Evaluating the Formation of Bacterial Chondronecrosis with Osteomyelitis in Broilers Raised on Portable Wire Floor Models

Alex David Gilley
University of Arkansas, Fayetteville

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EVALUATING THE FORMATION OF BACTERIAL CHONDRONECROSIS WITH OSTEOMYELITIS IN BROILERS RAISED ON PORTABLE WIRE FLOOR MODELS
EVALUATING THE FORMATION OF BACTERIAL CHONDRENCECROSIS WITH OSTEOMYELITIS IN BROILERS RAISED ON PORTABLE WIRE FLOOR MODELS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Poultry Science

By

Alex David Gilley
University of Arkansas
Bachelor of Science in Poultry Science, 2011

May 2013
University of Arkansas
Abstract

Rearing birds on wire flooring has proven to be an effective method for consistently triggering significant levels of lameness in broilers, and while the wire flooring model has been proven effective, more mobile wire floor models have been developed to compliment the facility management conditions within commercial genetic selection programs. The mobile wire floor models have been titled “speed bumps”, and through previous evaluation have resulted in significantly higher levels of lameness in broilers compared to litter floor shavings. The current experiment contains two studies (studies 1 and 2). Both studies involved comparing leg weakness between broiler strains; while in study 1 the main objective was to detect the optimal time to introduce speed bumps (SB) into litter flooring pens and study 2 involved developing additional SB models and determining which models were the most efficient at inducing significant levels of lameness. Broiler lines B and D (study 1) and A and B (study 2) were hatched at a commercial hatchery and brought to the University of Arkansas Research Farm where they were reared straight run for 56 days with water and feed *ad libitum* at a final stocking density of 0.47 square meters per bird. Study 1 involved raising broilers on 5 different floor treatments, including litter floor shavings, wire flooring, SB-14 (SB introduced into litter floor pens at 14 days of age), SB-28 (SB introduced into litter floor pens at 28 days of age, and SB-42 (SB introduced into litter floor pens at 42 days of age). In study 2 broilers were raised on 7 floor treatments: litter floor shavings, flat wire floor model, SB50, SBL50%, SBL66%, SBW50%, and PT. The latter 5 treatments were SB models introduced into litter flooring pens at 28 days of age. The results of both studies indicated that SB introduction was ideal at 28 days of age, and between the 5 SB models used in trial 2, SBW50% proved to induce the highest degree of lameness. From the findings of Study 1 and 2 it can be determined that the most effective use of a SB model for inducing BCO related lameness is through introduction of the SBW50%
model at 28 days of age.
This thesis is approved for recommendation to the Graduate Council.

Thesis Director:

Dr. Nicholas B. Anthony

Thesis Committee:

Dr. Robert F. Wideman Jr.

Dr. Young Min Kwon
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Introduction

Since the 1950s broiler breeding companies have been selecting for increased body weight and feed efficiency. When comparing a broiler strain from 1957 to a broiler strain from 1991, Havenstein et al. (1994a) reported an increase in the 42 day body weight of 420% and improvement in feed efficiency of 30%. Along with these improvements however the modern broiler also had higher post twenty-one day mortality due to increased leg disorders (Havenstein et al., 1994a). A more recent study evaluated 28 male and 19 female commercial flocks and found that 0.52% of males and 0.38% of females culled exhibited leg disorders (McNamee et al., 1998). Unfortunately, an accurate economic cost associated with leg disorders is difficult to assess (Sullivan, 1994). A study conducted in the US estimated that leg disorders cost the industry between 80 and 120 million dollars annually. These figures are based on 1.1% mortality caused by lameness with an estimated 2.1% decrease in body weight due to the birds’ inability to reach feed and water related to leg weakness (Morris, 1993).

The increased incidence of lameness for the broiler industry has become an economic and animal welfare concern. In 1992 the Farm Animal Welfare Council (FAWC) evaluated meat market birds from commercial farms and found numerous types and severities of leg weakness. In many cases birds become lame late in the production period, and lame birds were often allowed to remain in the flock with the hope they could survive to processing. However, when producers do not properly euthanize lame birds they are in violation of the Agriculture Act of 1968 by causing or allowing unnecessary pain and distress. In addition, estimating the level of pain is subjective and difficult. Kestin et al. (1992) developed one of the first methods for evaluating leg weakness in broilers. This method is referred to as gait scoring (GS) which involves visual observation of the birds’ ability to walk on a known surface. This GS method is
broken down into six categories: 0 (the bird moves soundly), 1 (bird moves at a rapid pace with a slight walking deficiency), 2 (bird moves at a rapid pace with a significant walking deficiency), 3 (bird is able to move quickly but exhibits an important walking deficiency), 4 (bird exhibits great difficulty when moving), 5 (bird rarely moves and uses its wings for crawling). In 2000 the European Commission Scientific Committee on Animal Health and Animal Welfare concluded that birds having a GS of 3 or higher must be experiencing considerable pain. Upon recognition of the subjective nature of gait scoring the committee concluded GS to be an invalid method for measuring leg weakness. A second method for evaluating leg strength in broilers is by individually placing birds on a force plate (FP), which is a platform fitted with load cells that records forces applied by each footstep (Sandilands et al., 2011). In biomechanical laboratories the FP provides an effective method to evaluate balance and motion characteristics within test subjects (Cross, 1998). Sandilands et al. (2011) investigated whether GS or FP measurements were valid indicators for determining leg health in 6 week old broilers. After comparing postmortem leg pathologies to GS and FP scores, the investigators concluded that neither of the two methods could effectively be used for diagnosing leg weakness in broilers (Sandilands et al., 2011). Since the proposed methods for measuring leg weakness have been determined ineffective, further research is needed.

Lameness and leg weakness are two terms that traditionally have been used when referring to a general category of leg problems. Butterworth (1999) stated that leg weakness is more appropriate for defining leg problems since, unlike lameness; leg weakness does not imply that a bird is unable to move but rather that its walking ability is compromised. Hereafter in this thesis leg weakness will be used instead of lameness, in recognition of the fact that in a majority of the cases the birds observed were not lame (e.g. immobilized) but instead were suffering from
leg weakness.

The pathological states that result in leg weakness are categorized as either non-infectious (Lynch et al., 1992; Kestin et al., 1994) or infectious disorders (Bradshaw et al., 2002). Non-infectious disorders are attributed to genetics, high growth rates, high feed efficiency, body conformation, lack of exercise, and inadequate nutrition. It is important to note that these categories are not independent and in many cases it may take more than one insult to induce lameness. Infectious leg disorders occur less frequently but typically with increased levels of severity.
Literature Review

None-Infectious Leg Disorders in the Modern Broiler

Intensive genetic selection for low feed conversion and rapid weight gain has resulted in a higher incidence of leg problems. As previously mentioned, the study by Havenstein et al. (1994a) showed a 420% increase in final body weight when the 1991 broiler was compared with a 1957 broiler. Approximately 90% of this increase in body weight was due to genetic progress through selection over many generations. The dramatic increase in body weight also resulted in increased mortality related to leg weakness. Several studies comparing strains with different growth rates revealed correlations between increased growth rates and leg weakness. Kestin et al., (2001) reared 13 strains of broilers known to differ in growth rates on full and restricted feeding regimes. Both high live weights and rapid growth rates were shown to increase the incidence of leg weakness (Kestin et al., 2001).

Tibial dyschondroplasia (TD) is one of the most common non-infectious leg disorders for modern broilers (European Commission, 2000). The occurrence of TD is a result of modern meat type broilers being genetically and nutritionally manipulated to attain exceptionally high growth rates (Orth and Cook, 1994). Broiler chicks are highly susceptible to forming TD during the first few weeks as a result of rapid bone growth (Wise, 1970; Marks, 1979; Bond et al., 1991) and their marginal ability to efficiently synthesize 1,25(OH)2 Vitamin D3, an important regulator of bone metabolism (Abbas et al., 1985; Vaiano et al., 1994). Broilers with TD develop an avascular cone of cartilage below the growth plate that extends into the metaphyseal region (Lowther et al., 1974; Gay and Leach, 1985; Farquharson and Jefferies, 2000). TD has been reported to occur in most long bones, but is most commonly reported in the proximal tibiotarsus.
As a result of numerous studies, TD is now accepted as a consequence of immature chondrocytes unable to undergo terminal differentiation, thereby inhibiting vascularization and mineralization (Farquharson and Jefferies, 2000). After subjective evaluation of gait scores in commercial broilers found to have TD, Kestin et al., (1994) concluded that 64% of the birds showed severe gait defects. However, due to the subjective nature of gait scoring new methods for detecting TD were needed. Hand-held fluoroscopes (Lixiscope) are now commonly used within the industry to detect birds with TD lesions. The development of the Lixiscope in combination with a moderate heritability for TD ranging from 0.22 to 0.44, have successfully led to a reduced incidence of TD lesions in broilers (Ducro and Sorensen, 1992).

Another prevalent non-infectious, long bone leg disorder shown to have genetic effects is valgus-varus deformity (European Commision, 2000). Valgus deformities are characterized by a bow-legged stance, while varus deformities result in a knocked kneed stance. The etiology of this leg disorder is unclear however high growth rate, genetic defects, and lack of exercise are thought to contribute to expression of the deformity. There are conflicting hypotheses as to whether the two deformities are of the same pathogenesis, but overall valgus and varus are associated with “displacement of the gastrocnemius tendon on the medial side of the distal tibiotarsus” (Leterrier and Nys, 1992). Birds affected with valgus-varus deformity (VVD) are found to have lower bone mineralization and defective gaits. Pattison (1992) indicated a considerable reduction of VVD in Europe. This reduction can be attributed to genetic selection against VVD since it is known to have a heritability of approximately 0.70 (Ducro and Sorensen, 1992).

In addition to changes in body weight and growth rate, genetic selection has altered body composition. Havenstein and coworkers (1994a) reported drastic increases in live weight for the
modern broiler with even greater increases revealed for breast muscle expressed as a percentage of total body weight. It was reported that the 1991 strain had a 3% greater breast yield when compared with the 1957 strain, showing that ongoing selection had disproportionally increased the percent breast meat. Other studies have looked at changes in skeletal development between less selected and modern, heavily selected broiler lines. A study by Williams et al. (2000) demonstrated that modern broiler lines had less mineralized and more porous cortical bone than a slower growing line. From his study Williams and coworkers (2000) concluded that the faster growing line had a more juvenile stage of skeletal maturation making the bones more susceptible to bending and breaking.

Lack of exercise may also contribute to leg disorders in broilers. Rutten (2000) showed that exercise on a treadmill improved bone density in the tibia. Thorp and Duff (1998) also demonstrated that exercise would improve vascular patterns in the proximal growth plate. The relationship between growth rate and exercise were evaluated in a study in which activity levels between broilers of different growth rates were compared (Reiter and Bessei, 1998). Faster growing broilers were found to have lower activity levels, and the incidence of leg disorders was reduced when birds were forced to exercise by increasing the distance between feeders and waterers. In another study Reiter and Bessei (2001), evaluated the effects of changes in body conformation and activity levels with decreased weight load on birds’ legs. Weight load was decreased by applying a device that alleviated 25% of the birds’ body weight. Birds with the device were found to have higher activity levels and lower torsional deformities. The increased activity levels were attributed to a reduction in pain from a lower body weight load (Reiter and Bessei, 2001).

Along with genetic changes, modern poultry diets generally contain higher levels of
protein and fat, and lower levels of calcium and phosphorous. Calcium and Phosphorous are two essential minerals for bone formation (Havenstein et al., 1994a). When broiler strains from 1991 and 1957 were fed a typical commercial diet for 1991, the growth rate for both lines increased by 18-26%, but the 1991 broiler strain was unable to sustain its growth rate post 42 days of age. This tapering off of growth rate was attributed to increased leg disorders (Havenstein et al., 1994a).

While it is difficult to determine the origin or sole cause of non-infectious leg disorders, it is known that slowing the growth rate and decreasing the weight load on the legs will increase activity levels and decrease the incidence of leg disorders in broilers. Husbandry practices also can be manipulated to improve leg health, including reduced stocking densities (Grashorn and Kutritz, 1991), diet manipulation (Long et al., 1984; Riddell and Pass, 1987), feed restriction (Duff and Thorp, 1985; Classen and Riddell, 1990; Robinson et al., 1992), and photoperiod adjustment (F.A.W.C., 1992; Sorensen, 1999). These improvements may reduce non-infectious leg disorders however many birds may still be affected by infectious leg disorders.

**Etiology of Bacterial Chondronecrosis with Osteomyelitis (BCO)**

Infectious leg disorders typically occur less frequent but with higher severity. In modern broilers bacterial chondronecrosis with osteomyelitis (BCO) is the most prevalent infectious disorder that causes leg weakness (Bradshaw et al., 2002). Typical examples of the gross (macroscopic) femoral and tibiae pathology associated with BCO are shown in Figures 1 and 2. McNamee and coworkers (1998) sampled several male and female flocks, and indicated that 0.52% of all culled males and 0.38% of all culled females were culled for leg problems. BCO accounted for the highest percentage of leg related mortality at 17.3%, not including birds culled for other reasons and found subsequently to have BCO. BCO is a severe degenerative disorder.
expressed by broilers between 14-70 days of age with the majority of cases occurring around 35 days of age (McNamee et al., 1999). Affected birds begin to use their wing tips for support by placing them on the ground (Nairn and Watson, 1972). Affected birds may also have a noticeable limp, and in advanced stages of BCO birds become fully immobilized and are unable to reach food or water (McNamee and Smyth, 2000). In most cases death results 2-5 days after the onset of clinical BCO symptoms. The inability to reach feed or water is an obvious welfare concern as well as an economic loss associated with slow growth and decreased yields. Mortality costs to the UK poultry industry has been estimated at 6 million dollars annually (Pattison, 1992).

BCO lesions are found in all bones but most commonly occur at the proximal growth plates of the femur and tibiotarsus (Nairn, 1973). Severe BCO lesions appear macroscopically as areas of caseus exudates or lytic areas that make the bone brittle and fragile. Only 40-67% of BCO lesions are detected macroscopically, with the rest being found through histological examination for basophilic bacteria in the epiphyseal and physeal blood vessels. These vessels are surrounded by poorly stained cartilaginous matrix containing necrotic chondrocytes (Emslie et al., 1983). Since histological examinations are time consuming and seldom conducted, it can be concluded that BCO is significantly under-diagnosed in most commercial flocks.

**Bacteria Associated with BCO**

BCO lesions are caused by a plethora of bacterial organisms such as *Staphylococcus xylosus*, *Escherichia coli*, *Myobacterium avium*, *Salmonella spp*, *Enterococcus*, and *S. aureus*. The bacteria most commonly found in BCO lesions are *S. aureus* (Bradshaw et al., 2002). *S. aureus* is commonly found in oral cavities, eyes, and cloaca of healthy birds as well as their environment, from the hatchery to the processing plant. It is not precisely known why *S. aureus*
has been found to cause so many BCO lesions; however the prevalence of S. aureus infection is thought to be related to the intrinsic characteristics that allow S. aureus to affect a large number of animal species (Lee, 1996). Most strains of S. aureus recovered from infected humans or animals contain capsules (Sompolinsky et al., 1985; Karakawa et al., 1988). The capsule is essential to the pathological function of S. aureus through its ability to impair phagocytosis as well as its association with virulence (Jonsson and Wadstrom, 1993). The capsule has also been hypothesized to enhance the bacteria’s ability to adhere to cartilage (Speers and Nade, 1985). This hypothesis may be crucial in understanding why S. aureus is abundant in the leg joints.

Because S. aureus is gram-positive, the bacteria contains surface proteins. The surface proteins influence the organism’s virulence as well as its bacterial adherence (Holderbaum et al., 1985; Ryden et al., 1989; Switalski et al., 1993; Foster and McDevitt, 1994). Protein A (S. aureus surface protein) increases the virulence of S. aureus by blocking the Fc portion of immunoglobulin (Kloos and Bannerman, 1995) and preventing accumulation of neutrophils around the bacteria (Colburn et al., 1980). Lefevre and Jensen (1987) found no direct relationship between protein A and its ability to adhere to tissue, however they did suggest that when combined with other factors protein A may aid in the ability of S. aureus to cause staphylococcal infections in avian species.

**Models Developed to Induce BCO Related Lameness**

The development and maturation of avian and mammalian growth plates exhibit many similarities, making chickens an appropriate animal model for the pathogenesis of acute haematogenous staphylococcal osteomyelitis in infants (Howlett, 1979). Since chickens are an appropriate model, substantial research and information has been attained from naturally occurring BCO and from studying experimental models that induce BCO (Emslie et al., 1983).
Even though much research has been conducted it still remains difficult to determine how bacteria reach the bloodstream and become translocated into the growth plates. One proposal is that the staphylococcal infection is correlated with high incidences of food pad lesions (Smith, 1954). It has also been proposed that bacteria may enter the chick’s body shortly after hatching, through the open navel (Harry, 1957). Researchers have tried to induce *S. aureus* invasion in chickens through tracheal inoculation. *S. aureus* colonization was found in the trachea but no further invasion into the remainder of the body occurred (Mutalib et al., 1983b).

Different models for inducing BCO have been tested in order to better understand the evolution of BCO. Because *S. aureus* is known to be present in the air, the aerosol inoculation route has been assessed as a potential mode of bacterial translocation. Early attempts were relatively unsuccessful, causing only one case of osteomyelitis in 10 birds that were exposed to high levels of *S. aureus*. *S. aureus* was found colonized in the trachea; however, it was concluded that additional unknown factors were necessary for translocation of the bacteria into the bloodstream leading to the development of BCO (Mutalib et al., 1983b). McNamee and coworkers (1999) exposed birds to *S. aureus* via aerosol at 1 day of age, and at 21 days of age birds were injected with immunosuppressive viruses, CAV and IBDV. Birds exposed to *S. aureus* at 1 day of age and injected with CAV and IBDV while being fed *ad libitum* developed the highest percent of BCO cases (McNamee et al., 1999). This study supported the hypothesis that viruses with immunosuppressive effects may enhance the pathogenesis of osteomyelitis as previously stated by Thorp et al., (1993). Mutalib et al. (1983a) investigated whether stressful conditions such as severe feed restriction, poor nutrition, overcrowding, or adverse weather conditions were associated with BCO formation. The investigators were unable to conclude a relationship between the mentioned conditions and BCO formation; however, it has been stated...
that stressful conditions may activate BCO formation in cases where birds have been exposed to
*S. aureus* at an early age (Mutalib et al., 1983a).

A more successful model for inducing BCO was developed by injecting *S. aureus* intravenously. Nairn (1973) determined that turkeys injected intravenously with *S. aureus* develop osteomyelitis indistinguishable from osteomyelitis developing spontaneously in the field. Emslie et al., 1983 used Nairn’s model (injecting bird’s intravenously with *S. aureus*) to further investigate the vascular destruction of osteomyelitis caused by *S. aureus*. They conducted the experiment by performing vascular perfusion in 10 birds at 24 hr, 2 and 4 days post intravenous injection of *S. aureus*. Through vascular perfusion with a Berlin blue solution they were able to identify three major blood vessels in the growth plate region. The most common blood vessels found were the metaphyseal vessels. Emslie et al., 1983, found these vessels to be “situated within tunnels that extended into the hypertrophic layer of the zone of cartilage transformation in the growth plate” (Emslie et al., 1983). A second group of vessels found in the growth plate region were epiphyseal vessels. They are located in cartilage canals passing from the epiphysis through the zone of growth reaching the hypertrophic layer of the zone of cartilage transformation. Some epiphyseal vessels or transphyseal vessels were found to pass completely through the growth plate. Transphyseal vessels travel parallel of the germinal layer of the growth plate in canals turning at right angles passing through the growth plate and joining the metaphyseal vessels. Within 24 hours post injection, macroscopic lesions were visible and vascular tunnels containing bacteria as well as the vessels adjacent to the lesion were not penetrated by the Berlin Blue dye. Non-penetration of the blue dye into the vessels confirms that the bacteria obstructed the blood supply to the infected region (Emslie et al., 1983). This observation gave insight into the lack of efficacy of administration of antibiotics to birds that
have developed osteomyelitis. Similarly Howlett (1980) conducted a detailed ultrastructural examination of the proximal tibia growth plate. Howlett found that chondrocyte lacunae are opened by small vascular saccules formed through the expansion of thin walled metaphyseal blood vessels. Bacteria are thought to come in contact with the physeal cartilage at the ends of the capillary tips of metaphyseal vessels where the endothelium is fenestrated. Long and narrow metaphyseal vessels cause sluggish circulation around the growth plate allowing bacteria to accumulate, increasing the opportunity for bacterial infection (Trueta, 1959). Along with increased bacterial accumulation, cartilage is deficient in macrophages making the metaphyseal vessels more susceptible to localization of bacteria (Thorpe et al., 1993). In addition to a macrophage deficiency, birds fed \textit{ad libitum} spend an average of 76\% of their time sitting (Weeks et al., 2000) causing restricted blood flow and poor circulation, and contributing to ischemia and necrosis around the growth plate (Thorp, 1988).

Raising birds on wire flooring is a proven model that does not involve exposing birds to known pathogens. Broilers raised on wire flooring express no changes in growth rate or activity levels when compared to those grown on litter flooring (Wideman et al., 2012). Rapid growth rates along with unstable footing imposes a mechanical stress to the leg joints of birds reared on wire flooring, resulting in microfractures to the epiphyseal and physeal cartilage. Bacteria translocating across the respiratory epithelium or gastrointestinal tract can then be translocated into the microfractures forming bacterial foci and lytic channels (Wideman et al., 2012). Necrosis begins when lytic channels progressively destroy the vascular system within the calcifying zone of the metaphysis. Once the vascular structure is destroyed, trabecular struts deteriorate eliminating structural support for epiphyseal and physeal cartilage (Emslie et al., 1983).
Prevention of BCO

As mentioned above, successful models for inducing BCO have been developed; however, development of antibiotics, vaccines, or management strategies for the prevention of BCO have been lacking. Emslie et al., 1983 observed that vascular tunnels become occluded with bacteria, leading to ischemia and the development of necrotic voids in the metaphysis. Bacterial occlusion in these vascular tunnels inhibits antibiotics and circulating immune cells from reaching the origin of the bacterial foci, thereby reducing the effectiveness of antibiotics. Also the use of vaccines for treating BCO has shown to be ineffective (Jungherr and Plastridge, 1939). Using probiotics prophylactically has proven to be successful. Prophylactic use of probiotics apparently aids in reducing BCO by decreasing intestinal populations of pathogenic bacteria, strengthening the intestinal wall, and priming the immune system to neutralize potential translocated bacteria. When comparing birds grown on wire flooring Wideman et al. (2012) found reduced incidences of BCO when the broilers were given feed supplemented with Probiotics (Wideman et al., 2012). At this time the problem with using Probiotics is their cost effectiveness for commercial use. Bacterial interference, where there is competition from the interfering bacterium for the same tissue receptor, has been found to be a successful method for prevention of staphylococcal infection in humans (Kluytmans et al., 1997) and turkeys (Jensen et al., 1987). Bacterial inference also has been successful in preliminary studies conducted with chickens (Nicoll and Jensen, 1987); however, further research on bacterial interference in chickens is needed.
Objective

The studies reported herein were designed to investigate additional wire flooring designs that also do not require exposing broilers to known pathogens. The models being evaluated are more easily portable versions of the wire flooring model introduced by Wideman et al. (2012). The model involves placing a speed bump (SB) in a pen between the feeders and waterers, thereby forcing birds to transverse the SB repeatedly to eat and drink. The SB is constructed of aluminum tubing and wire mesh. The SB is 1.54 meters long with a base 91.44 cm wide at the base and 22.86 cm tall with a 50% slope. The slope imposes mechanical stress on the bird’s legs. The slope of the SB is calculated by the following formula: (height ÷ (0.50 * base)). In a study done by Wideman (Unpublished, 2011) use of the 9” SB significantly increased leg weakness in birds compared to litter flooring.

The current experiment contains two parts. In the first part of the experiment we determined the optimal time to introduce the 22.86 cm SB model in order to induce the highest incidence of BCO lesions, the objective of the second experiment was to introduce additional SB models and compare which models caused the highest incidence of BCO lesions.
Materials and Methods

Study 1

Two broiler lines, B and D, were compared in study 1. Line B is known for rapid early growth whereas Line D exhibits slow early growth but has been selected for increased eight week body weight. Animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #08036). Chicks from both lines were hatched at a commercial hatchery and delivered to the University of Arkansas Poultry Research Farm. They were placed straight run (male and females together) at eighty-five birds per pen (3.05m x 3.05m) with an initial stocking density of 0.36 square meters per bird. Chicks from Line B were assigned to pens 1-11, while Line D chicks were assigned to pens 12-22 (Figure 3). Throughout the experiment birds were grown on twenty three hours of light and one hour of dark with feed and water provided *ad libitum*. A standard commercial diet series was provided. Industry starter feed was fed as crumbles for the first five weeks after which pelleted finisher feed was provided for the remainder of the eight week study. All diets met or exceeded the National Research Council’s requirements (NRC, 2012). Regarding house temperature, thermo-neutral temperatures were maintained starting at 32° C for Days 1 to 3, 31° C for Days 4 to 6, 29° C for Days 7 to 10, 27° C for Days 11-14 and 24° C for the remainder of the experiment. At fourteen days of age the population was reduced to 65 healthy birds per pen, resulting in a final stocking density of 0.47 square meters per bird.

In this experiment five floor treatments were studied. Figure 3 shows a diagram of the pens and the location of each floor treatment within the house. In six pens (three per line) birds were reared on flat wire flooring as described previously (Wideman et al., 2012) and in four pens (two per line) birds were reared on litter flooring, beginning on the day of hatch, and continuing...
to the end of the study. The wire panels used in the wire flooring treatment were constructed from 5 x 5 cm lumber and were 3.05 meters long and 1.52 meters wide with 5 x 5 cm cross members added for support. Hardware cloth (1.3 x 2.54 cm mesh, .063 gauge, galvanized welded wire cloth; Direct Metals, Kennesaw, Ga) was fastened to the top of the frame and cross members. Panels were elevated on 30 cm high masonry blocks allowing manure to pass through and accumulate underneath the wire surface. The remaining pens had litter flooring and Speed Bumps (SB) were introduced at three different ages. The SB used in this experiment (SB50%) were 1.54 meters long with a 91.44 cm base and 22.86 cm tall having a 50% slope (Figure 4). Since the pens were 3.05 x 3.05 meters two SB were needed in order to extend the full width of the pen. Speed bumps were placed in four pens (two per line) at fourteen, twenty-eight, and forty-two days of age (SB-14, SB-28, SB-42). Once the SB were introduced, they remained in the pen until the end of the experiment. To insure that each bird was repeatedly exposed to the mechanical stress of climbing up and down their respective SB, feeders and waterers were placed on opposite sides of the SB (Figure 5).

Since the objective of this experiment was to study morbity and mortality associated with lameness, chick mortality data for the first fourteen days of the experiment was not collected. Most early mortality, however, resulted from inability to find feed or water. On day fourteen all pens were culled heavily to retain the 65 largest and clinically healthy birds per pen. Thereafter, all mortality was recorded by date, pen number, and wing band number. From 14 days of age until the end of the experiment investigators walked through each pen to observe the birds at a minimum of four times a week. Walking through each pen with a broom stimulated the birds to stand and walk around the pen, thereby enabling researchers to identify candidates showing signs of clinical lameness. Birds showing early signs of lameness (reluctance to stand,
choppy gait, mild limping) were marked with spray paint, which allowed researchers to more closely observe these birds over the ensuing days. Birds unable to stand or move without a severe limp or regular dipping of one or both wings were considered clinically lame and were humanely euthanized and necropsied. All daily mortality was necropsied to assess the lesions associated with the rapid onset of bacterial chondronecrosis of osteomyelitis (BCO). At necropsy tibial and femoral heads of both legs were evaluated and assigned subjective scores that were used to determine the cause of lameness and/or death. The potential diagnostic categories are listed in Table 1 with a brief description of the criteria used for determining the correct category to be selected for the cause of lameness and/or death.

The categories listed in Table 1 were used to determine cause of lameness and/or death for each bird; however, specific attention was given to those categories associated with BCO. These categories are FHS, FHT, FHN, and THN (Figures 1 and 2). Femora were categorized as normal when there was no visual macroscopic damage to the femoral head. FHS was diagnosed when the femoral head was separated from the articular cartilage with no further damage to the growth plate (e.g., epiphyseal). FHT was diagnosed when FHS occurred in combination with ulceration, erosion or fracturing of the growth plate, as well as macroscopic evidence of bacterial colony formation. FHN was diagnosed when perforation, fracturing, and necrosis of the femoral head occurred (Wideman et al., 2012). Once both femur heads were diagnosed, the proximal tibial heads were cut transversely and categorized. The tibiae were categorized as normal when struts of trabecular bone in the metaphyseal zone were shown to fully support the growth plate. THN was diagnosed when necrotic voids and lytic channels in the metaphyseal zone led to microfractures in the growth plate. The THN category was further subdivided to include THNs (Proximal Tibia Head Necrosis, Severe) and THNc (Proximal Tibia Head Necrosis Caseous).
THNs was identified in birds with large necrotic voids and deep lytic channels that clearly compromised the support for the growth plate, providing no support for the articular cartilage when the broiler was standing. THNc is a more advanced stage of THNs with obvious bacterial infiltration and sequestrate. The progressive stages for proximal femoral and tibia lesions (Figures 1 and 2) were assigned a score associated with the degree of severity for each respective category. Lower scores were given to the less severe stages of BCO and higher scores given to the most severe stages (Table 2). The steps mentioned above were carried out each day from Day 14 to Day 55. On day 56 and 57 a study teardown was conducted where all remaining birds (clinically healthy) were euthanized and body weight, gender, and leg necropsy data was collected.

**Study 2**

In this study broiler Lines A and B were compared. The objective was to compare the susceptibilities of the two lines to lameness, and to assess five alternate SB designs to determine which could most successfully induce the highest incidence of BCO related lameness. Chicks from both lines were hatched at a commercial hatchery and delivered to the University of Arkansas Poultry Research Farm where they were placed straight run at eighty-five birds per pen (3.05 x 3.05 meters) with a stocking density of 0.36 square meters per bird. Chicks from Line B were assigned pens 1-11 and Line A chicks were assigned pens 12-22 (Figure 6). Rearing conditions for study 2 were the same as described in study 1. On day fourteen each pen was culled to leave 65 healthy birds per pen with a stocking density of 0.47 square meters per bird. Mortality again was not recorded until Day fourteen.

Seven floor treatments were investigated during this study. Figure 6 provides a diagram of pens and the location of each floor treatment within the house. Birds were reared in six (three
per line) flat wire flooring pens and six (three per line) litter flooring pens from hatch until the end of the experiment. At 28 days of age five SB models were introduced into pens having litter flooring. The different SB models had the same basic construction as used in study 1, with additional features designed to place further stress on the broilers’ legs. The “limbo bar” SB had a 50% slope with a wooden bar suspended over the apex (Figure 7). One vertical support was fastened at each end of the SB with a series of holes running down the support so the height of the wooden limbo bar over the apex of the SB could be adjusted as the birds grew (SBL50%). The wooden limbo bar’s purpose was to discourage the birds from trying to fly over the speed bump, and to force the bird to stoop and crouch underneath the bar while walking across the apex of the SB. Figure 8 shows another limbo bar SB model 30.48 cm tall with a 66% slope and an adjustable wooden bar placed over the apex (SBL66%); the purpose of this model was to impose a steeper incline than in the SBL50% model. The next SB model shown in Figure 9 uses the SB with a 50% slope, however in this arrangement the SB was placed directly under the nipple waterers forcing birds to balance on the apex of the SB or laterally straddle the apex and balance while drinking (SBW50%). The final SB model introduced in this experiment was the Pagoda Top (PT). The PT is a SB50% model with a ceiling suspended above and parallel to the SB apex, requiring birds to traverse up and down the SB in a crouched position (Figure 10).

Starting at fourteen days of age researchers walked daily through each pen looking for mortality and lame birds. The process of looking for and determining lame birds were consistent with study 1. Once morbid or lame birds were identified, the same categories for cause of death listed in Table 1 were used, and necropsy procedures along with assigned BCO scores in Table 2 remained consistent with study 1.
Statistical Analysis

As BCO is a relatively new pathological condition most of the early work has focused on characterizing the disease and developing models to induce the condition. Little effort has been placed on ways to quantitate and compare relative severity of the BCO lesions. In this manuscript two methods for analyzing data are used. The first method involves examining the proximal femur and tibia for the presence of BCO lesions. If BCO lesions are present in one of the four leg joints (right proximal femur, left proximal femur, right proximal tibia, and left proximal tibia) it is then determined that BCO is responsible for the bird’s lameness. The second method involves forming subset categories for the BCO condition and giving each subset a numerical score called a severity score, as shown in Table 2. This method allows for leg quality measurement scores to be calculated for femora, tibiae, and the combination of femora and tibiae. Leg quality measurement scores contain three categories. The first category is the average femur severity score (AvgF), which is found by calculating the sum of the left and right femur severity scores and dividing the sum by two \(((\text{left femur score} + \text{right femur score}) / 2)\).

Likewise, an average tibia score (AvgT) is calculated using severity scores given for left and right tibiae \(((\text{left tibia score} + \text{right tibia score}) / 2)\). The third category is the total leg score (LS). The LS is calculated by dividing the sum of AvgF and AvgT by two, \(((\text{AvgF} + \text{AvgT}) / 2)\). Leg quality measurement scores were analyzed by a generalized linear model in SAS 9.2 (2000) using line, floor treatment, sex, and mortality as main effects in study 1 and line, floor treatment, and sex as main effects in Experiment 2. Interactions were tested for statistical significance \((P \leq 0.05)\) using the error mean squares. The Chi-square method was used for means separation for BCO lesion incidences and genetic parameters were analyzed with DMU package for single trait analysis.
Results

Study 1

Mortality not related to lameness totaled 2.0 % for line B and 1.8 % for line D. In both lines, SDS was the main contributor to non-lame mortality (1.1 % in line B, 0.8 % in line D) followed by Ascites (0.5 % line B, 0.9 % in line D), and UNK (0.4 % line B, 0.0 % line D). With regard to broilers that became lame, line differences were found for lameness frequency and leg quality measurements. The wire flooring treatment induced the highest percentage of lameness while litter flooring induced the lowest percentage for both line B and D (Table 3). Overall Line B was found to be more susceptible to leg weakness, having higher severity scores for AvgT and LS; however, Line D was found to have a higher severity scores for AvgF (Figure 11). Females had higher LS severity scores compared to males (Figure 12). The wire floor model induced higher AvgF severity scores than L, SB-42, and SB-14 (Table 4). The highest AvgT severity scores were induced by the W, SB-28, and SB-14 models (Table 4). LS calculations demonstrated that the wire floor model induced the highest severity score, with litter flooring and SB-42 having the lowest severity scores (Table 4). Differences in leg quality measurements for Lame and Non-Lame broilers were obtained by comparing leg quality measurements between birds that developed lameness between 14-56 days of age to birds that showed no clinical signs of lameness throughout the experiment and were necropsied on days 56 and 57 (Figure 13). Lame birds had higher severity scores for all three leg quality measurements. Body weight data were only available for broilers that survived the trial to 8 weeks. The body weights ranged from 1.83kg to 5.09 kg, and within this range broilers were assigned to three weight classes. Broilers with body weights between 1.83 - 2.99 kg were categorized into class 1, body weights between 3.00 – 3.99 kg categorized into class 2, and body weights between 4.00 – 5.09 kg were
categorized into class 3. Based on these categories the AvgF severity scores were higher for broilers in class 3 than class 2 (Table 5). Regarding AvgT severity scores, class 1 expressed a higher score than class 2 followed by class 3. Similarly for LS class 1 had a higher severity score than class 2 followed by class 3 (Table 5).

There were a few interactions observed between line and sex for study 1 (Table 6). Females from Line B had higher AvgF severity scores compared to Line B males; however Line D males had higher AvgF scores than Line D females. The LS calculation resulted in line B females appearing to be more susceptible to leg weakness than Line B males while no differences for LS were found between Line D males and females (Table 6). Interactions between floor type and Line are shown in Table 7 with line B being the most susceptible to femoral lesions when reared on flat wire flooring and least susceptible on litter flooring, while line D lacked differences between floor treatments for AvgF severity scores. Also shown in Table 7 is the interaction between lame and non-lame birds raised on different floor treatments. AvgF severity scores in Lame birds were the highest for birds reared on the wire floor model with no differences being found between floor treatments in non-lame birds. Concerning AvgT severity scores in lame birds reared on wire floor, SB-14 and SB-28 models resulted in the highest severity scores. Once again no differences were found between floor treatments for non-lame broilers (Table 7). Heritability estimates were also evaluated in study 1, through a series of equations of which different weights were applied towards different traits (Table 8). The highest heritability estimates resulting from the series of equations for Lines B and D were $0.52 \pm 0.17$ and $0.21 \pm 0.12$ respectively.
**Study 2**

In study 2 broiler Lines A and B were evaluated. For this study mortality not related to lameness was comparable to that observed in study 1. In study 2 differences were found between floor treatments. The highest percentages of lameness were induced by the W, SBL66%, and SBW50%, models, while lower percentages of lameness were induced by L, SB50%, SBL50% and PT models (*Table 9*). When Lines A and B were separated out, the highest percentage of lameness in Line A was found in broilers raised on W and SBL66% models with the lowest frequency found in birds raised on L, SBL50%, and PT (*Figure 14*). Line B expressed similar results with W, SBL66%, and SBW50% models inducing the highest frequency of lameness and litter flooring resulting in the lowest incidence of lameness. Line differences were only found with Line B having a higher percentage of lameness in birds raised on the SBW50% model, compared to Line A (*Figure 14*). For AvgF severity scores, the SBW50% and flat wire floor model resulted in the most severe femoral lesions, while SBL66% and SBL50% had the least impact on femoral lesions (*Table 9*). Broilers reared on flat wire flooring resulted in highest AvgT scores, and the lowest AvgT scores occurred in broilers reared on litter flooring (*Table 9*). Similarly for the calculated LS, highest lesion scores were seen in birds raised on the flat wire and SB50% models. Again the lowest severity scores were seen in broilers raised on litter flooring. Sex differences in leg quality scores also were observed in study 2. AvgT was found to have a higher score for females (2.33 ± .8) than males (2.13 ± .8). The composite score (LS) was found to be higher in females (2.12 ± .6) than in males (1.99 ± .5). *Figure 15* represents the cumulative increase in percentage lameness with the increasing age of the birds. Lameness stays constant within the first three weeks of age and progressively increases from 28-56 days of age.
Discussion

The present experiment re-confirmed that raising broilers on flat wire flooring is an excellent experimental model for inducing significant levels of BCO lesions. These findings are consistent with observations previously reported by Wideman et al. (2012). Unfortunately, the flat wire flooring model creates facility management issues that are difficult to incorporate into most commercial genetic selection programs. Therefore a portion of this research was designed to investigate the efficacy of inserting various SB designs between the feed and water lines (a more practical model for the industry setting) and to identify the ideal ages for the SB to be introduced. The three ages at which the SB models were introduced resulted in similar lameness frequencies and severity scores; however SB-28 resulted in the numerically highest percentage of lameness regardless of line tested, along with the numerically highest severity scores for AvgF, AvgT, and LS. Based on these results it can be concluded that the ideal time for a SB to be introduced is at 28 days of age. Introduction of the SB at 28 days of age is likely most effective due to the birds approaching maximum daily gain (Scheuermann et al., 2003; Zuidhof, 2009) while trying to adjust to the added mechanical stress that the SB induces. Perhaps the SB-14 model did not prove to be as effective because the birds were introduced to the SB too early allowing them to adjust to the added mechanical stress before reaching the peak of their growth rate. The SB-42 model triggered an identical frequency of lameness compared to the SB-28 model but had slightly lower severity scores. This is likely due to the birds not being exposed to the SB-42 model over a long enough time period, suggesting that the breakdown is more rapid for the SB-42 birds which are heavier at the time of SB introduction. The birds exposed to the SB-28 models may be more tolerant early, however, the accumulation of lesions eventually weakens their structure thus resulting in lameness and higher lesion scores.
When considering gender differences, females were found to have higher severity scores for AvgF, AvgT, and LS. Even though males have higher body weights, females have been reported to have higher percent breast meat than males (Young et al., 2001). Increased breast percentage has been thought to result in altered body conformations (Lilburn, 1994). The increased percentage of pectoral muscle may place greater stress on the leg bones, intensifying the force placed on legs when walking. Females also gain weight at a slower rate compared to males (Zuidhof, 2009) and because their rate of gain is reduced, females may be able to withstand lesions of increased severity before expressing clinical signs of lameness. Thirdly, females may encounter higher levels of stress when subjected to the aggressive nature of the maturing male broiler. Although Mutalib et al. (1983a) were unable to conclude that environmental conditions causing stress to the bird are directly linked to the formation of BCO, these authors did state that stressful conditions such as overcrowding may activate BCO formation in birds that have been previously exposed to *S. aureus* at an early age. Also as females get pushed around as a result of the males’ aggressive behavior, micro fractures in the tibial and femoral heads are likely to develop allowing bacterial infiltration to begin.

One of the more notable findings in study 1 was the different responses of the two lines to the various floor treatments. Overall Line B, the faster growing line, was more susceptible to lameness; however the majority of lameness in line B was attributable to BCO lesions occurring in the tibiae while the majority of lameness in line D was caused by BCO lesions in the femora. It is unclear why different lines of birds are more susceptible in one joint when compared to another. It might be hypothesized that the locations of the blood vessels within the legs are slightly different causing differing susceptibilities for the two leg joints. The mapping of blood vessels between broiler lines may be beneficial in finding line differences as well as a cause for
the current pathological condition. As mentioned Line B, the faster growing line, expressed higher incidences of BCO related lameness than Line D, the slower growing line selected for 8 week body weight. These finding are consistent with research showing that slow growing lines have lower incidences of leg problems compared to faster growing lines, due to higher bone mineralization being found in the slower growing lines (Shim et al., 2012) as well as faster growing bones having thicker articular cartilage, which can result in focal ischemia making faster growing bones increasingly susceptible to degenerative necrosis (Aggrey, 2003).

Heritability estimates were also found to differ between the two lines. As mentioned previously, Line B overall exhibited a higher level of susceptibility towards leg weakness, especially for tibial lesions. It appeared likely based on an examination of the data that a weighting system could be beneficial for estimating heritabilities. Since tibial lesions in Line B were so prevalent and seemed to have sufficient variability in severity, the tibial scores were used as one trait within the weighting system. As previous research has proven, most cases of BCO develop after 35 days of age (McNamee et al., 1999). Those findings tended to be confirmed in the present experiment, and suggest the possibility that birds affected by leg weakness prior to 35 days of age may be genetically predisposed to leg weakness. To address this possibility a new trait was developed. The new trait, days to lame (DTL), took into consideration how early in the flock’s life the bird developed leg weakness. For example, if a bird began to show clinical signs of leg weakness at 20 days of age and the flock was to be processed at 56 days of age then the DTL for that bird would be 36. Tibial severity scores and DTL were the two traits used for estimating heritability in Line B. To determine the ideal weight that should be placed on each trait a series of four equations were evaluated for Line B as shown in Table 8. Originally it was thought to put equal weight on both traits; however when that did
not result in a favorable heritability the weighting scheme was adjusted to place more emphasis on DTL starting with 0.7 for DTL and 0.3 for the tibial head severity score. The equation was adjusted several times until it was concluded that the highest heritability was found when considering DTL as the only trait. When considering Line D a weighting system was also involved, however because Line D and Line B clearly differed in their susceptibility to BCO, the equation used for Line B was not useful in estimating a heritability for Line D. Since Line D initially exhibits slower growth, almost all birds from this line developed clinical signs of leg weakness after day 35, thereby reducing the weight placed on the DTL trait. However, knowledge that Line D was most susceptible to femoral lesions allowed heritability to be estimated by using the total femur score (right femur + left femur). In Table 8 it should be noted that the standard error for the estimated heritabilities are large; however, with a larger sample size and more uniformed experimental design the standard error should decrease. These preliminary heritability results show that there may be potential to decrease the incidence of this pathological condition through genetic selection.

This study clearly demonstrates that, although birds may not show signs of clinical lameness such as having a noticeable limp or wing dipping and are considered “healthy”, nevertheless many of these birds are still likely to have mild BCO lesions. The average severity scores in this study for “healthy” birds was over 1 (severity scores over 1 means a lesion was present in at least one of the four joints) for AvgF, AvgT, and LS. Since non-lame birds are frequently found with BCO lesions and because these lesions are known to be progressive, these observations support that different birds are able to withstand different thresholds of lesion severity before becoming completely immobilized. The difference in AvgF severity scores for lame and non-lame birds is 0.13. While this deviation is considered significant, it is much
smaller than the deviations between lame and non-lame birds for AvgT and LS, 0.61 and 0.37, respectively. This may suggest that many birds are capable of withstanding more severe femoral lesions before showing clinical signs of lameness.

When looking at the relationship between leg severity scores and body weights it is obvious that BCO is an animal welfare concern. We found the highest AvgT and LS severity scores are present in class 1 and class 2. This is clear indication that the more severe lesions are having a negative impact on the well being of the bird by perhaps reducing activity, feeding and watering behaviors, causing sub-clinically affected birds to gain weight at a slower rate or in some cases causing birds to lose weight. However, the highest AvgF severity scores were found in birds that fell within class three (the heaviest weight class), once again implying that birds may be able to withstand more severe femoral lesions while maintaining normal activity and eating habits.

Once it was established that inserting SB into pens at 28 and 42 days of age would induce lameness at rates similar to that of flat wire flooring, the next step was to adjust the design of the SB model in a way to induce a higher incidence of lameness. In Table 8 it is shown that the SB model inducing the most severe leg measurement scores in combination with a numerically high incidence of lameness was the SB model of which was placed underneath the nipple waterer line (SBW50%). The prolonged amount of time that was involved while balancing on the apex of the SB during drinking likely induced an additional amount of torque (twisting notion) on the broilers legs resulting in higher severity scores and incidence of lameness. The SBL66% model resulted in a 30 % incidence of lameness which is higher than the 25 % incidence of lameness induced by the SBW50% model; however, the leg measurement scores induced by the SBL66% model were among of the lowest severity scores between the five SB models. It was observed in
broilers had a tendency to attempt to fly off the SBL66% model instead of cautiously walking down. The increased steepness of the 66% slope altered the behavior of the bird, possibly resulting in lameness due to the traumatic force of repeatedly jumping off the SB instead of lameness related to the formation of BCO lesions. In the beginning it was hypothesized that the parallel ceiling of the PT model would force birds to traverse up and down the SB model in a crouched position putting additional stress on the leg joints. It was observed that the PT model failed to induce lameness at the high rates previously hypothesized. Throughout the study it was observed that birds raised with the PT model were forced to move up and down the model in the desired crouching (or duck walking) position; however, the required stress and torque needed to induce micro fractures in the leg joints that ultimately leads to lameness may have been absent due to the cautious behavior of the bird while crossing the SB.

As seen in both experiments the occurrence of BCO progressively begins to increase between 28-35 days of age (Figure 15). These results are consistent with previous studies finding the majority of BCO cases occurring around 35 days of age (McNamee et al., 1999) with the hypothesis that broilers’ leg bones simply don’t develop at a sufficient rate to support the growth potential of the modern broiler (Wise, 1970).
Conclusion

Many questions were answered in the present study. First it was determined that different commercial broiler lines develop BCO lesions at different frequencies in different proximal leg bones (i.e. tibiae vs. femora). Line B had a higher susceptibility to overall leg weakness and tibial head lesions, while line D was less susceptible to leg weakness but more prone to developing proximal femoral head lesions. The second question answered, involved determining the ideal time to introduce SB into litter flooring pens with the objective of inducing consistently high frequencies of lameness related to BCO. Introducing SB at 28 days of age proved to induce the highest lameness frequency in combination with the highest severity scores for all three leg measurements (AvgF, AvgT, and LS). After determining the ideal time to introduce SB into litter floor pens, the main objective of Experiment 2 was to develop an improved SB model that could consistently induce reasonably high incidences of BCO lesions. The SBW50% model was found to be an adequate model for consistently inducing BCO lesions as well as being a practical model for industry use. A scoring system also was developed to help quantitate BCO. Evaluating the frequency of lameness is adequate and advantageous when considering that BCO is a progressive disease. Analysis in this manner eliminates the progressive aspect of the condition by determining whether the bird has BCO in a “yes” or “no” manner. However, there are advantages to using the severity scoring system shown in Table 2. This method may prove to be useful for populations that are highly susceptible to BCO in which finding variation within the population is needed. Numerical values also prove to be more efficient when genetic parameters must be calculated.

Recent research as well industry selection has begun to place an increased emphasis on improving skeletal development. This effort has been rewarded with the reduction of TD and
VVD in commercial broiler flocks. It is important to continue pursuing improvements. Without constant attention being placed on skeletal development, the foundation of the bird, maximum growth potential cannot be achieved as the bird will be unable to support the increased weight load brought on by the selection for a higher yielding and more efficient broiler. Animal welfare issues must also be taken into account. Broilers affected from leg weakness are likely to be experiencing pain, thereby explaining why broilers exhibiting the highest AvgT and LS severity scores were found in the lower weight classes. Presumably the more severe lesions were affecting the birds’ eating and drinking habits. Further investigation is needed such as the mapping of blood vessels throughout leg bones, correlating bone density values with BCO lesions, comparing lower body yield versus upper body yield in relation to leg weakness, genetic studies to determine if the occurrence of the present condition can be decreased through genetic selection, as well as developing non-invasive methods for determining if a bird has developed BCO lesions.
<table>
<thead>
<tr>
<th>U</th>
<th>Unknown cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Femoral Head and proximal tibia are normal</td>
</tr>
<tr>
<td>Cull</td>
<td>Individuals unable to grow at expected rates</td>
</tr>
<tr>
<td>TD</td>
<td>Tibia Dyschondroplasisa</td>
</tr>
<tr>
<td>SDS</td>
<td>Sudden Death Syndrome (heart attack, flipover)</td>
</tr>
<tr>
<td>PHS</td>
<td>Pulmonary Hypertension Syndrome (Ascites)</td>
</tr>
<tr>
<td>KB</td>
<td>Kinky Back (Spondylolisthesis)</td>
</tr>
<tr>
<td>TW</td>
<td>Twisted Leg or Slipped Tendon</td>
</tr>
<tr>
<td>FHS</td>
<td>Femoral Head Separation (epiphyseolysis)</td>
</tr>
<tr>
<td>FHT</td>
<td>Proximal Femoral Head Transitional Degeneration</td>
</tr>
<tr>
<td>FHN</td>
<td>Proximal Femoral Head Necrosis</td>
</tr>
<tr>
<td>THN</td>
<td>Proximal Tibial Head Necrosis</td>
</tr>
</tbody>
</table>
Table 2. Severity Scores Associated with Respective Proximal Bone Category

<table>
<thead>
<tr>
<th>Femur Bone Category</th>
<th>Assigned Femur Score</th>
<th>Tibial Bone Category</th>
<th>Assigned Tibial Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td>FHS</td>
<td>2</td>
<td>THN</td>
<td>2</td>
</tr>
<tr>
<td>FHT</td>
<td>3</td>
<td>THNs</td>
<td>3</td>
</tr>
<tr>
<td>FHN</td>
<td>4</td>
<td>THNc</td>
<td>4</td>
</tr>
</tbody>
</table>

1N=Normal Proximal Femoral Head, FHS= Proximal Femoral Head Separation, FHT= Proximal Femoral Head Transitional Degeneration, FHN= Proximal Femoral Head Necrosis

2N= Normal Proximal Tibial Head, THN= Proximal Tibial Head Necrosis, THNs= Proximal Tibial Head Necrosis Severe (growth plate undermined), THNc= Proximal Tibial Head Necrosis with Caseaus Exudate
Table 3. Cumulative frequency of lameness by floor treatment and line

<table>
<thead>
<tr>
<th>Floor Treatment</th>
<th>Line B</th>
<th>Line D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter</td>
<td>13.1 % (17/130)c</td>
<td>10.8 % (14/130)b</td>
<td>0.704</td>
</tr>
<tr>
<td>SB-14</td>
<td>22.3 % (29/130)bc</td>
<td>12.3 % (16/130)ab</td>
<td>0.156</td>
</tr>
<tr>
<td>SB-28</td>
<td>23.1 % (30/130)b</td>
<td>15.4 % (20/130)ab</td>
<td>0.049</td>
</tr>
<tr>
<td>SB-42</td>
<td>23.1 % (30/130)b</td>
<td>15.4 % (20/130)ab</td>
<td>0.049</td>
</tr>
<tr>
<td>Wire</td>
<td>39.1 % (86/220)a</td>
<td>21.4 % (47/220)a</td>
<td>0.001</td>
</tr>
</tbody>
</table>

a-c Means by line within a column with no common superscripts are different ($P \leq 0.05$).
1Chicks from Line B and D were placed at one day of age in pens containing flat wire flooring (Wire) or clean wood shavings (Litter). Birds in the remaining three floor treatments were raised on clean wood shavings until 14 (SB-14), 28 (SB-28), or 42 (SB-42) days of age when SB with a 50% slope were inserted into the respective pens.
Table 4. Leg quality measurement scores for each floor treatment

<table>
<thead>
<tr>
<th>Floor Treatment</th>
<th>L</th>
<th>SB-14</th>
<th>SB-28</th>
<th>SB-42</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>AvgF²</td>
<td>1.77 ± .04b</td>
<td>1.82 ± .04b</td>
<td>1.87 ± .04ab</td>
<td>1.78 ± .04b</td>
<td>1.96 ± .04a</td>
</tr>
<tr>
<td>AvgT³</td>
<td>1.56 ± .04b</td>
<td>1.68 ± .04a</td>
<td>1.68 ± .04a</td>
<td>1.56 ± .04b</td>
<td>1.77 ± .03a</td>
</tr>
<tr>
<td>LS⁴</td>
<td>1.67 ± .02c</td>
<td>1.75 ± .03b</td>
<td>1.77 ± .02b</td>
<td>1.67 ± .02c</td>
<td>1.86 ± .02a</td>
</tr>
</tbody>
</table>

a-c Means by trait within a row with no common superscript are different \((P \leq 0.05)\)

¹Chicks from Line B and D were placed at one day of age in pens containing flat wire flooring (Wire) or clean wood shavings (Litter). Birds in the remaining three floor treatments were raised on clean wood shavings until 14 (SB-14), 28 (SB-28), and 42 (SB-42) days of age when SB with a 50% slope were inserted into the respected pens. ²Average Femur Score ((Right Femur + Left Femur) / 2). ³Average Tibia Score ((Right Tibia + Left Tibia) / 2). ⁴Overall leg score ((AvgF + AvgT) / 2).
<table>
<thead>
<tr>
<th>Trait</th>
<th>Class 1 (1.83-2.99kg)</th>
<th>Class 2 (3.00-3.99kg)</th>
<th>Class 3 (4.00-5.09kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AvgF²</td>
<td>1.89 ± .07ab</td>
<td>1.80 ± .02b</td>
<td>1.93 ± .03a</td>
</tr>
<tr>
<td>AvgT³</td>
<td>1.80 ± .06a</td>
<td>1.58 ± .02b</td>
<td>1.30 ± .03c</td>
</tr>
<tr>
<td>LS⁴</td>
<td>1.84 ± .04a</td>
<td>1.69 ± .01b</td>
<td>1.62 ± .02c</td>
</tr>
</tbody>
</table>

a-c Means by trait within a row with no common letter are different ($P \leq 0.05$)

1Body Weights (kg) ranged from 1.83 kg to 5.09 kg. Based on their body weight, broilers were assigned to Class 1 (1.83-2.99kg) Class 2 (3.00-3.99kg) Class 3 (4.00-5.09kg). 2 Average Femur Score (($\text{Right Femur} + \text{Left Femur}) / 2$). 3 Average Tibia Score (($\text{Right Tibia} + \text{Left Tibia}) / 2$). 4 Overall leg score (($\text{AvgF} + \text{AvgT}) / 2$).
Table 6. Line by Sex Interaction for AvgF and LS leg quality measurement scores

<table>
<thead>
<tr>
<th>Leg Quality</th>
<th>Measure</th>
<th>Line</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AvgF(^1)</td>
<td>B</td>
<td>1.62 ± .04 ***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>2.02 ± .03 *</td>
</tr>
<tr>
<td></td>
<td>LS(^2)</td>
<td>B</td>
<td>1.75 ± .02 ***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>1.65 ± .02</td>
</tr>
</tbody>
</table>

\(^1\)Average Femur Score (\(\text{Right Femur} + \text{Left Femur} / 2\)). \(^2\)Overall leg score (\(\text{AvgF} + \text{AvgT} / 2\)). Symbols between genders represent gender differences. Symbols between lines represent line differences. *, \(P \leq 0.02\) ***, \(P \leq 0.001\)
Birds were placed at 1 day of age in pens containing flat wire flooring (W) and clean wood shavings (L). Birds were placed in pens containing clean wood shavings and SB with a 50% slope were inserted into pens at 14 (SB-14), 28 (SB-28), or 42 (SB-42) days of age.

Lame, represents birds developing lameness between 14-56 days of age and Non-Lame represents birds that were humanely euthanized and necropsied at the end of the 8 week trial.

Average Femur Score ((Right Femur + Left Femur) / 2 )

** (P < 0.01)  *** (P < 0.001)

Table 7. Line by floor and mortality by floor interactions for leg quality measurement score

<table>
<thead>
<tr>
<th>Leg Quality</th>
<th>Main Effect</th>
<th>Floor</th>
<th>Line</th>
<th>SB-14</th>
<th>SB-28</th>
<th>SB-42</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AvgF</strong>2</td>
<td></td>
<td></td>
<td></td>
<td>1.68 ± .05bc</td>
<td>1.63 ± .05c</td>
<td>1.75 ± .05ab</td>
<td>1.58 ± .05c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td></td>
<td></td>
<td>1.85 ± .05</td>
<td>2.02 ± .05</td>
<td>1.98 ± .05</td>
<td>1.96 ± .05</td>
</tr>
<tr>
<td>Mortality3</td>
<td></td>
<td></td>
<td></td>
<td>1.73 ± .11b</td>
<td>1.88 ± .10b</td>
<td>1.74 ± .09b</td>
<td>1.72 ± .10b</td>
</tr>
<tr>
<td><strong>AvgF</strong>3</td>
<td></td>
<td></td>
<td></td>
<td>1.77 ± .04</td>
<td>1.81 ± .04</td>
<td>1.90 ± .04</td>
<td>1.78 ± .04</td>
</tr>
<tr>
<td>None-Lame</td>
<td></td>
<td></td>
<td></td>
<td>1.82 ± .13b</td>
<td>2.21 ± .10a</td>
<td>2.22 ± .10a</td>
<td>1.98 ± .10ab</td>
</tr>
<tr>
<td><strong>AvgT</strong>4</td>
<td></td>
<td></td>
<td></td>
<td>1.52 ± .04</td>
<td>1.55 ± .04</td>
<td>1.52 ± .04</td>
<td>1.45 ± .04</td>
</tr>
</tbody>
</table>

1Birds were placed at 1 day of age in pens containing flat wire flooring (W) and clean wood shavings (L). Birds were placed in pens containing clean wood shavings and SB with a 50% slope were inserted into pens at 14 (SB-14), 28 (SB-28), or 42 (SB-42) days of age. 2Average Femur Score ((Right Femur + Left Femur) / 2) 3Lame, represents birds developing lameness between 14-56 days of age and Non-Lame represents birds that were humanely euthanized and necropsied at the end of the 8 week trial. 4Average Tibia Score ((Right Tibia + Left Tibia) / 2). **, (P ≤ 0.01) *** (P ≤ 0.001)
Table 8. Heritability estimates

<table>
<thead>
<tr>
<th>Trait</th>
<th>Heritability</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3, 0.7</td>
<td>0.47</td>
<td>0.17</td>
</tr>
<tr>
<td>Line B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2, 0.8</td>
<td>0.49</td>
<td>0.17</td>
</tr>
<tr>
<td>0.1, 0.9</td>
<td>0.51</td>
<td>0.17</td>
</tr>
<tr>
<td>DTL</td>
<td>0.52</td>
<td>0.17</td>
</tr>
<tr>
<td>0.7, 0.3</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Line D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8, 0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>0.9, 0.1</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>TF</td>
<td>0.17</td>
<td>0.12</td>
</tr>
</tbody>
</table>

1Weighting scheme used for determining the value to give to each individual bird allowing the researcher to estimate heritability. 230% weight put on total tibia score (right tibia + left tibia) and 70% weight put on days to lame (DTL). 320% weight put on total tibia score (TT) and 80% weight put on DTL. 410% weight put on total tibia score (TT) and 90% weight put on DTL. 5DTL was the only trait used to estimate heritability. 670% weight put on total femur (TF) and 30% weight put on DTL. 780% weight put on total femur (TF) and 20% weight put on DTL. 890% weight put on total femur (TF) and 10% weight put on DTL. 9TF was the only trait used to estimate heritability.
Table 9. Leg quality measurement scores for each floor treatment and cumulative frequency

<table>
<thead>
<tr>
<th>Measure</th>
<th>L</th>
<th>PT</th>
<th>SB50%</th>
<th>SBL50%</th>
<th>SBL66%</th>
<th>SBW50%</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>AvgF$^2$</td>
<td>1.77 ± .04bcd</td>
<td>1.75 ± .8abcd</td>
<td>1.94 ± .70abc</td>
<td>1.64 ± .60cd</td>
<td>1.63 ± .60d</td>
<td>2.04 ± .90a</td>
<td>1.99 ± .80ab</td>
</tr>
<tr>
<td>AvgT$^3$</td>
<td>1.77 ± .60c</td>
<td>2.45 ± .04ab</td>
<td>2.21 ± .70b</td>
<td>2.14 ± .80b</td>
<td>2.22 ± .70b</td>
<td>2.19 ± .80b</td>
<td>2.51 ± .80a</td>
</tr>
<tr>
<td>LS$^4$</td>
<td>1.77 ± .50c</td>
<td>2.10 ± .50ab</td>
<td>2.08 ± .60a</td>
<td>1.89 ± .40bc</td>
<td>1.92 ± .50bc</td>
<td>2.12 ± .60ab</td>
<td>2.25 ± .60a</td>
</tr>
<tr>
<td>% Lame</td>
<td>18 %</td>
<td>17 %</td>
<td>22 %</td>
<td>18 %</td>
<td>30 %</td>
<td>25 %</td>
<td>33 %</td>
</tr>
<tr>
<td></td>
<td>(69/390)b</td>
<td>(22/130)b</td>
<td>(57/260)b</td>
<td>(24/130)b</td>
<td>(39/130)a</td>
<td>(66/260)ab</td>
<td>(85/260)a</td>
</tr>
</tbody>
</table>

$^1$Chicks in study 2 were placed at one day of age in pens containing flat wire flooring (W) or clean wood shavings (L). Birds were placed in pens containing clean wood shavings with SB models inserted at 28 days of age. Pagoda Top model (PT) with a ceiling line running parallel to the SB, original SB model with a 50% slope (SB 50%), SB model with a 50% slope and an adjustable bar over the apex (SBL50%), SB model with a 66% slope and an adjustable bar over the apex (SBL66%), SB model with a 50% slope placed under a nipple water line. $^2$ Average Femur Score ((Right Femur + Left Femur) / 2). $^3$ Average Tibia Score ((Right Tibia + Left Tibia) / 2). $^4$ Overall leg score ((AvgF + AvgT) / 2).
Figure 1. Figure 1. Progressive Stages of BCO Affecting the Proximal Femoral Head

Figure 1. Square 1 shows a normal (N) femoral head with all the articular cartilage attached. FHS or epiphyseal separation is shown in square 2 with the articular cartilage being separated without damage being done to the growth plate. Squares 3-5 show femoral heads diagnosed as FHT, separation of the articular cartilage along with erosion and ulceration of the growth plate. The last three squares (6-8) show femoral heads diagnosed as FHN, when perforation, fracturing, and necrosis of the femoral head occurred.
Figure 2. Progressive BCO Stages of Affecting the Proximal Tibia Head.

The figure above is showing the progressive stages of BCO that occur in the proximal tibia head. Starting on the top row going left to right, the first tibia head appears normal (N) with no macroscopic lesions. The second tibia is expressing the beginning stages of BCO, tibiae of this nature were diagnosed as Proximal Tibia Head Necrosis (THN) showing the beginning formations of necrotic voids and lytic channels. Squares 3-5 are tibiae diagnosed as Proximal Tibia Head Necrosis Severe (THNs), showing large necrotic voids and deep lytic channels clearly compromising the support for the growth plate. Squares 6-10 are examples of tibiae diagnosed as Proximal Tibia Head Necrosis, Caseous (THNc). THNc is a more advanced stage of THNs with obvious bacterial infiltration and sequestrate.
Figure 3. Study 1 Pen Diagram.

Figure 3. Line B was grown in pens 1-11 and Line D was grown in pens 12-22. Floor treatments are designated in this diagram for each pen. W= flat wire flooring, L= litter flooring, SB-14= Speed Bump inserted at 14 days of age (DOA), SB-28= Speed Bump inserted at 28 DOA, SB-42=Speed Bump inserted at 42 DOA.
Figure 4. Diagram of Speed Bump used in study 1 for floor treatments SB-14, SB-28, and SB-42. This Speed Bump has a 50% slope and is 1.54 m in length, 91.44 cm wide at the base, and 22.86 cm tall. Slope was found by using the following formula: \((\text{height} \div (0.50 \times \text{base}))\) or \((22.86 \div (0.50 \times 91.44))\).
Figure 5. Above is a top view layout of each pen in study 1 and 2 after SB were inserted at their respective times. Two tube feeders were placed in the front of the pen with the two SB joined together in the center and the nipple waterer in the back of the pen. Nipple waterers and tube feeders being placed on opposite sides of the SB forced birds to traverse over the SB in order to reach feed and water. Pen dimensions were 10 x 10 requiring two SB to be joined together in order to cover the full width of the pen.
Figure 6. Pen diagram for study 2. Line B was grown in pens 1-12 and Line A was grown in pens 13-24. Floor treatments are designated for each pen. W= flat wire flooring, L= Litter, SB50%= Speed Bump with 50% slope, SBL50%= Speed Bump with a 50% slope and an adjustable limbo bar placed over the apex, SBW50%= Speed Bump with a 50% slope place underneath water line, SBL66%= SB with a 66% slope and an adjustable limbo bar placed over the apex, PT= Speed Bump with a pagoda top ceiling running parallel to the Speed Bump.
Figure 7. SBL50% Diagram

Figure 7. This SB was used in study 2 and has the same dimensions as SB50% (Figure 2), only difference being the adjustable limbo bar over the apex of the speed bump.

Figure 8. SBL66% Diagram

Figure 8. Speed Bump used in study 2. Length and base width are the same as SB50% and SBL50%; however, this Speed Bump is slightly taller, increasing the percent slope to 66%. It was anticipated that the increased slope would increase the mechanical stress on the legs.
Figure 9. Speed Bump used in study 2. The apex of a SB50% was placed directly underneath a line of nipple waterer. This resulted in birds walking up the Speed Bump and balancing on the apex while drinking.

Figure 10. Speed Bump model used in study 2 with an adjustable ceiling suspended above and parallel to the apex of Speed Bump. The adjustable ceiling resulted in birds walking up and down the slope in a crouched position.
Figure 11. Study 1 Mean Leg Quality Scores for Line B and D

Figure 11. Line differences within severity scores represented by *** ($P \leq 0.001$).
Figure 12. Study 1 Mean Leg Quality Scores between Sexes

Figure 12. Sex differences within severity scores represented by ***(P < 0.001).***
Figure 13. Study 1 Mean Leg Quality Scores between Lame and Non-Lame

Figure 13. Differences represented by *** (P < 0.001)
Figure 14. Frequency of lameness in broilers in Line A and B when reared on different floor treatments. Differences represented by *** (P < .001)
Figure 15. Cumulative Percentage of BCO Related Lameness for all Floor Treatments on a Weekly Basis for Study 1 and 2 Combined

Figure 15. Cumulative frequency of lameness on a weekly basis with all birds and treatments from Study 1 and 2 being pooled.
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