Kinetic examination of femoral bone modeling in broilers

R. Prisby,*[1] T. Menezes,† J. Campbell,† T. Benson,† E. Samraj,† I. Pevzner,‡ and R. F. Wideman Jr.§

*Department of Kinesiology and Applied Physiology, University of Delaware, Newark 19716; †Department of Kinesiology, University of Texas, Arlington 76019; ‡Cobb Vantress Industries, Siloam Springs, AR 72761; and §Department of Poultry Science, University of Arkansas, Fayetteville 72701

ABSTRACT Lameness in broilers can be associated with progressive degeneration of the femoral head leading to femoral head necrosis and osteomyelitis. Femora from clinically healthy broilers were dissected at 7 (n = 35, 2), 14 (n = 32), 21 (n = 33), 28 (n = 34), and 42 (n = 28) d of age, and were processed for bone histomorphometry to examine bone microarchitecture and bone static and dynamic properties in the secondary spongiosa (IISP) of the proximal femoral metaphysis. Body mass increased rapidly with age, whereas the bone volume to tissue volume ratio remained relatively consistent. The bone volume to tissue volume ratio values generally reflected corresponding values for both mean trabecular thickness and mean trabecular number. Bone metabolism was highest on d 7 when significant osteoblast activity was reflected by increased osteoid surface to bone surface and mineralizing surface per bone surface ratios. However, significant declines in osteoblast activity and bone formative processes occurred during the second week of development, such that newly formed but unmineralized bone tissue (osteoid) and the percentages of mineralizing surfaces both were diminished. Osteoclast activity was elevated to the extent that measurement was impossible. Intense osteoclast activity presumably reflects marked bone resorption throughout the experiment. The overall mature trabecular bone volume remained relatively low, which may arise from extensive persistence of chondrocyte columns in the metaphysis, large areas in the metaphysis composed of immature bone, destruction of bone tissue in the primary spongiosa, and potentially reduced bone blood vessel penetration that normally would be necessary for robust development. Delayed bone development in the IISP was attributable to an uncoupling of osteoblast and osteoclast activity, whereby bone resorption (osteoclast activity) outpaced bone formation (osteoblast activity). Insufficient maturation and mineralization of the IISP may contribute to subsequent pathology of the femoral head in fast-growing broilers.

Key words: broiler, bone microarchitecture, bone histomorphometry

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INTRODUCTION

Lameness in broilers is an important issue in the poultry industry, and the industry is striving to understand and reduce the problem. A variety of etiologies contribute to osteochondrosis and the separation of the femoral epiphysis from the articular cartilage, conditions that are commonly referred to as epiphysiolysis (epiphyseal separation) or femoral head separation (**FHS**; Duff and Randall, 1987; Riddell, 1992; Thorp, 1994; Julian, 1998; McNamee and Smyth, 2000; Kajita et al., 2001; Wilson et al., 2005; Ytrehus et al., 2007; Dinev, 2009; Durairaj et al., 2009; Wideman et al., 2012). Initial damage to the femoral head can disrupt the physiological barrier to infection and often results in progressive and degenerative damage leading to femoral head degeneration and femoral head necrosis (**FHN**), which also is known as bacterial chondronecrosis with osteomyelitis (**BCO**; McNamee and Smyth, 2000; Durairaj et al., 2009; Wideman et al., 2012; Wideman and Prisby, 2013). The pathogenesis of FHN or BCO is complex and may be initiated by mechanical means. Although investigations in poultry are sparse, mechanical interruption of blood flow to subchondral bone presumably contributes to femoral head pathologies (Trueta and Amato, 1960) and rapid increases in BW may initiate such events in broilers (Thorp et al., 1993; Durairaj et al., 2009; Wideman et al., 2012). Excessive weight presumably causes twisting and compression at the ball and socket joint of the femur and restricts indispensable blood vessels servicing this anatomical location. Thus, chronic inadequate blood flow resulting from compression of the vascular network would limit delivery of nutrients and oxygen

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[1]Corresponding author: prisby@udel.edu

to bone tissue and initiate the progressive degenerative changes leading to terminal FHN.

Examples of inadequate blood flow leading to tissue necrosis have been noted in obese humans, such that repetitive mechanical strain on weight bearing joints is associated with FHN and avascularity of subchondral bone (Sambrook, 2006). Yet in opposition to human avascular necrosis, which involves subchondral bone, in broilers the problem appears to be initiated at the growth plate, with FHS, osteochondrosis, and the resulting infection being linked to rapid growth (Riddell, 1992; Thorp et al., 1993; Thorp, 1994; Julian, 1998; Julian, 2005; Durairaj et al., 2009). The question remains as to whether blood vessel compression limits blood flow to a degree that compromises bone (re)modeling, mass, and strength, which in turn progressively deteriorates to clinical lameness. Alternatively, and not exclusively, the etiologies associated with femoral head degeneration and FHN may reside in the skeleton (e.g., imbalances between bone resorptive and formative processes during development). In other words, the pathology associated with lameness in broiler chickens may be inherent to the cells of bone tissue (e.g., osteoblasts and osteoclasts). Thus, we sought to examine bone modeling as a function of development to provide kinetic insight for potential causes of lameness. The objectives of this experiment were to evaluate the microarchitecture and static and dynamic properties in the proximal femora of broilers during development (i.e., at 7, 14, 21, 28, and 42 d of age).

MATERIALS AND METHODS

Animal procedures were approved by the Universities of Arkansas and Texas Arlington Institutional Animal Care and Use Committees and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996). Commercial broilers were reared in standard-sized floor pens (3 m × 3 m). Each pen initially contained 73 chicks, and on d 14 the pens were culled to 62 of the largest, fastest growing chicks. The photoperiod was set for 23L:1D. Thermoneutral temperatures were maintained throughout with target temperatures set at 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 starter diet was a commercial corn and soybean-meal based chick starter (crumbles), and after d 35 all birds were switched to a pelleted commercial corn and soybean-meal based finisher diet. Feed was formulated without meat or animal byproducts to meet or exceed minimum NRC (1994) standards for all ingredients. Six days and again 1 d before being killed, broilers were injected with tetracycline (50 mg/kg of BW) to fluorescently label the bone tissue (Sanger and Holt, 1965; Chan et al., 1977). Birds were euthanized via CO_2 gas inhalation. Euthanized birds were necropsied within 5 min postmortem. Previously published photographs illustrate typical FHS, FHT, and FHN lesions of the proximal femora (Wideman et al., 2012; Wideman and Prisby, 2013). Femora were recorded as being macroscopically normal or having FHS, FHT, or FHN, after which they were cleaned of most soft tissue, stored in glass vials in 10% formaldehyde at 4°C for 7 d, and then transferred to 70% ETOH for long-term storage at −20°C.

Bone Histomorphometry

Femora were sequentially dehydrated in ETOH and embedded undecalcified in methylmethacrylate at low temperature for bone histomorphometric evaluation. The central plane of the proximal femur was sliced frontally with a microtome (Leica RM2255, Bannockburn, Buffalo Grove, IL). Two 10-μm-thick histological sections per broiler were stained with Goldner's Trichrome for the evaluation of several parameters in the secondary spongiosa (**IISP**) according to the ASBMR histomorphometry nomenclature (Dempster, 2008): bone volume to tissue volume ratio (**BV/TV**, %), mean trabecular thickness (**Tb.Th**, μm), mean trabecular number (**Tb.N**, /mm^2), and mean trabecular separation (**Tb.Sp**, μm). In addition, these slides were used to evaluate percent osteoid surface to bone surface ratio (**OS/BS** [%]), which represents new bone that has yet to be mineralized. Cancellous bone lined by osteoblasts was measured and expressed as osteoblast surface to bone surface ratio (**Ob.S/BS**, %). For bone resorption parameters, two 10-μm-thick sections stained with tartrate resistant acid phosphatase (**TRAP**) were analyzed per broiler. Cancellous bone lined by osteoclasts was to be measured and expressed as osteoclast surface per bone surface ratio (**Oc.S/BS**, %). Bone dynamic parameters were determined on 2 unstained, 10-μm-thick sections under UV light microscopy, that is, mineral apposition rate (**MAR**, μm/d), single-labeled surface to bone surface ratio (**sLS/BS**, %), and double-labeled surface to bone surface ratio (**dLS/BS**, %). Mineral apposition rate was calculated as the mean distance between 2 tetracycline labels divided by the time interval between tetracycline injections (i.e., 5 d). Mineralizing surface per bone surface ratio (**MS/BS**, %) was calculated by adding dLS/BS and 1/2 sLS/BS. Bone formation rate to bone surface ratio (**BFR/BS**, $\mu m^3/\mu m^2$ per d) was calculated as the product of MS/BS and MAR. All parameters of bone resorption and formation were measured with the OsteoMeasure computerized system (OsteoMetrics, Decatur, GA) connected to a personal computer and a UV light-capable microscope. All parameters were measured in the IISP of the proximal femoral metaphysis.

Statistical Analysis

One-way ANOVA was used to determine the significance of differences in body mass, bone microarchitecture, and bone static and dynamic properties among

ages. Post-hoc analysis was conducted using the Student-Newman Kuels test. Data are presented as mean ± SE. Significance was defined a priori as $P \leq 0.05$. Tendencies for significant differences ($P \leq 0.10$) are reported.

RESULTS

Of the 240 left and right femora collected, 59 presented with overt external pathology defined as FHS or FHN. This pathology was 24.5% of the total sample size. The majority of pathology was FHS, which accounted for 22.9% and the remainder was FHN, which accounted for only 1.6% of the observed pathology. Thus, the majority of femora did not show signs of overt pathology and were considered to be normal. Femora that did not show overt signs of external pathology were chosen for histological examination, and thus, the data were derived from bone tissue that macroscopically appeared to be normal. Final sample sizes for the different age groups were as follows: 7 d (n = 35), 14 d (n = 32), 21 d (n = 33), 28 d (n = 34), and 42 d (n = 28). As shown in Figure 1, body mass increased as a function of age, illustrating the rapid weight gain typical of broilers.

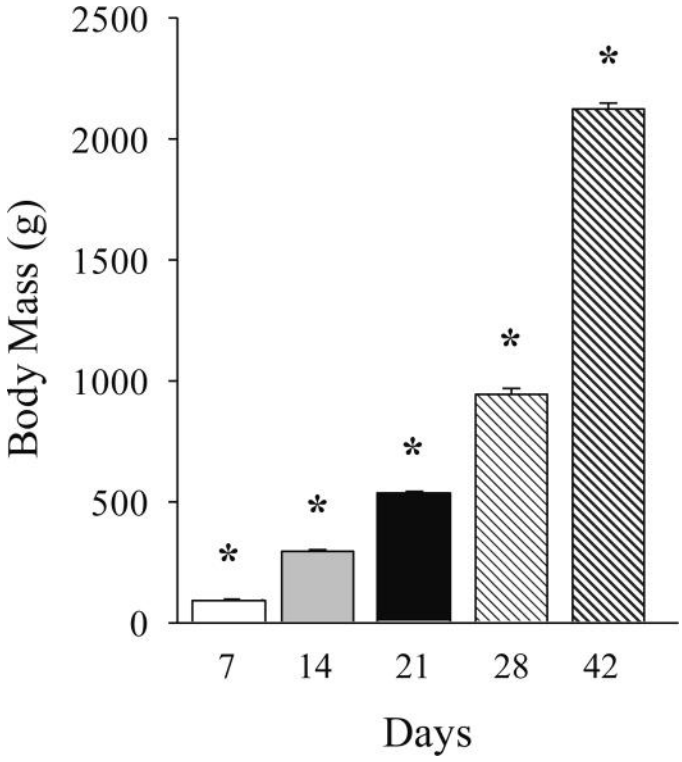

Figure 1. Body mass from 7 to 42 d of age. Values are means ± SE. *Denotes a significant ($P < 0.05$) difference from the other age groups.

Trabecular Bone Microarchitectural Properties

The BV/TV in the proximal femoral metaphysis peaked at d 28 (~8%) but declined to ~5% at d 42 (Figure 2). Generally speaking, alterations in BV/TV from d 7 to 28 reflect increases in both Tb.Th and Tb.N (Figure 2). Interestingly, declines in BV/TV at d 42 are attributable to reduced Tb.N because Tb.Th remained augmented. The Tb.Sp remained relatively constant from d 7 to 28 but significantly increased at d 42 versus the other time points (Figure 2).

Trabecular Bone Static Properties

Osteoid surface ratios were highest at d 7 and markedly fell by 43% at d 14, suggesting a decline in osteoblast function (Table 1). Osteoblast surfaces (Ob.S/BS) were similar and did not differ among ages (Table 1). Bone static properties for osteoclast activity (Oc.S/BS) were not measurable due to unusual TRAP staining patterns observed in all age groups. A representative photo illustrates this staining pattern in rat trabecular bone (Figure 3A) in comparison with staining patterns observed in broilers (Figure 3B). As observed in the broiler trabecular bone section, osteoclast activity is evident on the entire bone surface as opposed to bordering the surface of rat trabecular bone. Inability to separate trabecular bone surfaces from osteoclasts made measurement of osteoclast activity impossible. These staining patterns presumably represent dramatically enhanced osteoclast activity and, therefore, markedly high bone resorption in broilers from 7 to 42 d of age.

Trabecular Bone Dynamic Properties

The bone formation rate did not differ among ages (Figure 4); however, MS/BS at d 7 was significantly augmented in comparison with the other groups (Table 1). The amount of mineral laid down by osteoblasts per day (MAR) was consistent across age and did not differ (Table 1).

Other Observances Within the Proximal Femoral Metaphysis

A healthy femoral metaphysis is illustrated in Figure 5. In this example, bone tissue is stained green and osteoid (i.e., newly formed but yet to be mineralized bone tissue) is stained pink. The areas within the proximal femoral metaphysis identified as the primary spongiosa (**ISP**: immature bone tissue) and the secondary spongiosa (IISP: mature bone tissue) can be observed. As indicated previously, all measurements were made in the IISP. Figure 6 depicts complete destruction of bone tissue in the ISP, which extends into the IISP. As can be observed, the marrow space in the IISP appears normal and some bone tissue is evident below the destruction front. Figure 7 depicts a phenomenon often observed in avian bone, which is identified as elongated chondrocyte columns. In this example, the ISP appears

Table 1. Bone static and dynamic properties in 7- to 42-d-old broilers, including the osteoid surface to bone surface ratio (OS/BS), the osteoblast surface to bone surface ratio (Ob.S/BS), the mineral apposition rate (MAR), and the mineralizing surface per bone surface ratio (MS/BS)[1]

Age (d)	OS/BS (%)	Ob.S/BS (%)	MAR (μm/d)	MS/BS (%)
7	21.3 ± 2.7*	1.26 ± 0.23	0.70 ± 0.07	4.0 ± 0.5*
14	12.0 ± 1.6	0.76 ± 0.16	0.53 ± 0.06	2.6 ± 0.3
21	10.6 ± 1.8	1.23 ± 0.52	0.72 ± 0.09	2.9 ± 0.3
28	5.5 ± 1.0	0.54 ± 0.08	0.80 ± 0.17	2.5 ± 0.3
42	7.2 ± 1.7	0.45 ± 0.06	0.82 ± 0.21	2.1 ± 0.3

[1]Values represent means ± SE.

*Denotes a significant ($P < 0.05$) difference from all other ages.

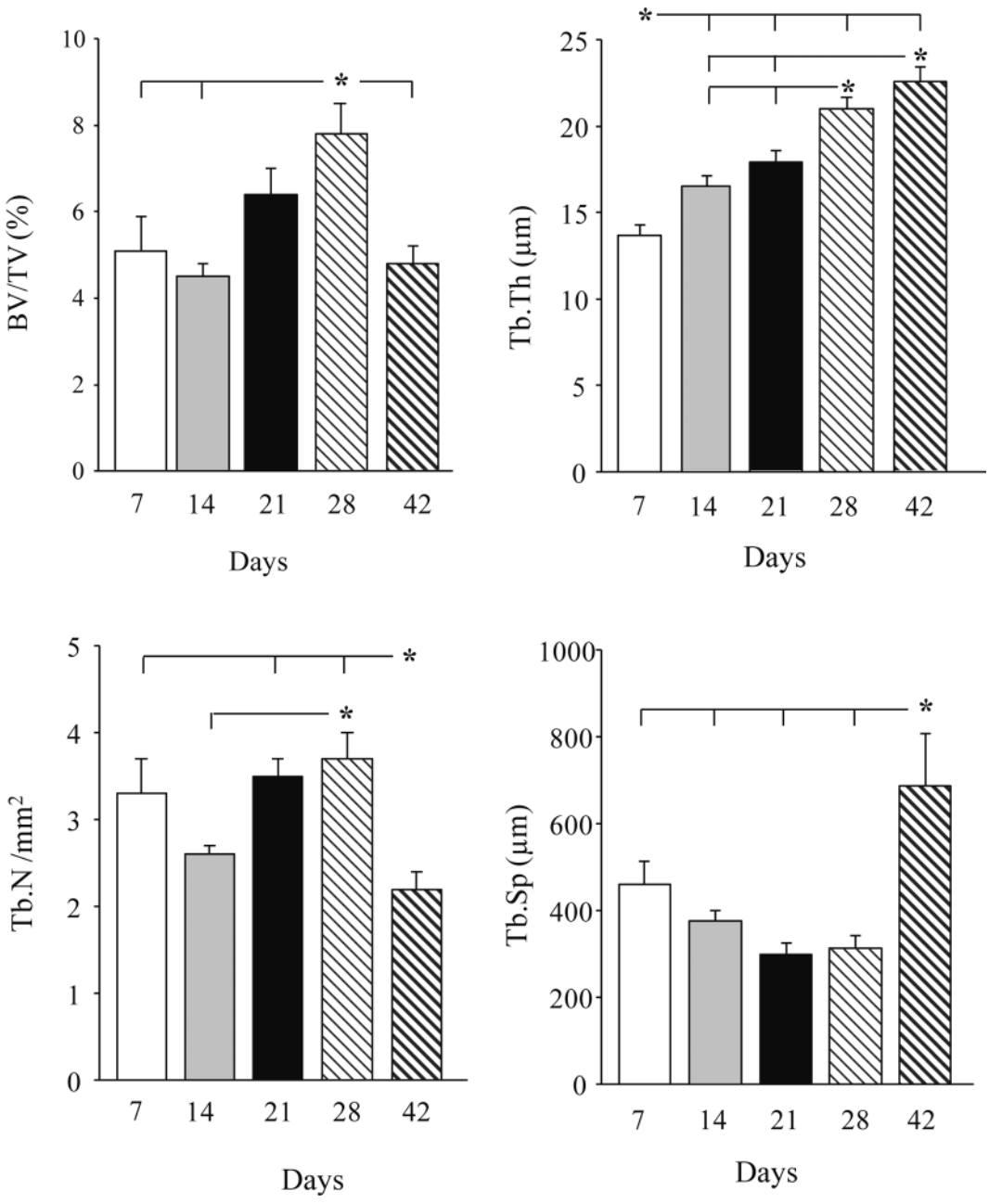

Figure 2. Bone microarchitectural properties in the proximal femoral metaphysis from 7 to 42 d of age, including the bone volume to tissue volume ratio (BV/TV), mean trabecular thickness (Tb.Th), mean trabecular number (Tb.N), and mean trabecular separation (Tb.Sp). Values are means ± SE. *Denotes a significant ($P < 0.05$) difference from indicated age groups.

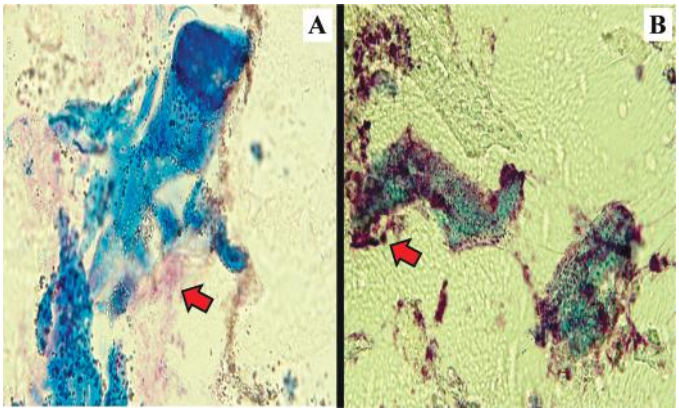

Figure 3. Photos depicting resorption of rat (A) and broiler (B) trabecular bone. Trabecular bone is stained blue. As can be observed from the representative photos, osteoclast activity (pinkish-purple cells denoted by red arrows) appears excessive on the broiler versus rat trabecular bone, such that overall activity was neither measurable nor quantifiable.

absent and the chondrocyte columns extend well into the IISP. Trabecular bone is sparse in the IISP (see middle of bone section).

DISCUSSION

The major finding of this investigation is that mineralization and maturation of the proximal femora apparently cannot keep pace with the rapid increases in body

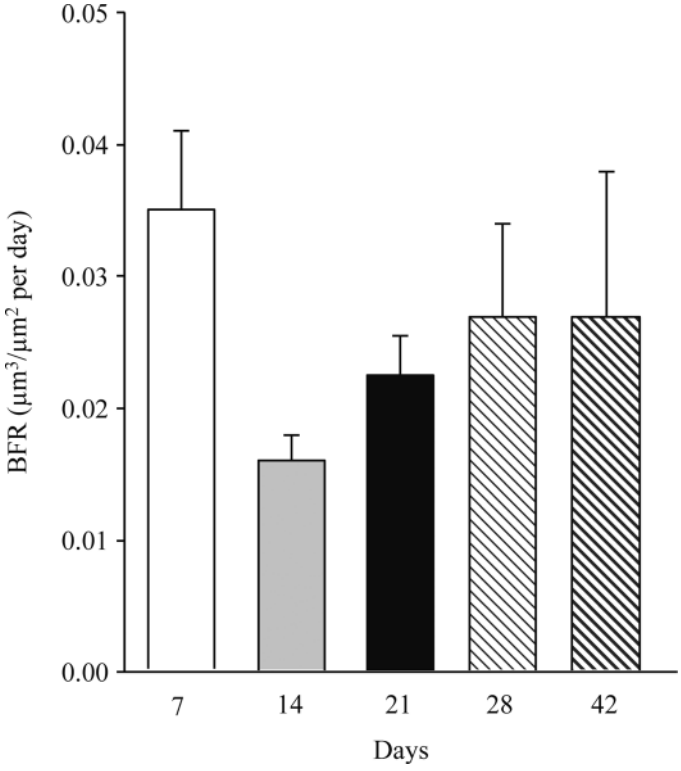

Figure 4. Bone formation rate (BFR) in the proximal femoral metaphysis of broilers from 7 to 42 d of age. Values are means ± SE.

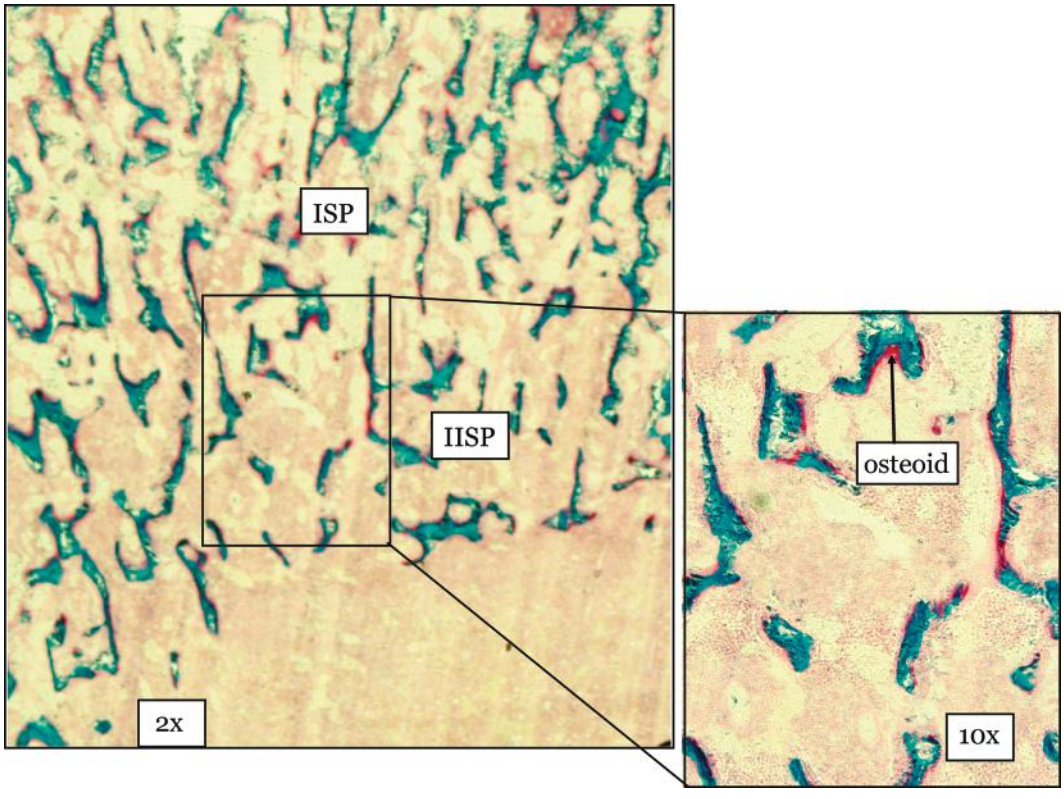

Figure 5. A photo representing healthy bone and bone marrow in the proximal femoral metaphysis of a broiler. Depicted in the photo are the primary (ISP; immature bone) and secondary (IISP; mature bone) spongiosae. Bone tissue is stained green, and osteoid seams are stained pink. Osteoid seams represent newly formed but unmineralized bone tissue.

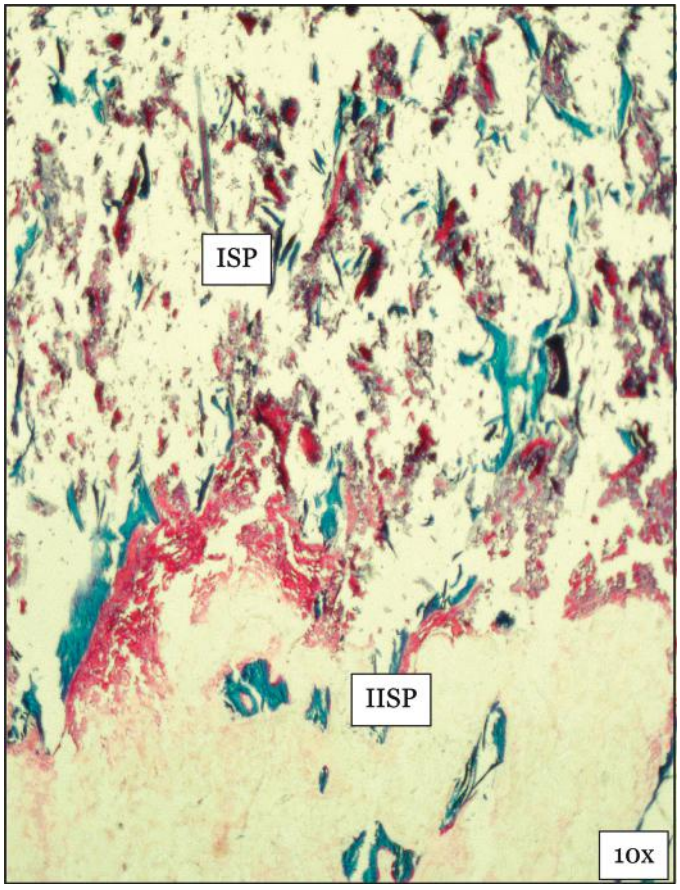

Figure 6. A photo representing destruction in the primary spongiosa (ISP) of the proximal femoral metaphysis of a broiler. As can be observed, destruction of bone tissue in the ISP extends into the secondary spongiosa (IISP). Normal bone and bone marrow is observed below the destruction front.

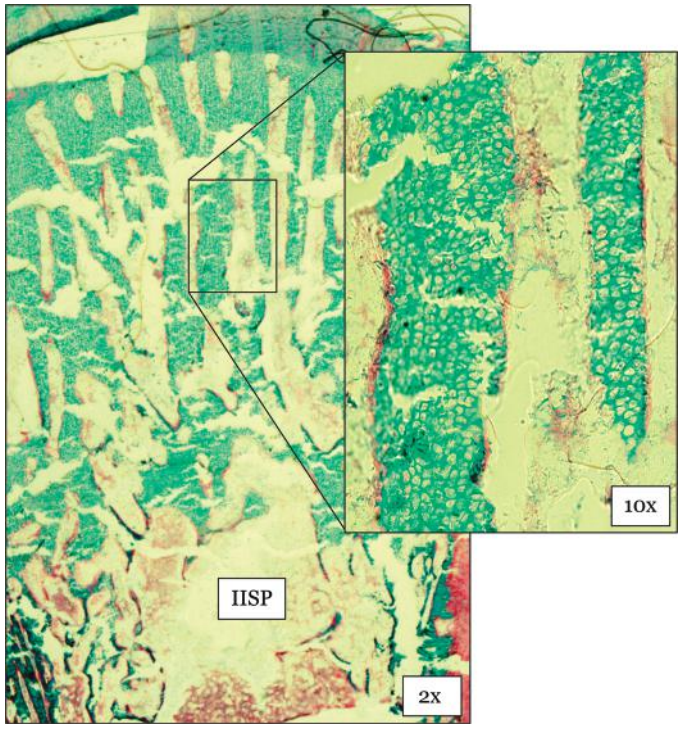

Figure 7. A photo representing elongated chondrocyte columns in the proximal femoral metaphysis of a broiler. As can be observed, chondrocyte columns extend into the secondary spongiosa (IISP). Little bone tissue is observed in both the primary and secondary spongiosae.

mass in broilers. An inability to augment bone volume appropriately may relate to the observed declines in osteoblast (Table 1 and Figure 4) and excessively marked osteoclast activities (Figure 3B) from d 7 to 42. It is important to recognize, however, that all measurements of bone microarchitecture and bone static and dynamic properties were made in apparently healthy femora and no lameness was noted in these animals. Thus, the stagnated femoral development during this period of rapid growth is not necessarily the impetus for lameness in broilers but may predispose their skeleton to enhanced risk, with mechanical perturbation presumably triggering the onset of lameness and subsequent bacterial infection.

Progressive degeneration of femora and tibiae in broilers often results in FHN and BCO. In fact, such lesions are routinely observed in commercial flocks (McNamee and Smyth, 2000; Wideman et al., 2012; Wideman and Prisby, 2013). However, as highlighted in this investigation, the lack of external macroscopic pathology is not evidence of normal bone metabolism, or even of a nonpathological interior. As observed in these macroscopically normal femora, the enzymatic reaction of broiler bone sections to tartrate resistance acid phosphatase is suggestive of excessive osteoclast activity. Coupled with the stagnant osteoblast activity observed as a function of development (i.e., bone formation rate), there appears to be an uncoupling of the osteoblast-to-osteoclast activity ratio that is necessary for significant bone accrual.

These data indicate that early development (i.e., d 7) was the most biologically active period, whereby newly formed bone tissue (i.e., OS/BS) and mineralizing surfaces (MS/BS) were highest. Yet, 7 d later, bone formative processes dramatically declined and is attributable to reduced osteoblast activity (OS/BS, bone formation rate, MS/BS) as opposed to the ability to recruit osteoblasts to bone surfaces (Ob.S/BS). Broiler chickens double and redouble body mass approximately 7 times in 8 wk (Wideman and Prisby, 2013) and from the day of hatch to d 42, femora have been demonstrated to increase 5.6 cm in length and to increase 6.9 cm in width at the mid-shaft (Applegate and Lilburn, 2002). Thus, skeletal disorders in broilers are theorized to result from an inability of the skeleton to mature at rates sufficient to mechanically support the rapid accrual of body mass (Classen and Riddell, 1989; Leterrier and Nys, 1992; Williams et al., 2000, 2004), particularly when rapid growth occurs during the first few weeks posthatch (Wideman et al., 2013).

Interestingly, bone microarchitectural data of the IISP (i.e., mature bone) suggest that while overall bone growth occurred (e.g., radial expansion of femora), development of mature trabecular struts in the IISP was retarded. This is evidenced by relatively consistent BV/

TV (i.e., 4.5–7.8%) from d 7 to 42. The components that comprise BV/TV are Tb.Th, Tb.N, and Tb.Sp. The number of trabeculae in the IISP remained relatively consistent from d 7 to 28, with a significant fall at d 42. However, trabeculae continued to thicken from d 7 to 42, suggesting that bone accrual did occur to some extent. In general, as trabecular thickness increases, the space between individual trabeculae diminishes. This was observed nonsignificantly in Tb.Sp from d 7 to 28. The decline in Tb.N in the face of consistently thick trabeculae (Tb.Th) at d 42 suggests that radial expansion of femora, which would serve to augment marrow volume and give the appearance of a decline in newly created trabecular struts, accounted for the dramatic rise in distance between individual trabeculae (Tb.Sp). Thus, radial expansion of femora presumably outpaced the ability to generate mature bone tissue in the IISP.

Susceptibility to lameness in broilers has also been attributed to unusually long chondrocyte columns at the proximal growth plates. We observed a similar phenomenon, whereby the chondrocyte columns appear to replace the ISP and extended well into a sparsely ossified IISP (Figure 7). Because large areas of chondrocyte columns are so plentiful in these birds, this could possibly coincide with a lack of blood vessel penetration that is necessary for bone development. In addition, vast areas of chondrocyte columns indicate that the bone tissue is still undergoing growth and development. Greater proportions of bone being composed of chondrocyte columns as opposed to IISP would serve to reduce bone strength and increase risk of fracture with augmenting body mass or load on the skeleton, leading to mechanical damage and lameness. Finally, it is important to note the microscopic damages observed in apparently healthy femora of broiler chickens during development. The destruction of bone tissue, as illustrated in Figure 6, was a common observance. In this case, it occurred in the ISP. The presence of this type of damage or necrosis within the marrow space even in the presence of a normal external bone appearance also may indicate inadequate bone perfusion that leads to tissue destruction or death.

In summary, the alterations in body mass were representative of rapid growth in broilers. The volume of trabecular bone remained relatively consistent during this time period. The lack of bone development was attributable to an uncoupling of osteoblast to osteoclast activity, whereby bone resorption outpaced bone formation. Osteoblast impairment was evident by the second week of development, such that newly formed but unmineralized bone tissue had diminished and the percentages of mineralizing surfaces were reduced. Osteoclast dysfunction presented as osteoclasts covering the entire trabecular bone surfaces with an inability to distinguish bone surfaces from osteoclast surfaces. The low bone volume observed in these broilers indicates that even as the birds were rapidly growing, the overall mature trabecular bone volume (i.e., IISP) remained relatively low. Such low mature bone volumes may arise from large areas of chondrocyte columns, large areas in the metaphysis composed of immature bone, bone tissue destruction, and perhaps reduced bone blood vessel penetration.

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REFERENCES

Applegate, T. J., and M. S. Lilburn. 2002. Growth of the femur and tibia of a commercial broiler line. Poult. Sci. 81:1289–1294.

Chan, M. M., R. S. Riggins, and R. B. Rucker. 1977. Effect of ethane-1-hydroxy-1,1-diphosphonate (EHDP) and dietary fluoride on biomechanical and morphological changes in chick bone. J. Nutr. 107:1747–1754.

Classen, H., and C. Riddell. 1989. Photoperiodic effects on performance and leg abnormalities in broiler chickens. Poult. Sci. 68:873–879.

Dempster, D. 2008. Histomorphometric analysis of bone remodeling, Pages 447–63 in Principles of Bone Biology. J. Bilezikian and T. J. Martin, ed. Elsevier, San Diego, CA.

Dinev, I. 2009. Clinical and morphological investigations on the prevalence of lameness associated with femoral head necrosis in broilers. Br. Poult. Sci. 50:284–290.

Duff, S., and C. J. Randall. 1987. Observations on femoral head abnormalities in broilers. Res. Vet. Sci. 42:17–23.

Durairaj, V., R. Okimoto, K. Rasaputra, F. D. Clark, and N. C. Rath. 2009. Histopathology and serum clinical chemistry evaluation of broilers with femoral head separation disorder. Avian Dis. 53:21–25.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773–1780.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry—A review. Vet. J. 169:350–369.

Kajita, Y., M. Takayasu, J. Yoshida, H. H. Dietrich, and R. G. Dacey. 2001. Vasodilatory effect of basic fibroblast growth factor in isolated rat cerebral arterioles: Mechanisms involving nitric oxide and membrane hyperpolarization. Neurol. Med. Chir. (Tokyo) 41:177–185.

Leterrier, C., and Y. Nys. 1992. Composition, cortical structure and mechanical properties of chicken tibiotarsi: Effect of growth rate. Br. Poult. Sci. 33:925–939.

McNamee, P., and J. A. Smyth. 2000. Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: A review. Avian Pathol. 29:477–495.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Riddell, C. 1992. Non-infectious skeletal disorders of poultry: An overview. Pages 119–145 in Bone Biology and Skeletal Disorders in Poultry. C. Whitehead, ed. Carfax Publishing Company, Abingdon, UK.

Sambrook, P. 2006. Glucocorticoid-induced osteoporosis. Pages 296–302 in Primer on the Metabolic Bone Disease and Disorders of Mineral Metabolism. M. Favus, ed. American Society of Bone and Mineral Research, Washington, DC.

Sanger, V. L., and J. A. Holt. 1965. Experimental tetracycline labeling in avian osteoporosis. Can. J. Comp. Med. Vet. Sci. 29:245–252.

Thorp, B. 1994. Skeletal disorders in the fowl: A review. Avian Pathol. 23:203–236.

Thorp, B., C. C. Whitehead, L. Dick, J. M. Bradbury, R. C. Jones, and A. Wood. 1993. Proximal femoral degeneration in growing broiler fowl. Avian Pathol. 22:325–342.

Trueta, J., and V. P. Amato. 1960. The vascular contribution to osteogenesis III. Changes in the growth cartilage caused by experimentally induced ischaemia. J. Bone Joint Surg. Br. 42B:571–587.

Wideman, R. F., Jr, A. Al-Rubaye, A. Gilley, D. Reynolds, H. Lester, D. Yoho, J. D. Hughes Jr., and I. Y. Pevzner. 2013. Susceptibility of four commercial broiler crosses to lameness attributable to bacterial chondronecrosis with osteomyelitis. Poult. Sci. 92:2311–2325.

Wideman, R. F., Jr, K. R. Hamal, J. M. Stark, J. Blankenship, H. Lester, K. N. Mitchell, G. Lorenzoni, and I. Pevzner. 2012. A wire flooring model for inducing lameness in broilers: Evaluation of probiotics as a prophylactic treatment. Poult. Sci. 91:870–883.

Wideman, R. F., and R. D. Prisby. 2013. Bone circulatory disturbances in the development of spontaneous bacterial chondronecrosis with osteomyelitis: A translational model for the pathogenesis of femoral head necrosis. Frontiers in Science (Front. Endocrinol.) 3:183. http://dx.doi.org/10.3389/fendo.2012.00183.

Williams, B., S. Solomon, D. Waddington, B. Thorp, and C. Farquharson. 2000. Skeletal development in the meat-type chicken. Br. Poult. Sci. 41:141–149.

Williams, B., D. Waddington, D. H. Murray, and C. Farquharson. 2004. Bone strength during growth: Influence of growth rate on cortical porosity and mineralization. Calcif. Tissue Int. 74:236–245.

Wilson, F., P. Stayer, L. Pace, and F. Muhammad. 2005. Histological determination of pathological or non artifact basis for epiphysis separation induced during coxofemoral disarticulation in clinically normal poultry. American Association of Veterinary Laboratory Diagnosticians, Hershey, PA.

Ytrehus, B., C. S. Carlson, and S. Ekman. 2007. Etiology and pathogenesis of osteochondrosis. Vet. Pathol. 44:429–448.