.

Table 4. Femur and Tibia Lesion Incidence (%) in 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	RF[3]	LF[4]	Avg[7] Femur	RT[5]	LT[6]	Avg Tibia
BSS-BSS	86.19_{a2}	85.71_{a}	85.95_{ab}	93.34_{a}	93.81_{a}	93.57_{a}
CT-BSS	85.24_{a}	76.67_{b}	80.95_{b}	93.33_{a}	92.38_{a}	92.86_{a}
BSS-CT	80.95_{a}	80.95_{ab}	80.95_{b}	90.48_{a}	95.72_{a}	93.09_{a}
CT-CT	87.62_{a}	87.14_{a}	87.38_{a}	92.38_{a}	90.0_{a}	91.19_{a}

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]Means within a column with different letters are significantly different at $P \leq .05$.
[3]Right Femur.
[4]Left Femur.
[5]Right Tibia.
[6]Left Tibia.
[7]Average.

Table 5. Average Femur and Tibia lesion severity score of 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	RF[3]	LF[4]	Avg[7] Femur	RT[5]	LF[6]	Avg Tibia
BSS-BSS	3.50_{a2}	3.48_{ab}	3.49_{ab}	3.75_{a}	3.86_{a}	3.80_{a}
CT-BSS	3.53_{a}	3.15_{c}	3.34_{ab}	3.92_{a}	3.92_{a}	3.92_{a}
BSS-CT	3.35_{a}	3.20_{bc}	3.28_{b}	3.73_{a}	3.84_{a}	3.79_{a}
CT-CT	3.60_{a}	3.51_{ab}	3.55_{a}	3.88_{a}	3.87_{a}	3.88_{a}

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]Means within a column with different letters are significantly different at P≤.05.
[3]Right Femur.
[4]Left Femur.
[5]Right Tibia.
[6]Left Tibia.
[7]Average.

Table 6. Histologic incidence of lesions of 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	Rickets %	Micro Frac[3] %	Chondrosis %	Osteomyelitis %	TD[4] %	Normal %
BSS-BSS	37.68[2]	15.94	27.54	5.80	1.45	11.59
CT-BSS	52.94	11.76	19.12	2.94	4.41	11.76
BSS-CT	38.03	14.08	19.72	4.23	5.63	12.68
CT-CT	33.80	18.31	12.68	4.23	14.08	12.8

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]No significant differences noted.
[3]Micro Fracture.

Table 7. Micro fracture severity in 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	# Samples	Micro Fractures	Level 1 %	Level 2 %
BSS-BSS	69	9	100.0	0.0
CT-BSS	68	8	100.0	0.0
BSS-CT	71	10	80.0	20.0
CT-CT	71	13	100.0	0.0

[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]No significant differences were noted.

Table 8. Chondrosis severity in 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	# Samples	Chondrosis	Level 1 %	Level 2 %	Level 3 %
BSS-BSS	69[2]	19	78.9	21.1	0.0
CT-BSS	68	13	92.3	7.7	0.0
BSS-CT	71	14	92.9	6.7	0.0
CT-CT	71	9	66.7	22.2	11.1

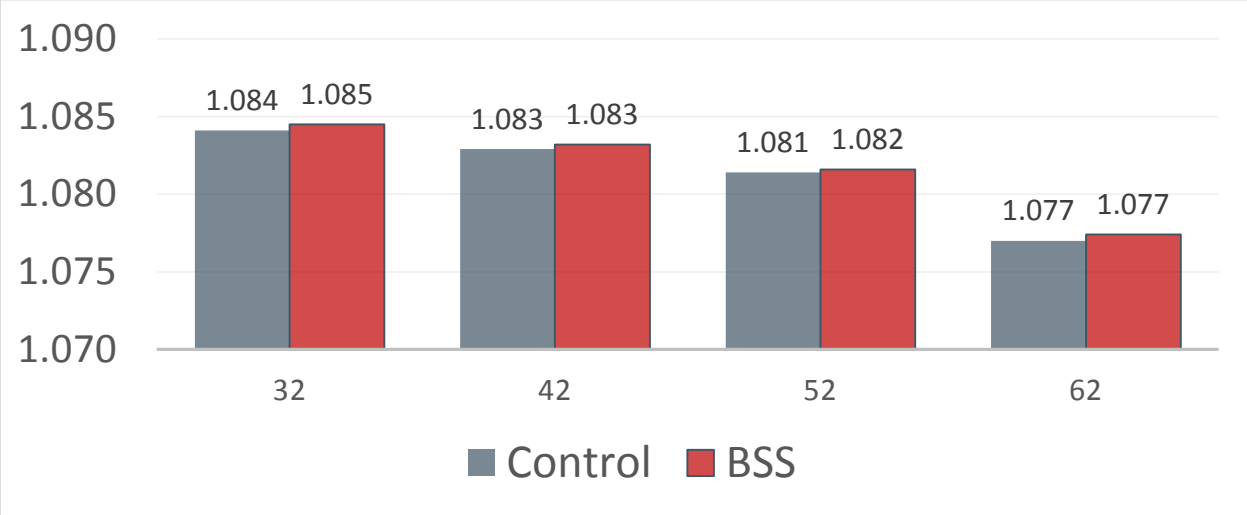
[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]No significant differences were noted.

Table 9. Osteomyelitis severity in 8 wk broilers from hens fed control or *Bacillus subtilis* diets in combination with like diets fed to progeny.

Treatment diets[1]	# Samples	Osteomyelitis	Level 1 %	Level 2 %	Level 3 %
BSS-BSS	69[2]	4	0.0	25.0	75.0
CT-BSS	68	2	0.0	0.0	100.0
BSS-CT	71	3	100.0	0.0	0.0
CT-CT	71	3	33.3	0.0	66.7

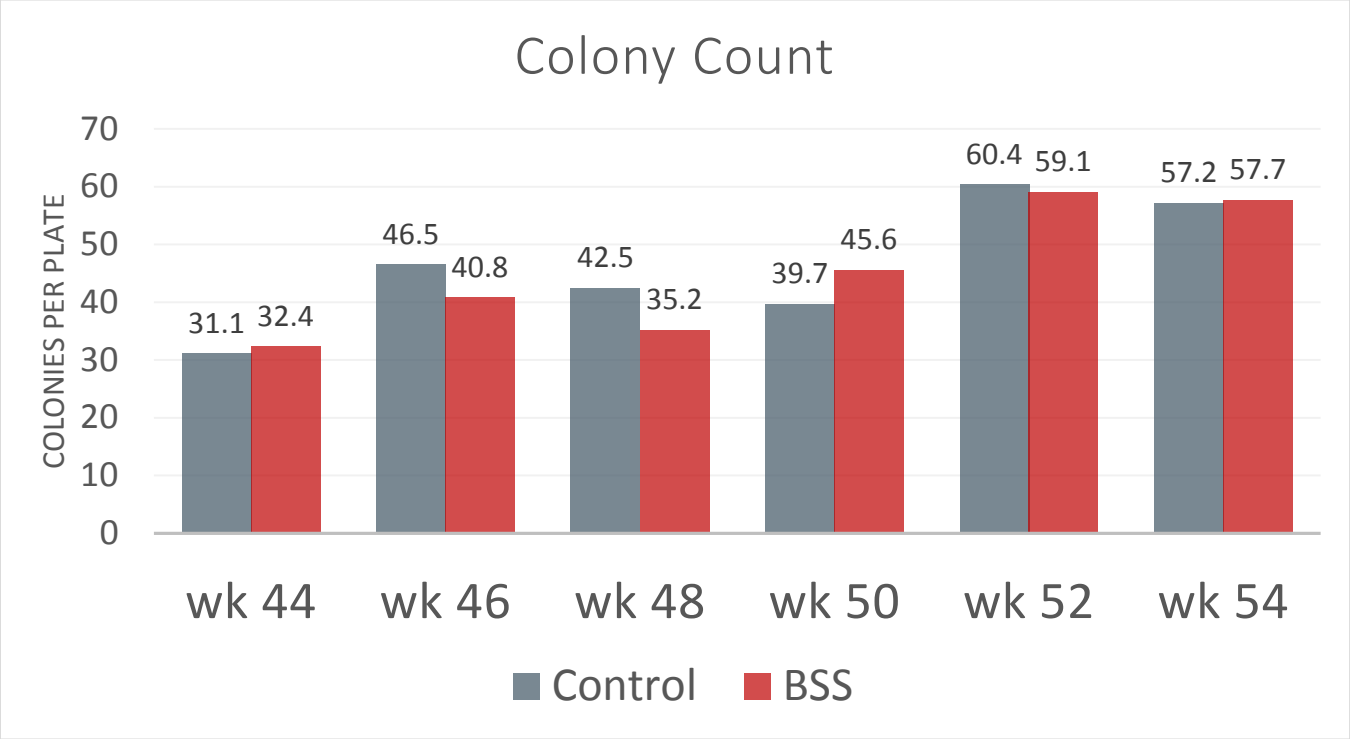
[1]Dietary treatments: first diet was fed to hen, second diet was fed to the broilers; CT=Control, BSS=*Bacillus subtilis*.
[2]No significant differences were noted.

Figure 1. Specific gravity of eggs from hens fed control or *Bacillus subtilis* diets[1].



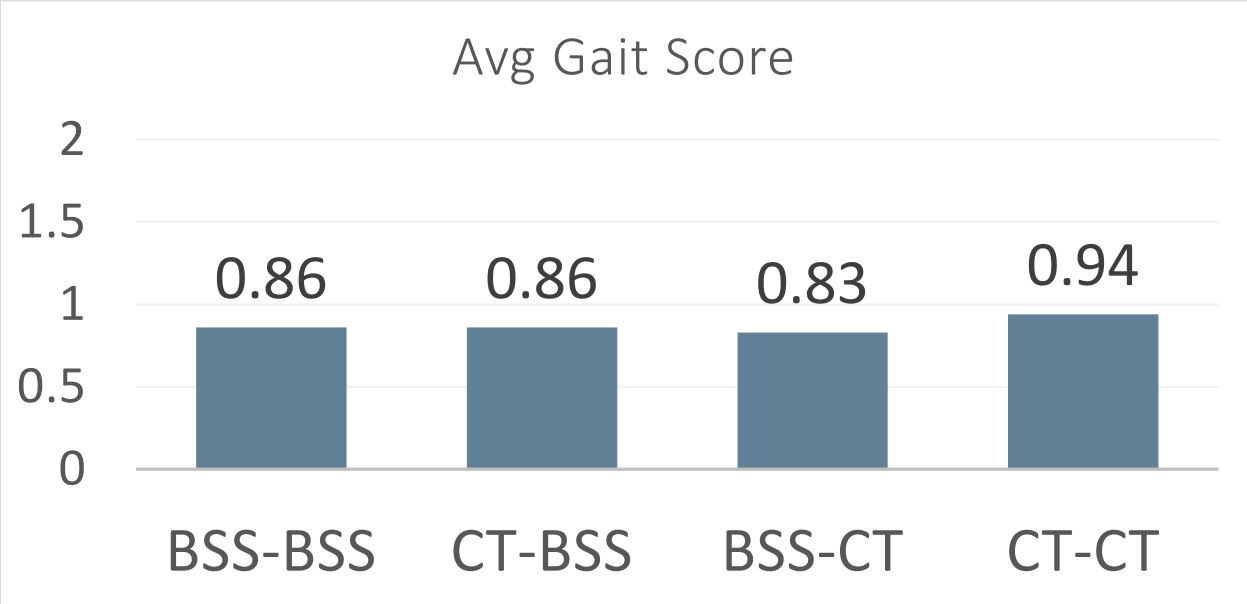
[1]Dietary treatments fed to hens; CT=Control, BSS=*Bacillus subtilis*.

Figure 2. Colony Count of bacteria on eggs from hens fed control or *Bacillus subtilis* diets[1].



[1]Dietary treatments fed to hens; CT=Control, BSS=*Bacillus subtilis*.

Figure 3. Gait score[1] in 8 week broilers from hens fed control or *Bacillus subtilis* diets[2] in combination with like diets fed to progeny.



[1]Gait score (n=30/treatment):
0=No visual signs of lameness, could walk 5m.
1=Slight limp, could walk 5m.
2=Severe limp, couldn't walk 5m.
[1]Dietary treatments fed to hens; CT=Control, BSS=*Bacillus subtilis*.

CHAPTER 4

INTERPRETIVE SUMMARY

Broiler lameness is a major problem for the poultry industry in both economic concerns and welfare concerns. Bacterial chondronecrosis with osteomyelitis is the majority of lameness seen in broilers today. Decreasing the amount of bacteria that infects the joints and causes necrosis in these heavy, fast growing broilers may be the key in reducing this disease. Previous studies have suggested that the bacteria found to inhabit these BCO lesions are commonly found in the breeder flock environments and hatchery environment. This previous work has lead us to search for a means of reducing bacterial contamination to broiler flocks via the breeder flock. In this study, broiler breeders were fed a Direct-fed microbial in hopes of reducing egg shell contamination of bacteria that leads to BCO in the offspring. Results from this study show that using a direct-fed microbial with broiler breeders does have a positive impact on the incidence and severity of BCO lesions in broiler progeny. A very high percentage of broilers in this study had signs of BCO due to the wire-floor ramps used to induce lameness. The natural incidence of BCO in commercial broiler flocks is relatively low considering the flock size, so future endeavors should focus on larger broiler breeder flocks and broiler offspring without wire-floor ramps.