Dexamethasone triggers lameness associated with necrosis of the proximal tibial head and proximal femoral head in broilers

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ABSTRACT Bacterial chondronecrosis with osteomyelitis (BCO) and turkey osteomyelitis complex (TOC) are characterized by bacterial infection and necrotic degeneration within the tibiae and femora. Stress and immunosuppression have been implicated in the pathogenesis of BCO and TOC. Immunosuppressive doses of dexamethasone (DEX) trigger high incidences of TOC in turkey poults. The present study was conducted to determine if DEX injections or heat stress can trigger BCO and lameness in broilers. In 3 independent experiments, broilers were weighed and either remained uninjected or received repeated injections of 0.9% saline or DEX dissolved in saline (0.45 to 1.5 mg of DEX/kg of BW). Across all 3 experiments, the incidences of lameness were 0% for uninjected controls, 0 to 8% in saline-injected groups, and 24 to 68% in groups injected with 0.9 to 1.5 mg of DEX/kg of BW. Growth was inhibited by DEX injections regardless of whether the birds became lame or survived. When compared with saline-injected groups, DEX injections consistently increased the incidence of severe proximal tibial head necrosis in lame birds as well as in survivors. The DEX injections also triggered a subset of lesions that are not considered pathognomonic for BCO (for example, avascular femoral head necrosis and fatty necrosis of the tibiae). In a fourth experiment, repeated episodes of heat stress did not trigger lameness, although the subclinical incidence of tibial head necrosis was substantially higher at 28 and 35 d of age in heat-stressed broilers when compared with broilers reared under thermoneutral conditions. Accordingly, stress and immunosuppression must be considered contributing factors in the pathogenesis of tibial and femoral lesions associated with lameness in broilers. A subset of the lesions triggered by repeated DEX injections did not precisely mimic the pathogenesis of BCO in broilers, and DEX consistently inhibited growth whereas BCO is associated with rapid growth. These caveats must be acknowledged when DEX is used to trigger lameness in broilers.

Key words: lameness, immunosuppression, glucocorticoid, osteomyelitis

INTRODUCTION

Bacterial chondronecrosis with osteomyelitis (**BCO**; formerly known as femoral head necrosis, proximal femoral degeneration, or bacterial chondronecrosis; **BCN**) is the most common cause of lameness in commercial broiler flocks. The term BCO encompasses necrotic degeneration and bacterial infection primarily within the proximal heads of the femora and tibiae, but also in the growth plates of other rapidly growing bones including the vertebrae (Carnaghan, 1966; Pattison, 1992; McNamee et al., 1998; Butterworth, 1999; McNamee and Smyth, 2000; Bradshaw et al., 2002; Dinev, 2009). Previously, BCO has been reproduced by injecting broilers

and turkeys with appropriate strains of *Staphylococcus* spp. (Carnaghan, 1966; Wise, 1971; 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). Recently, we demonstrated that broilers grown on wire flooring develop significant levels of BCO without being intentionally exposed to known pathogens (Wideman et al., 2012). Wire flooring imposes additional torque and shear stress on susceptible leg joints. In addition, flooring systems that deprive birds of access to floor litter stimulate chronic stress, including immunosuppression attributable to elevated blood corticosterone concentrations (El-Lethey et al., 2003). The net impact of growing broilers on wire flooring likely includes enhanced mechanical damage (microtrauma) to proximal leg joints combined with generalized immunosuppression and thus proliferation of hematogenously distributed bacteria. Environmental stressors and immunosuppression have been implicated

in the etiology of spontaneous BCO outbreaks in commercial flocks (Mutalib et al., 1983a,b; Butterworth, 1999; McNamee and Smyth, 2000).

Bacterial arthritis and infection of the proximal tibiae are characteristic symptoms of turkey osteomyelitis complex (**TOC**). Environmental stressors and immunosuppression contribute to the eruption of opportunistic pathogens harbored subclinically in the proximal tibial joints of turkeys that develop TOC (Wyers et al., 1991; Huff et al., 1998, 1999, 2000, 2006). The involvement of many different opportunistic microorganisms suggests susceptibility to TOC may be influenced more by deficiencies in the host immune response or stress-mediated immunosuppression rather than by the pathogenicity of any one organism (Huff et al., 2000, 2005, 2006). In an experimental setting, TOC can be triggered by injecting turkey poults with repeated immunosuppressive doses of dexamethasone (**DEX**), a synthetic glucocorticoid (Huff et al., 1998, 1999, 2000). Three intramuscular DEX injections on alternating days resulted in an 8% incidence of TOC as compared with a 0% incidence in untreated poults, and a second round of 3 DEX injections increased the TOC incidence to 64% as compared with a 0% incidence in untreated poults (Huff et al., 2000). Steroid-induced femoral head necrosis also has been demonstrated in adult Leghorn hens (Cui et al., 1997). Based on the etiological similarities between BCO and TOC, 3 experiments were conducted to determine if DEX injections can trigger BCO and lameness in broilers. In view of recent indications that the stress of overheating during incubation may compromise leg health (Oviedo-Rondón et al., 2008, 2009a,b; Shim and Pesti, 2011), we also evaluated the proximal femora and tibiae of heat-stressed broilers for the presence of BCO-type lesions.

MATERIALS AND METHODS

Animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol #11002). Environmental chambers (dimensions: 3.7 m long × 2.5 m wide × 2.5 m high) within the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm were used to conduct 4 experiments. The chambers use single-pass ventilation at a constant rate of 6 m^3 per min. The chambers were set up with clean wood shavings litter, tube feeders, and nipple waterers. Broiler chicks (mixed sex) from commercial hatcheries (Lines D or B) were placed at 80 per chamber at 1 d of age. Chicks received standard hatchery vaccinations for Marek's and tenosynovitis. The photoperiod was set for 23L:1D. Thermoneutral temperatures were maintained throughout, except as noted for experiment 4: 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. Commercial corn and soybean meal-based chick starter (crumbles) and finisher (pelleted) diets were formulated to meet or exceed

minimum National Research Council (1994) standards for all ingredients.

Experiment 1

At 29 d of age, 50 broilers from Line D were weighed and 25 were injected with DEX (Sigma-Aldrich Chemical Co., St. Louis, MO) prepared as described previously (Huff et al., 1998). A 200 mg/mL stock solution was prepared in absolute ethanol, and then 0.3 mL of the stock solution was suspended in 30 mL of 0.9% saline (0.9 g of NaCl per 100 mL of H$_2$O) to make a 2 mg DEX/mL injection solution. Each bird was injected intramuscularly (thigh muscle) with 1.5 mg of DEX/kg of BW. The remaining 25 broilers were injected intramuscularly with an equivalent volume of 0.9% saline alone. The injections were repeated on d 31 and d 33, with each bird receiving a total of 3 DEX (**DEX 1** group) or saline (**saline 1** group) injections. All birds were observed walking daily beginning on d 29. Lame broilers were reluctant to stand, exhibited an obvious limping gait while dipping one or both wing tips, and within 24 h, became immobilized. Lame birds were removed as soon as the onset of lameness was noticed and were euthanized via CO$_2$ gas inhalation. The birds were weighed on d 36 and d 42, and survivors on d 42 were euthanized via CO$_2$ gas inhalation and necropsied to assess subclinical lesion incidences in the proximal heads of the femora and tibiae. Also on d 42, broilers (n = 22) that had been reared in the same chambers but had not received any injections were necropsied to assess subclinical lesion incidences (uninjected group). The birds in the uninjected group were not weighed.

Experiment 2

At 36 d of age, 51 broilers from Line D were weighed and then 26 were injected intramuscularly (thigh muscle) with 1.0 mg of DEX/kg of BW. The remaining 25 broilers were injected intramuscularly with 0.9% saline alone. The injections were repeated on d 38, 40, 43, 45, and 47 for a total of 6 DEX (**DEX 2** group) or saline (**saline 2** group) injections per bird. Body weights were recorded on d 43 and d 49, and the survivors were necropsied on d 49 to assess subclinical lesion incidences in the proximal heads of the femora and tibiae. Uninjected controls were not evaluated in experiment 2.

Experiment 3

This experiment was conducted to determine if reducing the relative amount of DEX injected might permit the broilers to continue growing at an acceptable rate while still causing significant incidences of lameness. At 21 d of age, 100 broilers from Line B were weighed and 25 per group were injected intramuscularly (thigh muscle) with 0.9% saline (**saline 3** group); 0.45 mg of DEX/kg of BW (**Lo DEX 3** group); or 0.9 mg of DEX/kg of BW (**Hi DEX 3** group). The injections

were repeated on d 23, 25, 27, 29, and 31 for a total of 6 DEX or saline injections per bird. The 25 birds in the uninjected 3 group did not receive any injections. All birds were weighed on d 31 and d 42, and the survivors on were necropsied on d 42 to assess subclinical lesion incidences in the proximal heads of the femora and tibiae.

Experiment 4

Chicks from Line D initially were housed at 85 per chamber and were provided optimal early brooding temperatures at 32°C for d 1 to 3, 30°C for d 4 to 6, 28°C for d 7 to 10, and 26°C for d 11 to 14. Thereafter, the temperature in one chamber was set at 24°C through the end of the experiment (thermoneutral group). In a second chamber, the birds were exposed to 3 d (72 h) of heat stress per week. The temperature was increased to 33°C on d 22 to 24, d 29 to 31, and d 36 to 38 (heat stress group). The 33°C temperature was sufficient to induce sustained panting without causing mortality. Between the periods of heat stress, the temperature was returned to 24°C to allow the birds to recover, eat, and grow. At weekly intervals beginning on d 21, birds (n = 20 per chamber on d 21, 28, and 42; n = 15 per chamber on d 35) were euthanized and necropsied to evaluate the incidences of subclinical leg lesions. Final BW were recorded on d 42.

Necropsy Procedures

Euthanized birds were necropsied within 30 min postmortem. All birds that died or developed lameness were necropsied and assigned to one of the following diagnostic categories: **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; Riddel, 1976); **Normal F** = no macroscopic abnormalities of the proximal femur; **FHS** = proximal femoral head separation or epiphyseolysis; **FHT** = proximal femoral head transitional degeneration; **FHN** = proximal femoral head necrosis; **Normal T** = no macroscopic abnormalities of the proximal tibia; **TD** = tibial dyschondroplasia; or **THN** = proximal tibial head necrosis, a subcategory of BCO in the tibiotarsus (Butterworth, 1999). Previously published photographs illustrate typical BCO lesions of the proximal femora and tibiae (Wideman et al., 2012). Proximal femoral head lesions were categorized separately (FHS, FHT, or FHN) to emphasize the progressive development of BCO (Dinev, 2009; Durairaj et al., 2009; Olkowski et al., 2011; Wideman et al., 2012). Kinky back or spondylolisthesis was diagnosed based on the characteristic posterior paraparesis and hock-resting posture (Riddell, 1973; Riddel, 1976; Duff, 1990). The THN condition 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; Wideman et al., 2012). The total incidence of femoral lesions was calculated as Total Femur = (FHS + FHT + FHN). The total incidence of lameness was calculated as Total Lame = (KB + TW + TD + FHS + FHT + FHN + THN). The SigmaStat ANOVA package (Jandel Scientific, 1994, San Rafael, CA) was used to compare BW among experimental groups. No consistent differences were detected between right versus left legs or males versus females with regard to lameness incidences or lesion incidences (not shown); accordingly, data for both legs and both sexes were pooled for subsequent analyses. The SigmaStat Z-test procedure (Jandel Scientific) was used to compare lameness incidences and lesion proportions.

RESULTS

Experiments 1, 2, and 3

Within each experiment, the initial (pre-injection) BW did not differ between the saline, DEX, or uninjected groups. Thereafter, the saline-injected and uninjected broilers grew steadily, whereas DEX injections severely inhibited growth (Table 1). Lameness incidences for experiments 1 to 3 are shown in Figure 1. In experiment 1, 6 broilers (24% of the total) in the DEX 1 group became lame, with 5 developing lameness during the week following the first injection. In experiment 2, 11 birds (42.3% of the total) in the DEX 2 group became lame, with only one developing lameness within the week after the first injection. One bird in the saline 1 group (4%) and none of the birds in the uninjected 1 and saline 2 groups developed lameness. In experiment 3, none of the birds in the uninjected 3 group and 2 birds (8%) in the saline 3 group became lame. Five (20%) and 17 (68%) birds developed lameness in the Lo DEX 3 and Hi DEX 3 groups, respectively. Six injections at 1 mg of DEX/kg of BW (DEX 2) or 0.9 mg of DEX/kg of BW (Hi DEX 3) significantly elevated the incidence of lameness when compared with uninjected birds or birds that received 6 injections of 0.9% saline (saline 2 and 3) or 0.45 mg of DEX/kg of BW (Lo DEX 3; Figure 1). Across all 3 experiments, minimal mortality was attributed to causes other than lameness, including 2 saline-injected birds that developed PHS and 5 birds in the DEX-injected groups that died from SDS. All of the lameness was attributable to lesions of the proximal femoral or tibial heads, and no lameness was attributed to KB, TD, or TW.

Lesion incidences within the proximal femoral head and proximal tibial head diagnostic categories are summarized for experiment 1 in Figure 2. The saline 1 and uninjected 1 groups did not differ within any catego-

Table 1. Body weights for broilers that remained uninjected or that received 3 (experiment 1) or 6 (experiments 2 and 3) intramuscular injections of 0.9% saline or 0.9% saline containing the synthetic glucocorticoid dexamethasone (DEX)[1]

Experiment	Group	BW (g) and number of birds (n)		
		Day 29	Day 36	Day 42
1	Saline	$1,238 \pm 21^d$ (25)	$1,780 \pm 40^b$ (25)	$2,439 \pm 45^a$ (24)
	DEX	$1,345 \pm 24^d$ (25)	$1,302 \pm 33^d$ (24)	$1,509 \pm 65^c$ (18)
2		Day 36	Day 43	Day 49
	Saline	$1,733 \pm 35^c$ (25)	$2,433 \pm 56^b$ (25)	$3,041 \pm 70^a$ (25)
	DEX	$1,867 \pm 26^c$ (26)	$1,831 \pm 39^c$ (25)	$1,766 \pm 35^c$ (14)
3		Day 21	Day 31	Day 42
	Uninjected	764 ± 13^e (25)	$1,453 \pm 27^b$ (24)	$2,458 \pm 53^a$ (23)
	Saline	789 ± 14^e (25)	$1,540 \pm 27^b$ (22)	$2,452 \pm 48^a$ (21)
	Lo DEX	809 ± 15^e (25)	942 ± 26^d (22)	$1,283 \pm 57^c$ (18)
	Hi DEX	819 ± 14^e (25)	976 ± 39^d (10)	$1,472 \pm 57^b$ (7)

$^{a-e}$Different superscripts denote significant differences ($P \leq 0.05$) within an experiment.

[1]Initial BW were recorded before the first injection and final BW were recorded on the day when all survivors were necropsied.

ry. When compared with the saline 1 group, the DEX 1 group had fewer normal femora and fewer normal tibiae, combined with more femoral (Total = FHS + FHT + FHN) and more tibial (THN) lesions. Lesion incidences within the same diagnostic categories are shown for experiment 2 in Figure 3. When compared with the saline 2 group, the DEX 2 group did not differ in any of the femoral head lesion categories. The DEX 2 group had fewer normal tibiae and more tibiae exhibiting THN when compared with the saline 2 group. Lesion incidences for experiment 3 are shown in Figure 4. The uninjected 3 group had more normal femora and more normal tibiae than the saline 3 and Hi DEX 3 groups. When compared with the saline 3 group, the Lo DEX 3 group did not differ in any of the femoral head

or tibial head lesion categories. The Hi DEX 3 group developed higher incidences of FHN and THN when compared with the saline 3 group (Figure 4). Throughout the necropsies, THN lesions were recorded as being "severe" when the growth plate obviously was damaged, or when caseous exudates or bacterial sequestrae were macroscopically present. The incidences of severe THN lesions for the saline- and DEX-injected groups are compared for experiments 1 through 3 in Figure 5. Within each experiment, the DEX injections amplified the severity of THN lesions when compared with the relatively low incidence of severe THN in saline-injected and uninjected groups.
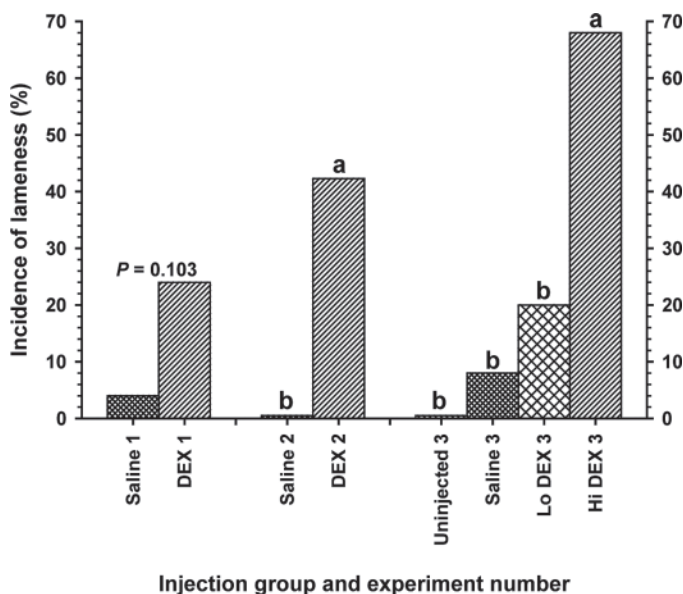

Figure 1. Incidence of lameness as a percentage of the total number of birds per group for broilers in experiments 1 through 3 that received 3 (Saline 1, DEX 1) or 6 (Saline 2, DEX 2, Saline 3, Lo DEX 3, Hi DEX 3) injections of saline or dexamethasone (DEX). ^{a,b}Values differed between the groups within an experiment ($P \leq 0.05$; SigmaStat Z-Test).
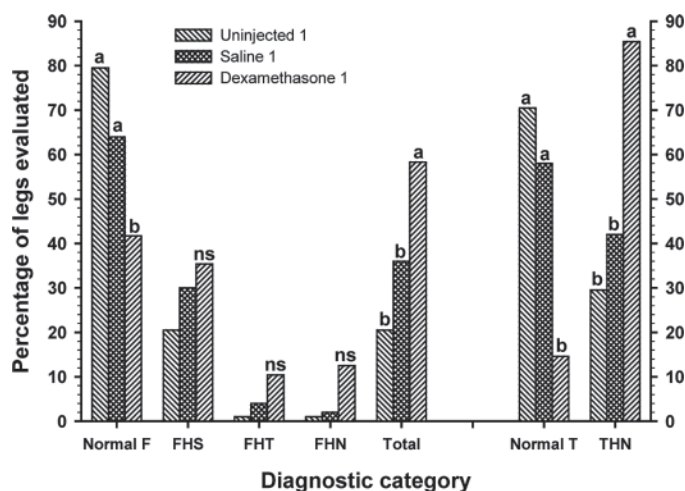

Figure 2. Incidences within proximal femoral or tibial head diagnostic categories for broilers in experiment 1 that remained uninjected (Uninjected 1) or received 3 injections of saline (Saline 1) or dexamethasone (Dexamethasone 1). The proximal femoral and tibial heads of both legs were evaluated and were diagnosed as being macroscopically normal (Normal F, Normal T; no apparent abnormalities) or they exhibited FHS (femoral head separation), FHT (femoral head transitional degeneration), FHN (femoral head necrosis), or THN (tibial head necrosis). Total = total femoral head lesions (FHS + FHT + FHN). Values reflect the percentages of all legs evaluated; within each group, the data for lame birds and survivors were pooled. ^{a,b}Values differed between groups within a diagnostic category ($P \leq$ 0.05; SigmaStat Z-test).
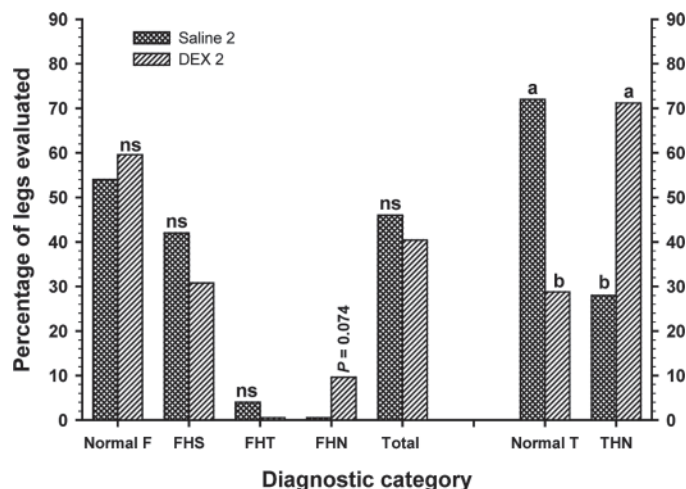
Figure 3. Incidences within proximal femoral or tibial head diagnostic categories for broilers in experiment 2 that received 6 injections of saline (Saline 2) or dexamethasone (DEX 2). The proximal femoral and tibial heads of both legs were evaluated and were diagnosed as being macroscopically normal (Normal F, Normal T; no apparent abnormalities) or they exhibited FHS (femoral head separation), FHT (femoral head transitional degeneration), FHN (femoral head necrosis), or THN (tibial head necrosis). Total = total femoral head lesions (FHS + FHT + FHN). Values reflect the percentages of all legs evaluated; within each group, the data for lame birds and survivors are pooled. [a,b]Values differed between groups within a diagnostic category ($P \leq 0.05$; SigmaStat Z-test).

The DEX injections distinctively altered the macroscopic appearance of 2 lesion categories, as shown in Figure 6. When FHS or epiphyseolysis was revealed during necropsies of uninjected or saline-injected broilers, the proximal surface of the growth plate was tan to
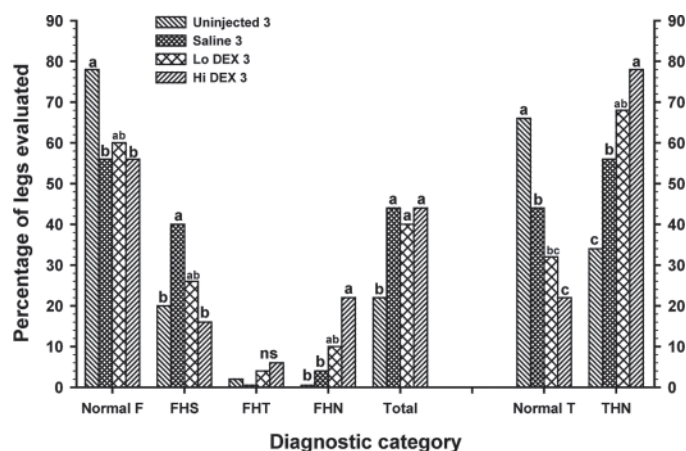

Figure 4. Incidences within proximal femoral or tibial head diagnostic categories for broilers in experiment 3 that remained uninjected (Uninjected 3) or received 6 injections of saline (Saline 3) or low or high dosages of dexamethasone (Lo DEX 3, Hi DEX 3). The proximal femoral and tibial heads of both legs were evaluated and were diagnosed as being macroscopically normal (Normal F, Normal T; no apparent abnormalities) or they exhibited FHS (femoral head separation), FHT (femoral head transitional degeneration), FHN (femoral head necrosis), or THN (tibial head necrosis). Total = total femoral head lesions (FHS + FHT + FHN). Values reflect the percentages of all legs evaluated; within each group, the data for lame birds and survivors are pooled. [a–c]Values differed between groups within a diagnostic category ($P \leq 0.05$; SigmaStat Z-test).
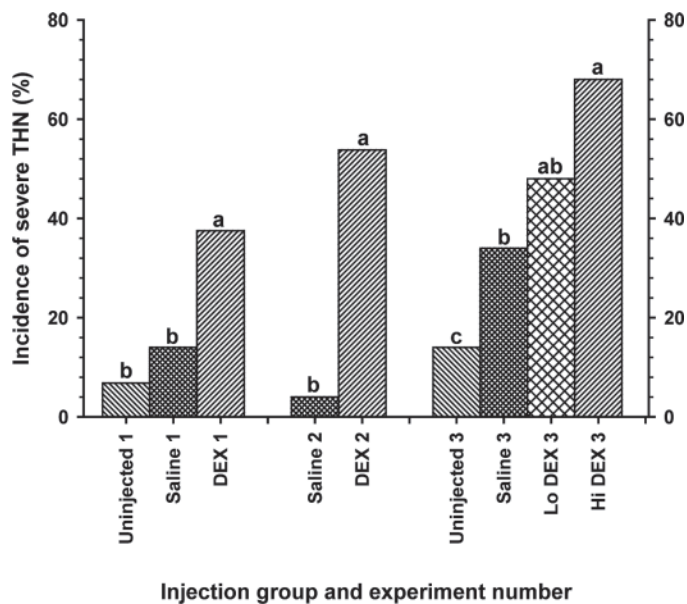

Figure 5. Incidences of "severe" tibial head necrosis (THN) for the uninjected and saline- or dexamethasone (DEX)-injected broilers are compared for experiments 1 through 3. Lesions that obviously had damaged the growth plate per se or that contained caseous exudates were recorded as being "severe." Values reflect the percentages of all legs evaluated; within each group, the data for lame birds and survivors are pooled. [a–c]Values differed between groups within a diagnostic category ($P \leq 0.05$; SigmaStat Z-test).

dark brown in color, typical of the appearance of FHS/epiphyseolysis in spontaneously lame birds (Figure 6A). However, in DEX-injected groups, FHS/epiphyseolysis occasionally revealed a colorless (white) growth plate that macroscopically appeared to be devoid of blood vessels in 19% of the DEX 1 group, 50% of the DEX 2 group, 8% of the Lo DEX 3 group, and 12% of the Hi DEX 3 group (Figure 6B). With regard to the tibiae, struts and spicules of trabecular bone in the metaphysis normally provide structural support for the growth plate (Figure 6C). For DEX-injected broilers in experiments 1 and 2 however, a diagnosis of THN frequently included observations of fatty necrosis in the core of the metaphysis as well as within the secondary center of ossification in the tibial epiphysis (Figure 6D). In experiment 3, a different macroscopic pathology was observed in the tibiae of 8% of the Lo DEX 3 group and in 14% of the Hi DEX 3 group, as shown in Figure 7. Immediately distal to the evidently unaffected resting zone of the physis, a pale (apparently avascular) band of necrosis developed in the prehypertrophic and hypertrophic zones of the growth plate, occasionally extending into the calcifying zone (degenerating hypertrophic zone) of the metaphysis. These bands of necrosis developed in tibiae having apparently normal metaphyseal trabecular bone (Figure 7A), as well as in tibiae exhibiting early (Figure 7B), intermediate (Figure 7C), and severe (Figure 7C) voids in the metaphyseal trabeculae (e.g., THN). Secondary centers of ossification also appeared to undergo fatty necrosis in affected broilers (Figure 7B,D). The tibiae shown in Figure 7A,C were preserved in 10% buffered formalin, decalcified, embedded in par-

affin, sectioned at 5 μm, and alternating sections were stained with hematoxylin and eosin or toluidine blue for histological evaluation. Microscopic examination of the tibia shown in Figure 7C revealed apparently normal cell histology in the epiphyseal cartilage and resting zone of the physeal growth plate (Figure 8A). However, the majority of cartilage cells in the prehypertrophic and hypertrophic zones of the growth plate had pyknotic nuclei indicative of necrosis. Swaths of necrotic cartilage cells also extended into the calcifying zone of the metaphysis, adjacent to metaphyseal blood vessels that contained numerous vacuoles (Figure 8A). At a higher magnification, granular cells (perhaps heterophils or eosinophils) also were abundant within the lumen of metaphyseal blood vessels that apparently

had been occluded by lipid microemboli (Figure 8B). Alternatively, lipid vacuoles developed in the perivascular connective tissue surrounding some of the epiphyseal vascular canals, but neither degenerative nor obstructive changes were evident in the transphyseal or metaphyseal blood vessels (Figure 9A,B; from the tibia shown in Figure 7A). In these sections, the resting and proliferating/prehypertrophic and hypertrophic zones of the growth plate appeared to be thickened with accumulations of cartilage cells exhibiting a normal appearance without pyknotic nuclei (Figure 9A).

Experiment 4

On d 42, males in the thermoneutral group weighed 2.65 ± 0.11 kg (mean ± SEM) and were not heavier
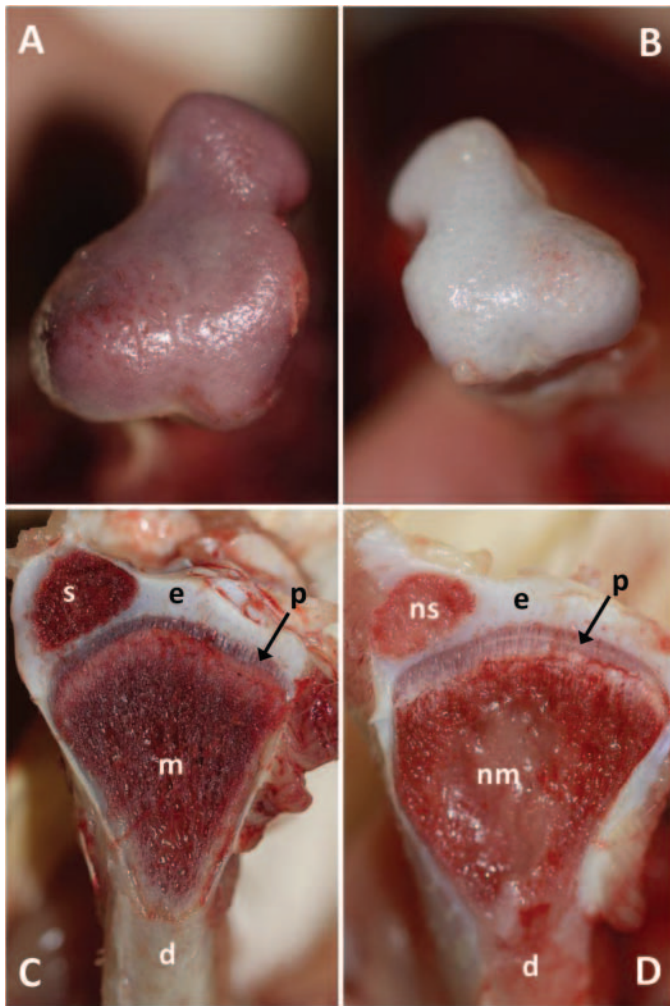

Figure 6. Epiphyseolysis in saline-injected broilers typically revealed a proximal surface of the growth plate that was tan to dark brown in color (A). In some dexamethasone-injected broilers, epiphyseolysis revealed a colorless (white) growth plate that macroscopically appeared to be devoid of blood vessels (B). In normal proximal tibiae, struts of trabecular bone in the metaphysis (m) transmit structural support from the shaft (diaphysis, d) to the growth plate (physis, p) and articular cartilage (epiphysis, e). A secondary center of ossification (s) develops in the tibial epiphyseal cartilage of broilers after (approximately) d 28 (C). In dexamethasone-injected broilers, the diagnosis of tibial head necrosis occasionally included observations of fatty necrosis in the core of the metaphysis (nm) as well as within the secondary center of ossification (ns; D).
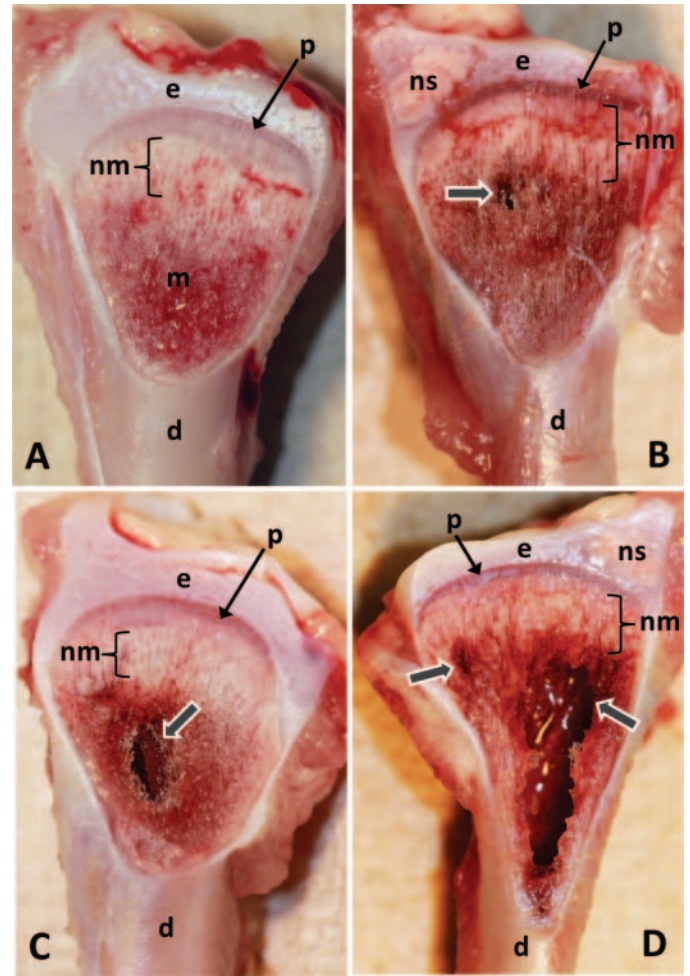

Figure 7. In experiment 3, 8% of tibiae in the low dexamethasone 3 group and in 14% of the tibiae in the high dexamethasone 3 group exhibited pale, apparently avascular bands of necrosis (nm) in the prehypertrophic and hypertrophic zones of the growth plate, occasionally extending into the calcifying zone of the metaphysis (m). The epiphysis (e), resting zone of the physis (p), and the shaft (diaphysis, d) appeared macroscopically unaffected. The bands of necrosis developed in tibiae having apparently normal metaphyseal trabecular bone (A), as well as in tibiae exhibiting minor (B), intermediate (C), and severe (C) voids within the metaphyseal trabecular bone (thick black arrows; e.g., tibial head necrosis). Secondary centers of ossification (ns) also appeared to undergo fatty necrosis in affected broilers (B, D).
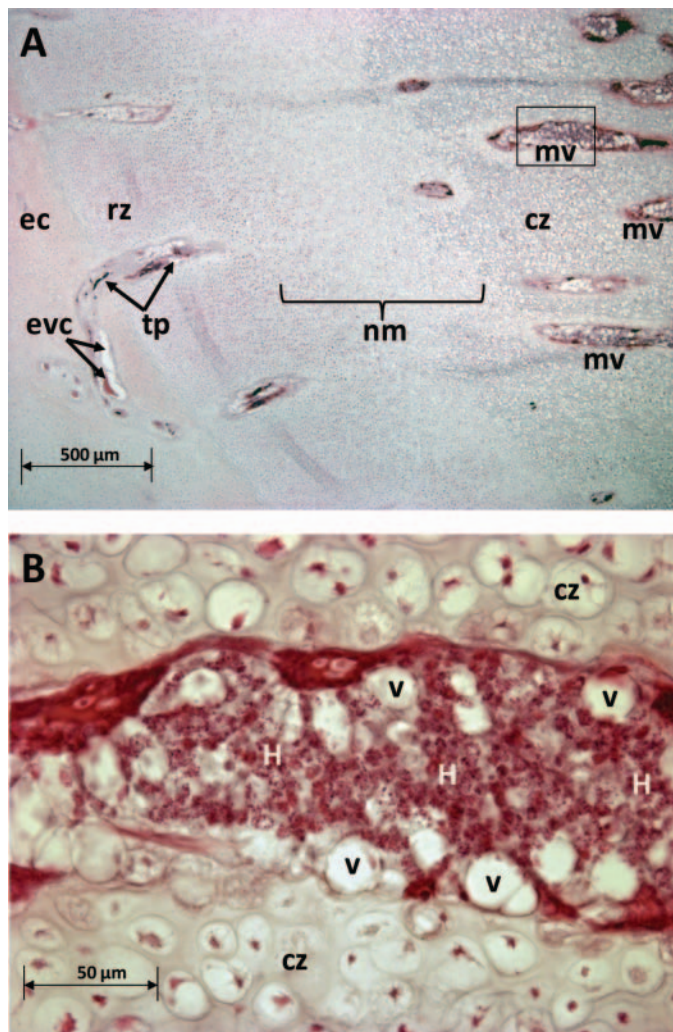
Figure 8. A) Histological section from the proximal tibia shown in Figure 7C, with the proximal epiphysis oriented to the left and the metaphysis to the right. The cells within the epiphyseal cartilage (ec) and resting zone of the growth plate (rz) have a normal appearance, and the epiphyseal vascular canals (evc) and transphyseal vessels (tp) also appear to be normal. Cartilage cells with pyknotic nuclei indicative of necrosis are prevalent in the prehypertrophic and hypertrophic zones of the growth plate (nm) as well as in the vicinity of metaphyseal blood vessels (mv) laden with lipid microemboli (vacuoles) in the calcifying zone (cz) of the metaphysis. The black rectangle in panel A outlines the region magnified in panel B, showing aggregations of granular cells (H) within the vacuole-laden (v) lumen of a metaphyseal blood vessel penetrating a necrotic swath of cartilage cells within the calcifying zone (cz). Five-micrometer thick paraffin section stained with hematoxylin and eosin. Microanatomical terminology is consistent with the established anatomical nomenclature for avian bones (Lutfi, 1970a,b; Howlett, 1979; Hunt et al., 1979; Ali, 1992).

Figure 9. A) Histological section from the proximal tibia shown in Figure 7A, with the proximal epiphysis oriented to the left and the metaphysis to the right. The cells within the epiphyseal cartilage (ec), resting zone (rz), and proliferating or prehypertrophic zone (ph) of the growth plate are normal in appearance. Cartilage cells within the calcifying zone (cz) of the metaphysis occasionally exhibit pyknotic nuclei. Transphyseal blood vessels (tp) and metaphyseal blood vessels (mv) appear unaffected, but numerous lipid vacuoles are evident in the perivascular connective tissues surrounding some of the epiphyseal vascular canals (evc). B) Magnification of the largest epiphyseal vascular canal (evc) from the upper panel, showing extensive lipid deposits (v) within the perivascular connective tissue. Toluidine blue was used to stain cartilage-associated mucopolysaccharides (5-μm thick paraffin section). Microanatomical terminology is consistent with the established anatomical nomenclature for avian bones (Lutfi, 1970a,b; Howlett, 1979; Hunt et al., 1979; Ali, 1992).

($P = 0.356$) than males in the heat stress group (2.54 ± 0.06 kg). Female BW also did not differ on d 42 ($P = 0.128$), with females in the thermoneutral group weighing 2.36 ± 0.12 kg and those in the heat stress group weighing 2.23 + 0.08 kg. Males were heavier than females in both groups ($P = 0.001$). No lameness developed in either group during this experiment. In the thermoneutral group, the subclinical incidence of THN remained constant at 20% or less between 21 and 42 d of age, whereas the incidence of subclinical femoral head lesions increased spontaneously from 10% on d
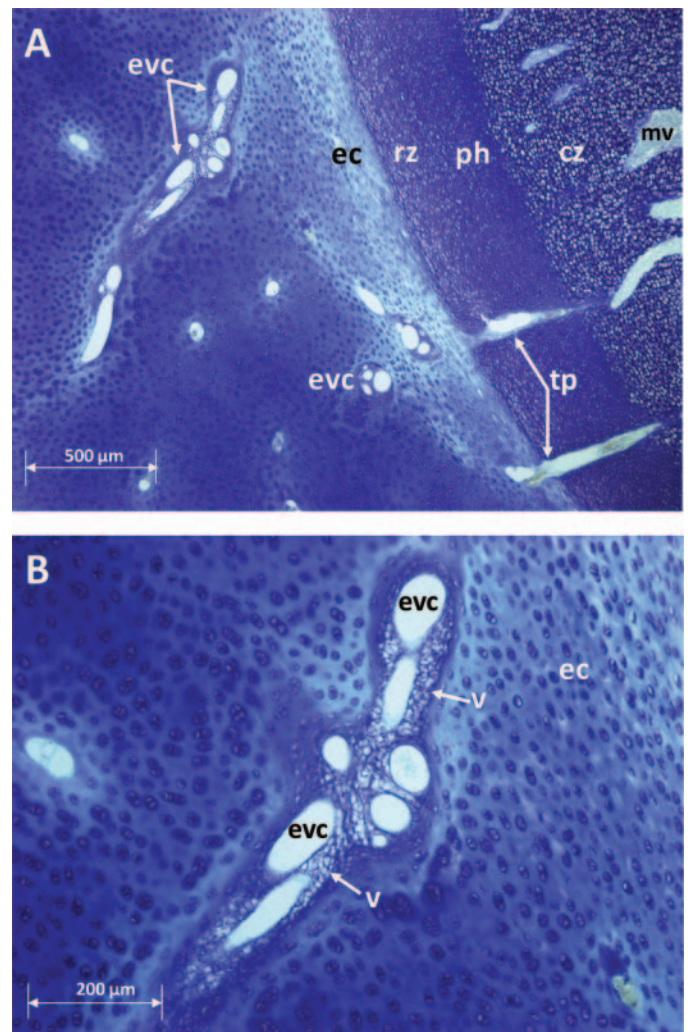
21 to 54% on d 42 (Figure 10). As shown in the upper panel, heat stress substantially increased the incidence of THN at 28 and 35 d of age, but not at 42 d of age when the THN incidences no longer differed between the heat stress and thermoneutral groups. Total femoral head lesion incidences (FHS + FHT + FHN) are shown in the middle panel. The femoral head lesion incidence was higher in the heat stress group than in the thermoneutral group on d 28, but not on d 35 or d 42. Combined incidences for all lesions of the proximal femora and tibiae are shown in the lower panel of Figure

10. The intermittent episodes of heat stress increased the combined lesion incidence at 28 d and 35 d of age.

DISCUSSION

Based on pathogenic similarities between BCO and TOC, and overwhelming evidence that synthetic glucocorticoid injections can trigger TOC in turkey poults (Huff et al., 1998, 1999, 2000, 2005, 2006), and FHN in adult Leghorn hens (Cui et al., 1997), the present study was conducted to evaluate the possibility that DEX injections might be used as an experimental model for triggering lameness attributable to BCO in broilers. We followed published protocols in which 3 injections of DEX (2 mg of DEX/kg of BW each) on alternating days caused an 8% incidence of TOC, whereas a second series of 3 DEX injections of the same dosage triggered TOC in 64% of the poults (Huff et al., 2000). Qualitatively similar responses were obtained for lameness in broilers. In experiment 1, 3 injections of 1.5 mg of DEX/kg of BW per injection caused a numerical increase ($P = 0.103$) in the incidence of lameness accompanied by increased incidences of macroscopic lesions in the proximal heads of femora and tibiae. The onset of lameness was remarkably rapid, with 5 of the 6 birds becoming lame within a week after the first injection. Lower doses of DEX were injected in experiments 2 and 3, and very few broilers developed lameness after the first 3 injections, whereas lame birds accumulated rapidly during the second week of DEX injections. In all cases, the DEX-induced increase in lameness was primarily associated with increased incidences of THN. As shown in Figures 1 and 5, broilers from Lines D (experiments 1 and 2) and B (experiment 3) exhibited qualitatively consistent responses to the DEX injections. A major point of concern was the severe reduction in growth rates in DEX-injected broilers. A corresponding attenuation of BW gain was reported for poults injected with DEX (Huff et al., 1998, 1999) and for laying hens injected with synthetic glucocorticoid (Cui et al., 1997). Even the lowest dose of DEX used in experiment 3 (0.45 mg of DEX/kg of BW) severely inhibited growth while only marginally tending to increase the incidence of lameness when compared with uninjected or saline-injected controls. It appears unlikely that the dosage of DEX injections can be adjusted to obtain significant levels of lameness without retarding growth in broilers.

The etiology of BCO in commercial broiler flocks likely includes the accumulation of osteochondrotic microfractures and clefts that serve as sites for colonization by opportunistic bacteria (Duff, 1989; Emslie et al., 1984; Duff and Randall, 1987; Hocking, 1992; Thorp et al., 1993), in combination with generalized immunosuppression and thus proliferation of hematogenously distributed bacteria (see Introduction). The pathogenesis of BCO is not instantaneous; therefore, broilers may not exhibit obvious symptoms of lameness even though subclinical lesions representing the earliest stages of BCO are present in their proximal femora and
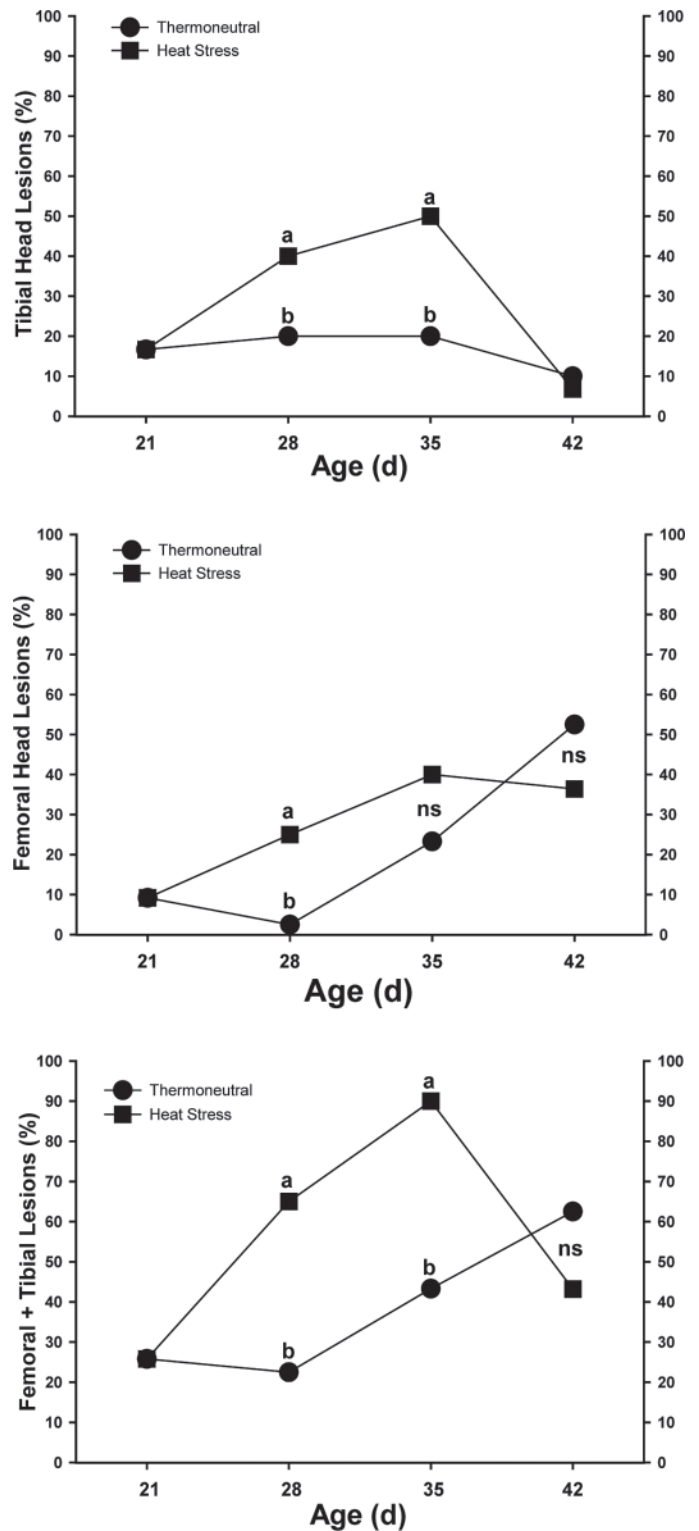

Figure 10. Incidences of tibial head lesions (upper panel), total femoral head lesions (proximal femoral head separation, proximal femoral head transitional degeneration, proximal femoral head necrosis; center panel), and both lesion sets combined (lower panel) for broilers in experiment 3 that were reared under thermoneutral temperatures or 3 weekly 72-h episodes of heat stress. [a,b]Values differed between groups within an age and diagnostic category ($P \leq 0.05$; SigmaStat Z-test).

tibiae. These observations suggest lameness is not necessarily caused by direct mechanical damage per se but rather by the severity of the ensuing bacterial infection (Wise et al., 1973; McCaskey et al., 1982; Riddell et al.,

1983; McNamee et al., 1998). Accordingly, broilers that remain fully capable of standing and walking with an acceptable gait nevertheless may possess early lesions that are consistent with the pathological progression leading to terminal BCO (Wideman et al., 2012). Early subclinical lesions were evident during final necropsies of the uninjected and saline-injected groups in all 3 experiments. In all of these experiments, FHS and THN were found in at least 30% of the femora and tibiae during necropsies of the uninjected or saline-injected survivors. In contrast, very few of these survivors exhibited FHN or the most severely abscessed stages of THN when compared with DEX-injected broilers.

Dexamethasone mimics the stress-mediated immunosuppression that has been implicated in the etiology of BCO and TOC in poultry (see Introduction), but several key responses to DEX in the present study did not mirror the typical characteristics of spontaneous BCO. Growth ceased almost immediately after the initial DEX injection regardless of whether the birds became lame or survived. In contrast, all of the uninjected and saline-injected broilers continued growing in spite of the fact that subclinical BCO lesions were present in some individuals. Previous studies indicated that subclinical BCO lesions may only minimally affect growth until immobilization due to lameness restricts access to feed and water (Wideman et al., 2012). Dexamethasone also altered the appearance of proximal femoral and tibial lesions. Epiphyseolysis (FHS) revealed an apparently avascular (white) growth plate in several of the DEX-injected broilers (Figure 6B). Fatty necrosis of the tibial metaphysis was observed in DEX-injected but not in saline-injected broilers (Figure 6D). A similar yellowish-colored fatty osteonecrosis of the metaphysis developed in the femur and humerus of rabbits injected with the corticosteroid methylprednisolone (Yamamoto et al., 1997). Steroid-induced FHN in Leghorn hens was attributed to adipocyte (fat cell) infiltration and an associated impairment of blood flow (Cui et al., 1997). Indeed, steroid-induced femoral head osteonecrosis in mammals is closely associated with reduced blood flow (ischemia) caused by fat emboli (lipid-loaded fibrin-platelet thrombi) that occlude the subchondral microcirculation or by hypertrophic adipocytes that are thought to compress the vascular supply to the growth plate (Wang et al., 1977; Yamamoto et al., 1997; Boss and Misselevich, 2003). Occlusion of the tibial metaphyseal vasculature by fat microemboli would account for the luminal vacuolation observed in DEX-injected broilers in experiment 3; vacuole-like spherical voids remain intact after fat droplets are dissolved by organic solvents used to process tissues for histology (Figures 8 and 9). In contrast, the focal ischemia associated with BCO in broilers has been attributed to mechanical compression, traumatic transection by physeal clefts or abscesses, or thrombotic occlusion of metaphyseal blood vessels (Duff, 1984, 1989; Emslie et al., 1984; Duff and Randall, 1987; Hocking, 1992; Thorp et al., 1993; Thorp, 1994; Wideman et al., 2012).

Accordingly, the pathogenesis of lameness caused by DEX does not appear to precisely recapitulate the pathogenesis of spontaneous BCO. Multiple systemic responses to DEX are likely to have contributed to the growth suppression and lesion formation in the present study. Future studies potentially might be designed to administer very low levels of DEX in the feed or drinking water, thereby mimicking the relatively constant stream of corticosterone produced endogenously when broilers are under chronic stress. Constant low levels of DEX administration in the feed or water might conceivably minimize growth suppression while potentially triggering proximal tibial and femoral lesions that are more pathognomonic for BCO. However, previous studies indicate that adding corticosterone to the feed suppresses growth and triggers hyperlipidemia in poultry, apparently without noticeably increasing the incidence of lameness (van Niekerk et al., 1989; Lin et al., 2006; Yuan et al., 2008).

In experiment 4, subclinical THN incidences for the thermoneutral group remained at ≤20% between d 21 and 42, whereas the femoral lesion incidences in the same birds increased spontaneously from 10% to >50% during the same period (Figure 10). It was against this background of reasonably constant tibial lesion incidences and gradually accumulating subclinical femoral lesions that the effects of repeated episodes of heat stress were contrasted. Heat stress did not cause lameness but did briefly accelerate the development of femoral lesions on d 28, and more persistently increased the development of tibial lesions on d 28 and 35. Perhaps acclimation to heat stress preempted ongoing differences between the thermoneutral and heat stress groups. The femoral and tibial lesions of both groups exhibited the typical pathognomonic macroscopic features of spontaneous BCO (Wideman et al., 2012) without any evidence of the avascular femoral growth plates or fatty tibial necrosis associated with DEX injections. Between d 35 and 42, the incidence of tibial lesions in the heat stress group receded back to control levels (Figure 10), suggesting new tibial lesions ceased forming and existing lesions may have been consolidated into the marrow of the elongating tibial diaphysis. Contemporaneously, femoral lesion incidences remained at their highest levels. Therefore, the trends in tibial and femoral lesion incidences were dissociated in experiment 4. Femoral and tibial lesion incidences also were dissociated in experiments 2 and 3, in which DEX injections did not increase the incidence of total proximal femoral lesions but did dramatically amplify the incidence of both THN and "severe" THN. These tendencies suggest differences may exist in the pathogenesis of femoral versus tibial lesion development and that tibial lesion incidences may increase preferentially over femoral lesions in broilers stimulated to release endogenous stress hormones (e.g., corticosterone during heat stress) or injected with a synthetic glucocorticoid such as DEX. The biological basis for a differential response remains to be clarified.

In conclusion, broilers that received 3 intramuscular injections of the synthetic glucocorticoid DEX at a dose of 1.5 mg of DEX/kg of BW per injection ceased growing after the first injection, and they developed a 24% incidence of lameness attributable to lesions of the proximal femora and tibiae. Reducing the injection dosage to ≤1.0 mg of DEX/kg of BW failed to attenuate the growth suppression, and the first 3 injections at this dosage caused a low (4%) incidence of lameness. The ensuing series of 3 injections at the ≤1.0 mg of DEX/kg of BW dosage caused >40% (experiment 2) and >60% (experiment 3) incidences of lameness, attributable predominately to severe lesions of the proximal tibiae, possibly caused by vascular occlusion by fat microemboli. Some of the femoral and tibial lesions triggered by DEX (e.g., avascular necrosis of the femoral growth plate and fatty necrosis of the tibial metaphysis) differed from the typical proximal tibial (THN) and femoral (FHS, FHT, FHN) lesions that are considered pathognomonic for BCO. Therefore, although DEX did cause lameness in a portion of the injected broilers (presumably the most "susceptible" individuals), nevertheless, DEX injections may not precisely mimic or recapitulate the pathogenesis of BCO. Repeated episodes of heat stress transiently increased proximal femoral lesions and more consistently increased proximal tibial lesions but did not cause lameness. Both DEX injections and heat stress preferentially increased the incidence of proximal tibial lesions, suggesting differences may exist in the pathogenesis of femoral versus tibial lesion development.

ACKNOWLEDGMENTS

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