



Impact of experimentally induced bacterial chondronecrosis with osteomyelitis (BCO) lameness on health, stress, and leg health parameters in broilers

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ABSTRACT Stress and lameness negatively affect the health, production, and welfare of broilers. Bacterial chondronecrosis with osteomyelitis (BCO) is a leading cause of stress and lameness in commercial broilers. External changes in skin temperature related to changes in blood flow can be detected with infrared thermography (IRT), offering a noninvasive tool to assess the health of animals. This study compared physiological and noninvasive measures of stress and lameness in clinically healthy and lame male broiler chickens between 25 and 56 d. Birds were raised in pens within separate environmental chambers containing either litter flooring (sound) or wire flooring, with the latter established to induce BCO lameness (lame). Physiological and noninvasive measures of stress and lameness were collected: body weight, (BW), relative bursa weight, core body temperature, corticosterone (CORT) concentrations in serum and feathers, surface temperatures of the head (eye and beak) and leg (hock, shank, and foot) regions by infrared

thermography (IRT), leg blood oxygen saturation (leg O₂), and BCO lesion severity scores of tibial head necrosis (THN) and femoral head necrosis (FHN). Lame birds exhibited greater FHN and THN lesion severities, core body temperatures, and serum CORT ($P < 0.05$), but had lower BW, relative bursa weight, leg O₂, and IRT surface temperatures of the beak, hock, shank, and foot compared with sound birds ($P < 0.05$). The difference in THN lesion severity between sound and lame birds decreased with age. Linear relationships between leg O₂ with IRT leg surface temperatures were positive and negative between leg O₂ with BCO lesion severity ($P < 0.05$). There were negative correlations between serum CORT with hock, shank and foot temperatures ($P < 0.001$), indicating that BCO is stressful. These results indicate that birds lame from BCO are stressed, have reduced oxygen saturation of blood in their legs, and that IRT surface temperatures can be used as noninvasive indicators of stress and lameness in broilers.

Key words: broiler, lameness, wire flooring, infrared thermography, pulse oximetry

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INTRODUCTION

Lameness impacts the production and health of broilers and is also a major welfare issue, especially if the bird feels pain and cannot reach feed and water (McNamee and Smyth, 2000; Bradshaw et al., 2002; Weeks et al., 2002). Poor leg health and lameness can occur from infectious and noninfectious origins (Gentle, 2011), and bacterial chondronecrosis with osteomyelitis (BCO) is a leading infectious cause of lameness in

broilers. Birds with BCO succumb to bacterial infiltration and subsequent hematogenous colonization and physisitis of the proximal femoral and tibiotarsal (tibial) growth plates; this leading to necrosis and eventually lameness (Wideman, 2016; Wijesurendra et al., 2017). Lameness is estimated to affect up to 1% of commercial broilers and BCO continues to be a leading cause, largely because the etiology remains unknown (McNamee and Smyth, 2000; Wideman, 2016).

Body conformation, biomechanics, and behavior of individual birds can further exacerbate BCO incidence. For example, the mechanical stress of walking may create microfractures in femoral and tibial growth plates, providing additional sites for bacterial colonization and subsequently micro- and macroscopic lesions (McNamee and Smyth, 2000; Wideman et al., 2012; Wideman, 2016). Ischemia and vascular occlusion of the blood supply to

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the legs has been hypothesized as another contributing factor to BCO lameness (Wideman and Prisby, 2013; Wideman, 2016). The arterial vasculature supplying blood to the legs curves around the proximal femoral and tibia heads, creating pinch points, and increased sitting behavior can further decrease blood flow and oxygen supply to the femoral and tibial head growth plates (Wideman, 2016). Research is needed to elucidate the predisposing factors that cause BCO in broilers as well as reliable methods to assess leg health.

It is logical to conclude that lameness is painful (McGeown et al., 1999; Nääs et al., 2009) and, consequently, stressful in broilers (Danbury et al., 2000; Weeks et al., 2000; Weimer et al., 2020). For example, Danbury and colleagues (2000) reported that, when given the choice, broilers with an impaired gait selected to consume feed supplemented with an analgesic (carprofen) more than broilers with a normal gait. Additionally, the pathogenesis of BCO typically develops over several days before clinical signs can be observed, making it a chronic stressor. Stressors activate the hypothalamic-pituitary-adrenal axis to release corticosterone (CORT), and CORT concentrations are a common measure of stress in animal welfare studies (Siegel, 1995; Mormède et al., 2007). However, the additional stress from blood draw and restraint can affect blood CORT levels (Mormède et al., 2007) and this warrants a need for valid noninvasive methods. Circulating CORT measures indicate a “snapshot” of acute stress at the time of collection whereas feather CORT is reported to be an integral measure of CORT throughout the life of the animal reflected in the growth of the feather measured (Bortolotti et al., 2008). Infrared thermography (IRT), a noncontact measure of surface temperatures emitted by an animal, can be used as a noninvasive method to measure health by identifying localized areas of inflammation and reduced blood flow in thermal images (Eddy et al., 2001).

The objective of the current study was to determine the associations between IRT body region surface temperatures and feather CORT with traditional stress and leg health measures of sound and lame male broilers. The current study is subsequent to previous studies that reported positive relationships between serum and feather CORT concentrations (Weimer et al., 2018), IRT as a noninvasive tool to detect BCO lameness (Weimer et al., 2019), and BCO lameness-related stress (Weimer et al., 2020) in male broilers. The second objective was to determine the relationships between leg blood oxygen saturation with the severity of BCO necrosis and with IRT leg surface temperatures.

MATERIALS AND METHODS

Animals and Facility

The University of Arkansas Institutional Animal Care and Use Committee (IACUC #16014) approved all procedures in this study. Male Cobb 500 byproduct broiler chicks (N = 720 chicks) were transported from a

commercial hatchery (Cobb-Vantress, Fayetteville, AR) to the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm. Chicks were randomly divided and placed into 1.5 m wide × 3 m long pens (60 chicks per pen; 0.08 m²/chick) within 12 separate environmental chambers (3.7 m long × 2.5 m wide × 2.5 m high; 1 pen per chamber). Pens had either new wood shavings litter flooring (N = 6 pens) or wire flooring (N = 6 pens). The details of the wire flooring have been described previously (Wideman et al., 2012; Weimer et al., 2019). Birds had *ad libitum* access to feed and water. Diets were formulated to industry standards for the age of the birds (NRC, 1994). A crumbled commercial starter diet was provided until d 28 when birds were switched to a pelleted commercial finisher diet and water was provided via nipple drinkers.

The light intensity in each chamber was 20 lux and the photoperiods were the following: 23L:1D for d 0 to 4; 20L:4D for d 5 to 14; and 18L:6D from d 15 through the end of trial. Chamber temperatures were measured daily and set to 33°C for d 0 to 3; 30°C for d 4 to 7; 26°C for d 8 to 14; and 24°C for d 15 through the end of the trial. The accuracy of the set temperatures was determined with an infrared thermometer (IRT657, General Tools & Instruments LLC, Secaucus, NY). The caretaker entered each chamber daily to evaluate bird health and mobility. On d 14, bird density was reduced to 50 clinically healthy chicks (0.09 m²/chick) per chamber to ensure the stocking density and opportunities for mobility were uniform as has been described in previous studies (Weimer et al., 2019, 2020). The study was conducted from October to December 2016.

Experimental Design

The study followed a matched pairs block design and the experimental unit was a pen of birds (N = 12 pens in 6 pair blocks of 2 pens). Each pair was matched by age and consisted of a block of 2 pens and each pen was in a separate environmental chamber. One pen had litter flooring (exclusive to the birds classified as sound) and one pen had wire flooring (exclusive to the birds classified as lame). Starting on d 25 (when the first wire flooring bird became clinically lame) and every day thereafter until d 56, birds identified as lame during daily observations were removed and sampled. Birds were determined to be lame if they would not step to walk away from the caretaker after gentle coaxing in the home pen during daily checks. For every lame bird that was sampled (wire flooring pen), so was a randomly selected sound (clinically healthy) bird from the sound environmental chamber in the matched pair block (both birds were sampled at the same age). Thus, each bird that became lame in a wire flooring pen (lame) was matched to a random bird from a designated litter flooring pen (sound). The rationale for pairing lame and sound birds from adjacent pens was to control for pen stocking density within each matched pair and to

compare measures on sound and lame broilers of the same age.

Premortem Data Collection

Once removed from the pen, a thermal image was taken of each bird's head and legs, blood was collected, and core body temperature and leg blood oxygen saturation were measured. Thermal images were taken with an IRT camera (Fluke Ti400, Fluke, Everett, WA). Details of the IRT image analysis have been previously reported (Weimer et al., 2019, 2020). Immediately after thermal image capture, blood was collected from each bird within 60 s of being removed from their home pen via brachial vein venipuncture. Each blood sample was immediately injected into serum separation tubes (SST Vacutainer, BD, Franklin Lakes, NJ). Serum was separated from blood via centrifugation (10 min at $1,500 \times g$), frozen at -20°C , and measured for CORT concentrations calculated from optical densities read with an absorbance microplate reader (BioTek, Winooski, VT) at 450 nm. Core body temperature was measured with a thermometer (TM99A Thermistor Temperature Instrument, Cooper Atkins, Middlefield, CT) inserted into the vent. Pulse oximetry is a noninvasive measure of arterial blood oxygen saturation. Blood oxygen saturation of the right and left leg (leg O_2 , %) was measured indirectly with a veterinary pulse oximeter (Model 2500A, Nonin Medical, Inc., Plymouth, MN) affixed with a flexible wrap sensor that was wrapped around the distal right and left shank and right and left values were averaged for analysis.

Postmortem Data Collection

Birds were humanely euthanized via rapid cervical dislocation, body weight (BW) was recorded, feathers and the bursa of Fabricius were collected, and right and left proximal femur and proximal tibiotarsus (referred to as tibia) heads were scored for BCO lesion severity. The third primary feathers were collected from the left wing. Bursas were excised and immediately placed into a 10% formalin solution, weighed (g), and normalized as a percentage of BW (bursa weight $\times 100/\text{BW}$).

The right and left femur and tibia were macroscopically scored for femoral head necrosis (FHN) and tibial head necrosis (THN) BCO lesion severity. Briefly, FHN and THN lesion severities was scored on a 0 to 3 scale of increasing severity where 0 indicated no abnormalities and 3 indicated severe necrosis. FHN was classified into the following categories: 0- no abnormalities (Normal); 1- separation of the head from the acetabulum (FHS; epiphyseolysis); 2- transitional degeneration (FHT); and 3- severe necrosis (FHN). THN lesion severity was also scored on a 0 to 3 scale into the following categories: 0- no abnormalities (Normal); 1- mild necrosis (THN); 2- severe tibial head necrosis (THNS); and 3- caseous THN (THNC). A quantitative FHN index was calculated by summing the right (0–3) and left (0–3) proximal FHN

lesion severity scores for a range of 0 to 6. Similarly, a THN index was calculated by summing the right (0–3) and left (0–3) proximal THN lesion severity scores for a range of 0 to 6. A Total Necrosis (Total N) index was calculated by summing FHN and THN category scores for a range of 0 to 12.

Serum and Feather CORT Assay

CORT was extracted from feathers using an adapted method (Bortolotti et al., 2008) with the details reported elsewhere (Weimer et al., 2018). Serum CORT (ng/mL) and feather CORT (pg/mm of feather length) were determined with an ELISA (Arbor Assays, Ann Arbor, MI). ELISA plate inter- and intra-assay CVs were $<7\%$.

Statistical Analysis

The study followed a matched pairs block design and the experimental unit was a pen of birds ($N = 12$ pens in 6 pair blocks of 2 pens). Wk 3 data had low sample numbers ($N = 12$ birds) and was excluded from analysis. Data from 128 sound and 128 lame birds ($N = 256$ total birds) was used for analysis.

Data was analyzed in JMP Pro (v14, SAS Institute Inc, Cary, NC). To analyze the daily lameness incidence for each wire flooring pen from wk 4 to 8, a logistic regression model for the binomial response of lame vs. sound by day was used. A paired t test was performed to compare the means of all measures. The delta differences (Δ) between each pair of lame and sound birds (calculated as lame minus sound for each matched pair, $N = 128$ matched pairs consisting of 1 sound and 1 lame bird) for the following measures: BW, relative bursa weight, leg O_2 , serum CORT, feather CORT, IRT surface temperatures of the eye, beak, hock, shank, and foot, THN, FHN, and Total N. An ANOVA with the fixed effect of week and random effect of pen was used to analyze weekly (wk 4, 5, 6, and 7 aggregates) mean delta (Δ) differences for each matched pair of birds. Linear regressions (R^2) were performed to analyze the relationship between leg O_2 with BCO necrosis lesion severity scores (FHN, THN, and Total N) and IRT leg region (hock, shank and foot) surface temperatures. Pearson's pairwise correlations (r) were performed to compare the linear relationships of all measures with each other. Data were considered significant at $P \leq 0.05$.

RESULTS

Lameness Incidence in the Wire Flooring Pens

No birds in pens with litter flooring became lame over the course of the study. The proportion of birds that became lame in the wire flooring pens was 45% ($N = 134$

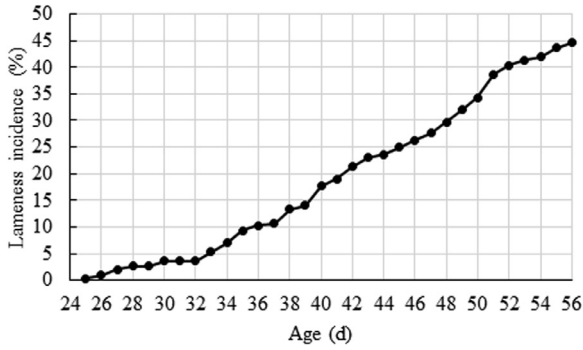


Figure 1. Cumulative proportion (%) of male broilers that became lame from d 25 to 56 in the wire flooring pens.

Table 1. Cumulative weekly percentages of lame male broilers in the wire flooring pens (N = 6 pens) at 4, 5, 6, 7, and 8 wk.

Wk (d)	Wire pen# ¹					
	1	2	3	4	5	6
4 (28)	4	2	0	4	0	6
5 (35)	10 ^b	4 ^b	0 ^b	14 ^{ab}	2 ^b	26 ^a
6 (42)	30 ^a	4 ^b	0 ^b	42 ^a	4 ^b	48 ^a
7 (49)	56 ^a	4 ^b	8 ^b	52 ^a	8 ^b	64 ^a
8 (56)	64 ^a	8 ^c	24 ^b	70 ^a	24 ^b	78 ^a

¹Each wire flooring pen had 50 birds at 4 wk.

^{a,b,c}Means not sharing the same letters across each row differ at $P \leq 0.05$.

birds; Figure 1) at the end of the study. However, lameness incidence varied drastically amongst the 6 wire flooring pens and final d 56 lameness incidence ranged from 8 to 78% (Table 1).

Means and Deltas

There were no differences between sound and lame bird feather CORT or IRT eye surface temperatures ($P > 0.05$; Table 2). Addressing stress physiology measures, lame birds had lower BW (2,616 vs. 3,481 g), relative bursa weight (0.08 vs. 0.11%), and IRT beak surface temperatures (29.9 vs. 32.8°C; $P < 0.05$; Table 2) compared with sound. In contrast, lame birds had elevated core body temperature (41.8 vs. 41.5°C) and serum CORT (14.56 vs. 7.71 ng/mL) compared with sound ($P < 0.0001$; Table 2). For leg health measures, lame birds had lower leg O₂ (78.0 vs. 81.2%), hock surface temperatures (36.3 vs. 36.9°C), shank surface temperatures (35.9 vs. 36.7°C), and foot surface temperatures (32.8 vs. 35.5°C) and more severe FHN (4.26 vs. 1.91), THN (3.16 vs. 2.31), and Total N (7.41 vs. 4.22) compared with sound ($P \leq 0.0004$; Table 2).

The weekly differences (delta [Δ]) between sound and lame chickens were calculated by subtracting the sound values from the lame values (mean lame minus mean sound values of each matched pair) for each measure in Table 3. The Δ of lame and sound matched pair of birds was similar for relative bursa weight, core body temperature, feather CORT, and hock, shank, and foot surface temperatures across wk 4 to 8 ($P > 0.05$; Table 3).

Table 2. Effect of leg health status (lame vs. sound) on stress and leg health related parameters of 4–8 wk old male broilers. Data shown as mean (\pm SE) body weight (BW, g), relative bursa weight (% of BW), core body temperature (°C), serum corticosterone (CORT) concentration (ng/mL), feather CORT concentration (pg/mm), leg blood oxygen saturation (leg O₂, %), eye, beak, hock, shank, and foot IRT surface temperatures (°C), and BCO lesion severities for FHN, THN, and Total N.

Measure ²	Status ¹	
	Sound	Lame
BW	3,481 \pm 64 ^a	2,616 \pm 58 ^b
Stress		
Relative bursa wt	0.108 \pm 0.004 ^a	0.084 \pm 0.002 ^b
Core body temp	41.5 \pm 0.03 ^b	41.8 \pm 0.05 ^a
Serum CORT	7.71 \pm 0.18 ^b	14.6 \pm 0.51 ^a
Feather CORT	1.89 \pm 0.08	2.18 \pm 0.36
Eye temp	32.8 \pm 0.07	32.7 \pm 0.11
Beak temp	32.8 \pm 0.15 ^a	29.9 \pm 0.34 ^b
Leg health		
Leg O ₂ ³	81.2 \pm 0.34 ^a	78.0 \pm 0.35 ^b
Hock temp	36.9 \pm 0.08 ^a	36.3 \pm 0.12 ^b
Shank temp	36.7 \pm 0.08 ^a	35.9 \pm 0.14 ^b
Foot temp	35.5 \pm 0.12 ^a	32.8 \pm 0.24 ^b
FHN	1.91 \pm 0.09 ^b	4.26 \pm 0.15 ^a
THN	2.31 \pm 0.06 ^b	3.16 \pm 0.08 ^a
Total N	4.22 \pm 0.12 ^b	7.41 \pm 0.19 ^a

¹N = 258 birds; sound broilers were raised on litter, lame birds were raised on wire flooring.

²Descriptions of the methods for sCORT, fCORT, FHN, THN, and Total N and IRT surface temperature determinations are reported in Weimer et al., 2019, 2020.

³Leg blood oxygen saturation (%) was measured with a pulse oximeter affixed with a flexible wrap sensor.

^{a,b}Means not sharing the same letters across each row indicate significant paired t test differences at $P \leq 0.001$.

Serum CORT Δ s were at least 4 ng/mL lower at wk 6 compared to wk 4, 5, and 8 ($P = 0.0007$). The Δ beak surface temperatures were very different from eye surface temperatures and this was most apparent at 4 wk, when the Δ beak surface temperature was -4.97°C and Δ eye surface temperature was -0.37°C (Table 3). The Δ leg O₂ increased with age from -5.92% at wk 4 to -1.98% at wk 8 ($P = 0.03$; Table 3). The Δ THN scores decreased with age from 1.14 at wk 4 to 0.40 at wk 8 ($P \leq 0.004$), while Δ FHN increased from 4 to 5 wk (1.92 to 2.88, respectively), then decreased to 1.48 at 8 wk (Table 3). The increase of Δ FHN scores was also reflected in the Δ Total N score increase from 4 to 5 wk (3.07 to 4.04, respectively) and the decrease to the lowest Δ at 8 wk (1.89; $P = 0.002$; Table 3).

Linear Relationships

There were weak, yet significant ($P < 0.05$) linear regressions between IRT leg surface temperatures (hock, shank, and foot), necrosis scores (FHN, THN, and Total N), and leg O₂ for sound and lame birds (Tables 4 and 5).

There were no significant linear regressions between BCO necrosis scores with IRT foot surface temperatures within sound birds only, or for any regressions within lame birds only. There were negative linear regressions between IRT leg surface temperatures (hock, shank, and

Table 3. Weekly differences (Δ) in stress and leg health parameters of lame and sound 4–8 wk old male broilers. Data shown as mean (\pm SE) body weight (BW, g), relative bursa weight (% of BW), core body temperature ($^{\circ}$ C), serum corticosterone (CORT) concentration (ng/mL), feather CORT concentration (pg/mm), leg blood oxygen saturation (leg O₂, %), IRT surface temperatures ($^{\circ}$ C) of the eye, beak, hock, shank, and foot, and BCO lesion severities for FHN, THN, and Total N.

Δ^2	Wk (d) ¹			
	4 (28–34)	5 (35–41)	6 (42–48)	7 (49–56)
BW	-699 \pm 98.0	-982 \pm 69.3	-844 \pm 75.1	-883 \pm 69.9
Stress				
Relative bursa wt	-0.03 \pm 0.01	-0.03 \pm 0.01	-0.02 \pm 0.01	-0.01 \pm 0.01
Core body temp	0.26 \pm 0.24	0.06 \pm 0.20	0.27 \pm 0.20	0.26 \pm 0.18
Serum CORT	9.21 \pm 1.60 ^a	8.21 \pm 1.17 ^a	3.30 \pm 1.21 ^b	7.38 \pm 1.00 ^a
Feather CORT	0.37 \pm 1.31	0.28 \pm 0.93	0.20 \pm 1.00	0.61 \pm 0.70
Eye temp	-0.37 \pm 0.35 ^{ab}	0.25 \pm 0.26 ^a	0.19 \pm 0.27 ^a	-0.65 \pm 0.23 ^b
Beak temp	-4.97 \pm 1.21 ^b	-3.41 \pm 0.97 ^{ab}	-2.01 \pm 1.00 ^a	-4.27 \pm 0.88 ^b
Leg health				
Leg O ₂	-5.92 \pm 1.34 ^b	-4.18 \pm 0.93 ^{ab}	-2.37 \pm 0.97 ^a	-1.98 \pm 0.79 ^a
Hock temp	-0.84 \pm 0.42	-0.90 \pm 0.32	-0.49 \pm 0.34	-0.68 \pm 0.28
Shank temp	-1.59 \pm 0.54	-1.12 \pm 0.45	-0.55 \pm 0.46	-1.06 \pm 0.41
Foot temp	-3.58 \pm 0.89	-2.75 \pm 0.70	-2.05 \pm 0.72	-3.52 \pm 0.62
FHN	1.92 \pm 0.59 ^{ab}	2.88 \pm 0.46 ^a	2.24 \pm 0.48 ^{ab}	1.48 \pm 0.41 ^b
THN	1.14 \pm 0.26 ^a	1.11 \pm 0.17 ^a	1.03 \pm 0.18 ^a	0.40 \pm 0.15 ^b
Total N	3.07 \pm 0.69 ^{ab}	4.04 \pm 0.52 ^a	3.32 \pm 0.54 ^a	1.89 \pm 0.45 ^b

¹The number of matched pair sound and lame birds for each week were as follows: wk 4 (N = 15 pairs), wk 5 (N = 36 pairs), wk 6 (N = 32 pairs), and wk 7 (N = 45 pairs).

²Delta difference (Δ) for each matched pair of measure values was calculated as lame minus sound.

^{a,b}Means not sharing the same letters across each row differ at $P \leq 0.05$.

foot) with necrosis scores (FHN, THN, and Total N; Table 4) within both sound and lame birds. Compared to hock and shank surface temperatures, IRT foot surface temperatures had the strongest negative regressions with FHN (slope = -0.210, $R^2 = 0.083$, $P < 0.0001$), THN (slope = -0.103, $R^2 = 0.088$, $P < 0.0001$), and Total N (slope = -0.313, $R^2 = 0.108$, $P < 0.0001$) within sound and lame birds but this was not significant within sound birds (Table 4).

There were no significant linear regressions between leg O₂ and IRT hock surface temperatures within sound and lame birds, nor for any regressions within sound birds only. Generally, leg O₂ had a positive linear regression with IRT leg surface temperatures and negative regressions with FHN, THN, and Total N. The strongest linear relationship between leg O₂ and IRT leg surface temperatures was with the foot within sound and lame

birds (slope = 0.207, $R^2 = 0.117$, $P < 0.0001$) and within lame birds only (slope = 0.179, $R^2 = 0.067$, $P = 0.003$). The strongest linear regressions between leg O₂ and BCO lesions severities was with THN within both sound and lame (slope = -0.070, $R^2 = 0.108$, $P < 0.0001$) and within lame birds only (slope = -0.062, $R^2 = 0.072$, $P = 0.002$; Table 5).

Table 6 shows the pairwise correlations (r) between stress indices, leg O₂, and IRT leg surface temperatures. BW was negatively correlated ($P < 0.01$) with core body temperature ($r = -0.19$) and serum CORT ($r = -0.38$) and positively correlated ($P < 0.01$) with leg O₂ ($r = 0.22$), beak surface temperature ($r = 0.25$), and foot surface temperature ($r = 0.24$). Serum CORT was positively correlated ($P < 0.05$) with core body temperature ($r = 0.13$) and feather CORT ($r = 0.27$), and negatively correlated ($P < 0.01$) with leg O₂ ($r = -0.18$), hock,

Table 4. Regression coefficient (R^2), slope, and 95% confidence intervals for the linear relationships between mean hock, shank, and foot IRT surface temperatures ($^{\circ}$ C) with BCO lesion severities for FHN, THN, and Total N at 4 to 8 wk for (A) sound birds raised on litter and lame birds on wire flooring and (B) sound birds on litter.

BCO Score	Leg region	(A) Sound and lame ¹				(B) Sound			
		R^2	Slope	Lower 95% ²	Upper 95%	R^2	Slope	Lower 95%	Upper 95%
FHN	Hock	0.022	-0.230	-0.267	-0.027	0.067	-0.288	-0.416	-0.089
	Shank	0.021	-0.193	-0.261	-0.021	0.062	-0.285	-0.405	-0.078
	Foot	0.083	-0.210	-0.400	-0.172	0.012	-0.148	-	-
THN	Hock	0.024	-0.112	-0.272	-0.032	0.113	-0.219	-0.482	-0.171
	Shank	0.036	-0.122	-0.306	-0.069	0.097	-0.216	-0.460	-0.144
	Foot	0.088	-0.103	-0.404	-0.180	0.014	-0.265	-	-
Total N	Hock	0.029	-0.342	-0.287	-0.048	0.121	-0.507	-0.493	-0.185
	Shank	0.032	-0.316	-0.295	-0.057	0.109	-0.501	-0.478	-0.167
	Foot	0.108	-0.313	-0.433	-0.214	0.020	-0.141	-	-

¹Regression models were run on individuals. (A) N = 256 birds (B) N = 128 birds.

²Lower and upper 95% confidence intervals are present at $P \leq 0.05$.

Table 5. Regression coefficient (R^2), slope, and 95% confidence interval values of the linear relationships between mean leg blood oxygen saturation (leg O_2) with hock, shank, and foot IRT surface temperatures ($^{\circ}C$) and with BCO lesion severities for FHN, THN, and Total N at 4 to 8 wk for (A) sound birds raised on litter and lame birds on wire flooring and (B) lame birds on wire flooring.

	(A) Sound and lame ¹				(B) Lame			
	R^2	Slope	Lower 95% ²	Upper 95%	R^2	Slope	Lower 95%	Upper 95%
Leg region								
Hock	0.003	0.014	-	-	0.001	0.009	-	-
Shank	0.046	0.069	0.093	0.328	0.024	0.064	-	-
Foot	0.117	0.207	0.229	0.447	0.067	0.179	0.089	0.413
BCO score								
FHN	0.051	-0.100	-0.340	-0.107	0.002	-0.020	-	-
THN	0.108	-0.070	-0.434	-0.215	0.072	-0.062	-0.422	-0.010
Total N	0.086	-0.169	-0.402	-0.177	0.022	-0.082	-	-

¹Regression models were run on individuals. (A) Sound and lame N = 256 birds (B) Lame N = 128 birds.

²Lower and upper 95% confidence intervals are present at $P \leq 0.05$.

shank, and foot surface temperatures ($r = -0.41$ through -0.34), eye surface temperature ($r = -0.14$), and beak surface temperature ($r = -0.41$). Leg O_2 was positively correlated ($P < 0.01$) with shank ($r = 0.21$) and foot surface temperatures ($r = 0.34$), but not hock surface temperature. All IRT leg surface temperatures were positively correlated with each other ($P < 0.01$; Table 6).

Table 7 summarizes the correlations (r) between BCO lesion severity and stress indices. There were no significant correlations between feather CORT and IRT eye surface temperature with BCO necrosis severity ($P > 0.05$). Necrosis severities of FHN, THN, and Total N were negatively correlated ($P \leq 0.01$) with relative bursa

weight (-0.23 through -0.37) and IRT beak surface temperature (-0.22 through -0.28) and positively correlated ($P < 0.01$) with core body temperature ($r = 0.18$ through 0.27 ; Table 7).

DISCUSSION

This study examined the differences in health and stress measures between lame and sound male broilers on the same day of age. The birds that became lame were raised in pens with a validated wire flooring model to experimentally BCO lameness. Our previous studies provide evidence that this experimentally induced BCO

Table 6. Correlations (r) of body weight (BW, g), relative bursa weight (% of BW), core body temperature ($^{\circ}C$), serum corticosterone (CORT) concentration (ng/mL), feather CORT concentration (pg/mm), and eye, beak, hock, shank, and foot IRT surface temperatures ($^{\circ}C$) of lame and sound male broilers at 4–8 wk.

Correlations (r) ¹											
	BW	Relative bursa wt	Core temp	Serum CORT	Feather CORT	Eye temp	Beak temp	Leg O_2	Hock temp	Shank temp	Foot temp
BW	1.00	-0.12	-0.19**	-0.38**	-0.09	-0.07	0.25**	0.22**	-0.10	0.02	0.24**
Relative bursa wt		1.00	-0.23**	-0.32**	-0.08	0.10	0.18**	0.00	0.23**	0.16**	0.17**
Core body temp			1.00	0.13*	0.03	0.29**	0.20**	-0.10**	0.35**	0.40**	0.17**
Serum CORT				1.00	0.27**	-0.14*	-0.41**	-0.18**	-0.34**	-0.34**	-0.41**
Feather CORT					1.00	-0.08	0.01	0.07	-0.12	-0.03	0.01
Eye temp						1.00	0.52**	0.03	0.60**	0.57**	0.38**
Beak temp							1.00	0.27**	0.58**	0.68**	0.69**
Leg O_2								1.00	0.12	0.21**	0.34**
Hock temp									1.00	0.82**	0.62**
Shank temp										1.00	0.85**
Foot temp											1.00

¹ r values with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ ** $P \leq 0.01$.

Table 7. Correlations (r) of femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis (Total N) with body weight (BW), core body temperature ($^{\circ}C$), relative bursa weight (% BW) serum corticosterone (CORT) concentration (ng/mL), feather CORT concentration (pg/mm), and eye and beak IRT surface temperatures ($^{\circ}C$) of lame and sound male broilers at 4–8 wk.

	Correlations (r) ¹						
	BW	Relative bursa wt	Core body temp	Serum CORT	FeatherCORT	Eye temp	Beak temp
FHN	-0.25**	-0.37**	0.26**	0.42**	0.00	0.02	-0.26**
THN	-0.24**	-0.23**	0.18**	0.26**	0.01	-0.02	-0.22**
Total N	-0.28**	-0.37**	0.27**	0.42**	0.00	0.01	-0.28**

¹ r values with asterisks indicate significant pairwise correlations ** $P \leq 0.01$.

lameness can be influenced by both mechanical (i.e., BW) and psychological (i.e., CORT) stressors (Weimer et al., 2019, 2020) and the current study builds on this work.

Substantial variation in the incidence of BCO lameness was observed in the current study. While the cumulative lameness was 45%, the average lameness incidence for the top 3 (out of 6) wire flooring pens was 71% vs. 19% for the 3 wire flooring pens with the lowest incidence. Previously, lameness incidence differed significantly between study replicates (52% vs. 14%) (Weimer et al., 2019). In the current study, pen-to-pen lameness incidence ranged from 8 to 78%. Each pen was in a separate environmental chamber and, although monitored daily, fluctuations in each chamber conditions could have contributed to the large variation in pen-to-pen lameness incidence. An alternative experimental design to use in future experiments could include both a wire flooring and litter flooring pen in each environmental chamber. On average, lame birds weighed about 25% less than sound throughout the current study, and similar results have been reported in other studies (Giley et al., 2014). One distinction between the current study and our previous studies is that lame bird BW was not collected in previous work (Weimer et al., 2019), while sound birds from the wire flooring pens were not sampled or weighed in the current study. Thus, direct comparisons of sound and lame bird measures from our current study cannot be compared to our previous work. Sound birds from the wire flooring pens were not sampled in the current study for several reasons. First, the incidence of lameness of birds in wire flooring pens was greater than 50% in previous studies (Weimer et al., 2019). Due to the spontaneous nature of BCO lameness incidence of birds on the wire flooring (Wideman and Prisby, 2013), an experiment designed to sample a lame and sound bird from each wire flooring pen each day would not have been possible. Second, clinically healthy birds in wire flooring pens had more severe BCO lesions than sound in previous studies (Weimer et al., 2019) and, to make the direct comparison of noninvasive measures of stress, IRT leg surface temperatures, and leg bone BCO necrosis severity in sound (litter raised) versus lame (wire flooring raised) male broilers, a dataset balanced for health status within age was curated.

If the magnitude of the difference (Δ) in any measure were to increase with age, we would expect this increase to signify an increase in severity and detriment to the bird, and the opposite to signify a lessening effect. Although sound birds weighed significantly more than lame, the magnitude of the difference (Δ) between the matched pair sound and lame birds did not differ across weeks. Raising broilers with fast growth rates to heavy body weights is commonly accepted as the predominant factor in the onset of BCO (Wideman, 2016). Body weight is influenced by many internal processes and body weight likely reflected the earlier, concerted changes in CORT and blood flow (core body temperature, leg O_2 , and IRT surface temperatures of the shank, foot, and beak).

Along with body weight, core body temperature and serum CORT concentrations were elevated, while relative bursa weight was depressed for lame birds compared with sound. The magnitude of the difference (Δ) in core body temperature between the matched pairs sound and lame birds did not differ across weeks. This may indicate that core body temperature is a good experimental measure for the physiological response to BCO lameness at any age. No research to date has measured circulating CORT concentrations in lame broiler chickens, nor compared them to sound broilers in the same study. Decreased immune organ weights (bursa, thymus, and spleen) are an accepted indicator of increased stress (Gross et al., 1980; Compton et al., 1990; Malheiros et al., 2003; Yang et al., 2015) and similar reports of the relationships between increased circulating CORT and decreased in body weight and relative bursa weight (Puvadolpirod and Thaxton, 2000; Kidd, 2004; Lara and Rostango, 2013) have been made. Increased CORT concentrations predispose chickens to have a greater susceptibility to stress (Siegel, 1995).

The decreased relative bursa weights may have reflected an inhibitory effect of CORT on immune function (Gross et al., 1980; Yang et al., 2015) or may have been the consequence of the bacterial infection in lame birds (Wideman, 2016). Therefore, lame birds were likely experiencing immunological stress along with physical (wire flooring) and psychological (i.e., CORT) stress. During the stress response, peripheral blood is sequestered to the core to increase core body temperature. Results from a small study reported increased core body temperature from handling stress on chickens (Cabanac and Aizawa, 2000). However, the fever that accompanies infectious diseases, like BCO, is essential in combating viral and bacterial infections because it slows the multiplication of infectious pathogens (Hart, 1988). The elevated core body temperatures of lame birds may have been reflective of the cytokine-induced febrile response to stress (Liu et al., 2015), or to BCO infection, or a combination of the two in the current study. The reduced relative bursa weight and elevated core body temperature in lame birds was likely more influenced by the immune response to the pathogenic bacteria, not stress from the wire flooring.

In previous work, we found feather CORT concentrations exhibited the same increasing dose-response as serum CORT to synthetic CORT administered in the drinking water (Weimer et al., 2018). Given the assumption that the pathology of BCO progressively leads to lameness, we hypothesized feather CORT concentrations would capture the additive stress of lame birds becoming lame. However, this was not the case in the current study. In fact, feather CORT concentrations were not a reliable indicator of stress in any of our comparisons. Although feather CORT followed the same time-course pattern as serum CORT in previous work, the coefficients of variation for feather CORT were twice as high as serum CORT (Weimer et al., 2018). Many biological factors such as keratin deposition, nutrition, and genetics affect CORT deposition into growing

feathers (Harris et al., 2017). The CORT extraction technique could also affect feather CORT concentration yield. Feathers were minced with scissors into pieces smaller than 2 mm for extraction method this process may not effectively disrupt the feather matrix enough for precise measurement (Ataollahi et al., 2020). There are other reports of the inadequacy of using feather CORT as a biomarker of avian stress reported in an avian wildlife study (Harris et al., 2017). Our results suggest that feather CORT was not a useful retrospective index of stress.

In addition to feather CORT, the IRT surface temperatures of the eye and beak were proposed noninvasive indicators of stress. There were no differences in the cumulative averages of the eye surface temperatures between lame and sound birds. Eye surface temperatures had weak negative correlations with body weight and relative bursa weight and weak positive correlations with serum and feather CORT. In another study, both eye and beak surface temperatures of sound birds were negatively correlated with serum CORT (Weimer et al., 2020). However, beak surface temperatures were a much stronger indicator of stress than eye surface temperatures in previous (Weimer et al., 2020) and the current study.

The IRT eye and beak surface temperatures of the birds in this study were lower than the birds in Weimer et al. (2020). The birds were raised in the same pens within environmental chambers and had thermal images taken with the same camera in the same location (hallway) within the housing facility. This could be because the aforementioned study was conducted in the spring-summer and the current study was conducted in the fall-winter months. While the environmental chambers were individually controlled for temperature, the hallway temperature may have differed in the previous study compared to the current study but was not recorded. The ambient temperature of the thermal image location should be controlled or recorded in future research to avoid this potential confounding factor. However, the magnitude of the differences (Δ) did follow the same pattern in the current study and in previous (Weimer et al., 2020), so some similarities can be drawn from the results of both studies. Given these similar findings, it is interesting that, although they are anatomically close in proximity, beak surface temperatures are more sensitive to the physiological stress response than eye surface temperatures.

Several factors could contribute to both the sensitivity of the bird's systemic response to BCO infection, localized responses to stress, and the process by which IRT measures surface temperature. These factors could include differences in the physical properties of the eye (mostly liquid) vs. the beak (skin) or because the upper beak region has both sympathetic and parasympathetic innervation (Kuenzel, 2007). Several studies have found handling stress to decrease eye surface temperatures in chickens (Edgar et al., 2013; Herborn et al., 2015), while others have found increases (Moe et al., 2017). Given the contradictory literature on eye temperatures as a

noninvasive stress measure, the results of this study indicate that compared to eye surface temperatures, beak surface temperatures serve as a more accurate indicator of the stress response in broilers.

Postmortem clinical diagnosis of BCO was confirmed after birds were visually identified as lame from the wire flooring pens. As expected, lame birds had more severe FHN, THN, and Total N compared with sound. As reported previously (Weimer et al., 2019), it is interesting that sound birds raised on litter also exhibited macroscopic evidence of BCO lesions. Particularly for THN, the deltas (Δ) decreased with age, indicating that THN incidence increased with age for sound birds in the current study. The development of subclinical THN lesions in sound broilers could have been due to the bacteria in the environment or introduced by the caretaker. Thus, we suspect that FHN may contribute more to the onset of clinical lameness than THN because clinically healthy birds exhibit subclinical THN in this and previous studies (Wideman and Pevzner, 2012; Weimer et al., 2019).

Lame birds had lower IRT hock, shank, and foot surface temperatures compared with sound in the current study. The leg regions in which IRT surface temperatures were measured were distal to the BCO infection sites (proximal femoral and tibial heads) and IRT surface temperatures decreased as the leg region became more distal. One study found broilers with lower footpad surface temperatures had more severe footpad dermatitis scores and the authors concluded that the decrease in footpad surface temperature was due to increase in tissue necrosis and decrease in blood flow to the footpad (Jacob et al., 2016). Reduced blood flow has been hypothesized to contribute to the pathogenesis of BCO leading to lameness in broilers (Wideman, 2016). In previous reports it was found that, compared to hock and shank, the foot surface temperatures were the coldest and it was concluded that the foot was the most sensitive to changes in upstream blood flow because it is the furthest distance blood must travel (Weimer et al., 2019). However, measures of blood flow were not collected in this previous study. An indirect indicator of health and blood flow is blood oxygen saturation measured with a pulse oximeter. Previous work has found that the blood oxygen saturation of broilers with ascites is lower than those with normal hearts (Julian and Mirsalimi, 1992). In the current study, a pulse oximeter measured the blood flow of the shank and, on average, lame broilers had 3% lower blood oxygen saturation in their legs compared to sound broilers, indicating that lame broilers have reduced blood flow to their legs.

The moderate positive linear relationships between serum CORT concentrations and beak surface temperatures with tibial and femoral head necrosis in this study further indicate that BCO lameness is indeed stressful in broilers. Zhang et al. (2017) injected the glucocorticoid methylprednisolone (20 mg/kg BW) from 8 to 15 d of age to physiologically induce FHN in broiler breeder chicks. At 42 d of age, birds with FHN had decreased chondrocyte proliferation and differentiation and increased chondrocyte apoptosis in the growth plate of

the femur compared with control birds injected with saline (Zhang et al., 2017). Stress due to dexamethasone injections leading to immunosuppression are implied contributors to the pathogenesis of BCO and turkey osteomyelitis complex (Huff et al., 1998; Wideman and Pevzner, 2012). The combination of high circulating CORT and reduced blood flow to the leg (as measured by blood oxygen saturation) may have exacerbated the proliferation of bacteria in the proximal femoral and tibial growth plates.

As with body weight, relative bursa weight, and core body temperature, the magnitude of the difference (Δ) for hock, shank, and foot surface temperatures did not change with age. The results from this study and Weimer et al. (2019) suggest the foot surface temperatures serve as reproducible noninvasive IRT indicator of BCO lameness and this may be due to reduced blood flow. This may indicate that IRT leg surface temperatures are good experimental measures for the noninvasive monitoring of BCO lameness at any age. However, these results are only significant in the conditions of the current study, that is, the proximal femoral and tibial heads of lame birds and sound birds were diagnosed for clinical signs of BCO. The experimental design of the current study is not feasible in field research.

There were unexpected relationships between the necrosis, IRT leg surface temperatures, and leg blood oxygen saturation data in the separate analyses for sound only, lame only, and both sound and lame birds, because these separate analyses did not follow the same patterns. There were negative regressions between BCO lesion severity with leg blood oxygen saturation and with IRT surface temperatures for sound and lame birds combined, indicating that as the severity of necrosis increased leg blood oxygen saturation and decreased IRT leg surface temperatures. Vascular occlusion by bacteria has been reported to be a component of BCO pathogenesis (McNamee and Smyth, 2000; Wideman, 2016) and this may be a factor in blood oxygen saturation. However, when separate analyses were run for sound birds only and lame birds only, these relationships were not as significant. The results from this study are not in full agreement with our previous work. While the regression coefficients were weak in the current and previous studies, the majority of the significant regression slopes between necrosis severity IRT leg surface temperatures were positive for sound birds only, with minimal negative slopes in a previous study (Weimer et al., 2019). This could be because the sound birds in the current study had more evidence of THN and reduced blood flow had already occurred, compared with the sound birds in previous work that may have had healthier legs or earlier stages of inflammation (Weimer et al., 2019).

CONCLUSIONS

The results of this study provide evidence that lame broilers experience more stress, with greater serum corticosterone concentrations and lower relative bursa

weights, than sound. The correlations between serum corticosterone concentrations, relative bursa weights, and core body temperatures with IRT beak surface temperatures support that beak surface temperatures are a more robust noninvasive measure of stress than eye surface temperatures in broilers. We suggest that measures of eye surface temperatures and feather corticosterone concentrations were not appropriate or sensitive enough to capture the stress effects of the wire flooring or BCO lameness in broilers. The IRT surface temperatures of hock, shank and foot are promising noninvasive measures, and possible predictors of, BCO lameness in broiler chickens. Linear regressions between leg blood oxygen saturation, IRT surface temperatures, and BCO necrosis severity add additional merit to the utility of IRT in lameness evaluation. Moreover, there is reduced blood oxygen saturation in lame broiler legs compared with sound, which may be symptomatic of reduced blood flow. These results indicate the utility of IRT as a noninvasive measure of broiler stress and lameness.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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