A wire-flooring model[1] for inducing lameness in broilers: Evaluation of probiotics as a prophylactic treatment

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ABSTRACT Bacterial chondronecrosis with osteomyelitis (BCO) is the most common cause of lameness in commercial broilers. Bacteria entering the blood via translocation from the respiratory system or gastrointestinal tract spread hematogenously to the proximal epiphyseal-physeal cartilage of rapidly growing femora and tibiae, causing BCO. We tested the hypothesis that rearing broilers on wire flooring should increase the incidence of BCO by persistently imposing additional torque and shear stress on susceptible leg joints. We also tested the hypothesis that probiotics might attenuate bacterial translocation and thereby reduce the incidence of BCO. In 5 independent experiments using 4 commercial lines, broilers grown on wire flooring developed lameness attributable predominately to BCO. The fastest-growing birds were not necessarily the most susceptible to lameness on wire flooring, nor did the genders differ in susceptibility in the 2 experiments that included both male and female broilers. The pathogenesis of BCO is not instantaneous, and accordingly, many broilers that did not exhibit lameness, nevertheless, did possess early pathognomonic lesions. These subclinical lesions were equally likely to develop in the right or left leg. The lesion status of the proximal femoral head did not determine the lesion status of the ipsilateral or contralateral proximal tibial head and vice versa. Broilers reared on wire flooring consistently had higher incidences of lameness than hatch-mates reared on wood-shavings litter. Adding probiotics to the diet beginning at 1 d of age consistently reduced the incidence of lameness for broilers reared on wire flooring. These experiments indicate that probiotics administered prophylactically may constitute an alternative to antibiotics for reducing lameness attributable to BCO. Rearing broilers on wire flooring provides an important new research model for investigating the etiology, pathogenesis, and treatment strategies for BCO.

Key words: broiler, lameness, osteomyelitis, flooring, probiotics

INTRODUCTION

Leg disorders and lameness attributable to a variety of etiologies adversely affect the performance and well-being of poultry and persistently elevate the morbidity and mortality in broiler flocks. Sporadic episodes of bacterial chondronecrosis with osteomyelitis (**BCO**; formerly known as femoral head necrosis, proximal femoral degeneration, or bacterial chondronecrosis, **BCN**) can cause substantial economic losses. The term BCO encompasses necrotic degeneration and microbial infec-

tion primarily within the proximal head (articular cartilage or epiphysis, growth plate or physis, and metaphysis) of the femur and tibiotarsus (hereafter referred to as the tibia), with the caveat that other rapidly growing bones, including the vertebrae, also may be affected (e.g., spondylopathy or spondylitis) (Carnaghan, 1966; Wise, 1971; Nairn and Watson, 1972; Nairn, 1973; McCaskey et al., 1982; Mutalib et al., 1983a; Griffiths et al., 1984; Duff, 1990a; 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). Bacterial chondronecrosis with osteomyelitis has been diagnosed in broilers in Australia, Canada, Europe, and the US and is considered the most common cause of lameness in commercial broilers (Pattison, 1992; McNamee et al., 1998; Butterworth, 1999; McNamee and Smyth, 2000; Bradshaw et al., 2002; Dinev, 2009). Multiple opportunistic organisms have been isolated from BCO lesions,

including predominately *Staphylococcus aureus* and *Escherichia coli*, often in mixed cultures with other microbes, including *Salmonella* spp. (Nairn and Watson, 1972; Andreasen et al., 1993; Tate et al., 1993; Thorp et al., 1993; McNamee et al., 1998; Butterworth, 1999; Joiner et al., 2005; Dinev, 2009; Kense and Landman, 2011).

A complex pathogenesis for BCO has been proposed (Butterworth, 1999; McNamee and Smyth, 2000). High growth rates impose excessive torque and shear stress on the structurally immature cartilaginous growth plates of the proximal leg bones and thoracic vertebrae. These mechanical forces cause microfracturing and cleft formation (osteochondrosis) within the epiphyseal and physeal cartilage. Osteochondrotic clefts often truncate blood vessels penetrating the columns of cartilage cells, contributing to focal ischemia and necrosis. Local ischemia also has been attributed to sluggish blood flow and thrombosis caused by mechanical compression of the cartilage layers, the resting posture and inactivity of fully fed broilers, and an excessive resistance to flow through long, narrow metaphyseal vascular channels (Wise, 1971; McCaskey et al., 1982; Riddell et al., 1983; Duff, 1984a,b,c, 1985, 1989a,b, 1990a,b; Julian, 1985; Duff and Randall, 1987; Thorp, 1988, 1994; Thorp and Duff, 1988; Riddell, 1992; Thorp et al., 1993; Thorp and Waddington, 1997; Thorp, 1994; McNamee et al., 1998; Bradshaw et al., 2002; Dinev, 2009). The arterioles supplying the hypertrophic zone of the growth plate terminate in the metaphyseal vascular plexus, consisting of a tuft of capillaries and venules undergoing rapid angiogenesis and possessing a fenestrated endothelium. The fenestrations are large enough to permit cellular elements of the blood to pass into spaces within the cartilaginous matrix (Beaumont, 1967; Lutfi, 1970; Hunt et al., 1979; Howlett, 1980; Emslie and Nade, 1983, 1985; Howlett et al., 1984). Bacteria transmitted to chicks from breeder parents, contaminated eggshells, or hatchery sources (Skeeles, 1997; McCullagh et al., 1998; Rodgers et al., 1999; McNamee and Smyth, 2000; Kense and Landman, 2011) or that enter the chick's circulation via the respiratory system or gastrointestinal tract (Mutalib et al., 1983a,b; Andreasen et al., 1993; Thorp et al., 1993; McNamee et al., 1999) spread hematogenously and can exit the bloodstream through the fenestrated endothelium at the tips of the metaphyseal vascular plexus (Emslie and Nade, 1983, 1985). The translocated bacteria adhere directly to the cartilage matrix, they colonize osteochondrotic clefts and zones of necrosis, and they form obstructive emboli in the metaphyseal vasculature (Emslie and Nade, 1983; Emslie et al., 1983; Speers and Nade, 1985; Alderson et al., 1986; Alderson and Nade, 1987; Thorp, 1988; Thorp et al., 1993; McNamee et al., 1998, 1999; McNamee and Smyth, 2000). Immune cells may not readily gain access to the isolated foci of bacteria, and immunosuppression caused by chicken anemia virus, infectious bursal disease virus, or environmental stressors can facilitate microbial proliferation (Mutalib et al., 1983a; Andreasen

et al., 1993; McNamee et al., 1998, 1999; Huff et al., 2000). The growth plates migrate longitudinally with the ends of growing bones, leaving behind previously formed bacterial sequestrae as focal zones of necrosis or large fibrinonecrotic abscesses in the metaphysis and diaphysis (Emslie and Nade, 1983; Emslie et al., 1983, 1984; Daum et al., 1990; Thorp et al., 1993; Skeeles, 1997). Lytic substances released at sites of bacterial colonization promote generalized necrosis within the calcifying zone of the metaphysis, destroying the vasculature and eliminating struts of trabecular bone that normally provide structural support for the epiphyseal and physeal cartilage (Emslie and Nade, 1983; Emslie et al., 1984; Wyers et al., 1991). Bacteria penetrating through the growth plate to the epiphysis via transphyseal vessels trigger septic arthritis of the hock and hip joints (Emslie et al., 1984; Emslie and Nade, 1985; Alderson et al., 1986; Alderson and Nade, 1987; Thorp, 1988; McNamee et al., 1998; Daum et al., 1990; Joiner et al., 2005).

It has been difficult to investigate the etiology, pathogenesis, and treatment strategies for BCO because the incidence typically is low in research flocks. Previously, BCO has been reproduced by injecting broilers and turkeys intravenously with appropriate strains of *Staphylococcus* spp. in quantities sufficient to sustain bacteremia without triggering overt septicemia (Carnaghan, 1966; Nairn, 1973; Emslie and Nade, 1983, 1985; Emslie et al., 1983; Mutalib et al., 1983a; Griffiths et al., 1984; Alderson et al., 1986; Daum et al., 1990). One objective of the present study was to create an experimental model for reliably triggering BCO in commercial broilers without purposefully exposing the flock to known pathogens. Leg disorders and lameness are commonplace when broilers are reared in cages with wire flooring. The categories of disorders associated with cage rearing include high incidences of breast blisters, subclinical reductions in overall skeletal mineralization, reductions in bone strength, valgus-varus deformities, and lameness caused by slipped tendons (twisted leg, perosis, or chondrodystrophy). These disorders have been attributed to the high stocking densities and lack of exercise in cage rearing systems. Cage rearing systems and alternative floor rearing systems apparently do not induce significant incidences of BCO (Haye and Simons, 1978; Andrews et al., 1990; Riddell et al., 1983; Bradshaw et al., 2002). Our wire flooring model reliably triggers BCO by rearing broilers at relatively low densities in pens large enough to permit normal levels of activity. It is our hypothesis that the sustained footing instability induced by wire flooring creates persistent additional mechanical torque and shear stress on susceptible leg joints. Presumably, the resulting microtrauma to the epiphyseal-physeal cartilage should facilitate colonization by blood-borne bacteria translocated from the respiratory and gastrointestinal tracts. With regard to the intestinal tract as a potential source of bacterial translocation, a second objective was to evaluate the effect of probiotics on the incidence of lameness.

In view of concerns regarding the development of anti-biotic resistance in bacteria commonly associated with osteomyelitis (McNamee and Smyth, 2000; Waters et al., 2011), probiotics potentially can provide a plausible alternative for prophylactically reducing the incidence of BCO. Probiotics may interfere with the development of osteomyelitis by attenuating intestinal populations of pathogenic bacteria, improving gut health to reduce bacterial leakage (translocation) across the gut wall, or by priming the immune system to better eliminate translocated bacteria.

MATERIALS AND METHODS

Animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocols #08036 and #11002). Environmental chambers ($3.7 \times 2.5 \times 2.5$ m, length $\times$ width $\times$ height) within the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm were used to conduct 5 independent experiments. The chambers use single-pass ventilation at a constant rate of 6 m^3/min. Each chamber contained a rectangular pen with dimensions of 3×1.5 m with flooring consisting of clean wood-shavings litter or raised wire panels. The wire panels were constructed from 5×5 cm lumber and were 3-m long and 1.5-m wide, with 5×5 cm cross-members added for support. Hardware cloth (1.3×2.54 cm mesh = 0.5 inch $\times$ 1 inch, 0.063 gauge, galvanized welded wire cloth; Direct Metals, Kennesaw, GA) was fastened to the top of the frame and cross-members. The panels were elevated on 30-cm high masonry blocks to permit manure to pass through and accumulate underneath the wire surface. Tube feeders were positioned at the front of the pen, and nipple waterers were positioned at the back of the pen, thereby forcing the chicks to traverse the length of the floor to eat and drink. Between experiments, the pens were disassembled and the chambers and wire-flooring panels were thoroughly cleaned using a pressure washer and detergent.

Broiler chicks from 3 different commercial hatcheries were placed at $\geq$60 per pen at 1 d of age (approximately 690 cm^2/chick). Chicks received standard hatchery vaccinations for Marek's, tenosynovitis, and infectious bursal disease virus. At 14 d of age, the population was reduced to between 50 and 55 of the largest, healthiest chicks per pen (approximately 900 cm^2/chick). The d 14 culling protocol was instituted because necropsies of runts during the first 10 d revealed macroscopic evidence of systemic bacterial infection, including osteomyelitis. The photoperiod was set for 23L:1D. Thermoneutral temperatures were maintained throughout: 32°C for d 1 to 3, 30°C for d 4 to 6, 28°C for d 7 to 10, 26°C for d 11 to 14, and 24°C thereafter. Feed and water were provided ad libitum. The control diet was a commercial 23% CP corn and soybean meal-based chick starter (crumbles) formulated to meet or exceed

minimum NRC (1994) standards for all ingredients. In experiments 1 to 4, the experimental diet consisted of control feed mixed with the Biomin probiotic PoultryStar (Biomin Holding GmbH, Herzogenburg, Austria; 22.7 kg of the control feed mixed with 12.5 g of probiotic). PoultryStar is a proprietary blend of dried *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici*, and *Lactobacillus reuteri*. In experiment 5, the experimental diet consisted of the control feed mixed with the Biomin IMBO probiotic (Biomin Holding GmbH; 22.7 kg of the control diet mixed with 12.5 g of probiotic). Biomin IMBO probiotic is a single-microbe probiotic [*Enterococcus faecium* DSM 3530 (Biomin IMB52)] that has been approved for use in Europe and therefore may be of use to reduce lameness in EU poultry flocks.

Birds in all pens were observed walking every 2 d beginning on d 14. Lameness typically began after d 35 and progressed rapidly in birds that 24 to 48 h previously appeared to be healthy. Affected broilers had difficulty standing, exhibited an obvious limping gait while dipping one or both wing tips, and finally were completely immobilized. Lame birds were removed as soon as the onset of lameness was noticed and were euthanized via CO$_2$ gas inhalation. As was indicated by Dinev (2009), lame birds with BCO can die quickly because they have difficulty accessing food and water. Therefore, birds found dead also were necropsied to assess leg lesions. All survivors on d 56 were considered to be clinically healthy. In 3 experiments, these survivors were weighed, killed via CO$_2$ gas inhalation, and necropsied to assess subclinical lesion incidences in the proximal heads of the femora and tibiae.

Experiment 1 (December 2009)

Pilot studies indicated that broilers reared in pens with full wire flooring (e.g., no access to litter) developed significantly higher incidences of lameness than broilers reared on clean wood-shavings litter (not shown). Wire flooring causes footing instability that imposes additional torque, stress, and strain on susceptible leg joints. It was not clear if wire flooring also triggers lameness by denying the birds access to litter materials, similar to the onset of osteopenia (cage layer fatigue) attributable to phosphorus deprivation when laying hens initially were housed in cages (Grumbles, 1959). In experiment 1, male broiler chicks from line C were housed in one pen with wood-shavings litter and in 3 pens with wire flooring. Control feed was provided in the pen with litter and in one pen with wire flooring. Control feed containing PoultryStar probiotic was provided continuously in the second pen with wire flooring, and control feed mixed with dry used litter (21.5 kg of feed + 1.13 kg of litter) was provided continuously in the third pen with wire flooring. The chicks were weighed on d 1 and at 2-wk intervals thereafter until the experiment was terminated on d 56.

Experiment 2 (March 2010)

The objectives of this experiment were to determine if the PoultryStar probiotic would reduce lameness in broilers reared on wire flooring and to compare the efficacy of the probiotic alone or in combination with clean wood shavings added to the feed as a prebiotic. Male and female broiler chicks from line B were housed in one pen with wood-shavings litter flooring and in 3 pens with wire flooring. Control feed was provided in the pen with litter flooring and in one pen with wire flooring. Control feed containing probiotic was provided continuously in the second pen with wire flooring, and control feed mixed with probiotic plus prebiotic (21.5 kg of feed + 1.13 kg of clean wood shavings) was provided continuously in the third pen with wire flooring. All survivors were weighed on d 56.

Experiment 3 (August 2010)

In the preceding experiments, the chicks were placed on wire flooring beginning at 1 d of age, and the probiotic was included prophylactically in the feed beginning on d 1. The objective of this experiment was to determine if the PoultryStar probiotic could effectively reduce lameness when added therapeutically to the feed 28 d after placing the chicks on wire flooring. Male broiler chicks from line B were housed in one pen with wood-shavings litter and in 3 pens with wire flooring. Control feed was provided in the pen with litter and in one pen with wire flooring. Control feed containing probiotic was provided continuously beginning at 1 d of age in the second pen with wire flooring or beginning at 28 d of age in the third pen with wire flooring.

Experiment 4 (October 2010)

In this study, 10 environmental chambers were used to evaluate the reproducibility with which the PoultryStar probiotic might reduce the incidence of lameness in broilers reared on wire flooring. Male broiler chicks from line D were housed in chambers numbered 1 through 10, all of which contained pens with wire flooring. Control feed was provided in the odd-numbered chambers and control feed containing probiotic was provided continuously beginning at 1 d of age in the even-numbered chambers. No pens with litter flooring were used in this experiment. Survivors were weighed on d 56.

Experiment 5 (April 2011)

In this study, the efficacy of the Biomin IMBO probiotic was assessed. Male and female broiler chicks from line G were housed in one pen with wood-shavings litter and in 4 pens with wire flooring. Control feed was provided in the pen with litter and in 2 pens with wire flooring. Control feed containing the IMBO probiotic was provided continuously beginning at 1 d of age in the remaining 2 pens with wire flooring. Survivors were weighed on d 56.

Necropsy Procedures

Euthanized birds were necropsied within 30 min postmortem. In experiments 1 and 2, all birds that died or developed lameness were necropsied and assigned to one of the following diagnostic categories: cull = runts and moribund individuals that failed to thrive; **UNK** = unknown cause of death; **SDS** = sudden death syndrome (flipover, heart attacks); **PHS** = pulmonary hypertension syndrome (ascites); **KB** = kinky back or spondylolisthesis; **TW** = twisted leg or slipped tendon (perosis, chondrodystrophy; Riddell, 1976, 1992; Thorp, 1992); **TD** = tibial dyschondroplasia; **LAME-UNK** = lameness for undetermined reasons; **FHS** = proximal femoral head separation or epiphyseolysis; **FHT** = proximal femoral head transitional degeneration; and, **FHN** = proximal femoral head necrosis. In all 5 experiments, the proximal femoral head lesions were categorized separately (FHS, FHT, or FHN) to emphasize the progression of BCO lesions (Dinev, 2009; Durairaj et al., 2009), as represented in Figure 1. Kinky Back or spondylolisthesis was diagnosed based on the characteristic posterior paraparesis and hock-resting posture (Riddell, 1973, 1976; Duff, 1990a). In the first 2 experiments, appreciable numbers of lame birds were assigned to the LAME-UNK category. Accordingly, based on evidence that broilers can have necrosis and BCO in the proximal head of the tibia even when the proximal head of the femur appears to be normal (Mutalib et al., 1983a; Thorp and Waddington, 1997; McNamee et al., 1998, 1999; McNamee and Smyth, 2000), in subsequent experiments, the proximal tibial heads from clinically lame birds also were evaluated macroscopically for evidence of tibial head necrosis (**THN**) as a subcategory of BCO in the tibiotarsus (Butterworth, 1999). Femoral and tibial heads that appeared to be normal macroscopically were not routinely evaluated microscopically. As illustrated in Figure 2, THN was diagnosed according to established macroscopic criteria, including the presence of necrotic voids and lytic channels extending proximally from the diaphysis or metaphysis to the growth plate, thereby undermining the metaphyseal trabecular bone that normally provides structural support for the physeal growth plate and epiphyseal articular cartilage (Nairn, 1973; Mutalib et al., 1983a; Andreasen et al., 1993; Skeeles, 1997; McNamee and Smyth, 2000; Joiner et al., 2005). Broilers that developed clinical lameness associated with THN in the absence of macroscopic femoral head lesions routinely exhibited evidence of osteomyelitis in the form of fibrinonecrotic (caseous) exudates or bacterial sequestrae in the tibiae (Figure 2). Also in experiments 3 to 5, clinically healthy survivors were humanely killed on d 56 and necropsied to evaluate the incidence of subclinical macroscopic lesions in each proximal femoral head and each proximal tibial head. The total incidence of
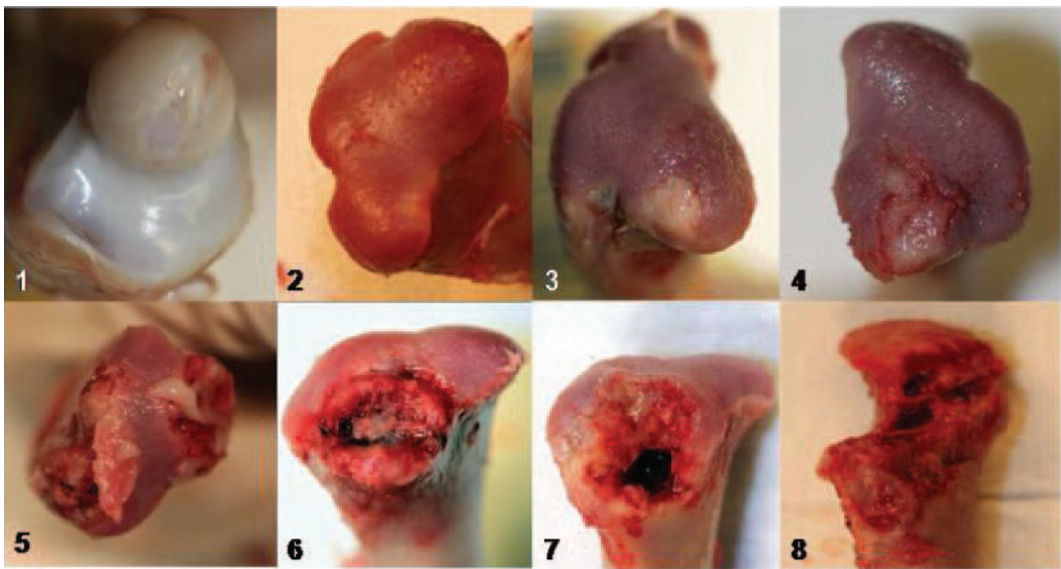
Figure 1. Stages of proximal femoral head degeneration leading progressively to bacterial chondronecrosis with osteomyelitis (BCO): 1, normal proximal femoral head; 2, femoral head separation (FHS: epiphyseolysis); 3–5, progressive necrosis, ulceration, erosion, and fracturing of the growth plate (femoral head transitional degeneration, FHT); 6–8, perforation, fracturing, and necrosis/osteomyelitis of the femoral head (femoral head necrosis, FHN).

femoral lesions was calculated: Total femur = (FHS + FHT + FHN). The total incidence of lameness was calculated: Total lame = (KB + TW + LAME-UNK + TD + FHS + FHT + FHN + THN). The SigmaStat ANOVA package (Jandel Scientific, San Rafael, CA) was used to compare BW among experimental groups, treatments, and genders. For comparisons of lesion and lameness incidences, the individual bird was used as the experimental unit, and the SigmaStat Z-test procedure was used to compare proportions.

RESULTS

Examples of tibiae exhibiting mild to severe THN lesions were preserved in 10% buffered formalin for histological evaluation. Microscopic examination of THN
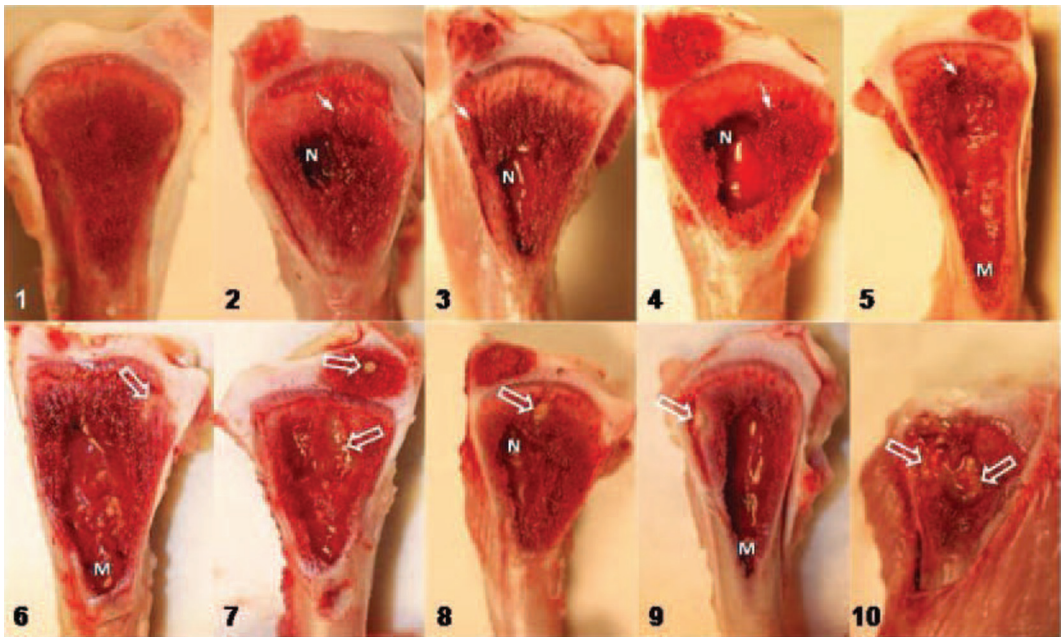

Figure 2. Stages of proximal tibial head necrosis (THN) leading progressively to bacterial chondronecrosis with osteomyelitis (BCO): 1, normal proximal tibial head with struts of trabecular bone in the metaphyseal zone fully supporting the growth plate; 2–5, necrotic voids (N) in the metaphyseal zone undermine the support of the growth plate, leading to microfractures of the growth plate. Necrotic voids typically were filled with fibrinonecrotic exudate. Lytic channels (small arrows) penetrate from the necrotic voids into the growth plate. Bacterial infiltration and sequestrae (open arrows) provide macroscopic evidence of osteomyelitis. Sequestrae occasionally can be seen in the secondary ossification center of the epiphysis (upper open arrow, stage 7). Necrotic voids can communicate with precocious ectopic extensions of the marrow cavity (M).
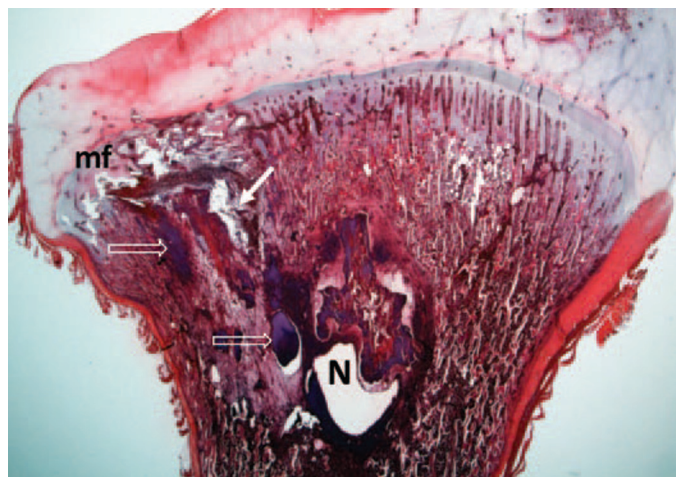
Figure 3. Proximal tibial head necrosis (THN), a category bacterial chondronecrosis with osteomyelitis (BCO). Voids containing liquefied necrotic tissue (N, fibrinonecrotic exudate) in the metaphyseal zone extend via lytic channels (closed arrow) to microfractures (mf) in the growth plate. Bacterial infiltration and sequestrae (open arrows) provide evidence of osteomyelitis.

voids revealed foci of liquefied fibrinonecrotic exudate associated with extensive purulent inflammation. Microfractures of the epiphyseal-physeal interface were apparent upon histological examination of tibiae with large THN voids (Figure 3).

Experiment 1

The BW for 56-d-old birds fed the control diet alone tended to be heavier than those for hatch-mates fed diets containing the probiotic or feed mixed with litter, regardless of floor type: $4,063 \pm 82^a$; $3,778 \pm 149^{ab}$; $3,406 \pm 116^b$; and, $3,617 \pm 53^b$ g for the litter-control, wire-control, wire-probiotic, and wire-used litter groups, respectively ([a,b]Means with different super-

scripts are significantly different, $P < 0.05$). To address the possibility that the fastest-growing individuals more readily developed lameness on the wire flooring, the individual BW for all birds grown on wire (regardless of diet treatment) were plotted by age in the separate panels of Figure 4. The BW of birds that remained clinically healthy (normal) were separated from those that eventually developed lameness. Across all 4 age groups, it was not the heaviest individuals that consistently became lame (Figure 4). None of the birds in this experiment died from unknown causes; one bird died from PHS and 2 from SDS. No lameness was attributable to KB or TW; one lame bird had TD and 3 were diagnosed as LAME-UNK. The most prevalent diagnoses were femoral head lesions (FHS, FHT, and FHN), thus the values for total lameness shown in Figure 5 overwhelmingly consist of femoral head lesions. The lowest lameness incidence (12%) occurred in the pen with wood-shavings litter (litter-control group), whereas the highest incidence (68%) occurred in birds fed the control diet while being reared on wire flooring (wire-control group). When compared with the wire-control group, adding the probiotic to the control diet reduced ($P < 0.01$) the incidence of lameness to 38% (wire-probiotic group), whereas the wire-litter group had an intermediate (48%) incidence (Figure 5).

Experiment 2

All survivors were weighed on d 56. Within a gender, the BW did not differ between floor type or diet treatment ($P > 0.09$). Males averaged $2,901 \pm 66$ g; $2,934 \pm 90$ g; $2,853 \pm 82$ g; and $2,725 \pm 57$ g and females averaged $2,571 \pm 71$ g; $2,357 \pm 140$ g; $2,516 \pm 74$ g; and $2,360 \pm 72$ g (mean $\pm$ SEM) for the litter-control, wire-control, wire-probiotic, and wire-prebiotic groups, respectively. When pooled independent of diet
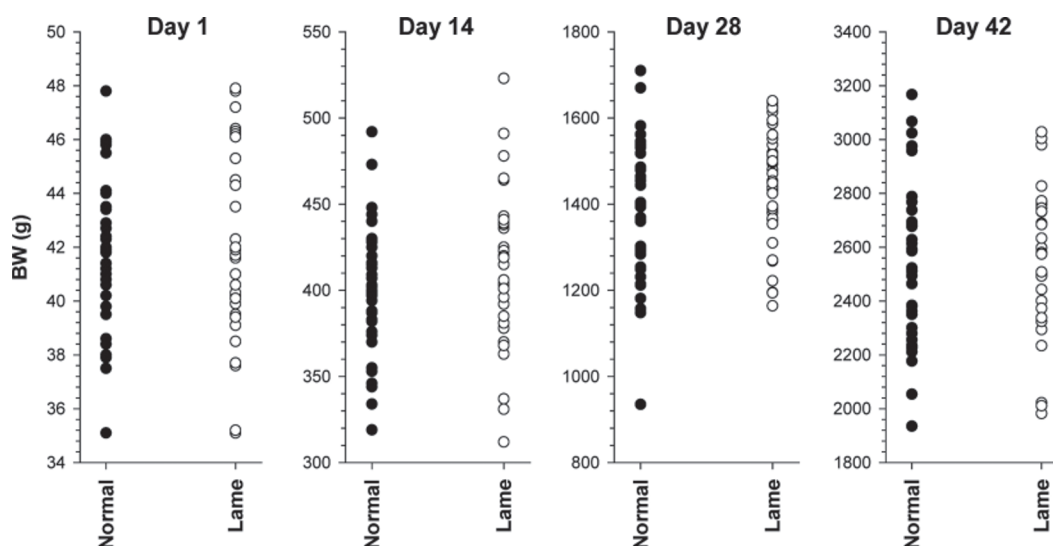

Figure 4. Individual BW for all male broilers in experiment 1 that were grown on wire (regardless of diet treatment) plotted to separate the values for individuals that did not become lame through d 56 (normal) from those of individuals that did (lame). Across all 4 ages, it was not the heaviest individuals that consistently developed lameness by d 56.
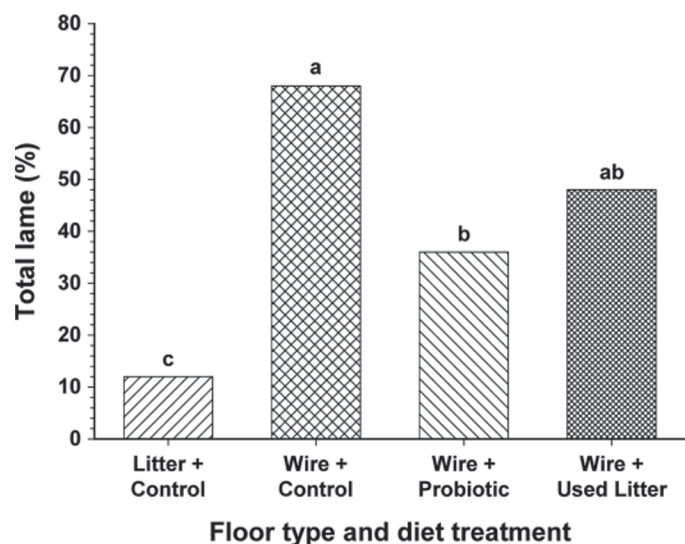
Figure 5. Total lameness through d 56 for male broilers in experiment 1 that were reared on clean wood-shavings litter and fed a control diet (litter-control) or for broilers reared on wire flooring and fed the control diet alone (wire-control), the control diet mixed with the PoultryStar probiotic (wire-probiotic; Biomin Holding GmbH, Herzogenburg, Austria), or the control diet mixed with used litter (wire-used litter). [a–c]Values with different letters differed significantly at $P \leq 0.05$ using repeated Z-tests (SigmaPlot) to compare all proportions.

or floor treatment, the males averaged 2,853 ± 74 g and females averaged 2,451 ± 90 g (mean ± SEM; $P = 0.001$). Two birds died from PHS. No lameness was attributed to TD or TW, 2 lame birds had KB, and 5 birds were diagnosed as LAME-UNK. The most prevalent diagnoses were femoral head lesions (FHS, FHT, and FHN). The lowest incidences of total lameness occurred in the litter-control and wire-probiotic groups (8% each), whereas the highest incidence (28%) occurred in the wire-control group. The lameness incidence for the wire-prebiotic group (16%) was intermediate when compared with the wire-control and wire probiotic groups. The incidence of lameness did not differ between genders (16% for females vs.14% for males with floor and diet treatments combined).

Experiment 3

Body weights were not recorded in this experiment. None of the birds died from unknown causes, PHS, or SDS. One lame bird was assigned to the TW category. This was the first experiment in which a consistent effort was made to detect THN. The most prevalent diagnoses for lame birds were THN (10 birds), FHN (9 birds), and lame-UNK (5 birds). When the categories were pooled as total lame, the highest incidence (22%) occurred in birds that were fed the control diet while being reared on wire flooring (wire-control group), whereas birds fed the same diet but grown on wood shavings (litter-control group) had the lowest incidence (2%; $P = 0.006$). Adding probiotic to the feed beginning on d 1 reduced the total incidence to 10% for broilers grown on wire flooring (wire-probiotic D1 group), which was

intermediate ($P = 0.173$) between the shavings-control and wire-control groups. Adding probiotic to the feed at 28 d of age (wire-probiotic D28 group) resulted in 18% total lameness, which did not differ from the 22% value for the wire-control group ($P = 0.803$). Twenty-five birds per pen that survived to d 56 were necropsied to evaluate the incidence of subclinical macroscopic lesions in each proximal femoral and tibial head. When pooled by diagnostic category independent of treatment group, there was no evidence that any of the subclinical lesions preferentially formed in either the left or right leg (not shown). Within each diagnostic category, there was no evidence that subclinical lesions formed differentially among the treatment groups (not shown).

Experiment 4

All survivors were weighed and necropsied on d 56. The BW did not differ between diet treatments (control = 3.35 ± 0.03 kg, n = 155; control + probiotic = 3.28 ± 0.03 kg, n = 175; $P = 0.108$, mean ± SEM). The diagnostic categories for all lame birds through 56 d of age are summarized as percentages of the total lameness in Figure 6. The most prevalent diagnoses for lame birds were THN, FHN, and FHS. Incidences of spondylolisthesis, FHT, and FHN did not differ significantly between the diet treatments ($P = 0.117$, 0.242, and 0.282, respectively). The percentage of lameness attributable to THN was significantly higher for broilers fed the control feed than for broilers fed the control feed + probiotic ($P = 0.004$). When pooled by diagnostic category independent of diet treatment, no tendency was revealed for lesions to form preferentially in either the left or right leg (not shown, $P \geq 0.489$; Z-test).

Figure 7 shows total lameness for the individual environmental chambers (control feed: odd-numbered chambers; control feed + probiotic feed: even-numbered chambers) or pooled by diet treatment groups (all control vs. all probiotic). Some variability in the responses observed among individual chambers was evident regardless of diet treatment. For example, the incidence of lameness in control chamber 7 was not different from the incidence in any of the probiotic chambers (e.g., 18 vs. 10%; $P = 0.387$, comparing chambers 7 vs. 10 using a Z-test). Nevertheless, the difference in lameness between the diet treatments (chambers pooled) was significant ($P = 0.001$), with 18% of the broilers fed the probiotic feed developing lameness when compared with 32.4% for broilers fed the control feed.

All survivors on d 56 were necropsied to evaluate subclinical macroscopic lesions in each proximal femoral and tibial head. When pooled by diagnostic category independent of treatment group, there was no evidence that any of the subclinical lesions preferentially formed in either the left or right leg (not shown). When pooled by diagnostic category independent of left or right leg, there was no evidence that any of the subclinical lesions preferentially formed in either of the diet treatments (not shown). Regardless of whether the proximal fe-
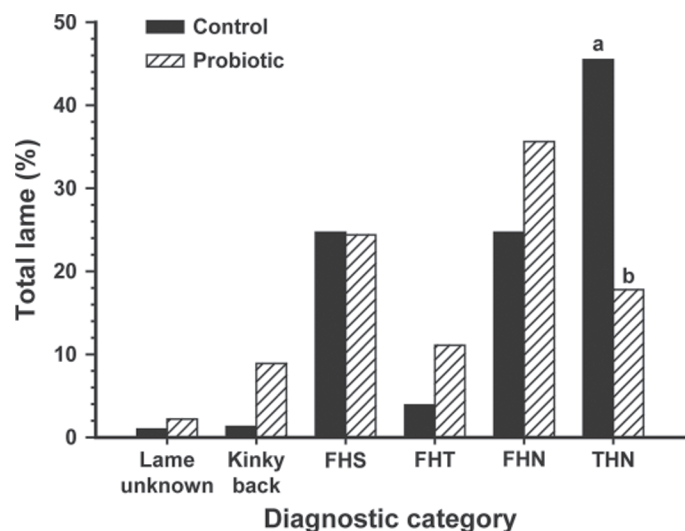
Figure 6. Diagnostic categories for lame male broilers in experiment 4 that were reared on wire flooring and were fed the control feed or the control feed mixed with the PoultryStar probiotic, including lame unknown (cause of lameness could not be determined); kinky back (spondylolisthesis); FHS (femoral head separation); FHT (femoral head transitional degeneration); FHN (femoral head necrosis); and THN (tibial head necrosis). [a,b]Tibial head necrosis incidences differed significantly between the control and probiotic diet treatments ($P = 0.004$; Z-test, SigmaPlot). Incidences of spondylolisthesis, FHT, and FHN did not differ significantly between the diet treatments ($P = 0.117$, 0.242, and 0.282, respectively).

mur head was normal or exhibited FHS, FHT, or FHN within the same leg (right and left legs combined), the ipsilateral (same-side) proximal tibial head was significantly (femur normal, femur FHS, femur FHT) or nu-
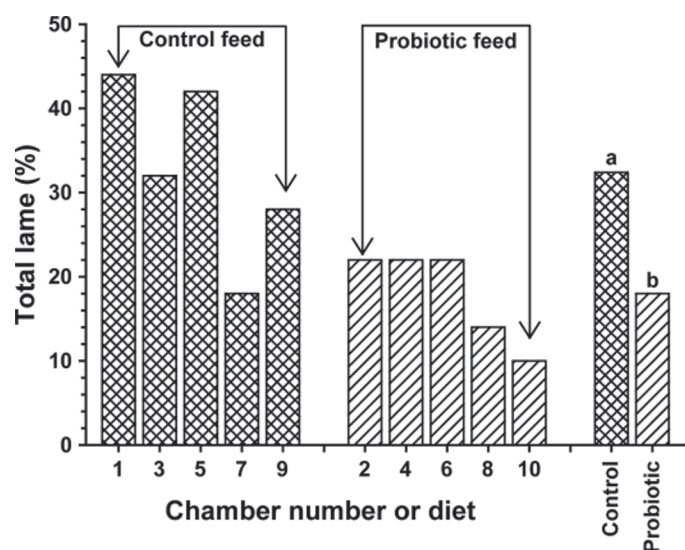
merically (femur FHN) more likely to remain normal than to exhibit macroscopic indications of THN (Figure 8). The principal diagnostic categories (diet treatments and left and right legs pooled) are compared in Figure 9 for all lame birds versus all survivors on d 56. Within the femoral head diagnostic categories, the survivors had higher incidences of FHS but lower incidences of FHN when compared with lame broilers ($P = 0.001$). In the tibial head categories, the survivors had higher incidences of normal tibiae and lower incidences of THN when compared with lame broilers ($P = 0.001$). Indeed, 58.2% of the tibiae in lame birds exhibited THN and only 41.8% appeared to be normal. For the survivors, 13.6% of the tibiae exhibited THN whereas 86.4% appeared to be normal ($P = 0.001$), providing direct evidence of a significant relationship between THN and lameness (Figure 9).

Experiment 5

All survivors were weighed on d 56. The BW of females did not differ between floor type or diet treatment ($P = 0.112$). The average BW for females (2,838 $\pm$ 34 g) was lower ($P = 0.001$) than the average BW for

Figure 7. Male broilers in experiment 4 were reared on wire flooring in environmental chambers and were fed either a control diet (control feed, odd-numbered chambers) or the control diet mixed with the PoultryStar probiotic (Biomin Holding GmbH, Herzogenburg, Austria; probiotic feed, even-numbered chambers). Incidences of total lameness are shown by individual chamber (numbers 1–10) as well as with the chambers pooled by diet treatment (control diet vs. probiotic diet). [a,b]The lameness incidences for all chambers pooled by diet treatment differed significantly ($P = 0.001$; 77/238 for the control diet, 45/250 for the probiotic diet) based on a Z-test (SigmaPlot).
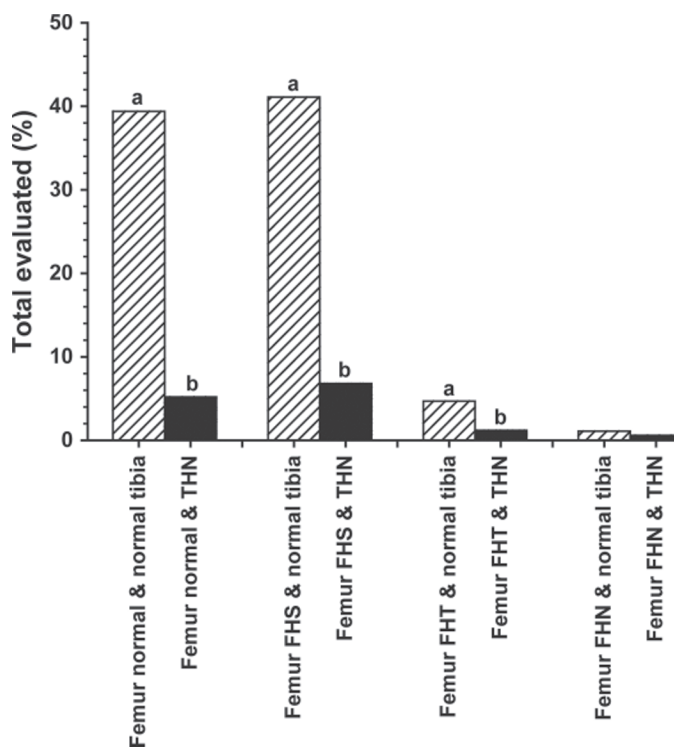

Figure 8. Male broilers in experiment 4 that did not develop clinical lameness were necropsied on d 56 to evaluate the incidence of subclinical macroscopic lesions in proximal femoral and tibial heads (660 right and left legs combined for both diet treatments). Regardless of whether the proximal femur head was normal or exhibited FHS (femoral head separation) or FHT (femoral head transitional degeneration), within the ipsilateral leg the proximal tibial head was significantly more likely to remain normal than to exhibit macroscopic indications of THN (tibial head necrosis). FHN = femoral head necrosis. [a,b]Values with different letters differed significantly at $P \leq 0.05$ using repeated Z-tests (SigmaPlot) to compare proportions.
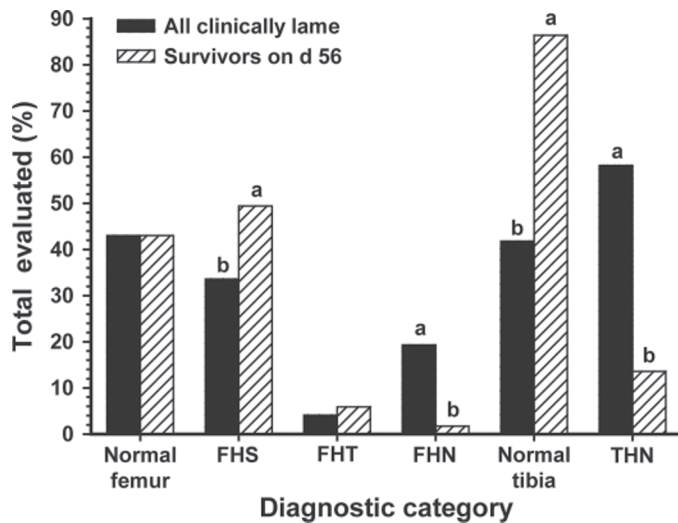
Figure 9. Male broilers in experiment 4 were reared on wire flooring through 56 d of age and were fed a control diet or the same diet supplemented with the PoultryStar probiotic (Biomin GmbH, Herzogenburg, Austria). Combining both diet treatments, 122 birds succumbed to clinical lameness and 330 survived to d 56 without developing clinical lameness. Combining left and right legs, 244 and 660 legs were evaluated within the clinically lame and survivors groups, respectively. Diagnostic categories for the femur include normal, FHS (femoral head separation), FHT (femoral head transitional degeneration), and FHN (femoral head necrosis). Diagnostic categories for the proximal tibial head include normal and THN (tibial head necrosis). [a,b]Values with different letters differed significantly at $P \leq 0.05$ using repeated Z-tests (SigmaPlot) to compare all proportions.

males (3,363 ± 53 g). The BW of females were consistently lower than for males within each diet and treatment group ($P = 0.01$). The BW of males in the litter-control group (3,477 ± 55 g) was higher ($P = 0.027$) than the BW of males in the wire-control group (3,244 ± 36 g), whereas males in the wire-probiotic group had intermediate BW (3,369 ± 59 g). Three birds died of SDS, which was the only cause of mortality not associated with lameness. The chicks from line G were remarkably healthy and exhibited an exceptionally low susceptibility to the onset of lameness on wire floor-

ing. One bird was lame for unknown reasons, and no lameness was attributable to TD, TW, or KB. Total lameness for broilers in the litter-control group (4%) and the wire-probiotic group (5%; 2 pens pooled) did not differ ($P = 0.89$), whereas both of these groups had lower incidences of lameness than the wire-control group (15.5%, $P \leq 0.02$; 2 pens pooled). Table 1 summarizes the leg lesion incidences by treatment and diagnostic category for all lame birds. The overall incidence of lameness was low and thus lesion categories did not differ among the groups. For the wire-control group, THN was the most prevalent lesion diagnosis (70.7%), followed by FHT and FHS. The incidence of lameness did not differ between genders ($P = 0.07$). Necropsy observations for survivors on d 56 are provided in Table 2. None of the proximal femoral head or tibial head diagnostic categories differed when sorted either by gender (all males vs. all females) or by leg (all right vs. all left legs; not shown). When the femoral head categories were pooled regardless of gender or leg, only the incidence of FHT differed among the floor type and diet treatment groups. The incidence of FHT was lower in the wire-probiotic and wire-control groups than in the litter-control group. Total femoral head lesions did not differ significantly among the groups. When the tibial head diagnostic categories were pooled regardless of gender or leg, the wire-probiotic group had the most normal tibiae (79.3%) and the lowest incidence of THN (20.7%) when compared with both groups fed the control diet regardless of floor type (Table 2). The necropsy data for survivors on d 56 reflect low incidences of FHT and THN in broilers fed the probiotic.

Meta-Analysis of Experiments 1–5

As shown in Figure 10, feeding broilers the control diet while rearing them on wire flooring consistently resulted in the highest incidences of lameness when compared with hatch-mates fed the same control diet while being reared on wood-shavings litter (wire-con-

Table 1. Leg diagnostic incidences for broilers in experiment 5 that developed lameness while being reared on wood-shavings litter or wire flooring and that were fed a control diet or the control diet mixed with the Biomin IMBO probiotic (Biomin Holding GmbH, Herzogenburg, Austria; probiotic diet)

	Floor type and diet treatment (%)		
Bone and diagnosis[1]	Litter flooring + control diet	Wire flooring + control diet	Wire flooring + probiotic diet
Femur			
Normal	25 (1/4)[2]	44.1 (15/34)	58.3 (7/12)
FHS	0	23.5 (8/34)	33.3 (4/12)
FHT	75 (3/4)	29.4 (10/34)	8.3 (1/12)
FHN	0	2.9 (1/34)	0
FHS + FHT + FHN	75 (3/4)	55.9 (19/34)	41.7 (5/12)
Tibia			
Normal	75 (3/4)	29.4 (10/34)	41.7 (5/12)
THN	25 (1/4)	70.6 (24/34)	58.3 (7/12)

[1]The proximal femoral and tibial heads of both legs were evaluated (right and left legs pooled; sexes pooled) and were diagnosed as being macroscopically normal (no apparent abnormalities) or as exhibiting FHS (femoral head separation), FHT (femoral head transitional degeneration), FHN (femoral head necrosis), or THN (tibial head necrosis).

[2]Lesion incidences did not differ by diagnostic category among the groups due to the low numbers of clinically lame individuals (repeated Z-tests for proportions; SigmaPlot).

Table 2. Leg diagnostic incidences for broilers in experiment 5 that did not develop lameness while being reared on wood-shavings litter or wire flooring and that were fed a control diet or the control diet mixed with the Biomin IMBO probiotic (Biomin Holding GmbH, Herzogenburg, Austria; probiotic diet)

Bone and diagnosis[1]	Diet and floor treatment (%)		
	Litter flooring + control diet	Wire flooring + control diet	Wire flooring + probiotic diet
Femur			
Normal	27.8 (30/108)	30.9 (58/188)	37.9 (75/198)
FHS	31.5 (34/108)	41.4 (78/188)	41.9 (83/198)
FHT	38 (41/108)[a]	25.5 (48/188)[b]	19.7 (39/198)[b]
FHN	2.8 (3/108)	2.1 (4/188)	0.5 (1/198)
FHS + FHT + FHN	72.2 (78/108)	69.1 (130/188)	62.1 (123/198)
Tibia			
Normal	50.9 (55/108)[c]	67.6 (127/188)[b]	79.3 (157/198)[a]
THN	49.1 (53/108)[a]	32.4 (61/188)[b]	20.7 (41/198)[c]

[a–c]Different superscripts denote a significant difference ($P \leq 0.05$) between the groups within a diagnostic category (SigmaPlot Z-test).

[1]The proximal femoral and tibial heads of both legs were evaluated (right and left legs pooled; sexes pooled) on d 56 (right and left legs pooled; sexes pooled) and were diagnosed as being macroscopically normal (no apparent abnormalities) or as exhibiting FHS (femoral head separation), FHT (femoral head transitional degeneration), FHN (femoral head necrosis), or THN (tibial head necrosis).

trol vs. litter-control groups; experiments 1, 2, 3, and 5). Adding probiotics to the control feed consistently reduced the incidence of lameness for broilers reared on wire flooring (wire-probiotics groups) to levels that did not differ from those of the litter-control group (experiments 2 and 5) or to levels that were intermediate between those of the litter-control and wire-control groups (experiments 1 and 3). Only lines C, B, D, and G were used for these 5 probiotic experiments, and no additional probiotic experiments were conducted using these or any other lines.

DISCUSSION

Growing broilers on wire flooring provides a reliable experimental model for reproducibly triggering significant levels of lameness attributable to BCO. In 5 independent experiments using 4 broiler lines, the highest incidence of lameness developed when broilers were fed the control diet while being reared on wire flooring, whereas hatch-mates fed the same diet but grown on wood-shavings litter consistently developed the lowest incidences of lameness (Figure 10). The wire-flooring model promotes high growth rates and normal levels of activity at lower stocking densities when compared with cage-rearing systems while using unstable footing to impose additional torque and strain on susceptible leg joints. Based on our current understanding, excessive shear stress causes osteochondrosis of the epiphyseal-physeal cartilage accompanied by mechanical truncation or thrombotic occlusion of metaphyseal blood vessels (McCaskey et al., 1982; Riddell et al., 1983; Duff, 1984b, 1989a,b; Duff and Randall, 1987; Thorp and Duff, 1988; Thorp et al., 1993). Local biomechanical stress and impaired blood flow to the epiphyseal-physeal cartilage also contribute to the formation of osteochondrotic lesions in a variety of species (Trueta and Amato, 1960; Riddell, 1975; Ytrehus et al., 2004, 2007). Osteochondrotic clefts and damaged or obstructed metaphyseal vessels are thought to facilitate bacte-

rial colonization, leading to lameness attributable to BCO (see Introduction). Indeed, BCO of the proximal femoral head (FHS + FHT + FHN) and proximal tibial head (THN) constituted $\geq 85\%$ of the lesions identified in clinically lame birds that had been reared on wire flooring in all 5 experiments. Most of the lameness developed between 6 and 8 wk of age, as has been reported for field outbreaks of BCO (McNamee et al., 1998; Dinev, 2009). Lameness progressed very rapidly in broilers that had appeared to be healthy during the preceding 24 to 48 h, as previously reported by Joiner et al. (2005). Diagnoses of TD, TW, and KB were relatively scarce and did not appear to be influenced by floor treatment. These results are consistent with sur-
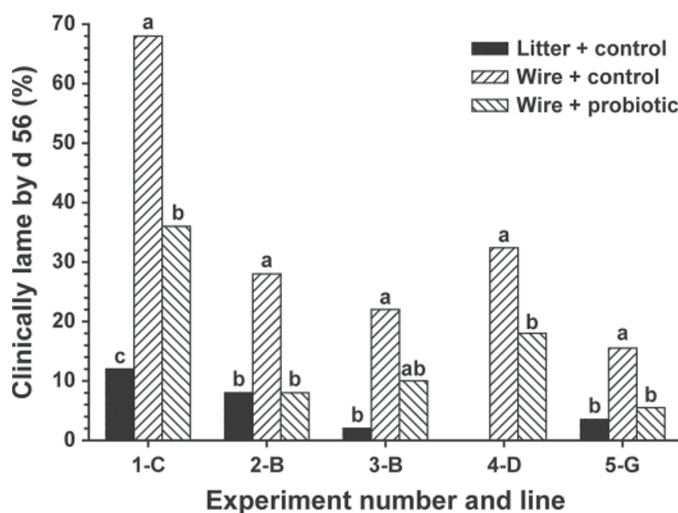

Figure 10. Meta-analysis of total lameness through 56 d of age for broilers in experiments 1–5 from lines C, B, D, and G that were reared on clean wood-shavings litter and fed a control diet (litter-control) or reared on wire flooring and fed the control diet alone (wire-control) or the control diet mixed the PoultryStar (experiments 1–4) or IMBO (experiment 5; Biomin Holding GmbH, Herzogenburg, Austria) probiotic beginning on d 1 (wire-probiotic). [a–c]Values with different letters within an experiment differed significantly at $P \leq 0.05$ using repeated Z-tests (SigmaPlot) to compare proportions.

veys of commercial broiler flocks in which BCO was the most prevalent cause of lameness, with minor contributions from TD, TW, KB, and valgus-varus deformities (Pattison, 1992; Thorp and Waddington, 1997; Butterworth, 1999; McNamee and Smyth, 2000; Dinev, 2009).

Management strategies (e.g., alternative lighting and feed restriction programs) that tend to reduce the early growth rates of broiler flocks also tend to reduce the incidence of lameness and skeletal disorders (Riddell, 1983; Bradshaw et al., 2002). Maximal growth rates increased the incidence of BCO in broilers exposed experimentally to *Staphylococcus aureus* (McNamee et al., 1999). However, absolute BW per se cannot be the primary determinant of susceptibility to BCO in the present studies. In experiment 1, broilers in the heaviest BW cohorts were not disproportionately predisposed to the subsequent onset of lameness. As expected, males were significantly heavier than females in experiments 2 and 5, but the genders did not differ in their susceptibility to lameness when reared on wire flooring. The genders also did not differ in subclinical lesion incidences when the survivors were necropsied on d 56. In 2 of the 5 experiments, broilers grown on wire flooring tended to have lower final BW than those grown on wood-shavings litter, but wire flooring nevertheless triggered the highest incidence of lameness. Broilers experiencing leg discomfort may not stand and walk to the feeders as frequently as their healthier flock mates (Kestin et al., 1992; Sørensen et al., 2000; Weeks et al., 2000), suggesting improvements in leg health should substantially improve flock uniformity and growth performance. In a variety of mammalian species, the incidence of lameness attributable to osteochondrosis is poorly correlated with growth rates or absolute BW among individuals within the same genetic line. Instead, genetic susceptibility to osteochondrosis appears to be more closely associated with differences in the anatomic conformation of susceptible joints (Ytrehus et al., 2007). Joint conformations and misalignments also may contribute to the susceptibility to osteochondrosis and lameness in broilers (Riddell et al., 1983; Julian, 1985). These observations suggest that lameness resembles other metabolic diseases, such as PHS, in that the overall incidence often is highest in the fastest-growing flocks or genetic lines, but the fastest-growing individuals in those flocks are not necessarily the most susceptible (Wideman and Kirby, 1995; Roush and Wideman, 2000).

The wire-flooring model consistently produces broilers in which the continuum of BCO lesion progression is readily apparent, as illustrated in Figures 1 and 2. In the case of proximal femora, epiphyseolysis/FHS has been attributed to the use of excessive force to disarticulate the femur from the pelvis, to postmortem degenerative changes in birds that died several hours before being necropsied, or to acute trauma caused by mishandling of the legs during catching (Riddell et al., 1983; Julian, 1985; Duff and Randall, 1987; Duff, 1990b). We concur that the epiphyseal cartilage often

does not detach from the physeal growth plate until the femur is disarticulated at necropsy. The same can be said for overt fracturing of the femur head leading to a diagnosis of FHN during necropsy. In this very limited sense, both epiphyseolysis/FHS and FHN might be considered artifacts. In the present studies, epiphyseolysis/FHS typically was observed immediately posteuthanasia, often unilaterally rather than bilaterally, and with similar frequency regardless of the force applied to disarticulate the femur. Necropsies of broilers that were in full rigor after succumbing to SDS nevertheless failed to produce a disproportionate incidence of epiphyseolysis (data not shown). Epiphyseolysis tends to occur more frequently in older broilers fed ad libitum than in feed-restricted broilers (Riddell et al., 1983). McNamee et al. (1999) reported the incidence of recovery of *S. aureus* from proximal femora and tibiae was greater than the incidence of BCO diagnoses based on macroscopic and histologic examinations, suggesting femora and tibiae that appear normal nevertheless can be infected with BCO-causing microorganisms. Indeed, *Staphylococcus aureus* and *E. coli* were isolated from proximal femora that succumbed to epiphyseolysis during necropsies of lame broilers from commercial flocks (Thorp et al., 1993). Osteochondrotic clefts at the epiphyseal-physeal boundary and within the physeal cartilage are considered to predispose broilers to epiphyseolysis/FHS, which in turn is considered an early or initial macroscopic manifestation of BCO (Duff, 1984b, 1989a; Duff and Randall, 1987; Duff, 1990b; Thorp et al., 1993; Bradshaw et al., 2002). Accordingly, we concur with numerous previous investigators that most epiphyseolysis should be attributed to underlying traumatic (osteochondrosis) or infectious (osteomyelitis) femoral head pathology (Duff, 1984a,b,c, 1986, 1990a,b; Duff and Hocking, 1986; Duff and Randall, 1987; Thorp et al., 1993; Durairaj et al., 2009).

In the case of the proximal tibiae, we initially considered the possibility that the earliest necrotic voids and lytic channels (Figure 2) might represent precocious ectopic extensions of the marrow cavity, particularly when caseous exudates and bacterial sequestrae were not visible. This possibility was contradicted by observations that within the same bird one tibia could appear completely normal while the contralateral tibia exhibited large voids extending from the midmetaphysis to the hypertrophic zone of the growth plate. The voids were so large that the metaphyseal vasculature supplying the growth plate must have been substantially eliminated, as indicated by Emslie et al. (1984). Histologic examinations revealed microbial foci within the metaphyseal parenchyma adjacent to necrotic voids and lytic channels, in agreement with previous reports (Mutalib et al., 1983a; McNamee and Smyth, 2000). Voids undercutting the bony support structure facilitate microfracturing of the epiphyseal-physeal cartilage (Figure 3). Finally, when all of the stages shown in Figure 2 were consolidated within the single diagnostic category of THN, many of the lesions did not exhibit

obvious (macroscopic) bacterial sequestrate; nevertheless, THN was directly associated with lameness. In experiment 4, for example, FHN and THN were the predominant diagnostic categories that consistently were associated with lameness, whereas much lower incidences of FHN and THN were detected in d 56 survivors. Thorp and Waddington (1997) previously reported high incidences of BCO in the tibiae and femora of lame broilers from commercial flocks, with little evidence of tibial or femoral BCO in apparently healthy individuals. The BCO lesions in the proximal tibia were more common than lesions of the proximal femur in commercial broilers from Holland but not in commercial broilers from Scotland or Northern Ireland (Thorp and Waddington, 1997). Previous reports also indicate that field outbreaks of BCO may affect only one leg while the contralateral leg appears macroscopically normal (McNamee et al., 1998; Dinev, 2009). When induced experimentally, bacterial sequestrae and zones of necrosis develop adjacent to the physeal growth plate and within the metaphysis of the proximal tibia, occasionally in the absence of macroscopic lesions in the femur (Emslie et al., 1983; McNamee et al., 1999). Based on the cumulative evidence, we consider the patterns of lesion progression shown in Figure 2 to be pathognomonic for BCO of the proximal tibia.

Mild to severe osteochondrosis can be observed in the epiphyseal-physeal cartilage of leg bones and thoracic vertebrae of apparently healthy broilers exhibiting no symptoms of infectious or traumatic spondylolisthesis or of lameness (Wise et al., 1973; McCaskey et al., 1982; Riddell et al., 1983; McNamee et al., 1998), suggesting lameness is not necessarily caused by direct mechanical damage per se but rather by the ensuing bacterial infection (McNamee et al., 1998). The pathogenesis of BCO cannot be instantaneous; therefore, logic dictates that many susceptible broilers may not exhibit obvious symptoms of lameness but still may possess subclinical lesions primarily consisting of osteochondrosis and the earliest stages of BCO. Accordingly, survivors necropsied on d 56 remained fully capable of walking yet they often exhibited early BCO lesions (Figures 8, 9; Table 2). In experiment 4, sufficient numbers of survivors were available on d 56 to permit ipsilateral versus contralateral comparisons of all 4 proximal growth plates. Subclinical lesions were equally likely to develop in left and right legs, and the status of the proximal femoral head did not determine the status of the ipsilateral or contralateral proximal tibial head and vice versa. These observations are consistent with the interpretation that subclinical mechanical damage to one or more proximal leg bones need not trigger overt lameness until the damaged area becomes infected. The resulting bacterial proliferation, immunological assault by responding phagocytes (macrophages and heterophils), and widespread lysis and necrosis of the metaphyseal trabecular bone and vasculature would then culminate in clinical lameness (Howlett, 1980; Duff, 1984b; Thorp et al., 1993). Broilers completely immobilized by lameness typically exhibited the most severe FHN and THN lesions. Presumably the wire flooring model imposes increased torque or stress on proximal leg joints and accelerates the onset of BCO by increasing the availability of osteochondrotic clefts and thrombosed blood vessels for bacterial colonization.

It also is plausible that wire flooring per se constitutes a significant stressor contributing to generalized immunosuppression, and thus, bacterial proliferation. For example, chronic stress and immunosuppression were induced in Leghorn hens by housing them on slats and depriving them of access to deep litter (El-Lethey et al., 2003). Glucocorticoid-induced femoral head necrosis has been demonstrated in adult Leghorn hens (Cui et al., 1997). Environmental stressors and immunosuppression clearly contribute to the eruption of opportunistic pathogens harbored subclinically in the proximal tibial joints of rapidly growing turkeys that develop turkey osteomyelitis complex (Wyers et al., 1991; Huff et al., 2000, 2006). The involvement of many different opportunistic microorganisms suggests the susceptibility to turkey osteomyelitis complex may be influenced more by deficiencies in the host immune response or by stress-mediated immunosuppression rather than by the pathogenicity of any one organism (Huff et al., 2000). Important roles for environmental stressors and immunosuppression clearly must be considered in investigations of the spontaneous etiology of BCO (Mutalib et al., 1983a,b; Butterworth, 1999; McNamee and Smyth, 2000). In the present study, general necropsy observations consistently failed to reveal external symptoms of infection with chicken anemia virus (e.g., pale combs and wattles caused by anemia), nor was fatty degeneration of the marrow (yellow marrow) detected through 56 d of age (McNulty, 1991; Kuscu and Gurel, 2008).

Acknowledging the role of bacterial translocation and bacteremia in the pathogenesis of BCO, probiotics theoretically might reduce the onset of lameness by improving the health of the gastrointestinal tract or by priming the immune system. Our wire-flooring model combines single-pass ventilation (excellent air quality) plus wire flooring (reduced contact with fecal material) plus continuous probiotic delivery in the feed (sustained re-inoculation) to create ideal conditions under which a probiotic might be expected to elicit beneficial responses. In this environment, it initially seemed plausible that wire flooring might enhance the incidence of lameness, in part, by preventing the ingestion of naturally occurring probiotics or the re-ingestion of nutrients from the litter. However, adding used or clean litter to the feed as a prebiotic or probiotic in experiments 1 and 2 did not markedly reduce the incidence of lameness. In contrast, adding probiotics prophylactically to the feed beginning at 1 d of age did consistently reduce the incidence of lameness in broilers reared on wire flooring. Probiotics are not antibiotics and are unlikely to be effective if administered therapeutically only after lameness has developed in a flock. Indeed, starting the probiotic administration at 28 d of age did not reduce

the incidence of lameness markedly below that of the wire-control group in experiment 3. These observations support administering probiotics continuously and beginning at a very early age. Necropsy results for lame birds and for survivors on d 56 did not consistently reveal differential incidences within the lesion categories among the treatment groups, revealing no tendency for probiotics to attenuate the earliest femoral head or tibial head lesions elicited by wire flooring. In view of the obvious association between THN and clinical lameness in experiments 3 and 4 (Figure 9), it was intriguing that in experiment 5, the Biomin IMBO probiotic significantly reduced the incidence of THN in survivors on d 56, when compared with either the litter-control or wire-control groups (Table 2). Overall, the probiotics apparently delayed the progressive deterioration of early lesions into the grossly degenerative abscesses that are associated with terminal lameness in broilers. These experiments indicate that bacterial translocation from the gastrointestinal tract is likely to be a significant route contributing to hematogenous infection, and that probiotics administered prophylactically can provide a plausible alternative to antibiotics for reducing the incidence of BCO.

ACKNOWLEDGMENTS

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