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Noninvasive Measures of Stress and Lameness in Broilers

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Noninvasive Measures of Stress and Lameness in Broilers

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ABSTRACT

The concept of broiler chicken welfare has evolved from a component of animal husbandry to a label on a chicken product package. Recent attention from the public has led to the need for higher welfare standards for animal production. A primary concern of broiler production is low activity/locomotion attributed to fast growth rates leading to poor leg health. To collect the scientific data necessary to determine conditions providing the best welfare for the bird, multiple methods of assessment are required. Stress is influenced by external (temperature, humidity, lighting, stocking density) and internal (metabolism, thermoregulation, hormonal balance) factors. This series of studies evaluated measures of health, stress and behavior. The main objective was to investigate noninvasive measures of broiler stress and lameness. The two primary noninvasive methods were extraction of the stress hormone corticosterone (CORT) from feathers to evaluate stress and infrared thermography (IRT) to evaluate stress as well as lameness attributed to bacterial chondronecrosis with osteomyelitis (BCO). First, an ELISA was used to measure the effects of CORT administration in the drinking water over a 72 hour period on CORT from serum, cecal contents, excreta and two feather types. The next series of studies evaluated light intensity and flooring type effects on broiler stress and leg health. Results from these studies indicate light intensity did not consistently affect the stress and leg health parameters that were measured. Rearing broilers on wire flooring is an effective method for inducing BCO lameness. While the wire flooring did induce lameness, it did not consistently affect stress or leg health parameters measured on sound broilers. The final study combined stress and lameness measures from previous studies to compare lame and sound broilers. In this study, statistical models were evaluated for their potential use in predicting lameness. The results from these studies suggest 1) the current method of extracting CORT from feathers is not useful

to evaluate stress; 2) IRT measures of beak surface temperatures may be a useful method to evaluate stress; and 3) IRT measures of leg region surface temperatures may be a useful method to detect/predict lameness attributed to BCO in broilers.

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CHAPTER 1 Literature Review

1.1 INTRODUCTION

Some consumers are willing to pay more for products from companies who have a reputation of being more sustainable or 'green'. The definition of 'green' covers a broad spectrum ranging from environmental issues to social responsibility for the ethical treatment of food animals (Smith and Bower, 2012). People define 'animal welfare' by different standards because their quality of life notions are shaped by cultural and personal values (Johnson, 2009). One definition is "*the welfare of an individual is its state in regards to its attempts to cope with its environment*" (Broom, 1986). According to Broom (2011), animal welfare assessments are criteria for measuring sustainability because the public will not accept animal products from systems that cause poor welfare.

The discipline of animal welfare started in European Union in 1965 when the Brambell Committee published the "*Five Freedoms*" (Brambell, 1965). The Brambell Committee Freedoms described the possibility of livestock animals to not suffer and be productive with acute, transient stressors so long as they are comfortable, fear, stress and pain-free, have feed and water and can express normal behavior. Some freedoms are easy to measure and maintain, such as access to feed and water. However, freedoms such as comfort may be more difficult to measure and maintain because of individual animal differences and human subjectivity. Some animal welfare research focuses on the development of welfare assessment methods in different environments while other research focuses on the fundamental biology of welfare and stress (review: Carenzi and Verga, 2009). Thus, valid research is necessary to measure animal welfare.

In June 2016, Perdue Farms Inc. announced the launch of their "*2016 and Beyond: Next Generation of Perdue Commitments to Animal Care*" program initiative (Purdue Farms Inc.,

2016). In August 2016, the Humane Society of the United States (HSUS) Senior Director of Food Policy sent letters to the major broiler processing CEOs announcing the organization's focus "*will likely shift toward broiler welfare issues*" (Keefe, 2016). It seems HSUS is holding Perdue Inc.'s initiative to be "*precedent-setting*" and the new standard to which all broiler producers will be held to. HSUS will focus their slander to those who do not comply. The initiatives are likely to have larger economic costs and may reduce broiler poultry production in the United States.

Public concern has forced many food service companies to require stricter production standards for growing animals destined for food. Unfortunately, many of these demands lack scientific validation or are too complicated for commercial use (Grandin, 2014). A major hurdle animal welfare assessors face is how to measure individual welfare at the group level. Large group sizes make individual assessment very difficult (Daigle, 2014). Animal welfare evaluations must be performed in a short period of time to result in the most benefit for the cost involved (review: Czycholl et al., 2015). To collect the scientific data necessary to determine conditions providing the best welfare for the animal, multiple methods of assessment are required. These assessments continually strive to objectively measure the health, physiology, natural behavior and affective state (Johnson, 2009; review: Czycholl et al., 2015).

With the recent press attention on broiler chicken welfare, the need for scientific research into valid measures of broiler stress is increasingly imperative. These measures will yield welfare assessments providing meaningful information on the welfare status for the broilers within their house environment, not how effectively we can appease activist demands.

1.1.2 Broiler Stress and Welfare

Stress is a broad term contextually used to describe stimuli challenging homeostasis, emergency responses and the chronic state of imbalance (Blas, 2015). Stress can be defined as "*the biological response elicited when an individual perceives a threat to its homeostasis*" (review: Moberg, 2000). The stress response is essential for survival and malfunction of the stress response leads to decreased welfare. Eustress is a response to normal or moderate stressors that results in benefits for the individual. Distress is a response to stressors that results in harm or injury for the individual. The purpose of welfare science is to determine when the stress response ('*eustress*') becomes destructive ('*distress*') to the welfare of the individual (review: Moberg, 2000).

Measuring stress is difficult because unlike most diseases, stress has no defined etiology or prognosis (review: Moberg, 2000). The primary physiological measure of stress in avian research is determining the circulating concentrations of the glucocorticoid hormone corticosterone (CORT).

1.1.3 Hypothalamo-pituitary-adrenal (HPA) Axis Secretion of Corticosterone (CORT)

The hypothalamo-pituitary-adrenal (HPA) axis is the primary organ system mediating the stress response. In 1950, Hans Selye described the "*General Adaptation Syndrome*" (GAS) as the physiological response regulating homeostasis for "*adaptation energy*" of a species to adapt to changes in their environment. Major unpredictable perturbations elicit the "*fight or flight*" (Cannon, 1935) response, during which the autonomic system repartitions energy to combat a stressor. During the alarm reaction the autonomic nervous system immediately releases the catecholamines epinephrine (E) and norepinephrine (NE) to supply the burst of energy needed to combat the stressor (Blas, 2015). Catecholamine production is dependent on glucocorticoids and

an action of catecholamines is to stimulate the HPA axis to increase glucocorticoid secretion (Carsia, 2015).

Hormone signaling is essential to maintaining homeostasis and specific stressors affect essentially every endocrine system (Matteri et al., 2000). Circulating glucocorticoids are the key mediator of the stress response but do not act alone. Corticosterone (CORT) is the primary glucocorticoid hormone secreted in response to a stress-inducing event in birds (deRooos, 1961). In mammals, the stress hormone is cortisol. Compared to cortisol, corticosterone is analogous in every way, structurally differing only by the absence of a hydroxyl group on carbon 17. The secretion of CORT is the result of hypothalamic stimulation to release corticotropin-releasing hormone (CRH). Released from the hypothalamus, CRH travels via the median eminence (ME) to stimulate, proopiomelanocortin (POMC) derived, adrenocorticotropic hormone (ACTH; review: Mormède et al., 2007) secretion from the anterior pituitary gland. ACTH then travels in the blood to stimulate the adrenal gland cortex to secrete CORT into circulation (Blas, 2015; **Figure 1.1**).

Cholesterol is the major precursor of CORT synthesis (Kime and Norymberski, 1976). Hypertrophy of the liver is a result of stress due to increased lipid content and decreased moisture content (Puvadolpirod, 1997; review: Virden and Kidd, 2009). Adaptation energy is created through gluconeogenesis (Olanrewaju et al., 2007). CORT has a significant effect on protein, carbohydrate and lipid metabolism. In wild and domestic species, this effect is not consistent because the increase in fat depots sometimes compensates for protein loss (Carsia, 2015). As CORT concentrations increase, the proportions of heterophils and lymphocytes are altered and circulating concentrations of cholesterol and glucose also increase (Siegel, 1980;

Puvadolpirod, 1997; Scanes, 2009). Increased circulating glucose leads to increases in cardiac output, heart rate, blood pressure, and respiration rate (review: Siegel, 1995; Puvadolpirod, 1997). Increases in CORT concentrations also inhibit skeletal calcification and induce osteoporosis in adults (Siegel and Latimer, 1970).

In the wild, the stress response is essential to prey species' survival in situations such as escaping from a predator. When animals anticipate stressors, basal glucocorticoid concentrations fluctuate naturally and follow a circadian rhythm (Blas, 2015). Some stressors, such as seasonal fluctuations and photoperiod, are anticipated and cause physiological adjustments (Blas, 2015).It is pertinent to consider the stress response of wild species stress when evaluating domestic animal welfare.

With broilers, short term stress events are of minimal impact to overall flock welfare (review: Virden and Kidd, 2009). In contrast, chronic stress elicits permanent negative effects on broiler production. Negative stress effects may include impaired immunity, reduced body weight, shifted metabolism, and less desirable carcass characteristics (Lin et al., 2006; Shini et al., 2009; review: Virden and Kidd, 2009).

1.1.4 Stress Response Measure Considerations

The difficulty in measuring avian stress and welfare is that "*we must integrate behavioral, biochemical and physiological measures to determine one outcome*" (review: Moberg, 2000). According to Dawkins in 2004(a), assessing animal welfare should answer two questions: 1) are the animals healthy?; and, 2) do they have what they want? Preference test results may negatively influence performance. For example, a study by Dawkins and colleagues (2004b) evaluated two groups of laying hen pairs in pens connected by a doorway to a test pen.

One group was provided with an enriched environment (wood shaving flooring and a box of sprouting wheat) while the other group was provided with a barren environment (wire flooring). Birds provided access to the enrichment spent significantly more time in the enriched environment than the birds with access to the barren environment. However, the enriched birds had higher concentrations of fecal CORT and had thinner eggshells. The physiological measures would indicate distress and the preferential behavioral measures would indicate eustress. A housing system providing environmental enrichment may result in birds having elevated CORT concentrations.

Studies have shown increases in plasma CORT concentrations in pigeons (Rees and Harvey, 1987), ducks (Rees et al., 1983) and chickens (Rees et al., 1984) subjected to treadmill exercise compared to those not exercised. The magnitude of circulating CORT concentrations to exercise is positively correlated with workload (Rees and Harvey, 1987). Rees and colleagues (1983) exercised ducks on a treadmill for 60 minutes and found that CORT concentrations increased at both 30 minutes and 60 minutes compared to baseline. Additionally, CORT concentrations were higher at 30 minutes than at 60 minutes. A different response was reported in chickens, with CORT concentrations being higher at 60 minutes than 30 minutes of treadmill exercise (Rees et al., 1984). The authors also reported higher plasma concentrations of epinephrine, norepinephrine and dopamine after 60 minutes of exercise. These studies suggest increased activity increases HPA axis activity and resultant CORT production.

Increased CORT during activity may have health benefits. For instance, β-endorphin and ACTH are both synthesized from POMC and are secreted concomitantly from the pituitary gland (Guillemin et al., 1977). In 1988, McCormack and Denbow studied the effects of β-endorphin

injections of (ranging from 0 to 6 micrograms) on fast (Rock-Cornish) and slow (Single-Comb White Leghorn) growing lines of chickens. The authors found that low concentrations of β-endorphin injections increased feeding, drinking and body temperature in both lines. Moderate exercise may have health and welfare benefits in broilers.

1.2 Blood Measures of HPA Activity

1.2.1 Heterophil to Lymphocyte Ratio (H:L)

Stress changes hematological proportions of blood cells. Perception of environmental changes can determine the degree of immunosuppression in broilers (Gross and Siegel, 1981). The heterophil to lymphocyte (H:L) ratio is an accepted valid indicator of stress effects on the immune system of broilers (Gross and Siegel, 1983; Scanes, 2015). However, some hematologists caution the use of standard H:L ratios as a direct stress indicator, as deviations from typical lymphocyte morphologies may indicate a compromised health status (Cotter, 2016). Heterophils and lymphocytes are two (out of five) types of chicken white blood cells (leukocytes). Heterophils are considered equivalent to human neutrophils with similar phagocytic functionality. Lymphocytes (T and B cells) are the leukocytes involved in adaptive immunity, which respond to repeated exposure to an antigen (Abbas et al., 2014). In chickens, leukocytes can secrete ACTH to stimulate the adrenal cortex (review: Marsh and Scanes, 1994). In response to CORT, lymphocytes adhere to blood vessel wall endothelial cells and migrate into tissues such as lymph nodes, spleen, bone marrow and skin (review: Davis et al., 2008).

In a review of stress in chickens, Siegel and Gross (2000) report a normal H:L ratio to be 0.4 and a ratio of 0.6 to 1.2 indicating higher stress levels. Exposure to CORT in water (Post et al., 2003; Shini et al., 2009) causes populations of heterophils to increase and lymphocytes to

decrease (p 177, Scanes, 2015), which consequently increases the ratio of heterophils to lymphocytes. Caution must be taken when employing the H:L ratio as a physiological measure of stress as leukocyte populations are also strongly influence by health status. Health status of the bird needs to be considered when interpreting the H:L ratio as a measure of stress (review: Maxwell, 1993).

1.2.2 Plasma or Serum CORT

Blood plasma and serum samples are widely used to assay CORT in animal stress and welfare studies (review: Mormède et al., 2007). Basal concentrations of CORT fluctuate through development and display a circadian rhythm (Majsa et al., 1976). An issue with CORT assays are the large amount of variation due to differences in individual stress perception and physiological response.

1.2.3 Restraint and Handling During Blood Draw for CORT Measure

Plasma CORT concentrations are elevated immediately following aversive stimuli, such as handling and restraint, and can vary depending individual bird previous experiences (Kannan and Mench, 1997). The human-animal interaction is an important component of broiler welfare. Handling is an acute stressor in broilers (Chloupek et al., 2011). The stressful experience of handling and blood sampling to measure circulating CORT concentrations may inherently alter CORT concentrations. Handling broilers in an upright position is less stressful than handling in an inverted position (Kannan and Mench, 1997). In 1994, Hemsworth and colleagues measured plasma CORT concentrations in handled and non-handled broilers immediately following 5 different handling period lengths (ranging from 3 min to 15 min). Except for the 3-minute handling treatment, the authors found lower plasma CORT concentrations in handled birds compared to non-handled. The findings suggest the previous handling experience did not reduce

the stress of handling for blood sampling. Chloupek and colleagues (2011) handled broilers using two different techniques (gentle and rough handling) and found that regardless of handling technique, plasma CORT concentrations were higher for birds handled for 150 and 180 seconds than those handled 30 to 120 seconds. Drawing blood longer than 2 minutes after bird capture will yield blood samples with higher CORT concentrations (review: Mormède et al., 2007; Chloupek et al., 2011).

The problems with stress measurements in domestic poultry are further complicated as genetic selection of broilers may have altered their stress responses. In two studies in 2011, Soleimani and colleagues compared body temperature, H:L ratios and plasma CORT concentrations of Red Jungle Fowl and commercial broilers under heat stress (36°C for 3 h). The authors found that commercial broilers have significantly increased body temperature, H:L ratios, and plasma CORT concentrations under heat stress compared to Red Jungle Fowl. However, throughout both experiments Red Jungle Fowl showed lower H:L ratios, higher plasma CORT concentrations, and higher heat shock protein 70 gene expression than commercial broilers. These results suggest genetic selection from the Red Jungle Fowl may have resulted in lower basal CORT concentrations, yet higher plasma CORT concentration sensitivity related to heat stress. The authors concluded domestication and selective breeding has led to individual broilers being more susceptible to stress. Therefore finding less invasive, quicker and less expensive measures of stress will help lead to a better understanding of the commercial bird's optimal welfare.

1.3 Noninvasive Physiological Measures of the HPA

1.3.1 Fecal CORT

CORT measures from fecal material are an accepted noninvasive measure of stress. Fecal collection does not require handling of birds, although the presences of humans during fecal collection may stress the birds. Metabolized CORT is deposited in excreta and can be measured in the feces in chickens (Rettenbacher et al., 2004; Rettenbacher et al., 2006; Alm et al., 2014). Typically, fecal CORT metabolite measures pool multiple samples to reduce or dilute high individual variation and fluctuations. Sex, diet, gut microbiota, and metabolism can all contribute to individual variation in CORT concentrations (Goymann, 2012). On the flock level, fecal CORT can be correlated with mortality and both fecal CORT concentrations and mortality are reported to be higher in the winter compared to the summer season (Dawkins et al., 2004c).

Rettenbacher and colleagues (2004) reported the median percentages of radiolabeled CORT in multiple individual broiler tissues and other samples at 1, 2, 4 and 8 h after i.v. administration of radiolabeled CORT. The authors also found that blood CORT decreased with time and fecal CORT increased with time. This makes sense as blood CORT concentrations are an "instant" measure of CORT and fecal CORT accumulates after digestion over time. Fecal CORT measures are a useful noninvasive stress measure. However, variations in secretory patterns may attenuate CORT concentrations (review: Morméde et al., 2007).

1.3.2 Feather CORT

Feathers function to protect the skin, provide a barrier to pathogens and aid in thermoregulation of birds. In addition, good feather coverage optimizes energy metabolism and

feed efficiency (Leeson and Walsh, 2004b). Feather weight has been reported to be significantly correlated to body weight during the 5th to 7th week of age of production (Fisher et al. 1981).

Keratin is the primary protein in feathers which serves as a repository for CORT deposits (Bortolotti et al., 2008a). Feather elongation and growth occurs in a radial pattern around the feather follicle, as does the deposition of CORT (Bortolotti et al., 2008b). Resultantly, different anatomical sections of the feather may contain different concentrations of CORT related to differences in keratin density. Bortolotti and colleagues (2008b) found that the highest proportion of CORT deposited per milligram of feather weight was in the vanes.

There are a variety of reports of CORT concentrations in feathers for wild and domestic birds which indicate that this may provide a noninvasive tool for routine use (Bortolotti et al., 2008, Faihurst et al., 2011; Carbajal et al., 2014). Given that the keratin becomes inert when fully developed, feathers can be painlessly clipped for sample collection (Bortolotti, 2010). Feather CORT assays are becoming more popular in avian ecological research, specifically in evaluating the effects of human-mediated environmental changes to bird physiology over long time periods (Berk et al., 2016). To date, the only published research on feather CORT in broilers is a small-scale research note published by Carbajal and colleagues in 2014. The feather chosen to extract CORT was a body feather from the interscapular area from 22 randomly selected broilers in a commercial setting. No correlations were found between feather CORT and sex, weight or feather fault bars.

It is unclear how quickly CORT can be deposited in the feather and how well this is associated with circulating CORT concentrations. Fairhurst et al. (2013) measured plasma CORT concentrations and feather CORT concentrations in Tree Swallows given CORT in feed at 7, 9

and 11 days of age compared to controls. The only correlation between feather and plasma CORT concentrations was on day 9, and this day was the most variable among individuals. There are still many avenues of investigation needed to validate feather CORT concentrations as an avian stress index, such as hormonal regulation, keratin content, and types of feathers extracted. When compared with baseline concentrations, stronger correlations in stress-induced red-legged partridges have been found between feather CORT concentrations with circulating CORT concentrations (Bortolotti et al., 2008). However, circulating CORT concentrations indicate a snapshot of acute stress at the time of collection whereas feather CORT concentrations indicate cumulative stress throughout the growth of the feather (Bortolotti et al., 2008).

Feather CORT concentrations can be affected by variations within and across individuals. This makes environmental measures of stress difficult to evaluate. In 2015, Harris measured feather CORT concentrations from right and left flight feathers (primaries P2 and P6, secondaries S2 and S4 and retrices R1 and R5) on the same individual adult Tree Swallows (N = 12). The same feathers on opposing sides of the bird did not have the same feather CORT concentrations. These results indicate a low repeatability of feather CORT concentrations from the same individual and CORT incorporation and depletion may be different in different feathers.

Another factor affecting feather CORT concentrations is the extraction methodology. The accepted paper validating the extraction method was published by Bortolotti and colleagues in 2008. Feather samples were first washed with or without soap to remove debris. Studies have shown using hexane or ethanol to wash feathers decreases CORT concentrations (Bortolotti et al., 2008b; Harris, 2015). Methanol is the extraction substrate. Methanol causes both swelling of the feather to liberate the analyte by diffusion and readily solubilizes steroid hormones (Meyers

et al., 2012). No differences have been reported when times for methanol extraction incubation (Botolotti et al., 2008b) or methanol volumes (Berk et al., 2016) were altered.

Feather CORT concentrations are typically normalized per milligram of feather mass or per millimeter feather length. Bortolotti and colleagues (2008b) caution feather CORT normalization by feather mass, as feather keratin density can vary considerably and can introduce inconsistencies in the expression of feather CORT concentrations. Berk and colleagues (2016) found a negative quadratic relationship between feather length and mass and CORT concentrations, with shorter and lighter feathers having higher CORT concentrations.

1.4 Environmental Effects on Productivity and Stress

House conditions and seasonal fluctuations have major impacts on broiler flock productivity, health and welfare (Dawkins et al. 2004, review: Olanrewaju et al., 2006). The welfare state of an individual broiler is affected by multiple environmental contributors such as house environment (Dawkins, 2004), social relationships (Gross and Siegel, 1981; Gross, 1984) and immune status (Gross and Siegel, 1981).

In a large-scale (2.7 million) European Union study conducted by Dawkins and colleagues (2004), producers stocked broilers at varying densities ranging from 30 to 46 kg/m^{-2}. Environmental, production, physiological, behavioral and health parameters were measured. Of all parameters measured, the authors found that regardless of stocking density, house temperature and humidity contributed more to overall broiler flock welfare (Dawkins et al., 2004). Certain locations within the house may create fearful stimuli and have a negative effect on flock welfare. In a Brazilian commercial broiler study, Miragliotta and colleagues (2006) measured vocalizations and found inlet and outlet sectors of the house to be more stressful due to higher

temperatures, noise levels and light intensities. The authors also found the highest mortalities to be in zones with exhaust fans. Thus, these studies suggests environmental factors are the most important for overall broiler welfare.

Stressed birds increase their water intake (Puvadolpirod, 1997) and therefore excrete more moisture, leading to decreased litter quality and higher ambient ammonia (NH_3) volatilization (Miles et al., 2011). This finding support Dawkins' statement that overall environmental conditions have most impact on broiler welfare. Lighting throughout the environment, as well as light provided incidentally via vents, also have a significant impact on broiler welfare.

1.4.1 Light Intensity

Sight is considered to be the dominant sense in the broiler chicken, as the occipital lobes are extremely enlarged compared to mammals and comprise a large portion of their brain (Cobb, 1960). Compared to human vision, broilers have a heightened visual perception of illuminance, which is the amount of light reflected from a surface (Prescott et al., 2004). Broilers have a high proportion of cones in their retinas compared to humans. This indicates they have better vision in brighter than dim light, and dimly lit environments may deprive them of some sensory input (Manser, 1996).

Light is important to broiler growth, reproductive development, behavior and welfare. Typically, producers keep light intensity high during the brooding phase so chicks can find feed and water, maximizing growth and production. Light intensity then decreases after the first week of life to reduce activity (Kestin et al., 1992, Bizeray et al., 2002) and to improve production

measures such as feed conversion (Lien et al., 2008) and weight gain (Gross and Siegel 1981; Lien et al., 2008).

According to Manser (1986), the four main lighting research areas in broilers are light source, photoperiod (dark and light periods), light color and light intensity. Light intensity is an environmental parameter that strongly influences broiler health, productivity and welfare (Gross and Siegel, 1981). It is suggested that 5 lux is the minimum light intensity needed for good welfare (Deep et al., 2013, NCC, 2014). Lower light intensities are thought to reduce broiler productivity and compromise welfare by comparing eye development (Blatchford et al., 2012; Deep et al., 2013). For this reason, in the European Union, 20 lux with 80% illumination at bird eye level is a regulatory requirement (Council of the European Communities, 2007).

Traditionally, lower light intensities have been shown to improve production performance in feed conversion (Lien et al., 2008) and weight gain (Gross and Siegel, 1981; Lien et al., 2008) as well as behavioral benefits such as decreased aggression and cannibalism (Leeson and Walsh, 2004b). Conversely, Deep and colleagues (2013) found broilers raised in low levels of light intensity (0.5 and 1 lux) had lower body weight gain and carcass yield as a proportion of live weight, while eye weight and diameter were greater, indicating poor eye health. Olanrewaju and colleagues (2014) found no difference in plasma CORT or glucose concentrations when broilers were provided five light intensities ranging from 0.2 to 25 lux. Some scientists report higher light intensities do not increase aggression or cannibalism (Deep et al., 2010). One reason could be that broiler chickens are juveniles and do not yet secrete high enough concentrations of reproductive hormones associated with aggression.

Light intensity may also affect broiler leg health. Some research has demonstrated no difference in gait scores in different commercial light intensities (Kristensen et al., 2006, Deep et al, 2013) while other research found gait scores of broilers raised in 200 lux had better gait scores than broilers raised in 1 lux (Blatchford et al., 2014). However, the light intensities in the research reported by Blatchford and colleagues (2014) were extremely different and not reflective of commercial conditions. Kendall's correlation coefficients were analyzed in a study investigating leg health and environmental parameters (Kumari et al., 2015). When lameness, hock and footpad scores were recorded on a binomial scale (absence or presence), significant negative correlations were found between lameness and relative humidity (75.6%) as well as footpad score and light intensity (83.9%). Further research is necessary to establish optimal light intensities that promote the best overall welfare in broilers.

1.5 Broiler Lameness

In a 2017 study written by Donald Broom titled "*Animal Welfare in the European Union*", Broom states "*…the greatest animal welfare problem in the world is broiler chicken leg disorders and related problems*". The study was commissioned by the Policy Department for Citizens' Rights and Constitutional Affairs upon request of the Committee on Petitions to review the past, present and future of animal welfare legislature in the European Union (Broom, 2017). The strength of Broom's statement recognizes the need for scientific research to improve broiler leg health.

Broiler lameness is a major welfare issue if the bird cannot reach feed and water. Broilers today grow three to four times faster than previous generations (Alltech, 2016). The 2013 average market weight of broilers was about 6 pounds (2.72 kilograms) (Alltech, 2016). In 1995,

the average body weight of a commercial broiler was 4.66 pounds (Alltech, 2016). This is a 22% increase in body weight in an 18-year period (MacDonald, 2014; **Figure 1.2**). Mortalities of up to 2% have been attributed to lameness, costing the industry approximately 4 billion dollars globally (Alltech, 2016). One hypothesis is the lack of activity and high proportion of time spent sitting by the modern broiler (Bizeray et al., 2002; Weeks et al., 2002; Ruiz-Feria et al., 2014). Other contributors to broiler lameness may include leg morphologies, nutritional inadequacies, disease, management, and environmental factors.

Modern broiler leg conformation may predispose individuals with extreme morphologies (hip width, hip and leg angle) to lameness (Paxton et al., 2010). In a study comparing the gait dynamics of modern broilers to the ancestral Jungle Fowl, it was found that modern broilers have a lower pelvic limb muscle mass (Paxton et al., 2010). The broiler walks slowly, with a wide base of support and large lateral ("waddle") motions to cope with their instability due to heavy weight (Paxton et al., 2013). However, the lateral force of the broiler gait may be a natural component of avian gait rather than a negative result of selection (Corr et al., 2006).

Bizeray and colleages (2002) reported that healthy, non-lame adult broilers spend about 30% of their time standing and 70% sitting. Ruiz-Feria and colleagues (2014) investigated increasing the activity of broilers to improve leg health within their environment by providing ramps and increasing the distance between feeders and waterers. The authors found no difference in bone breaking strength and the ramps decreased tendon breaking strength. The results from this study suggest the current broiler is raised for the environment it has been selected for and additions to the environment may not enhance welfare.

In 2016 commercial broiler production study in Southern Brazil, Federici and colleagues used the Welfare Quality® protocol to assess the status of the industry's broiler welfare. On a 100-point scale, with 100 being the best (most desirable) welfare score, footpad dermatitis and lameness were amongst the lowest scores (below 20). The median proportion of lame broilers (with a lameness score of 4 or 5) was 14%. The authors concluded the high lameness incidence is a major welfare concern for the Brazilian broiler industry.

1.5.1 Bacterial Chondronecrosis with Osteomyelitis (BCO) Lameness

First reported in chickens in 1972, bacterial chondronecrosis with osteomyelitis (BCO) is a current major contributor to broiler lameness (review: McNamee and Smyth, 2000). In a 2015 review, Wideman suggests the increase in broiler lameness in recent years is related to "*the disproportionately large increase in body mass accretion to the smaller progress of skeletal maturation*". This suggestion indicates the current broiler skeleton cannot physiologically handle the weight it now carries. Today, a broiler chick weighs 40 g at hatch and in 8 weeks weighs over 4000 g. If this growth rate was applied to humans, a 3 kg baby would weigh 300 kg in 8 weeks (Wideman and Prisby, 2013). Physical shear stress from heavy broiler weights may lead to micro fractures in the proximal femur and tibiotarsus (referred to as tibia). These micro fractures provide areas for bacteria to proliferate and thus degrade the proximal growth plate. Bacterial proliferation in BCO may be amplified by behavioral factors leading to lameness. The ischiadic artery is the major blood supply to the leg in avian species (Dzialowski and Dane, 2015). The ischiadic artery curves around distal femoral head, at the coxofemoral joint, and flows down to the knee-where it connects with the femoral artery. The large proportion of time broilers spend sitting may create a pinch point at these two arterial locations, reducing the rate of

blood supply to the legs (review: Wideman, 2015). BCO lameness is a substantial broiler welfare issue and decreasing lameness will require further research.

1.5.2 Behavioral/Visual: Gait Scoring and Latency to Lie (LTL)

The most practical way to assess broiler lameness at the flock level is by gait scoring individuals. A normal gait is an integrated function of the nervous, muscular and skeletal systems and a failure in one of these components will result in leg weakness or lameness (review: Manohar et al., 2015). Kestin and colleagues (1992), published the first broiler gait scoring system with six categories ranging from normal to immobile. In this study, the authors concluded that 90% of broilers surveyed had a detectable gait abnormality, with 26% having abnormalities severe enough to consider their welfare poor. The authors also concluded that genetics are an important determinant of leg weakness. However, gait scores were based on any sort of "uneven gait" observed, i.e. any score greater than zero. The Kestin gait scoring system is used in the European Welfare Quality® assessment protocol for poultry. In a commercial broiler study using the Welfare Quality® assessment, a report published results indicating the United Kingdom as having the lowest lameness incidence, followed by the Netherlands, with Italy having the highest lameness incidence (Welfare Quality® Report, 2010).

National Chicken Council (NCC, 2014) welfare assessors watch broiler movement but are not required to gait score. If gait scoring is deemed appropriate by the assessor, the U.S. Gait Scoring System is employed to assess 100 broilers. This system uses a 0 - 2 category scoring system with 0 defined as "*bird should walk at least 5 feet, and while the bird may appear ungainly, there are no visible signs of lameness*", 1 defined as "*bird should walk at least 5 feet, but appears awkward, uneven in steps*", and 2 defined as "*bird will not walk 5 feet without sitting*

down or there is obvious lameness". The number of birds unable to walk or move after gentle encouragement is recorded. Gait scoring is a qualitative method to assess broiler lameness. Therefore, this method creates opportunities for intra- and even inter-observer variation (Garner et al., 2002). Lameness assessment must be objective and repeatable to produce practical information.

In addition to gait scoring, the latency to lie (LTL) test is a method to assess lameness. The LTL test provides an objective, quantitative measure of broiler lameness. This is an advantage in comparison to the inherent subjectivity of gait scoring (Weeks et al., 2002). The test involves placing birds to an enclosure filled to a 30 mm depth of tepid (32°C) water. LTL measures the time elapsed between water introduction and the bird actually sitting in the water. If a bird does not sit within 10 minutes (Ruiz-Feria et al., 2014; Berg and Sanotra, 2003) or 15 minutes (Weeks et al., 2002), the LTL test ends and the bird's time is recorded. The purpose of the water is to provide a novel, aversive stimulus that the broiler must choose to sit in above other motivations (Weeks et al., 2002; Ruiz-Feria et al., 2014). For example, if a broiler is experiencing leg pain, the bird will have to choose between sitting in water versus experiencing the pain associated with continuing to stand. Normally, broilers do not experience their feet touching water and will not sit or preen in water (Weeks et al., 2002).

Ruiz-Feria and colleagues (2014) investigated associations in two studies evaluating the effects of distance (3 or 8 m) between feeders and waterers with and ramp provision on LTL. In the first study, there was no effect of distance on LTL. In the second study, on d 49, broilers not provided ramps (NR) had shorter LTLs than broilers provided ramps (WR) when the distance between feeders and waterers was 3 m. However, when the distance between feeders and

waterers was 8 m, NR broilers had longer LTLs than WR broilers. Tendon breaking strength weaker in WR broilers. The results from this study suggest distance between resources may have limited effects on leg health.

Rault and colleagues (2017) evaluated the effect of 5 lux and 20 lux light intensity on mixed sex broiler behavior, welfare and productivity. LTL times were measured in combination with leg and foot condition measures (footpad dermatitis, hock burn and leg straightness) at 46 d of age. Although feed intake did not differ, broilers kept at 20 lux weighed less than broilers kept at 5 lux at 46 d of age. These productivity measures may have been reflective of the reported increased activity of broilers kept at 20 lux during the light period than broilers at 5 lux. The authors found no differences in LTL times. The results of this study suggest light intensities of 20 lux and 5 lux have no effect on broiler leg health.

1.5.3 Broiler Leg Pain

Broilers may be experiencing pain due to lameness without behaviorally displaying their pain. Assessing pain in chickens is a difficult task, especially in a practical setting. The stoic nature of broilers could affect LTL test performance. Weeks and colleagues (2000) found broilers with higher gait scores spent more time sitting with a limb extended. Long periods of leg extension in lame birds may be a method to increase blood flow to the leg (review: Wideman, 2015) or relieve pain (Weeks et al., 2000). Müller and colleagues (2015) found relative adrenal weights of broilers with gait problems to be higher than broilers with normal gaits, indicating lameness increases physiological stress.

Changes in motivation can change pain-related behavior in layers transiently induced (by sodium urate injection in to the ankle joint) with gouty arthritis (Gentle and Corr, 1995; Gentle

and Tilston, 1999; Gentle, 2001). In a 1995 study, Gentle and Corr found birds caged individually exhibited more pain-related behavior than birds housed in group pens after sodium urate injection. Pain related behavior was classified as standing on one leg, limping when walking, or prolonged sitting. Social influences may have increased pain guarding and decreased behavioral display of pain. In a 1999 study, Gentle and Tilston (1999) found pain-related behavior of birds (induced by sodium urate injection) decreased after they were placed in a novel environment. Furthermore, when surface skin temperature of the injected ankle joint was measured, peripheral inflammation decreased when broilers were moved from their individual cages and placed in a novel pen. Work by Gentle and colleagues (1999; 2001) suggests novelty or environmental enrichment could distract the bird from focusing on pain and therefore reduce clinical lameness. Another area of broiler pain-related behavior research is the use analgesics in feed.

1.5.4 Analgesic Choice and Lameness Research

Lame broiler chickens may experience hypoalgesia, where lame broilers have decreased pain perception due to increased stress. Studies investigating stress-induced algesia in mammals have shown that exposure to stressful stimuli can suppress pain (Butler and Finn, 2009). Carprofen, a non-sterioidal anti-inflammatory drug (NSAID), has been used in broiler pain studies. These studies hypothesize that if lame broilers are given the choice of normal feed or feed containing carprofen, and they select the caprofen feed, they are in pain and self-medicating. Danbury and colleagues (2000) found that when lame and sound broilers were given the choice of normal or carprofen-laced feed, lame birds selected more carprofen-laced feed than sound broilers. However, when this study was replicated, no differences were found (Siegel et al., 2011). Hothersall and collegues (2016) found that lame broilers injected with carprofen or

meloxicam had longer LTL times than lame broilers given a saline injection. Further research on methods or technology to objectively evaluate broiler lameness is necessary.

1.6 Infrared Thermography (IRT) Utility in Biological Research

Infrared thermography (IRT) is a noninvasive, non-contact method of creating a pictorial representation of surface heat (infrared radiation) from an object (review: Eddy et al., 2001). IRT has been utilized on over 30 species of birds in avian science for over 50 years (review: McCafferty, 2013). Animal and environmental limitations must be considered when utilizing IRT in biological research. IRT surface temperatures may be affected by blood vessel location, physical obstructions to thermal signals (especially feathers in chickens) malaise, biorhythm, age, stress and environment (Loughmiller et al., 2000; review: McCafferty, 2013). External factors that may affect IRT temperatures include ambient air temperature, humidity, and focal distance (review: McCafferty, 2013). Controlling these factors are essential to the collection of viable IRT data (Rekant et al., 2016).

A metabolism study in cattle utilized IRT to correlate heat production with body surface temperature (Gomes, 2016). Gomes and colleagues (2016) found correlations between heat production and maximum average skin temperatures (65%) and average ocular surface temperatures (69%) in young bulls. Previous studies have utilized IRT as a tool to detect injury and disease related to lameness in horses (review: Eddy et al., 2001; Douthit et al., 2014) pigs (Amezcua et al., 2014) and dairy cows (Nikkhah et al., 2005; Oikonomou et al., 2014; Stokes et al., 2012). Hypotheses tested in IRT research are centered around detecting peripheral blood flow. IRT measures of increasing surface temperature on a body region may be an indicator of vasodilation for immune cells to respond to local inflammation (Oikonomou et al., 2014, Stokes

et al., 2012). Relatedly, the decrease in surface temperature on a body region may be an indicator of vasoconstriction and decreased blood flow (Jacob et al., 2016).

IRT can be used to investigate broiler stress, (Edgar et al., 2013; Herborn et al., 2015; Moe et al., 2017) heat loss (Yahav and Giloh, 2012) and early infection (Wilcox, 2009) by focusing on specific anatomical areas. Thermoregulatory control is mediated by the hypothalamus (Esmay, 1978). High correlations ($R^2 = 0.83$) between body temperature and whole facial temperature in broilers have been reported (Giloh et al., 2012; Yahav et al., 2012). Wilcox and colleagues (2009) found an 83% correlation between thermal images temperatures of food pads and broilers with bumblefoot 14 days later. Bumblefoot (footpad dermatitis) is clinically characterized as the swelling of the plantar metatarsal or digital pads of the foot in broilers (Wilcox et al., 2009).

Heat production can be an indicator of broiler stress. Broilers lack sweat glands and have a relatively high core body temperature compared to other agriculture animals (107.5°F or 41.9°C). Thus, heat stress can quickly impair broiler welfare. With little intramuscular fat and feathers to provide insulation, broilers depend on panting as their only mechanism to dissipate heat in a hot environment. In a 2012 heat stress study, Yahav and Giloh (2012) compared surface temperatures of broiler comb, wattle, face, leg (shank), toes, neck and whole body regions. Facial surface temperature exhibited the highest positive correlation ($R^2 = 0.83$) with core body temperature.

IRT has been utilized to measure the stress response in broilers. Stress-induced hyperthermia occurs within 10 to 15 minutes of a perceived stressor, in which the core body temperature drops 0.5 to 1.5°C. Edgar and colleagues (2013) focused on the comb and eyes to

investigate stress-induced hyperthermia. The bird was caught and placed in a holding box where thermal images were taken in 1-minute intervals. The combs of the broilers dropped 2°C in response to the handling treatment and eye temperature dropped initially, then overshot to temperatures higher than baseline. In another stress-induced hypothermia study, Moe et al. (2017) evaluated handling stress for 10 min on broiler head and footpad regions on d 30, 36 and 37 of age. The authors found that manual handling restraint in a vertical position lead to increased head surface temperatures decreased footpad surface temperatures. Additionally, footpad temperatures were higher on d 36 compared to d 30. The authors report stress may influence body surface temperatures and must be considered when employing IRT to evaluate broiler health status. Thus, IRT technology is a promising application as an indicator tool of broiler health, stress and welfare.

1.7 SUMMARY

Many environmental parameters influence the welfare of broilers. To reliably measure broiler stress, the method employed must not itself evoke stress. To reliably measure lameness, methods employed must be objective and the factors affecting avian gait must be considered. Once scientific data validates objective, repeatable noninvasive measures of stress and lameness, on-farm application would significantly improve technicians' ability to evaluate and improve broiler health and welfare.

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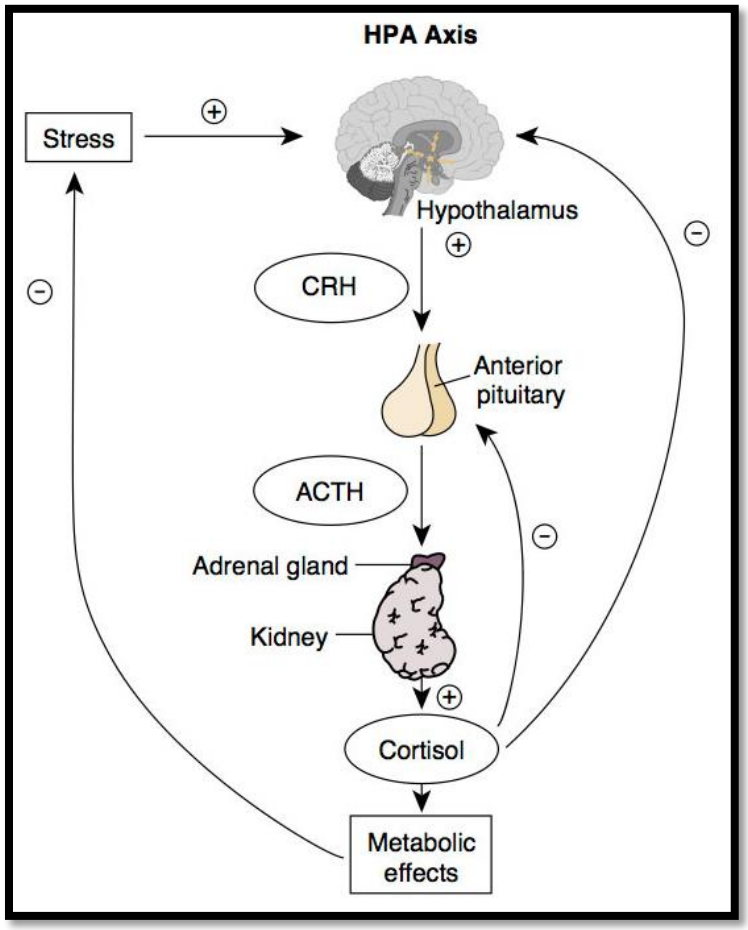

Figure 1.1. Mechanism of the hypothalamo-pituitary-adrenal axis: + indicates a stimulation effect and – indicates an inhibitory effect. In the chicken, corticosterone is produced instead of cortisol. Adapted from: https://embryology.med.unsw.edu.au/embryology/images/4/4f/HPA_axis.jpg

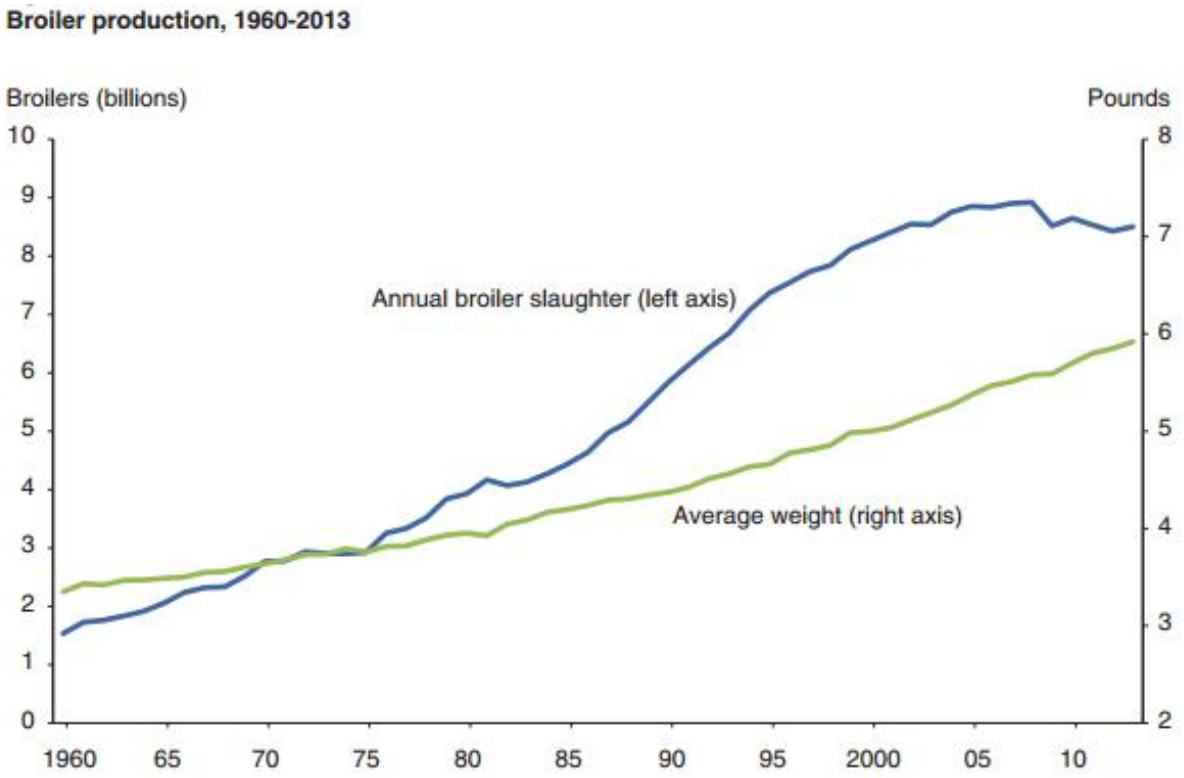

Figure 1.2. Broiler production annual broiler slaughter and average live weight at slaughter from 1960 to 2013. Adapted from: USDA, National Agricultural Statistics Service, chickens slaughtered under federal inspection.

CHAPTER 2 Effect of corticosterone on traditional and noninvasive indices of stress

2.1 ABSTRACT

There are distinct advantages of noninvasive measures of stress when compared to the restraint models used to measure stress. The present proof-of-concept study was designed to compare heterophil:lymphocyte (H:L) ratios, serum corticosterone (CORT) and cecal CORT contents with feather CORT concentrations for broiler chicks receiving CORT in the drinking water and in controls. At the onset of the photoperiod on d 28, male commercial broilers (N=140) were provided either normal tap water (C) or water containing 20 mg/L of CORT (CS) for up to 72 h. CS birds were provided CORT water using a carboy system. Body weight (BW), blood, feathers and cecal samples were collected from 10 randomly chosen broilers from each treatment group at 0, 6, 12, 24, 30, 48, and 72 h. Blood samples were drawn within 60 s of capture. Birds were euthanized, weighed and feather and cecal samples collected. Data were analyzed with a JMP Pro ANOVA. Mean comparisons were analyzed on significant treatment effects using *post hoc* test slice t-tests to compare C and CS measures within each sampling time point. Pearson's pairwise correlations for all data were analyzed. Differences were considered significant at $P \leq 0.05$. Time, treatment and the time by treatment interaction had a significant effect on BW, H:L ratio, serum, cecal and feather CORT concentrations. CS broilers weighed less than C at 30 h and and 72 h. CS broiler H:L ratios were higher than C starting at 12 h through 72 h. Serum CORT in the CS group was elevated from 6 h through 72 h; while CS cecal CORT concentrations were elevated starting at 24 h through 72 h compared to C. Body feather CORT increased with time for both CS and C group. CS bird primary feather CORT concentrations were higher than C from 6 h through 72 h. The results indicate that noninvasive measures of CORT have the potential for estimating stress in chickens. The techniques required for sample collection and storage may provide a useful management tool to evaluate environmental stressors and increase productivity in commercial broiler production systems.

2.2 INTRODUCTION

Consumer interest in the origin of their food is growing (Smith and Bower, 2012). Food service companies have responded to consumer pressure by requiring strict production standards for poultry and livestock. Unfortunately, many of the public concerns lack a scientific foundation (Grandin, 2014). To collect the data necessary for evaluation of conditions that are best for the animal, multiple methods of assessment are required. These assessments include performance, behavior and physiological responses. The physiological measures commonly employed to evaluate environmental effects on animals include restraint and invasive techniques to take blood and tissue samples.

Environmental stressors elicit a cascade of biochemical responses, resulting in the release of corticosterone (CORT; Blas, 2015). Assays measuring CORT concentrations in serum or plasma provide indications of the individual bird's stress status at the time the blood was drawn. However, blood source measures of CORT are only a measure of acute stress (Bortolotti et al., 2008; Blas, 2015) which can be confounded by fear of humans (Hemsworth et al., 1994) and the stress of handling restraint (Kannan and Mench, 1996; Bortolotti et al., 2008; Alm, et al., 2014). Circulating concentrations of CORT follow a circadian rhythm (de Jong et al., 2001) and time of sampling must be considered. Chicken serum or plasma CORT concentrations are often measured in combination with heterophil to lymphocyte (H:L) ratios, as the increase in H:L ratio is an accepted valid indicator of the response of the immune system to chronic stress (Gross and Siegel, 1983; Shini et al., 2008). Finding objective, repeatable noninvasive measures of stress would greatly improve our ability to evaluate various animal stressors (Post et al., 2003a; Bortolotti et al., 2008, Fairhurst et al., 2013).

Chronic stress can have detrimental impacts in the broiler industry (review: Virden and Kidd, 2009). Noninvasive measures of avian CORT in feces and feathers have been evaluated, but have not been compared within the same bird. Growing feather cells are highly vascularized and CORT is deposited in the keratin structure (Bortolotti et al., 2008). Given that feathers are not living tissue, they can be painlessly clipped for sample collection (review: Bortolotti, 2010). However, it is unclear how quickly CORT is deposited in the feather and how closely this follows circulating concentrations of CORT. CORT, *per se* or following metabolism, can be deposited in the excreta of chickens (Rettenbacher et al., 2004; 2006; Alm et al., 2014). The present study was designed to determine if noninvasive measures are comparable to traditional measures of stress. Reports of feather CORT concentrations in wild and domestic birds indicate this method may provide a noninvasive approach with potential for meeting the requirements for routine use (Bortolotti et al., 2008, Faihurst et al., 2011; Carbajal et al., 2014).

Administration of CORT in the drinking water provides an experimental model to examine whether noninvasive measures of CORT and hence, stress status can be monitored by noninvasive measures (Post et al., 2003a; Shini et al., 2008). We compared different sources of CORT sampled from the same individual bird. Therefore, our objective was to investigate the validity of noninvasive measures of broiler stress with commonly used methods.

2.3MATERIALS AND METHODS

2.3.1 Birds, Housing, and Feed

Male broiler chicks (N = 140) from a commercial hatchery (Cobb, Fayetteville, AR) were randomly divided into two environmental chambers (3.7 m long x 2.5 m wide x 2.5 m high) in the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research

Farm (IACUC #140005). The photoperiod was set to 23L:1D for d 0-4; 20L:4D for d 5-14 and 18L:6D for d 15 through the end of trial. Chamber temperature was set to 32.2°C for d 0-3, 29.4°C for d 4-7, 25.6°C for d 8-14 and 23.9°C d 15 through the end of trial. The caretaker walked through each room every day to acclimate birds to the routine presence of a human.

Birds were raised on litter flooring with *ad libitum* access to feed and water. The diet was a starter diet formulated to meet minimum industry standards (NRC, 1994). Water was provided via nipple drinkers. On d 21, a ladder was placed in the right corner of both rooms to acclimate birds to the novel object. The ladder served as the location of the elevated CORT water carboy system to administer CORT in the treatment chamber (CS). The ladder-carboy system was constructed so the gravitational force would provide the same water pressure to the nipple drinkers as that from the tap water provided in the control (C) chamber. Synthetic CORT (C2505, Sigma Aldrich, St. Louis, MO) was dissolved in ethanol and added to the water. At the beginning of the photoperiod on d 28, treatment began in the CS chamber at a constant concentration of 20 mg/L (Post et al., 2003a;, Shini et al., 2008) until the end of trial.

2.3.2 Sampling and Analysis

Blood, cecal and feather samples were collected from 10 randomly selected broilers from both chambers at seven time points for a total of 140 sampled birds. Baseline (0 h) samples were collected on d 27, 24 h prior to the start of CS treatment. Considering 0800 on d 28 as the CS treatment start time, samples were collected at six additional time points. Samples were collected at 6 h, 12 h, 24 h, 30 h, 48 h, and 72 h throughout CS treatment administration. Birds were removed from their chamber and a blood sample was drawn from the brachial vein within 60 s of capture with a 21 g X 1 ½ inch needle attached to a 3 mL syringe. Each blood sample collected

was immediately divided into two sub-samples; the first into a sample tube for serum separation and the second into sample tube coated with K_2 EDTA for heterophil and lymphocyte determination. Birds then were humanely euthanized via cervical dislocation, body weight was recorded, and cecal and feather samples were collected. Cecal samples were collected by expelling the contents of a ceca during necropsy into a conical tube and stored at -20°C. Feather samples were collected from the intrascapular area (body feather) and third primary on the left wing (primary feather), and stored at -20°C in a freezer storage bag.

Heterophil to lymphocyte (H:L) ratio was determined using a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer within 4 h of blood draw. Serum separation tubes were centrifuged at 1500 x g for 10 min to separate blood serum from whole blood. Serum was frozen at -20°C until assay. Cecal CORT was extracted using an ethanol-based method reported by Alm and colleagues (2014). Feather CORT was extracted using a methanol-based method reported by (Bortolotti et al., 2008). Serum, cecal and feather CORT concentrations were measured by a DetectX® Corticosterone EIA kit (Arbor Assays, Ann Arbor, MI).

2.3.3 Statistical Analysis

Data were analyzed using JMP Pro (version 13, SAS Institute Inc, Cary, NC), with the individual bird as the experimental unit. The statistical model fit treatment, time, and the interaction of treatment and time as fixed effects. BW fit a normal distribution, while H:L ratio and all CORT data fit a lognormal distribution and were analyzed within their respective distributions. Mean comparisons were analyzed on significant treatment effects using *post hoc* test slice t-tests to compare C and CS measures within each sampling time point. Results are

reported as LSMeans and back-transformed LSMeans for abnormally distributed data. Pearson's pairwise correlations were evaluated using the JMP Pro Multivariate platform. Differences were considered significant at $P \leq 0.05$.

2.4 RESULTS AND DISCUSSION

These results compared common and noninvasive measures of stress from the same individual broiler chicken. Previous studies have compared plasma CORT concentrations with H:L ratio (El-Lethey et al., 2003; Post et al., 2003a; Shini et al., 2008), fecal CORT concentrations, (Dehnhard et al., 2003), cecal CORT concentrations, (Rettenbacher et al., 2006), and feather CORT concentrations (Lattin et al., 2011; Fairhurst et al., 2013) in various avian species. However, no studies have evaluated the relationships of these measures in domestic chickens. The present study was the first to compare H:L ratios and CORT concentrations in serum, cecal contents, body feathers and primary feathers within the same individuals.

There were main effects of time ($P = 0.0146$), but not treatment ($P = 0.70$) for BW (**Table 2.1**). T-test comparisons revealed CS birds had reduced body weight compared to C at 30 h ($P < 0.0001$) and 72 h ($P < 0.0001$). Reduced growth is a common response to high CORT concentrations (Thaxton and Puvadolpirod, 2000; El-Lethey et al., 2003; Post et al., 2003a; Virden et al., 2007; Shini et al., 2009).

There were the main effects of time ($P < 0.0001$), treatment ($P = 0.05$) and treatment by time interaction ($P < 0.0001$) for H:L ratios. C birds had higher ($P = 0.05$) H:L ratios than CS prior to CORT treatment administration at 0 h (**Table 2.1**). Once CS birds began receiving CORT, H:L ratios increased with time and was higher at 12 h ($P = 0.02$), 24 h ($P < 0.0001$), 30 h ($P = 0.003$), 48 h ($P < 0.0001$), and 72 h ($P < 0.0001$) compared to controls (**Table 2.1**). CORT

and H:L ratio measures have a strong relationship indicating an increased stress response (Gross and Siegel, 1983; Post et al., 2003b; Shini et al., 2008). Serum or plasma CORT concentrations are often measured in combination with heterophil to lymphocyte (H:L) ratios. The increases in H:L ratio in the present study agrees with previous reports of the effect of increased CORT concentrations and chronic stress on the immune system of broilers (Gross and Siegel, 1983; Shini et al., 2008, Shini et al., 2009).

Turning to serum CORT, there were main effects of time ($P < 0.0001$), treatment ($P = 0.05$) and treatment by time interaction ($P < 0.0001$) (**Figure 2.1**). The CS group had higher CORT concentrations than controls at all six time points ($P < 0.0001$) measured after the onset of CORT treatment compared to controls. There was a decrease ($P < 0.0001$) in serum concentrations of CORT between 48 and 72 h in CS chickens. The results in the present study determining CORT in serum showed a similar pattern to those in plasma CORT reported by Post and colleagues (2003a). In the present study, there was a rapid increase after provision of CORT in the drinking water (**Figure 2.1**). The CORT concentrations determined in the present study were, however, higher than those reported in Post and colleagues (2003a). Confounding environmental stressors can mask or match the stress response to a CORT treatment. El-Lethey and colleagues (2003) showed that the stress of foraging material deprivation matched that of feeding CORT when measuring decreased immune response in laying hens.

There were significant effects for cecal CORT concentrations with main effects of time ($P < 0.0001$), CS treatment ($P = 0.05$) and treatment by time interaction ($P = 0.004$). Cecal CORT concentrations in the CS group were higher at 24 h ($P = 0.04$), 30 h ($P < 0.0001$, 48 h ($P = 0.04$) and 72 h ($P = 0.001$) compared to controls (**Figure 2.2**). Cecal CORT was only higher in

CS broilers beginning at 24 h compared to controls and could have been due to the observed increase in drinking behavior of CS birds. The cecum is an important organ involved in water absorption (Svihus, 2014), CORT concentrations are rarely measured in cecal contents as it is invasive to collect samples, whereas fecal CORT samples can be collected noninvasively. In a an 8 h time-course study comparing CORT concentrations in blood, feces and tissues of six layer chickens, Rettenbacher et al. (2006) found more CORT (ng/g) in feces than in cecal contents. CORT concentrations in the present study were similar to those in the aforementioned study. Typically, fecal samples are pooled for CORT assay, whereas in the present study we examined individual cecal samples. Pooling samples eliminates individual variation and fluctuations of cecal CORT in the present study could be due the inherent high variation in individual CORT concentrations.

The concentrations of CORT in feathers were normalized by feather length to reflect cumulative feather growth over time (Bortolotti et al., 2008) and are presented as CORT pg/mm of feather length. There were main effects of time ($P < 0.0001$), treatment ($P = 0.05$) and treatment by time interaction ($P < 0.0001$) for body feather CORT concentrations. There were higher concentrations of CORT in body feathers at 6 h, ($P < 0.0001$) 12 h, ($P < 0.0001$) and 24 h ($P < 0.0001$) time points in CS compared to C birds. Later time points showed no differences between CS and C body feather CORT concentrations, due to an increase in feather concentrations of CORT in the C group (**Figure 2.3a**). Similarly, there were main effects of time ($P = 0.01$), treatment ($P < 0.0001$) and treatment by time interaction ($P < 0.0001$) for CORT concentrations in primary feathers. There was a stronger linear increase in CORT concentrations in primary feathers in CS ($R^2 = 0.53$; $P < 0.0001$) compared to C birds ($R^2 = 0.45$; $P < 0.0001$). As with serum CORT concentrations, C bird primary feather CORT concentrations were higher

(P = 0.01) than CS prior to CORT treatment. After, CORT administration, CS primary feather CORT concentrations were higher (P < 0.0001) than C broilers at all 6 time points (**Figure 2.3b**).

There was a negative correlation between BW with H:L ratio (r = -0.25; P = 0.003), serum CORT (r = -0.25; P = 0.003), and primary feather CORT concentrations (r = -0.26; P = 0.004). There were positive correlations (P ≤ 0.05) between serum CORT, cecal CORT, body feather CORT and primary feather CORT concentrations (**Table 2.2**). There was a positive correlation between body feather CORT and primary feather CORT concentrations (r = 0.62; P < 0.0001). **Table 2.3** shows the CV (%) of CORT sources. Serum CORT had the lowest CV compared to cecal CORT, body feather CORT and primary feather CORT concentrations.

Studies investigating the utility of CORT from feathers in wildlife avian species most commonly utilize a primary feather (Bortolotti et al., 2008). The concentrations of CORT reported for body feathers from the intrascapular area in the present study for control birds were greater than concentrations reported in Carbajal et al. (2014). This could be due to the differences in birds or the environment they were raised in as their samples were randomly collected in a commercial setting versus the experimental setting of the present study. Concentrations of CORT from both feather types in CS and control birds increased with time (**Figures 2.3a; 2.3b**). This trend was unlike the response in C serum concentrations of CORT, which sustained low concentrations of CORT (~3 ng/mL) after CORT treatment onset. Control group body feathers showed an unexpected increase in CORT concentrations with time. This continued increase may be due to the combined effect of human presence in combination wtih social hierarchy disruption by repeated removal of conspecifics (Gross and Colmano, 1971). While body feathers from CS birds had high, constant concentrations of CORT, control CORT

was naturally produced by the broiler and concentrations accumulated in the body feathers. This suggests that body feathers may serve as a more sensitive measure for environmental stressors.

Primary feathers from CS birds showed a linear increase in CORT concentrations over time, with much higher concentrations than the controls. Investigating changes in CORT patterns over time is important to compare across and within all CORT samples (Bortolotti et al., 2008). The positive linear relationship between serum CORT with primary feather CORT concentrations ($r = 0.39$; $P < 0.0001$) was stronger than with body feather CORT ($r = 0.22$; $P = 0.01$) concentrations.

Feather CORT may provide a historical record of an individual's stress level (Bortolotti et al., 2008). The acute stress response can result in either adaptive or non-adaptive long-term response, with long term, non-adaptive responses leading to increased mortality and decreased reproduction. Adaptive stress responses lead to increased overall fitness (Wingfield et al., 1997). Measures of circulating CORT concentrations determine stress levels at the time of sample collection whereas feather CORT measures determine an average of CORT over the growth of the feather (Bortolotti et al., 2008). Feather CORT measures cannot be assumed to be correlated with circulating CORT (Lattin et al., 2011; Fairhurst et al., 2013). The linear relationship of body feather CORT and primary feather CORT was moderately strong ($r = 0.62$, $P < 0.0001$). Differences in feather growth and keratin deposition may have contribute to the differences in CORT concentrations in body and primary feathers from the same individual chicken. Additionally, two individuals with the same feather CORT concentrations may have vastly different stressor experiences (several short, high stress events within a primarily low stress environment vs. constant, moderately stressful environment).

Feather CORT measures are the only retrospective method discovered to measure avian stress to date (Bortolotti et al., 2008). Primary feather CORT measures showed a linear increase in CORT concentrations, which was most reflective of the response to providing CORT in the drinking water. Thus, feather CORT shows to be a promising tool for both research and on-farm applications to measure broiler stress.

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Table 2.1. Effect of CORT on growth and heterophil to lymphocyte ratio (H:L) of broiler chickens. Data shown are body weight (g) ± SEM and H:L ± SEM of 4 wk old male broilers receiving normal tap water (C) or a CORT in the drinking water treatment[1] (CS) for baseline (0 h) and six treatment time points (N=10 birds/treatment/time point).

Sampling	BW (g)[2]			H:L ratio		
Hour	C	CS	P value	C	CS	P value
0	1604 ± 24.5	1579 ± 36.7	0.70	0.94 ± 0.57[a]	0.44 ± 0.20[b]	0.05
6	1632 ± 33.8	1653 ± 61.6	0.75	0.39 ± 0.12	0.41 ± 0.18	0.88
12	1745 ± 43.3	1714 ± 40.9	0.64	0.23 ± 0.03	0.55 ± 0.33	0.02
24	1695 ± 64.4	1608 ± 40.4	0.20	0.30 ± 0.13[a]	1.48 ± 0.61[b]	0.0001
30	1811 ± 54.2[a]	1550 ± 42.0[b]	0.0001	0.36 ± 0.17	1.17 ± 0.34	0.003
48	1635 ± 56.7	1512 ± 44.3	0.07	0.25 ± 0.07[b]	2.53 ± 1.68[a]	0.0001
72	1832 ± 47.9[a]	1557 ± 53.5[b]	0.002	0.44 ± 0.11[b]	4.96 ± 1.43[a]	0.0001

[1]CS CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study.
[2]The ANOVA main effect of treatment was not significant (P = 0.70). The table shows t-test comparisons of CS and C birds within each time point.
[ab] Means within the same row followed by uncommon superscripts differ at $P \leq 0.05$.

Table 2.2. Pairwise correlations between body weight (BW), heterphil to lymphocyte ratio (H:L), serum corticosterone (CORT) concentrations (ng/mL), cecal CORT (ng/g dry wt), body feather CORT (pg/mm feather length) and primary feather CORT (pg/mm feather length) of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment[1] (CS) measured at baseline (0 h) and six treatment time points (N=10 birds/treatment/time point).

	Correlations (r)					
	BW	H:L	Serum CORT	Cecal CORT	Body feather CORT	Primary feather CORT
BW	1.00	-0.25**	-0.25**	-0.18	-0.14	-0.25**
H:L		1.00	0.21*	0.24*	0.26**	0.39**
Serum CORT			1.00	0.24*	0.22**	0.36**
Cecal CORT				1.00	0.40**	0.30**
Body feather CORT					1.00	0.62**
Primary feather CORT						1.00

[1]CS CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study.

Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 2.3. CV (%) of serum corticosterone (CORT) concentrations (ng/mL), cecal CORT (ng/g dry wt), body feather CORT (pg/mm feather length) and primary feather CORT (pg/mm feather length) of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment[1] (CS) measured at baseline (0 h) and six treatment time points (N=10 birds/treatment/time point).

	Time (h) after treatment onset							CV	
CORT source	0	6	12	24	30	48	72	Mean	SD
Serum									
C	27.17	8.20	32.98	35.10	20.24	49.50	25.06	28.32	12.90
CS	27.45	37.90	39.67	48.21	41.53	44.12	60.65	42.79	10.17
								35.56	11.54
Ceca									
C	-	-	34.01	48.31	47.81	27.06	128.16	57.07	40.77
CS	-	-	60.89	78.02	91.02	89.54	107.14	85.32	17.15
								71.20	28.96
Body feather									
C	75.52	231.08	149.76	186.84	16.10	39.18	14.99	101.92	87.31
CS	92.57	113.59	55.83	116.06	62.67	52.38	32.24	75.05	32.51
								88.49	59.91
Primary feather									
C	137.84	50.19	68.19	226.32	27.30	70.91	21.63	86.05	72.79
CS	42.26	100.70	37.93	59.52	51.61	69.42	57.94	59.91	20.90
								72.98	46.85

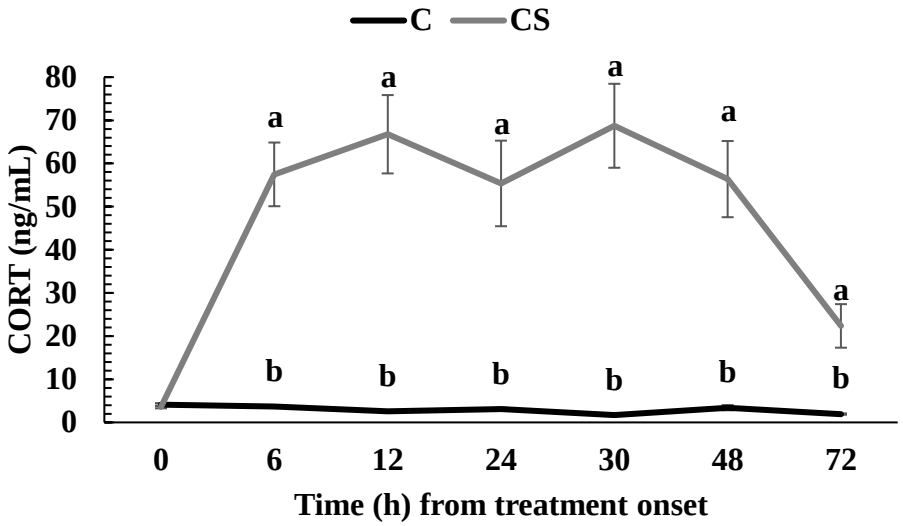


Figure 2.1. Effect of CORT in the drinking water on serum concentrations of CORT. Data shown are average serum corticosterone (CORT) concentrations (ng/mL) ± SEM of 4 wk old male broilers administered normal tap water (C) or CORT water treatment (CS;) measured at baseline (0 h) and six treatment time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

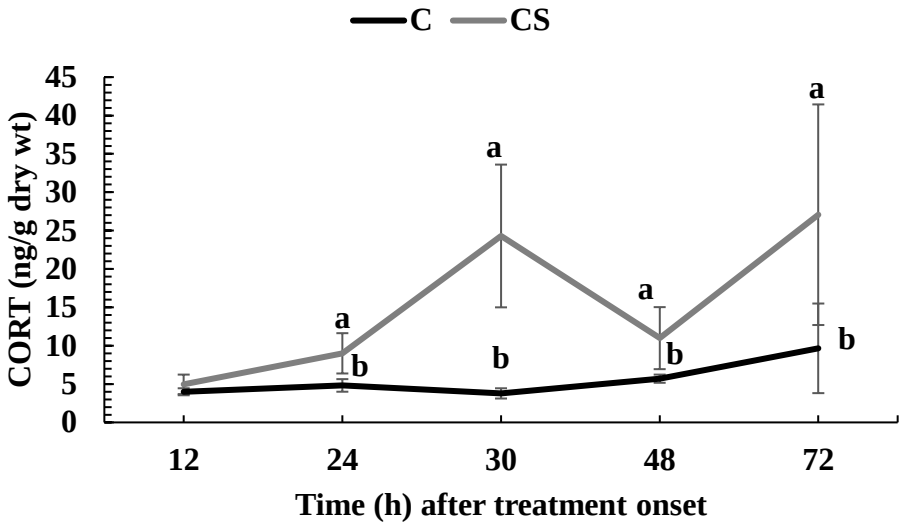
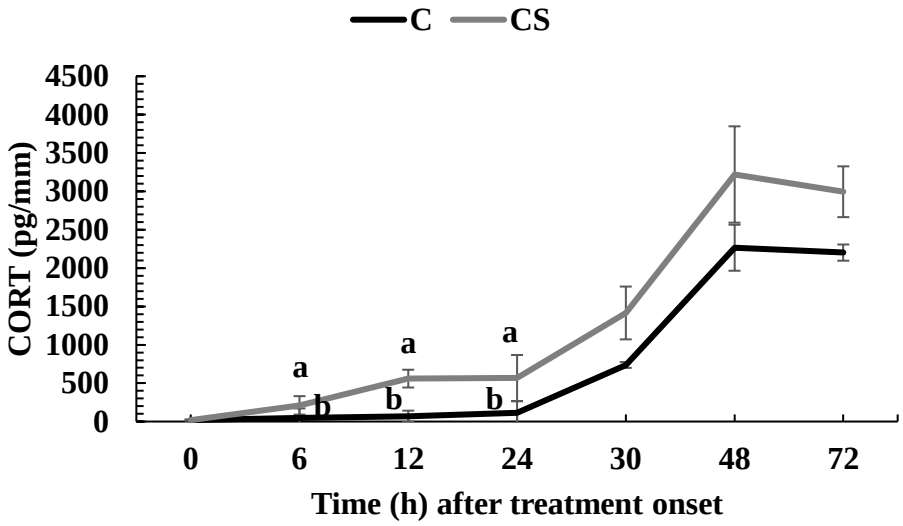


Figure 2.2. Effect of CORT in the drinking water on cecal concentrations of CORT. Data shown are average cecal corticosterone (CORT) concentrations (ng/g dry weight) ± SEM of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment (CS) measured at baseline (0 h) and six additional time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

A



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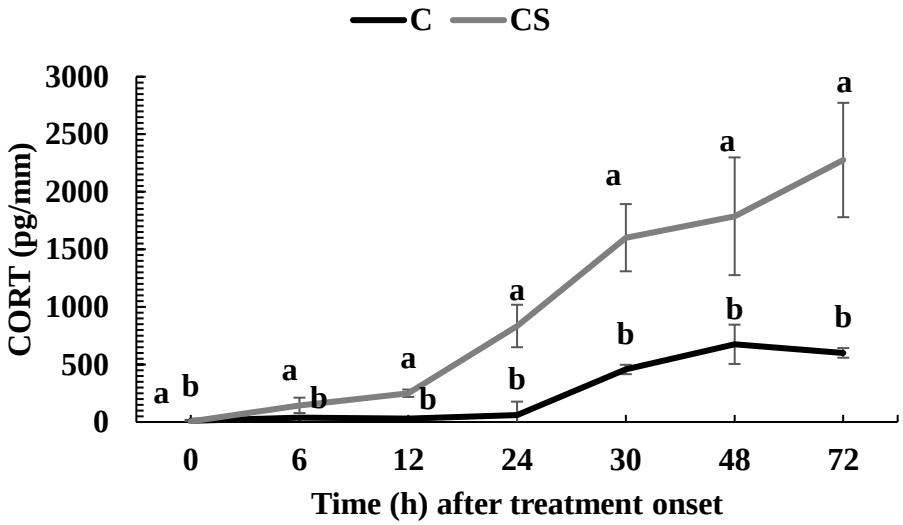

Figure 2.3. Effect of CORT in the drinking water on feather concentrations of CORT. Data shown are average (A) body feather corticosterone (CORT, pg/mm) (B) and primary feather CORT (pg/mm) concentrations ± SEM of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment (CS) measured at baseline (0 h) and six additional time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

CHAPTER 3 Effect of corticosterone on traditional and noninvasive indices of stress

3.1 ABSTRACT

There are distinct advantages of noninvasive measures of stress when compared to the restraint models to measure stress. Infrared thermography (IRT) is a noninvasive measure of infrared radiation from an object. IRT analysis in biological research can evaluate clinical health status. The present proof-of-concept study was designed to compare heterophil to lymphocyte (H:L) ratios, serum corticosterone (CORT) and excreta CORT concentrations with feather CORT concentrations and eye and beak surface temperatures for broiler chicks receiving CORT in the drinking water and control non-supplemented water. At the onset of the photoperiod on d 28, male commercial broilers (N=140) were provided either normal tap water (C) or water containing 20 mg/L of CORT (CS) for 72 h. Birds were provided water using an elevated carboy system. Body weight (BW), blood, feathers and excreta samples were collected from 10 randomly chosen broilers from each treatment group at 0, 6, 12, 24, 30, 48, and 72 h. Water consumption was measured. Data were analyzed with JMP Pro ANOVA. Mean comparisons were analyzed on significant treatment effects using *post hoc* test slice t-tests to compare C and CS measures within each sampling time point. Pearson's pairwise correlations for all data were analyzed. Differences were considered significant at $P \leq 0.05$. Time, treatment and the time by treatment interaction had a significant effect on H:L ratio, as well as serum, excreta and feather CORT concentrations. Time, treatment and the time by treatment interaction had a significant effect on H:L ratio and CORT concentrations from serum, excreta and feathers. BW was lower in for CS birds at 48 and 72 h compared to C. The H:L ratios for CS birds were higher than C at 6 h through 72 h. Compared to C birds, serum, excreta and body feather CORT concentrations in the CS group were elevated from 6 h through 72 h. Primary feather CORT concentrations in CS birds were elevated at the same time points, with the exception of 30 h. Primary feather CORT increased with time for both CS and C birds. The results indicate that noninvasive measures of

CORT have potential for estimating stress in chickens. The techniques required for sample collection and storage may provide a useful management tool to evaluate environmental stressors and increase productivity in commercial broiler production systems.

3.2 INTRODUCTION

Consumer interest in the origin of their food is growing (Smith and Bower, 2012). Food service companies have responded to consumer pressure by requiring stricter production standards for poultry and livestock. Unfortunately, many of the public concerns are not founded scientific contributions (Grandin, 2014). To collect the data necessary for evaluation of conditions that are best for the animal, multiple methods of assessment are required. These assessments include performance, behavior and physiological responses. The physiological factor commonly employed to evaluate environmental effects on animals include animal restraint and invasive techniques to take blood and tissue samples.

Environmental stressors elicit a cascade of biochemical responses, resulting in the release of corticosterone (CORT; Blas, 2015). Assays measuring CORT concentrations in broiler serum or plasma provide indications of the individual bird's stress status at the time the blood was drawn. However, blood source measures of CORT are only a measure of acute stress (Bortolotti et al., 2008; Blas, 2015) which can be confounded by fear of humans (Hemsworth et al., 1994) and the stress of handling restraint (Kannan and Mench, 1996; Bortolotti et al., 2008; Alm, et al., 2014). Circulating concentrations of CORT follow a circadian rhythm (de Jong et al., 2001) and time of sampling must be considered. Serum or plasma CORT concentrations are often measured in combination with heterophil to lymphocyte (H:L) ratios as the increase in H:L ratio is an accepted indicator of the effects of chronic stress on the immune system (chicken: Gross and Siegel, 1983; Shini et al., 2008). Finding objective, repeatable noninvasive measures of stress would greatly improve our ability to evaluate animals under potentially stressful conditions (Post et al., 2003a; Bortolotti et al., 2008, Fairhurst et al., 2013).

Chronic stress can have detrimental impacts in the broiler industry (review: Virden and Kidd, 2009). Noninvasive measures of avian CORT in feces and feathers have been evaluated, but have not been compared within the same bird. Growing feather cells are highly vascularized and CORT is deposited in the keratin structure (Bortolotti et al., 2008). Given that feathers are not a living tissue, they can be painlessly clipped for sample collection (review: Bortolotti, 2010). However, it is unclear how quickly CORT is deposited in the feather and how closely this follows circulating concentrations of CORT. CORT, *per se* or following metabolism, can be deposited in the excreta of chickens and other animals (Rettenbacher et al., 2004; 2006; Alm et al., 2014). The present study set out to determine if these noninvasive samples are comparable to traditional measures of stress. Reports of CORT concentrations in feathers for wild and domestic birds indicate this method may provide a noninvasive approach with potential for meeting the requirements for routine use (Bortolotti et al., 2008, Faihurst et al., 2011; Carbajal et al., 2014).

Infrared thermography (IRT) is a noninvasive, non-contact method of determining surface heat (infrared radiation) from an object (review: Eddy et al., 2001). Previous studies have used IRT technology to evaluate metabolic heat production (Ferreira et al., 2011; Yahav and Giloh 2012), footpad dermatitis (Wilcox et al., 2009; Jacob et al., 2016) and handling stress (Edgar et al., 2013; Moe et al., 2017) in chickens. IRT surface temperatures of the eye and beak were evaluated as a noninvasive measure of stress in this chapter.

CORT administration in the drinking water provided an experimental model to examine physiological stress (Post et al., 2003a; Shini et al., 2008). Therefore, our objective was to compare the results of noninvasive versus commonly used methods of assessing broiler stress over a 72 h period.

3.3 MATERIALS AND METHODS

3.3.1 Birds, Housing, and Feed

Male broiler chicks (N = 140) from a commercial hatchery (Cobb, Fayetteville, AR) were randomly divided into two environmental chambers (3.7 m long x 2.5 m wide x 2.5 m high) in the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm (IACUC #16014). The photoperiod was set to 23L:1D for d 0-4; 20L:4D for d 5-14 and 18L:6D for d 15 through the end of trial. Chamber temperature was set to 32.2°C for d 0-3, 29.4°C for d 4-7, 25.6°C for d 8-14 and 23.9°C d 15 through the end of trial. The caretaker walked through each chamber every day to acclimate birds to the repeated presence of a human.

Birds were raised on litter floor with *ad libitum* access to feed and water. The starter diet was formulated to meet minimum industry standards (NRC, 1994). Water was provided via nipple drinkers. On d 21, a ladder was placed in the right corner of both chambers to acclimate birds to the novel object. The ladder was used to elevate the water carboy system to administer CORT in the treatment room (CS) and normal tap water for birds in the control chamber (C). Water consumption was measured for comparison in both rooms. The ladder-carboy system was constructed so the gravitational force would provide the same as the water pressure to the nipple drinkers as that from the tap water. Synthetic CORT (C2505, Sigma Aldrich, St. Louis, MO) was dissolved in ethanol and added to the water. At the beginning of the photoperiod on d 28, treatment began in the CS chamber at a constant concentration of 20 mg/L (Post et al., 2003a;, Shini et al., 2008) until the end of trial.

3.3.2 Sampling and Analysis

Blood, cecal and feather samples were collected from 10 randomly selected broilers from each chamber at seven time points for a total of 140 sampled birds. Baseline (0 h) samples were collected on d 27, 24 h prior to the start of CS treatment. Considering 0800 on d 28 as the CS treatment start time, samples were collected at six additional time points. Samples were collected at 6 h, 12 h, 24 h, 30 h, 48 h, and 72 h throughout CS treatment administration. Birds were removed from their chamber, a thermal image was taken of the head, and a blood sample was drawn from the brachial vein within 60 s of capture with a 21 g X 1 ½ inch needle attached to a 3 mL syringe. Thermal images were captured with a thermal imaging camera (Fluke Ti400, Fluke®, Everett, WA). The camera background temperature was 22°C, emissivity was 0.95 and with 100% transmission. The focal distance of the camera from the bird was 3.3 to 3.6 m. Images were uploaded to a computer and analyzed using SmartView® (v 2.8) software. Each individual pixel (76, 800 total) within the thermal image had an associated temperature recorded. Within each head image, shapes were made to isolate pixels of the eye pupil and the beak region anterior to the nostril (**Figure 3.1**). The averages of the pixel temperatures within each shape of the bird eye and beak regions were recorded and used in all subsequent temperature calculations. Each blood sample was immediately divided into two sub-samples; the first into a serum separation tube for CORT assay and the second into K_2 EDTA-coated blood tube for heterophil and lymphocyte determination. Birds then were humanely euthanized via cervical dislocation, body weight recorded, and excreta and feather samples collected. Excreta samples were collected during necropsy by expelling the contents of the large intestine into a conical tube and stored at -20°C. Feather samples were collected from the intrascapular area (body feather) and second and third primary on the left wing (primary feather) and stored at -20°C in a freezer storage bag.

Heterophil to lymphocyte (H:L) ratio was determined using a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer within 4 h of blood draw. Serum separation tubes were centrifuged at 1500 x g for 10 min to separate serum from whole blood. Serum was frozen at -20°C until assay. Excreta CORT was extracted using an ethanol-based fecal extraction reported by Alm and colleagues (2014). Feather CORT was extracted using a methanol-based method reported by (Bortolotti et al., 2008). Serum, cecal and feather CORT concentrations were determined by a DetectX® Corticosterone EIA kit (Arbor Assays, Ann Arbor, MI).

3.3.3 Statistical Analysis

Data were analyzed using JMP Pro (version 13, SAS Institute Inc, Cary, NC), with the individual bird as the experimental unit. The statistical model fit treatment, time, and the interaction of treatment and time as fixed effects. BW, eye surface temperatures and beak surface temperatures fit a normal distribution, while H:L ratios and CORT data fit a lognormal distribution, and were analyzed within their respective distributions. Mean comparisons were analyzed on significant treatment effects using *post hoc* test slice t-tests to compare C and CS measures within each sampling time point. Results are reported as LSMeans and back-transformed LSMeans for abnormally distributed data. Pearson's pairwise correlations were evaluated using the JMP Pro Multivariate platform. Differences were considered significant at $P \leq 0.05$.

3.4 RESULTS AND DISCUSSION

This experiment compared commonly employed and noninvasive measures of stress from the same individual broiler chicken. Previous studies have compared plasma CORT concentrations with H:L ratio (El-Lethey et al., 2003; Post et al., 2003a; Shini et al., 2008), fecal

CORT, (Dehnhard et al., 2003), cecal CORT, (Rettenbacher et al., 2006), and feather CORT concentrations (Lattin et al., 2011; Fairhurst et al., 2013) in various avian species. However, none have evaluated these relationships in domestic chickens. The present compared H:L ratios and CORT concentrations in serum, excreta, body feathers and primary feathers within the same individuals.

Water consumption (per bird) was adjusted for bird density and calculated for both rooms throughout CORT treatment administration. CS birds consumed more water than C birds by 6 h after CORT water administration began. CS birds consumed 26% more water at 6 h, 42% more at 12 h, 48% more at 24 h, 60% more at 30 h, and 49% more at 48 h compared to C birds. By the end of the 72 h CORT water administration CS birds consumed an average of 45% more water than C birds. In a study investigating continuous administration of ACTH (8 IU ACTH/kg BW/d) for 7 d via implant pump, ACTH treated birds consumed 88% more water than controls (Puvadolpirod et al., 2000). The dramatic increase for CS bird water intake agrees with previous work (Puvadolpirod et al., 2000).

There were no main effects of treatment ($P = 0.33$) or time ($P = 0.10$) for BW (**Table 3.1**). T-test results showed C birds weighed more than CS birds at 24 h ($P = 0.05$), 48 h ($P = 0.0009$) and 72 h ($P < 0.0001$). Reduced growth is a common response to high CORT concentrations (Thaxton and Puvadolpirod, 2000; El-Lethey et al., 2003; Post et al., 2003a; Virden et al., 2007, Shini et al., 2009).

There were main effects of time ($P < 0.0001$) treatment ($P < 0.0001$) and treatment by time interaction ($P < 0.0001$) for H:L ratios. T-tests revealed that CS bird H:L ratios increased with time in the CS group and were higher ($P < 0.05$) at all 6 time points after CORT treatment

onset compared to C (**Table 3.1**). CORT and H:L ratio measures have a strong relationship indicating an increased stress response (Gross and Siegel, 1983; Post et al., 2003b; Shini et al., 2008). Serum or plasma CORT concentrations are often measured in combination with heterophil to lymphocyte (H:L) ratios. The increases in H:L ratio in the present study agrees with previous reports of chronic stress effects on the immune system of broilers (Gross and Siegel, 1983; Shini et al., 2008, Shini et al., 2009).

Turning to serum CORT, there were main effects of time ($P < 0.0001$), treatment ($P < 0.0001$) and treatment by time interaction ($P < 0.0001$) (**Figure 3.2**). The CS group had higher ($P < 0.05$) CORT concentrations than controls at all six time points measured after the onset of treatment. The results in the present study determining CORT in serum showed a similar pattern compared to plasma CORT concentrations reported by Post and colleagues (2003a). In the present study, there was a rapid increase in serum CORT concentrations for CS birds after provision of CORT in the drinking water (**Figure 3.2**). The CORT concentrations determined in the present study were, however, higher than those reported in Post and colleagues (2003a). Confounding environmental stressors can mask or match the stress response to a CORT treatment. El-Lethey and colleagues (2003) showed that the stress of depriving birds of foraging material matched that of feeding CORT when measuring the decrease in immune response in laying hens.

There were significant main effects of time ($P < 0.0001$), CS treatment ($P < 0.0001$) and treatment by time interaction ($P < 0.0001$) on excreta CORT concentrations. CORT concentrations in the CS group were higher ($P < 0.05$) at all six time points after CORT treatment administration compared to controls (**Figure 3.3**). Fecal CORT samples can be

collected noninvasively; however, excreta samples were collected invasively in this study for comparison within the same individual. In a study comparing plasma CORT and fecal glucocorticoid concentrations after ACTH, dexamethasone and saline injections on five individual chickens, plasma CORT and fecal CORT metabolite concentrations did not follow the same pattern (Dehnhard et al., 2003). However, the present study did reveal a similar pattern in serum CORT and excreta CORT concentrations, as reflected by a moderate positive correlation ($r = 0.60$; $P < 0.01$) (**Table 3.2**).

The concentrations of CORT in feathers were normalized by feather length to reflect cumulative feather growth over time (Bortolotti et al., 2008) and are presented as CORT pg/mm of feather length. There were main effects of time ($P < 0.0001$), treatment ($P < 0.0001$) and treatment by time interaction ($P < 0.0001$) for body feather CORT. There were higher ($P < 0.05$) concentrations of CORT in CS body feathers at all six time points compared to C birds after CORT administration began (**Figure 3.4a**). Similarly, there were significant main effects of time ($P < 0.0001$), treatment ($P < 0.0001$) and treatment by time interaction ($P = 0.0004$) for CORT concentrations in primary feathers. CORT concentrations in CS birds had a more pronounced linear increase ($R^2 = 0.53$; $P < 0.0001$) compared to C birds ($R^2 = 0.45$; $P < 0.0001$). CS primary feather CORT concentrations were higher ($P < 0.05$) than C with the exception of 30 h ($P = 0.08$) (**Figure 3.4b**).

CS bird eye surface temperatures were higher at 6 h ($P = 0.0005$) and 72 h ($P = 0.03$) compared to C birds (**Figure 3.5a**). CS bird beak surface temperatures were higher at 6 h ($P = 0.0006$) compared to C birds (**Figure 3.5b**).There was a positive correlation between BW and beak surface temperature ($r = 0.17$, $P = 0.04$). There was a negative correlation between body

weight with H:L ratio (r = -0.23; P = 0.008) serum CORT concentrations (r = -0.17; P = 0.05) and excreta CORT concentrations (r = -0.27; P = 0.004). There was a positive correlation between serum CORT and eye surface temperature (r = 0.26, P = 0.002). There were positive correlations ($P \leq 0.05$) between serum CORT, excreta CORT, body feather CORT and primary feather CORT concentrations (**Table 3.2**). There was a positive correlation between body feather CORT and primary feather CORT (r = 0.62; P < 0.0001). There was a negative correlation between beak surface temperature and primary feather CORT (r = -0.17, P = 0.05). There was a positive correlation between eye surface temperature and beak surface temperature (r = 0.41, P < 0.0001) (**Table 3.2**).

Studies investigating the utility of CORT from feathers in wildlife avian species most commonly utilize a primary feather (Bortolotti et al., 2008). The concentrations of CORT reported for body feathers from the intrascapular area in the present study for control birds were greater than those reported in Carbajal et al. (2014). This could be due to the differences in birds or environment. Carbajal et al. (2014) randomly collected their feathers from birds raised in a commercial environment versus our experimental setting. Concentrations of CORT from both feather types in CS birds increased with time (**Figures 3.4a; 3.4b**). This trend was unlike the reponse with serum concentrations of CORT in C birds, which maintained low concentrations of CORT throughout the experiment. Body feathers from the C group also maintained low CORT concentrations whereas primary feather CORT concentrations increased with time. This continued increase may be due to the combined effect of human presence as well as social hierarchy disruption by repeated removal of conspecifics (Gross and Colmano, 1971). While body and primary feathers from CS birds had high, constant concentrations of CORT, control CORT was naturally produced by the broiler and both concentrations accumulated in the body

feathers. This suggests that primary feathers are a more sensitive measure for experimental stressors.

Body feathers from CS birds showed a steady linear increase in CORT concentrations at time measures after baseline, with much higher concentrations than the controls. Investigating changes in CORT patterns over time is important to compare across and within all CORT sample concentrations (Bortolotti et al., 2008). The positive correlation between serum CORT with body feather CORT concentrations ($r = 0.43$; $P < 0.0001$) was stronger than with primary feather CORT ($r = 0.35$; $P < 0.0001$) concentrations. **Table 3.3** shows the CVs of CORT sources. Serum CORT concentrations had the lowest variation compared to excreta, body feather and primary feather CORT concentrations.

Feather CORT may provide a historical record of an individual's stress levels (Bortolotti et al., 2008). The acute stress response can result in either adaptive or non-adaptive long-term response, with long term non-adaptive responses leading to increased mortality and decreased reproduction. Adaptive stress responses lead to increased overall fitness (Wingfield et al., 1997). Measures of circulating CORT concentrations determine stress levels at the time of sample collection whereas feather CORT measures determine an average of CORT over the growth of the feather (Bortolotti et al., 2008). Feather CORT measures cannot be assumed to be correlated with circulating CORT (Lattin et al., 2011; Fairhurst et al., 2013). The linear relationship of body feather CORT and primary feather CORT was moderately strong ($r = 0.62$, $P < 0.0001$). Differences in feather growth and keratin deposition may have contribute to the differences in CORT concentrations in body and primary feathers from the same chicken. Additionally, two individuals with the same feather CORT concentrations may have different patterns of stressor

experiences. One individual may experience several short, high stress events within a primarily low stress environment whereas another individual may experience a constant, moderately stressful environment.

Feather CORT measures are the only retrospective method available to measure cumulative avian stress to date (Bortolotti et al., 2008). Primary feather CORT concentrations in CS birds showed a linear increase, which was most reflective of the response to the CORT water treatment. Thus, feather CORT may be a promising tool for both research applications measuring broiler stress.

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Table 3.1. Effect of CORT on growth and heterophil to lymphocyte ratio (H:L) of broiler chickens. Data shown are mean body weight (g) ± SEM and mean H:L ± SEM of 4 wk old male broilers receiving normal tap water (C) or a CORT in the drinking water treatment[1] (CS) for baseline (0 h) and six treatment time points (N=10 birds/treatment/time point).

Sampling	BW (g)[2]			H:L ratio[3]		
Hour	C	CS	P value	C	CS	P value
0	1223 ± 31.1	1294 ± 58.2	0.33	0.58 ± 0.38	0.44 ± 0.33	0.46
6	1249 ± 53.1	1355 ± 34.9	0.15	0.64 ± 0.29^{b}	1.60 ± 1.24^{a}	0.02
12	1333 ± 45.0	1372 ± 47.7	0.60	0.35 ± 0.19^{b}	1.36 ± 0.59^{a}	0.0004
24	1303 ± 61.6^{a}	1158 ± 58.2^{b}	0.05	0.84 ± 0.39^{b}	4.28 ± 1.41^{a}	0.0001
30	1298 ± 61.3	1299 ± 37.7	0.99	0.38 ± 0.22^{b}	2.53 ± 0.53^{a}	0.0001
48	1404 ± 52.5^{a}	1157 ± 58.8^{b}	0.0009	0.36 ± 0.30^{b}	3.18 ± 1.48^{a}	0.0001
72	1516 ± 64.3^{a}	1221 ± 42.6^{b}	0.0001	0.44 ± 0.26^{b}	4.09 ± 0.86^{a}	0.0001

[1]CS CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study.
[2]The ANOVA main effects of treatment (P= 0.33) and time were not significant (P = 0.10). The table shows t-test results.
[3]The ANOVA main effect time was not significant (P = 0.46). The table shows t-test comparisons of CS and C birds within each time point.
[ab] Means within the same row followed by uncommon superscripts differ at $P \leq 0.05$.

Table 3.2. Pairwise correlations between body weight (BW), heterphil to lymphocyte ratio (H:L), serum corticosterone (CORT) concentratiosn (ng/mL), excreta CORT (ng/g dry wt), body feather CORT (pg/mm feather length) and primary feather CORT (pg/mm feather length) of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment[1] (CS) measured at baseline (0 h) and six treatment time points (N=10 birds/treatment/time point).

	Correlations (r)							
	BW	H:L	Serum CORT	Excreta CORT	Body feather CORT	Primary feather CORT	Eye surface temp	Beak surface temp
BW	1.00	-0.23**	-0.17*	-0.27**	-0.11	-0.09	0.05	0.17*
H:L		1.00	0.41**	0.41**	0.50**	0.31**	0.05	-0.07
Serum CORT			1.00	0.60**	0.43**	0.35**	0.26**	0.04
Excreta CORT				1.00	0.37**	0.31**	0.06	-0.15
Body feather CORT					1.00	0.62**	0.03	-0.03
Primary feather CORT						1.00	-0.06	-0.17*
Eye surface temp							1.00	0.41**
Beak surface temp								1.00

[1]CS CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study.

Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 3.3. CV (%) of serum corticosterone (CORT) concentrations (ng/mL), cecal CORT (ng/g dry wt), body feather CORT (pg/mm feather length) and primary feather CORT (pg/mm feather length) of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment (CS)[1] measured at baseline (0 h) and six treatment time points (N=10 birds/treatment/time point).

	Time (h) after treatment onset							CV	
CORT Source	0	6	12	24	30	48	72	Mean	SD
Serum									
C	9.43	9.98	17.40	18.73	27.14	37.78	46.58	23.86	14.08
CS	31.50	30.02	31.22	53.76	34.26	29.60	45.64	36.57	9.39
								30.22	18.78
Excreta									
C	96.68	90.45	40.84	28.88	33.39	35.86	41.99	52.58	28.40
CS	108.25	43.03	28.18	23.13	46.36	46.86	47.46	49.04	27.88
								77.10	28.14
Body feather									
C	62.50	34.28	104.59	112.94	120.57	110.39	100.18	92.21	31.67
CS	55.18	67.78	76.06	48.04	55.70	51.65	51.94	58.05	10.09
								75.13	20.88
Primary feather									
C	99.71	67.35	90.25	59.78	81.93	81.61	96.15	82.40	14.66
CS	134.76	58.36	39.37	78.95	51.74	27.02	28.01	59.74	37.76
								71.07	26.21

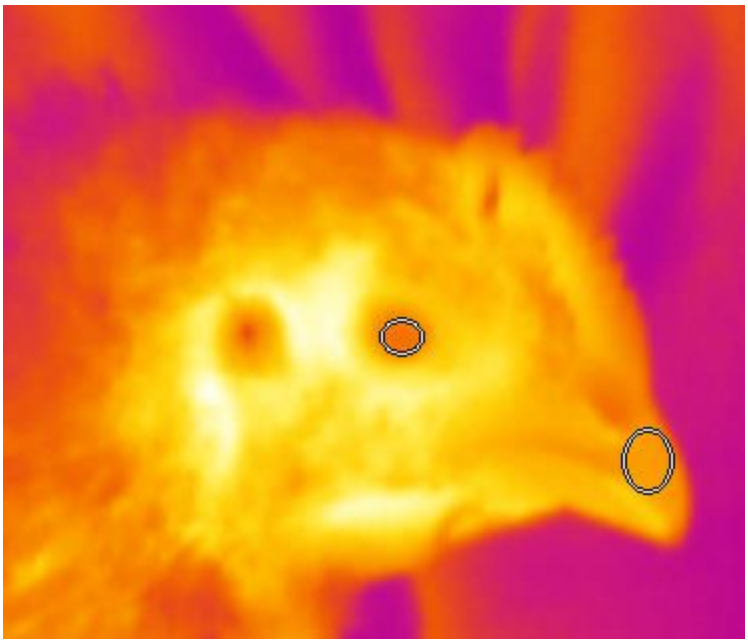


Figure 3.1. Pixels of the eye and beak were isolated (white circles) on the thermal image of the bird head for analysis.

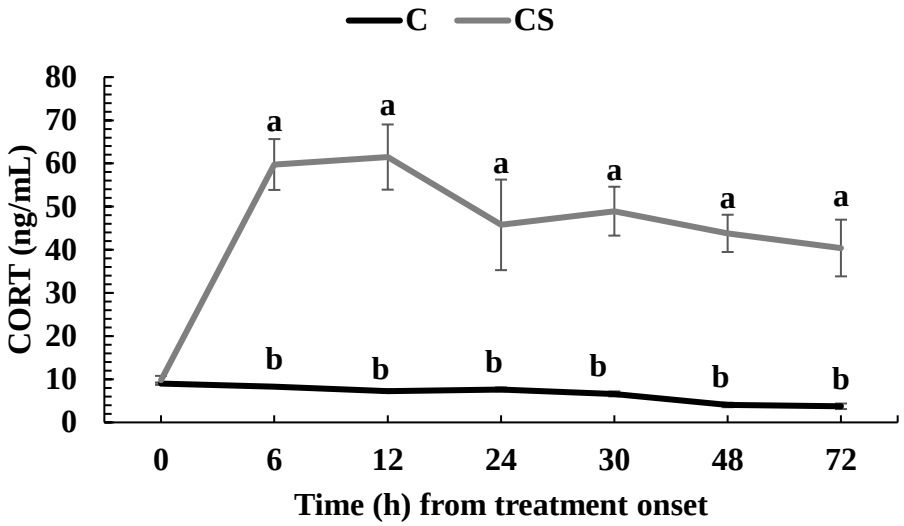


Figure 3.2. Effect of CORT in the drinking water on serum concentrations of CORT. Data shown are mean serum corticosterone (CORT) concentrations (ng/mL) ± SEM of 4 wk old male broilers administered normal tap water (C) or CORT water treatment (CS;) measured at baseline (0 h) and six treatment time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

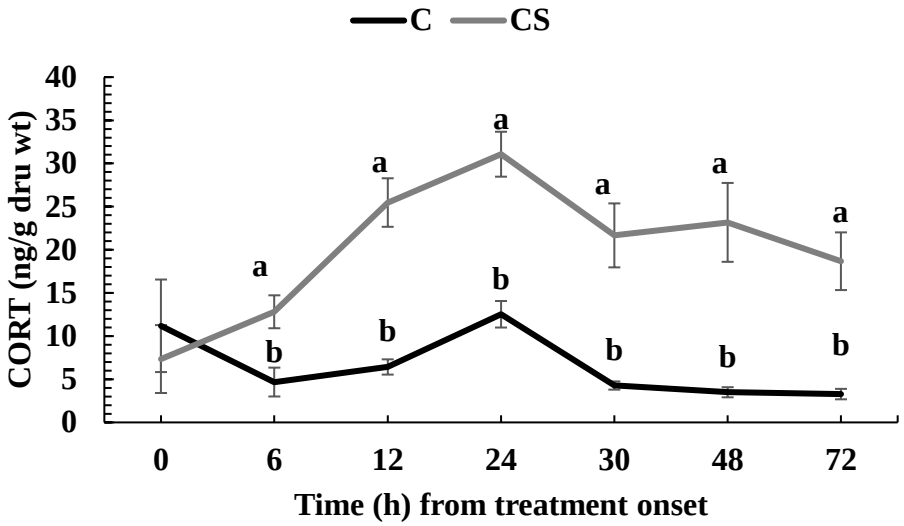
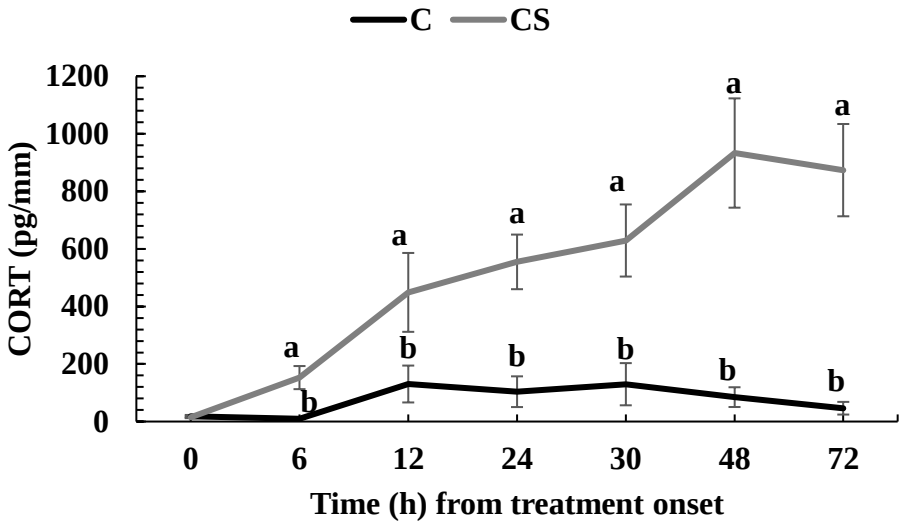


Figure 3.3. Effect of CORT in the drinking water on excreta concentrations of CORT. Data shown are mean excreta corticosterone (CORT) concentrations (ng/g dry weight) ± SEM of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment (CS) measured at baseline (0 h) and six additional time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

A



B

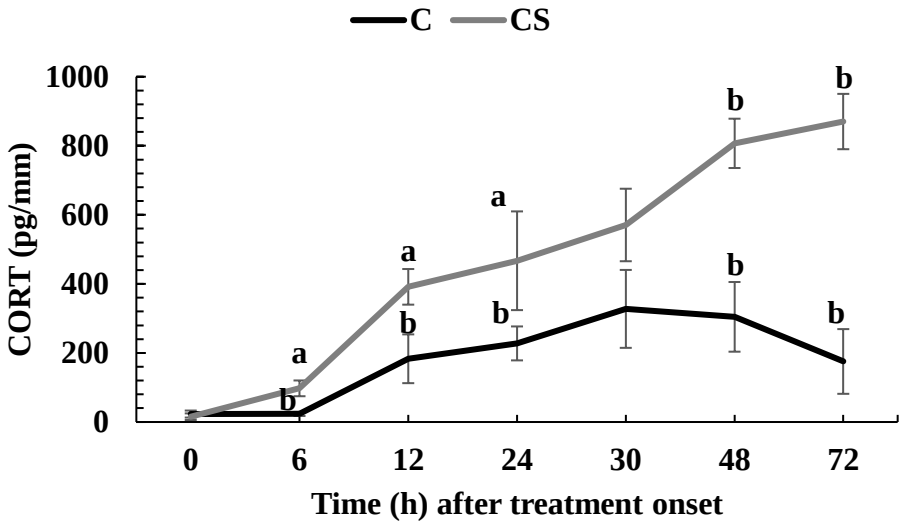
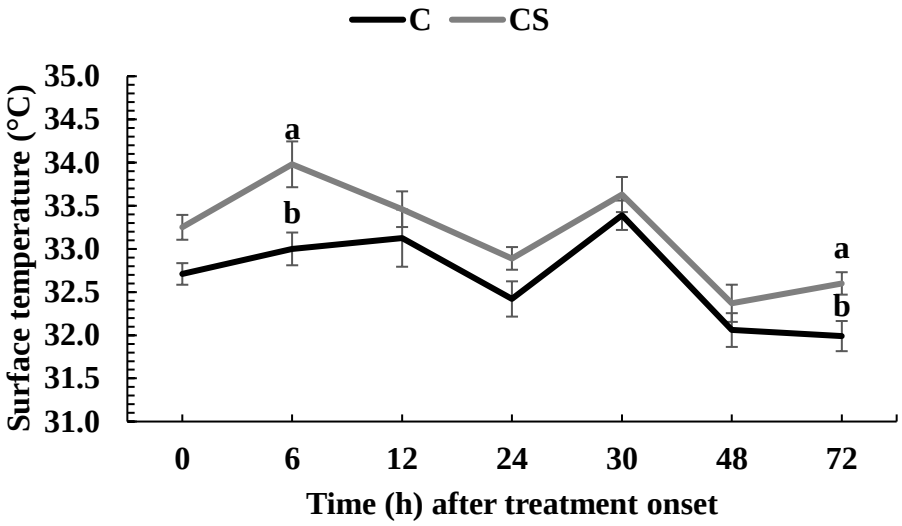


Figure 3.4. Effect of CORT in the drinking water on feather concentrations of CORT. Data shown are mean (A) body feather corticosterone (CORT, pg/mm) (B) and primary feather CORT (pg/mm) concentrations ± SEM of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment (CS) measured at baseline (0 h) and six additional time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

A



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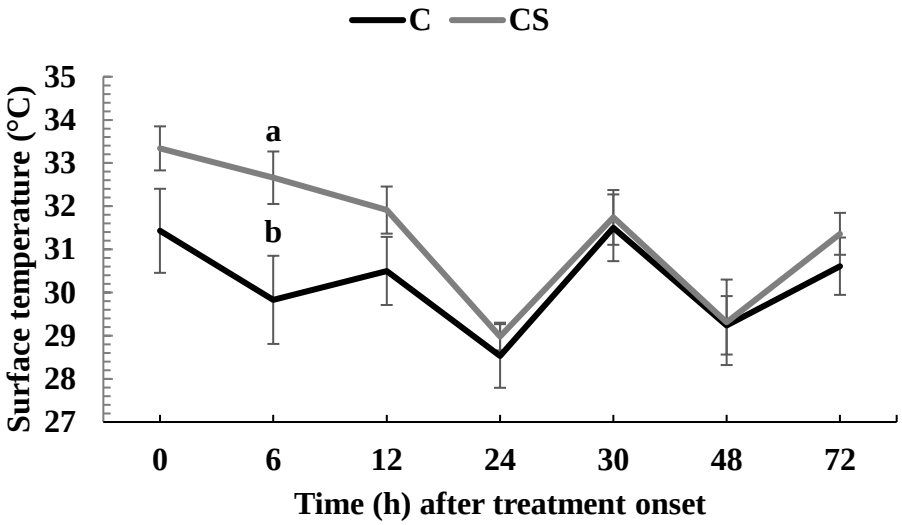

Figure 3.5. Effect of CORT in the drinking water on head region surface temperatures. Data shown are mean (A) eye surface temperatures (°C) and (B) beak surface temperatures (°C) ± SEM of 4 wk old male broilers administered normal tap water (C) or a CORT water treatment (CS) measured at baseline (0 h) and six additional time points (N=10 birds/treatment/time point). CORT water treatment was administered at a concentration of 20 mg/L at the beginning of the photoperiod on d 28 until the end of study. Means with different superscripts within each time point differ at $P \leq 0.05$.

CHAPTER 4 Infrared thermography (IRT) as a noninvasive method to detect lameness and lesions attributed to bacterial chondronecrosis with osteomyelitis (BCO)

4.1 ABSTRACT

Bacterial chondronecrosis with osteomyelitis (BCO) is a leading cause of lameness in broilers. Currently, subclinical BCO lesions can only be diagnosed *post-mortem*. Infrared thermography (IRT) is a noninvasive measure of infrared radiation from an object. IRT analysis in biological research can evaluate clinical health status. It was our hypothesis that IRT surface temperatures at key locations of the legs may detect BCO noninvasively. Two experiments (exp) were conducted with Cobb 500 byproduct male broilers. Birds were raised in pens within environmentally controlled chambers having litter or wire flooring at light intensities of either 2, 5 or 10 lux. Light intensity treatments were applied to six pens starting on d 8 (d 7 in exp 2) and through the duration of the experiment. On d 29 (d 28 in exp 2) half of the birds from each litter pen were moved to a wire flooring pen having the same light intensity as the corresponding litter pen. On d 7, 9, 28, 30, 38 and 56 in exp 1 and d 6, 8, 27, 29, 40 and 55 in exp 2, five clinically healthy (sound) birds from each pen had thermal images taken of their legs. An additional thermal image of each birds' head was taken in exp 2. Birds were then euthanized, body weight (BW) was recorded, and right and left proximal femora and tibiae and scored for femoral head necrosis (FHN) and tibial head necrosis (THN) lesion severity. A total necrosis category (Total N) was calculated by adding the four leg bone necrosis scores. The same thermal images were taken and leg bones were scored for birds that developed lameness from d 29 to 57. All birds that developed lameness were on wire flooring and no birds went lame on litter flooring. Pixels representing body regions of the eye, beak, and left and right hocks, shanks and feet were isolated on the thermal image for analysis. On d 28 in exp 1, hock surface temperatures increased ($P = 0.01$) with light intensity. There were no effects of flooring type (wire or litter) differences ($P > 0.05$) on FHN, THN or Total N lesion severity scores at any age. The total percentage of birds that went lame in exp 1 was 52% and 14% in exp 2. Surface temperatures of the hocks,

shanks and feet of sound birds were higher ($P < 0.0001$) than for lame birds in both experiments. Therefore, IRT surface temperatures of broilers may be useful to detect lesions attributed to bacterial chondronecrosis with osteomyelitis (BCO).

4.2 INTRODUCTION

Lameness has been estimated to affect up to 1% of all market weight broilers (Wideman, 2014). Broiler growth rates have increased over three fold in the past 50 years (Knowles et al., 2008). There is recent public concern that this rapid growth rate may contribute to impaired locomotion and increased lameness in broilers (Knowles et al., 2008). Lameness is logically assumed to be painful and consequently stressful. Another reason lameness is a major welfare issue is if the bird cannot reach feed and water. Lameness is practically evaluated by visually scoring the gait of the bird (NCC, 2014). However, inter-observational bias influences evaluation and visual assessment does not provide quantitative data to make reasonable comparisons to determine the welfare status of the birds (Marchewka et al., 2013).

Some studies have reported relationships between broiler mortality, growth rate and light intensity (Deep et al., 2013), whereas others have not (Lien et al., 2007; Fidan et al., 2015). Light intensity may affect an individual's activity and time spent walking (Newberry et al., 1988; Alivno et al., 2009; Deep et al., 2012). Kumari and colleagues (2015) found a negative correlation between light intensity and foot pad dermatitis.Studies have demonstrated no difference in gait scores for broilers kept at different commercial light intensities (Kristensen et al., 2006, Deep et al, 2013). In contrast, Blatchford and colleagues (2014) found broilers raised at intensities of 200 lux had better gait scores than broilers raised at 1 lux. However, these extreme differences in light intensities and are not reflective of commercial conditions. The 2014 NCC broiler welfare guidelines suggests 5 lux as the minimum light intensity necessary for broiler chickens (NCC, 2014). However, further research may be needed to establish an optimal light intensity that promotes the best overall welfare in broilers.

Infrared thermography (IRT) is a noninvasive, non-contact method of determining surface heat (infrared radiation) from an object (review: Eddy et al., 2001). IRT has been utilized on over 30 species of birds for over 50 years (review: McCafferty, 2013). Recent research has utilized IRT in domestic chickens as a noninvasive tool to measure metabolic heat production (Ferreira et al., 2011; Yahav and Giloh 2012), footpad dermatitis (Wilcox et al., 2009; Jacob et al., 2016) and handling stress (Edgar et al., 2013; Moe et al., 2017). However, no studies have utilized IRT to measure broiler lameness as it has in dairy cattle (Nikkhah et al., 2005; Stokes et al., 2012; Oikonomou et al., 2013), swine (Amezcua et al., 2014) and horses (review: Eddy et al., 2001; Douthit et al., 2014). While IRT cannot determine specific pathologies, it can detect localized areas of increased heat due to inflammation (review: Eddy et al., 2001; Wilcox et al., 2009; Amezcua et al., 2014) or decreased heat due to reduced blood flow (Jacob et al., 2016). IRT surface temperatures may be affected by blood vessel location, malaise, biorhythm, age, stress and environment (Loughmiller et al., 2000). One study used IRT to evaluate metabolic heat loss of broiler chicks fed diets with different energy levels. Total radiant heat loss, calculated by IRT image surface temperatures, revealed chicks had greater metabolic heat losses on higher energy diets (Ferreira et al., 2011). Recently IRT has been used to explore the relationship between footpad surface temperatures and visual footpad dermatitis scores. A decrease in footpad surface temperature was associated with increasing footpad dermatitis scores (Jacob et al., 2016). The decrease in footpad surface temperature was described to be related to the increased tissue necrosis and reduced blood flow to the footpad (Jacob et al., 2016).

Bacterial chondronecrosis with osteomyelitis (BCO) is a bacterial infection of the growth plates in the leg bones, leading to necrosis and eventually lameness (review: Wideman, 2015; 2016). The mechanical stress of walking may create micro fractures to the femora and tibiae

thereby creating wound sites for bacteria to colonize in the growth plates (review: Wideman 2015, 2016). Vascular occlusion and reduced blood flow have been hypothesized as a contributing factor in the pathogenesis of BCO lameness (review: Wideman and Prisby, 2013; review: Wideman 2015, 2016).

It was our hypothesis that changes in IRT surface temperatures at key locations of the legs would allow the detection of BCO noninvasively. We utilized a wire flooring model used in previous research (Wideman et al., 2012, Gilley et al., 2014). Wire flooring is an unstable walking surface that increases shear stress and mechanical torque on the growth plates in leg bones, thereby exacerbating BCO and the incidence of lameness (Wideman et al., 2012, review: Wideman, 2015). Two additional body regions, the eye and beak, also were evaluated with IRT to compare the surface temperature relationships of head region surface temperatures with those of the hock, shank and foot. In addition, eye and beak surface temperatures are related to core body temperatures (Stewart et al., 2008; Weschenfelder et al., 2013; Yahav and Giloh, 2012). Relatedly, eye temperatures may be an index of pain (Stewart et al., 2008). Stewart and colleagues (2008) found IRT eye temperatures of disbudded dairy calves were lower without local anesthetic than those that received local anesthetic during disbudding. The authors concluded the rapid drop in eye temperature was related to the vasoconstricive pain response of the sympathetic nervous system (Stewart et al, 2008). We hypothesize the wire flooring is uncomfortable to walk on and IRT may reveal an index of the pain experienced by broilers developing BCO on wire flooring. Previous research using IRT has revelaed noninvasive measures for identifying peaks in physiological events in humans and animals (Nääs et al., 2010).

The studies examined the environmental effects of three light intensities (2 lux, 5 lux and 10 lux) and two flooring types (litter and wire) on IRT surface temperatures of the hock, shank and foot. Surface temperatures taken *pre-mortem* were compared to proximal femoral and tibial head BCO lesion severity scores taken *post-mortem* in both clinically healthy (sound) and lame broilers. The data collected to address the relationship between BCO and surface temperatures also allowed determination of any effects of lameness on surface temperatures.

4.3 MATERIALS AND METHODS

4.3.1 Animals and Facility

Male Cobb 500 byproduct broiler chicks (N = 720) were obtained from a commercial hatchery (Cobb, Fayetteville, AR). They were randomly placed into six 1.5 m wide x 3 m long pens (120 per pen) within environmental chambers in the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm (IACUC # 16014). Birds had *ad libitum* access to feed and water. Diets were formulated to meet minimum industry standards (NRC, 1994). A crumbled commercial starter diet was provided until d 28 when birds were switched to a pelleted commercial finisher diet. Water was provided via nipple drinkers. The photoperiod was 23L:1D for d 0-4; 20L:4D for d 5-14 and 18L:6D from d 15 through the end of trial. Chamber temperatures were set at 33°C for d 0-3, 30°C for d 4-7, 26°C for d 8-14 and 24°C for d 15 through the end of trial. The accuracy of the set temperatures was determined by measuring ambient temperatures with a thermometer (TM99A Thermistor Temperature Instrument, Cooper Atkins, Middlefield, CT) and an infrared thermometer (IRT657, General Tools & Instruments LLC, Secaucus, NY). The trial duration was from September – November 2015.

4.3.2 Experiment 1

On d 8, six pens were assigned to one of three incandescent light intensity treatments (2 lux, 5 lux and 10 lux) (N = 2 pens per light intensity treatment). Initially the light intensity was 20 lux. Light intensity of each pen was measured daily at bird level with a digital light meter (Model LT300, Extech, Nashua, NH). On d 14, bird density was reduced to 105 clinically healthy chicks per pen. Birds were culled when they walked with an awkward gait or had a markedly smaller body size compared to their pen mates. The early culling protocol was instituted because previous studies revealed macroscopic evidence of BCO in runts and culls during the first 2 wk of age (Wideman et al., 2012). Birds were raised in pens with wood shavings litter flooring until d 29. On d 29, fifty birds from each litter flooring pen (N = 6) were moved to one of six pens with wire flooring (N = 6) in separate chambers. The wire flooring pens had the same pen dimensions, bird density and light intensity as the source litter pens. Thus, there were a total of 12 pens. Wire flooring panels consisted of hardwire cloth (1.3 x 2.54 cm mesh, 0.063-guage galvanized welded wire cloth; Direct Metals, Kennesaw, GA) attached to 5 cm x 5 cm lumber and were 1.5 m wide by 3 m long. The wire panels were elevated 30 cm high on masonry blocks. Birds from litter flooring also were moved to pens with the same light intensity to control for the stress of handling and introduction to a novel pen.

Five randomly selected birds from each pen had a thermal image taken of the head and legs on days 7 (N = 6 pens), 9 (N = 6 pens), 28 (N = 6 pens), 30 (N = 12 pens), 38 (N = 12 pens) and 56 (N = 12 pens). Thermal images were captured with a thermal imaging camera (Fluke Ti400, Fluke®, Everett, WA). The camera background temperature was 22°C, emissivity was 0.95 and with 100% transmission. The focal distance of the camera from the bird was 3.3 to 3.6 m. Images were uploaded to a computer and analyzed using SmartView® (v 2.8) software. Each

individual pixel (76, 800 total) within the thermal image had an associated temperature recorded. Within each head image, shapes were made to isolate pixels of the eye pupil and the beak region anterior to the nostril (**Figure 4.1**). Within each leg image, shapes were made to isolate pixels of the right and left hock joints (intertarsal joint; distal tibiotarsus and proximal tarsometatarsus), shanks (tarsometatarsus) and feet (metatarsus and phalanges; **Figure 4.2**). The averages of the pixel temperatures within each shape of the body regions were recorded and used in all subsequent temperature calculations. The pixel averages within thermal images of each bird's legs representing the right and left hock joint, shanks and feet were averaged used for data analysis.

Birds were humanely euthanized via cervical dislocation, body weight (BW) was recorded (final weights were recorded on d 57 and 58) and BCO lesion severity was macroscopically diagnosed for proximal femoral head necrosis (FHN) and proximal tibiotarsus (referred to as tibia) head necrosis (THN) lesion severity. The scoring scheme was a modified version of the methods described in previous research (Wideman et al., 2012, review: Wideman and Prisby, 2013; Wideman et al., 2013; 2014; Gilley et al., 2014; Wideman, 2014). FHN lesion severity was scored on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). THN lesion severity was also scored on a 0-3 scale in the following categories: 0- no abnormalities of the proximal tibia (normal); 1- mild proximal tibial head necrosis (THN); 2- severe tibial head necrosis (THNS); and 3- caseous THN (THNC; **Figure 4.3**)

A quantitative FHN index was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6. A quantitative THN index was created by summing the right (0-3) and left (0-3) proximal THN scores of increasing BCO lesion severity for a range of 0-6. A quantitative Total Necrosis (Total N) index was created by summing FHN and THN category scores of increasing BCO lesion severity for a range of 0-12.

Beginning on d 29 until d 57, birds in all pens were observed daily for lameness. Birds were considered lame if they would not step to walk when gently coaxed and when they used wing tips for support to stand. Lame birds were removed when the onset of lameness was observed, a thermal image was taken of their legs, they were euthanized via cervical dislocation and scored for BCO lesion severity as described above.

4.3.3 Experiment 2

Exp 2 followed the same design as exp 1 with the following minor exceptions. The five randomly selected birds from each pen had a thermal image taken of the head and legs on days 6 (N = 6 pens), 8 (N = 6 pens), 27 (N = 6 pens), 29 (N = 12 pens), 40 (N = 12 pens) and 55 (N = 12 pens). Six pens were assigned to one of three light intensity treatments on d 7 (2 lux, 5 lux and 10 lux) (N = 2 pens per light intensity treatment). Birds were transferred to wire flooring pens on d 28 (compared to d 29 in exp 1). Core body temperatures were measured with a thermometer (TM99A Thermistor Temperature Instrument, Cooper Atkins, Middlefield, CT) inserted 5 cm into the cloaca. Final BW was recorded on d 56 and 57. Beginning on d 29 until d 56, all birds were observed daily for lameness as in exp 1. Thermal images of lame bird heads were taken for eye and beak surface temperature analysis. The trial duration was from April – June 2016.

4.3.4 Statistical Analysis

Data were statistically analyzed using JMP software (version 13, SAS Institute Inc, Cary, NC), with the pen as the experimental unit. For the comparison of lame and sound birds within the same pen, the individual bird was the experimental unit. All continuous data were analyzed for distribution normality using the JMP distribution histogram platform. Body weight (BW) and surface temperature data were normally distributed. Linear regression models analyzing the main effects of light intensity (2 lux, 5 lux and 10 lux), flooring type (litter vs wire) and their interaction on BW, the degree of BCO necrosis of the left and right proximal femoral and tibial heads (FHN, THN and Total N), core body temperature (exp 2) and body region [eye, beak (exp 2) and mean left and right hock, shank and foot] surface temperatures for each age sampled (exp 1: d 7, 9, 28, 30, 38 and 56-58; exp 2: d 6, 8, 27, 29, 40 and 55-57) were evaluated. Linear regression models were also utilized to evaluate the effect of age and body region on surface temperatures. The effect of health status (sound vs. lame) on body region surface temperatures and on high or low FHN, THN, and Total N lesion severity was analyzed using regression modeling. Significant means were separated *post-hoc* with LSMeans (two analysis factors or less) and Tukeys (more than two analysis factors) where appropriate. Simple linear regression relationships between light intensity and necrosis severities were analyzed. To analyze the effects of light intensity and flooring type on lame bird percentages, a chi-square analysis was performed. Data were considered significant at $P \leq 0.05$.

4.4 RESULTS

4.4.1 Experiment 1

There were no effects of light intensity (d 7, 9, 28, 30, 38 and 57/58) on body weight (**Table 4.1**). Similarly, there was no effect of or flooring type on body weight on d 30 and 38

(**Table 4.1**). However, at d 57-58 of age, birds raised on wire flooring weighed less (P = 0.01) than birds raised on litter flooring (**Table 4.1**).

Table 4.2 summarizes surface temperatures (eye, beak, hock, shank and foot) in sound birds before the imposition of the wire flooring treatment (at d 7, 9 and 28). There was one apparently anomalous observation with on d 7, chicks in pens that were later to receive 10 lux light intensity had higher (P = 0.04) eye temperatures than birds in other the light intensity treatment groups (**Table 4.2**). By d 9 these differences were no longer observed (**Table 4.2**). There were no light intensity effects (P > 0.05) on eye, beak, shank or foot surface temperatures on birds 9 d of age. On d 28 of age, there were no light intensity effects (P > 0.05) on the surface temperatures of the eye, beak, shank or foot (**Table 4.2**). However, there were differences with hock surface temperatures of 28 d old birds (**Table 4.2**). Hock surface temperatures in birds in pens with 10 lux light intensity were higher (P = 0.01) than birds in pens with 5 lux or 2 lux on d 28 (**Table 4.2**). Moreover, the hock surface temperatures in birds in pens with 5 lux light intensity had higher (P = 0.04) hock temperatures than birds in pens with 2 lux on d 28 (**Table 4.2**). Regression analysis revealed a strong linear increase (R^2 = 0.93, P < 0.0001) in hock temperatures with light intensity on d 28 (**Figure 4.4**).

Eye, beak, shank, hock and foot surface temperatures of sound birds at d 30, 38 and 56, after the imposition of the wire flooring treatment and the continuation of the three light intensities, are shown in **Table 4.3**. There were no effects of light intensity (P > 0.05) on surface temperatures of the shank, hock foot or eye at any age (**Table 4.3**). There were marked differences shank surface temperatures with flooring in birds 30 d of age in pens with wire flooring had higher (P = 0.01) shank surface temperatures than birds raised on litter flooring

(37.3°C vs. 36.0°C, respectively) (means calculated from **Table 4.3**). In contrast, foot surface temperature in birds 30 d of age raised on litter were higher (P =0.01) than those on wire (36.4°C vs. 34.1°C, respectively) (means calculated from **Table 4.3**).

There were no effects of light intensity or flooring treatment (P > 0.05) on eye, beak, hock, shank or foot body region surface temperatures at d 38 of age (**Table 4.3**). On d 56 of age, birds raised on wire flooring had lower (P = 0.01) eye surface temperatures than birds raised on litter flooring (32.1°C vs. 33.4°C, respectively) (means calculated from **Table 4.3**). Beak surface temperatures on d 56 of age were lower (P = 0.01) for birds raised in pens with wire flooring than birds on litter (32.0°C vs. 35.2°C, respectively) (means calculated from **Table 4.3, Figure 4.5**). Wire flooring pens with 5 lux light intensity had the lowest (P = 0.004) beak surface temperatures compared to all other light intensity and flooring type treatments (**Table 4.3, Figure 4.5**).

Data were analyzed by combining light intensity and floor types across treatment groups to examine possible age differences in surface temperatures (**Table 4.4**). There were differences in eye, beak, shank and foot body region surface temperatures across ages (P < 0.05) but none for hock surface temperature (P > 0.05). Eye surface temperature decreased with age (P < 0.0001) while shank and foot surface temperatures increased (P = 0.0006; P < 0.0001, respectively) with age (**Table 4.4**). Beak surface temperature was lowest (P = 0.009) for birds on d 9 of age (**Table 4.4)**.

Data were also analyzed to determine whether there were differences in surface temperature between the five body regions. These data are summarized **Table 4.5**. There were differences (P < 0.05) within broiler age for eye, beak, hock, shank and foot body region

temperatures (**Table 4.5**). At each of the ages examined, hock temperatures were either amongst the highest ($P < 0.0001$) or were numerically the highest (**Table 4.5**). On d 7, hock and shank surface temperatures were highest, followed by eye surface temperature, while foot surface temperature and beak surface temperatures were the lowest ($P < 0.0001$) (**Table 4.5**). On d 9, hock and shank surface temperatures were the highest, followed by eye surface temperature, while beak and foot surface temperatures were the lowest ($P < 0.0001$) (**Table 4.5**). On d 28, hock and shank surface temperatures were the highest, followed by foot surface temperature, while eye and beak surface temperatures were the lowest ($P < 0.0001$). On d 30, hock surface temperature was the highest, followed by foot surface temperature, and eye, beak and shank surface temperatures were the lowest ($P < 0.0001$). On d 38 and 56, hock and shank surface temperature were the highest, followed by foot surface temperature, while eye and beak surface temperatures were the lowest ($P < 0.0001$) (**Table 4.5**).

There were no effects (light intensity or flooring type) on FHN, THN or Total N necrosis lesion score severities for sound birds 30, 38 and 56-57 d of age ($P > 0.05$) (**Table 4.6**). FHN BCO lesion scores were more severe ($P < 0.0001$) for sound birds on d 56-57 of age compared to sound birds d 30 and 38 d of age (**Figure 4.6a**). THN BCO lesion scores severity increased ($P = 0.003$) with age (**Figure 4.6b**). Relatedly, Total N BCO lesion severity scores increased ($P =0.03$) with age (**Figure 4.6c**).

4.4.2 Incidence of lameness

No birds raised on litter flooring developed lameness, whereas significant ($P < 0.0001$) numbers of birds developed lameness on wire flooring (**Figure 4.7**). The total percentage of birds raised on wire flooring that became lame was 52% (**Figure 4.7**). From d 35 to 38, a higher

($P < 0.05$) percentage of birds raised on wire flooring at 10 lux went lame compared to 2 and 5 lux. However, this difference was no longer apparent by d 39, and there were no light intensity differences in the percentage of birds that went lame throughout the rest of the trial (**Figure 4.8**).

4.4.3 Effect of health status on necrosis and surface temperatures

Compared to sound birds raised in the same wire flooring pens, birds who became lame had more severe ($P < 0.05$) FHN, THN and Total N BCO lesion severity scores (**Table 4.7**). Additionally lame birds had lower hock ($P < 0.0001$), shank ($P < 0.0001$) and foot ($P < 0.0001$) surface temperatures (**Table 4.9**). The data were also analyzed using mean BCO lesion severities and surface temperatures for lame and sound birds at the pen level. FHN, THN, and Total N BCO lesion severity scores were 200%, 61%, and 113% more severe in lame birds compared to sound birds, respectively (**Table 4.8**). Delta hock, shank and foot surface temperatures were lower ($P < 0.0001$) in lame than sound birds by, respectively, 3.1°C for hock, 2.7°C for shank and 6.2°C for the foot (**Table 4.10**). Delta foot surface temperatures were the lowest ($P < 0.0001$) compared to hock and shank surface temperatures for sound and lame birds by 0.8°C and 4.1°C, respectively (**Figure 4.9**).

4.4.4 Effect of necrosis severity on surface temperatures

Data were analyzed to determine whether there were differences in leg surface temperatures with the extent (high or low) of BCO lesion severities femoral head necrosis (FHN), tibial head necrosis (THN) or aggregate necrosis (Total N). These data are summarized in **Tables 4.11, 4.12 and 4.13.** There were consistently lower leg surface temperatures (shank, hock and foot) in lame than in sound birds irrespective of the degree of BCO lesion severity (**Tables 4.11, 4.12 and 4.13**).There were no differences effects of severity of BCO necrosis

(FHN or Total N) on hock, shank or foot surface temperatures (**Tables 4.11 and 4.13**). This was observed irrespective for sound or lame birds for FHN (**Table 4.11**) or Total N (**Table 4.13**). Similarly, there were no effects of THN on shank or foot temperatures (**Table 4.12**). However, independent of health status (sound vs. lame), hock temperatures were higher ($P = 0.04$) with increased BCO lesion severity of THN (**Table 4.12**).

4.4.5 Experiment 2

Table 4.14 summarizes body weights of the flooring and light intensity treatments. There were no effects ($P > 0.05$) of either light intensity (d 6, 8, 27, 29, 40, and 56-57) or flooring type (d 29, 40 and 56-57) on body weight (**Table 4.14**). Similarly, there were no light intensity effects ($P > 0.05$) on core body temperatures or surface temperatures (eye, beak, hock, shank and foot) in sound birds at d 6, 8 and 27 before the imposition of the wire flooring treatment (**Table 4.15**). Moreover, there were no light intensity or flooring type effects ($P > 0.05$) on core body temperatures or eye, beak, hock, shank and foot surface temperatures in sound birds at d 29, 40 and 55 after the imposition of the wire flooring treatment (**Table 4.16**).

Table 4.17 shows data combined across treatment groups to examine possible age differences in body core and eye, beak, hock, shank and foot surface temperatures. Core body temperature increased ($P < 0.0001$) with age (**Table 4.17**). Eye surface temperatures were the lowest ($P < 0.0001$) at 27 d of age compared to 6, 8, 29, 40 and 55 d of age (**Table 4.17**). Eye, beak, hock, shank and foot surface temperatures were the highest ($P < 0.0001$) at 55 d of age compared to younger ages (**Table 4.17**).

There were differences within broiler age for core body and eye, beak, hock, shank and foot body region surface temperatures (**Table 4.18**). Core body temperature the highest ($P <$

0.0001) compared to body region surface temperatures (**Table 4.18**). Beak surface temperatures were the lowest ($P < 0.0001$) compared to eye, hock, shank and foot surface temperatures for broilers 6, 8, 29, 40 and 55 d of age (**Table 4.18**). On d 27 of age, beak and foot surface temperatures were not different ($P > 0.05$) and these two body region surface temperatures were lower ($P < 0.0001$) than eye, hock and shank surface temperatures (**Table 4.18**).

There were no light intensity or flooring type effects on FHN, THN or Total N lesion score severities for birds 29, 40 and 56-57 d of age (**Table 4.19)**. FHN necrosis lesion score severity increased ($P < 0.0001$) with age (**Figure 4.10a**). THN BCO lesion severity scores were more severe ($P < 0.0001$) at 40 and 56-57 d of age compared to 29 d of age in sound broilers(**Figure 4.10b**). Relatedly, Total N necrosis lesion scores were more severe ($P < 0.0001$) at 40 and 56-57 d compared to 29 d of age in sound broilers (**Figure 4.10c**).

4.4.6 Incidence of lameness

No birds raised litter flooring developed lameness and therefore more ($P < 0.0001$) birds went lame raised on wire flooring. In contrast, there was a moderate incidence of lameness in birds on wire flooring (**Figure 4.11**). The total percentage of birds raised on wire flooring that became lame was 14% (**Figure 4.11**). There were no light intensity effects ($P > 0.05$) on the percentage of birds that went lame raised on wire flooring throughout the trial (**Figure 4.12**).

4.4.7 Effect of health status on necrosis and surface temperatures

Compared to sound birds raised in the same wire flooring pens, birds who became lame did not have consistently more severe ($P < 0.05$) FHN, THN and Total N BCO lesions (**Table 4.20**). In fact, lame birds only had more severe FHN and THN in 1 pen, and 2 pens with more severe Total N compared to sound birds (**Table 4.20**). Lame birds had lower hock, ($P < 0.05$)

shank ($P < 0.05$) and foot ($P < 0.05$) surface temperatures than sound birds, except for shank and foot temperatures in pen 5 (**Table 4.22**). Eye surface temperatures were lower ($P < 0.05$) for lame birds in pens 2, 3, 5 and 6 while beak surface temperatures were lower ($P < 0.05$) for pens 1, 2, 3 and 4 (**Table 4.22**). The data were also analyzed using mean necrosis and surface temperatures for lame and sound birds at the pen level. Cumulative FHN, THN and Total N BCO lesions were 42%, 16%, and 27% more severe in lame birds compared to sound birds, respectively (**Table 4.21**). Delta eye surface temperatures were 1.0°C lower ($P < 0.0001$) and beak surface temperatures were, more prominently, 2.4°C lower ($P < 0.0001$) in lame birds compared to sound birds (**Table 4.23**). Delta hock, shank and foot surface temperatures were lower ($P < 0.0001$) in lame than sound birds by 2.2°C for hock, 3.7°C for shank and 3.1°C for the foot (**Table 4.23**). Delta foot surface temperatures were the lowest ($P < 0.0001$) compared to hock and shank surface temperatures for sound and lame birds by 1.3°C and 1.5°C, respectively (**Figure 4.13**).

4.4.8 Effect of necrosis severity on surface temperatures

Tables 4.24, 4.25 and 4.26 summarize data when were analyzed for both lameness and the extent of BCO lesion severity scores (high or low) for, respectively, femoral head necrosis (FHN), tibial head necrosis (THN) and aggregate necrosis (Total N). There were no differences effects of tibial head necrosis severity (THN aggregate) on hock, shank or foot surface temperatures (**Table 4.25**). There were differences for femoral head necrosis (FHN) and aggregate necrosis (Total N) (**Tables 4.24 and 4.26**). This was observed irrespective for sound or lame birds for FHN (**Table 4.24**) or Total N (**Table 4.26**). Independent of health status (sound vs. lame), surface temperatures were higher for the hock ($P = 0.02$) and shank ($P = 0.03$) with increased severity of FHN (**Table 4.24**). Relatedly, independent of health status (sound vs.

lame), shank temperatures were higher (P = 0.02) with increased severity of Total N aggregate necrosis (**Table 4.26**).

4.5 DISCUSSION

The utility of infrared thermography (IRT) in biological research can have many applications. Specifically in the area of lameness detection, IRT has been utilized to measure inflammation leading to lameness in horses (review: Eddy et al., 2001; Douthit et al., 2014) foot lesions in dairy cattle (Nikkhah et al., 2005; Stokes et al., 2012; Oikonomou et al., 2013) and lameness in swine (Amezcua et al., 2014). However, no studies have utilized IRT to evaluate broiler lameness. These present studies examined whether light intensity (2 lux, 5 lux and 10 lux) and flooring (litter versus wire) influences surface temperatures as determined by IRT. Moreover, it was possible to examine the relationship between thermal image measures of leg hock, shank and foot regions taken *pre-mortem* to proximal femoral and tibial head necrosis lesion severity scores attributed to bacterial chondronecrosis with osteomyelitis (BCO) taken *post-mortem.* In addition, it was possible to examine differences in leg surface temperatures between in clinically healthy (sound) and lame broilers.

There were no effects of light intensity or flooring-type on body weight in exp 2 (**Table 4.14**) and only at until d 56 of age in exp 1 (**Table 4.1).** It was expected that wire flooring treatment would depress body weights. One report supports this conjecture with two studies evaluating the effects of flat wire flooring, litter shavings, and wire flooring speed bump ramps with 50% slope implemented at varying ages (Gilley et al., 2014). Body weights of 8 wk old male broilers raised on wire flooring were the lowest compared to the other flooring types (Gilley et al., 2014). It is difficult to explain the lack of a consistent effect of wire versus litter flooring on body weight of sound birds on wire flooring in the present study (**Tables 4.1 and**

4.14). A possible explanation could be the daily culling of lame birds resulting in lower stocking densities. Stocking densities at 8 wk of age for litter vs. wire birds was much different between the two experiments. Average stocking density for birds in litter pens in exp 1 at d 56 was 0.96 m^2 and 1.92 m^2 for wire pens. In exp 2 d 55 average litter pen stocking density averaged 0.97 m^2 and 1.17 m^2 for wire pens. The stocking density difference in wire flooring pens between exp 1 and exp 2 was 39%. Male broilers grown at lower stocking densities have been reported to have higher body weights compared to broilers grown at higher stocking densities (Puron et al., 1995, Feddes et al., 2002, Dozier et al., 2005; Abudabos et al., 2013). In contrast, some studies have shown no stocking density effects on body weight (Bolton et al., 1972; Buijs et al., 2009). The present study is in agreement with the latter reports.

Abudabos et al. (2013) evaluated stocking density effects on female broiler production characteristics and stress as measured by blood characteristics and IRT surface temperatures of the head, neck, wing, body and shank. IRT surface temperatures were the lowest for birds raised at lower stocking densities. Stocking density differences due to lameness incidence differences may have affected surface temperatures of the eye, beak, hock, shank and foot, which were numerically lower on d 56 in exp 1 (**Table 4.4)** compared to d 55 in exp 2 (**Table 4.16**).

In the present studies, light intensity had no effects on broiler leg surface temperatures (**Tables 4.2, 4.3, 4.15, and 4.16**). Higher light intensities have been found to be associated with increased broiler activity (Newberry et al., 1988; Gordon and Tucker, 1996; Alvino et al., 2009; Rault et al., 2017). However no previous studies have compared broiler activity and body region surface temperatures.

Light intensity and flooring type had no effects on BCO lesion severity scores of the proximal femora and tibiae (**Tables 4.6 and 4.19**). Studies evaluating light intensity effect on broiler leg health have found no differences (Gordon and Tucker, 1996; Kristensen et al., 2006; Deep et al., 2010; Blachford et al., 2012). The results of this study follow these results where light intensity had minimal effect on lameness incidence in exp 1 (**Figure 4.8**) and no effect in exp 2 (**Figure 4.12**). There were flooring type effects on leg surface temperatures in exp 1 (**Table 4.3**) but not in exp 2 (**Table 4.16**). This may have been due to the inherent higher susceptibility and resultant higher incidence of lameness (52%) of the birds in exp 1 (**Figure 4.7**) compared to lower (14%) lameness incidence in exp 2 (**Figure 4.11**). In other words, 38% more birds developed lameness raised on wire flooring in exp 1 than in exp 2. This may have been due to inherent qualities of the chicks, seasonality, humidity, or bacterial communities present in the air, water or feed. *Staphlococcus aureus* has been identified as a dominant bacterium recovered from BCO lesions but the presence of *Staphlococcus aureus* pathogen does not always lead to infection of growth plates (McNamee et al., 1998; review: McNamee and Smyth, 2000).

At 30 d of age sound birds on wire flooring had higher shank and foot surface temperatures yet no difference in hock surface temperatures than birds on litter in exp 1 (**Table 4.3**). All birds had been previously raised on litter flooring and had only been in the wire flooring pens for about 24 h. In 2009, Wilcox and colleagues challenged laying hens by injecting *Staphlococcus aureus* in each metatarsal foot pad to induce bumblefoot. Thermal images of each bird's feet were taken for 7 d post-injection and the temperature difference from the middle toe across the metatarsal footpad was recorded. Foot temperature differences for hens with mildly clinical bumblefoot increased on d 1 and 2, then decreased to sound hen temperatures by d 7. The increased shank and foot temperatures on d 30 for birds raised on wire flooring may be due

to the acute inflammatory stress response of the shanks and feet to the increased mechanical torque and shear stress on the leg bones. Once birds became lame hock, shank and foot surface temperatures were lower compared to sound birds on wire flooring both at the individual level within the pen and at the pen level in exp 1 (**Tables 4.9 and 4.10**, respectively) and exp 2 (**Tables 4.22 and 4.23**, respectively). It should be noted that in exp 2, the shank and foot temperatures were not different for one pen out of the six wire flooring pens (**Table 4.22**). This may be due to the low incidence of lameness from the wire flooring imposition. The mean number of individuals that went lame in exp 1 was 24/ pen and 7/pen in exp 2 (data calculated from **Tables 4.9 and 4.22**, respectively).

Five body region surface temperatures were measured on sound birds at each age. It was assumed that body region surface temperatures may vary across the body and with age. That was the *raison d'etre* for the initial decision to measure IRT surface temperatures at five different body regions (eye, beak, hock, shank and foot). It was initially hypothesized that eye and beak temperature would be an indicator of bird welfare (Edgar et al., 2013; Moe et al., 2017) where changes in eye and beak temperature would indicate increases in stress. Eye, beak, hock, shank and foot surface temperatures tended to increase with age in both exp 1 (**Table 4.4**) and exp 2 (**Table 4.15**). It is interesting that higher/lower body region surface temperatures fluctuated with age and hock surface temperatures tended to increase in exp 1 (**Table 4.4**). Eye and beak surface temperatures were lower than leg region surface temperatures at older ages in exp 1 (**Table 4.5**) and exp 2 (**Table 4.17**). In exp 1, eye and beak surface temperatures of 56 d old broilers were lower for birds on wire flooring than those on litter (**Table 4.3; Figure 4.5**). Currently, there has been no research investigating the beak as a possible noninvasive measure of stress. IRT comb surface temperatures of broilers have been utilized to investigate stress-induced hypothermia,

where a perceived stressor shunts peripheral blood to increase core body temperature (Edgar et al, 2013). The combs of the broilers dropped 2°C in response to the handling treatment and eye temperature dropped initially, then overshot to temperatures higher than baseline. Other studies have reported increases in head region surface temperatures and decreases in footpad surface temperatures after handling stress (Moe et al., 2017). Although this response is attributed to acute stress, the chronic stressor of the wire flooring may have prolonged this phenomenon.

Ambient temperature of the environment is an important area of management in broilers as heat stress is detrimental to production. Sensible heat loss (amount of heat removed to maintain homeostatic core body temperature) is more efficient in featherless areas (Nääs et al., 2010). The data collected allowed the testing of the validity of the assumptions. The peripheral blood vasomotor response may cause vasoconstriction or vasodilation (Yahav and Giloh, 2012) to varying degrees of different body regions. In a 2012 heat stress study, Yahav and Giloh (2012) compared surface temperatures of broiler comb, wattle, face, leg (shank), toes, neck and whole body regions. Facial surface temperature exhibited the highest positive correlation ($R^2 = 0.83$) with core body temperature. Nääs et al. (2010) found a high positive correlation ($R^2 = 0.80$) between ambient temperature and broiler featherless regions. In exp 2, we found core body temperatures increased with age (**Table 4.17**) and obviously higher compared to body region surface temperatures (**Table 4.18**). Ambient air temperatures were not recorded during IRT image capture and may have influenced results, especially on d 55 in exp 2 during the summer season.

It was hypothesized that leg region surface temperatures would be lower in lame chickens. This was the case in both experiments both within (**Tables 4.7 and 4.22**) and across pens (**Tables 4.8 and 4.23**). The difference in sound versus lame bird leg surface temperatures

was the greatest in the foot in exp 1 (**Figure 4.9**) and exp 2 (**Figure 4.13**). Furthermore, foot surface temperatures were lower than shank and hock temperatures for lame and sound birds in both studies. Sound broilers have been reported to spend over 70% of their time lying and lame broilers (gait score > 3) have been reported to spend over 80% off their time lying (Weeks et al., 2000). Surprisingly, there were no light intensity or flooring type differences in FHN, THN or Total N lesion score severity within age for sound birds after the imposition of the wire flooring treatment in both exp 1 (**Table 4.6**) and exp 2 (**Table 4.19**). Previous research has also found FHN and THN BCO lesions in sound broilers raised on litter flooring (Wideman et al., 2012; Wideman et al, 2015). FHN, THN and Total N lesion score severity increased with age in exp 1 (**Figure 4.6**) and exp 2 (**Figure 4.10**). In a BCO review, McNamee (2000) reported the incidences of FHN attributed to BCO peak around 35 d of age.

Bacterial proliferation in BCO may be amplified by behavioral factors leading to lameness. There were differences in exp 1 and exp 2 hock, shank and foot surface temperatures for sound and lame birds categorized as having high or low FHN, THN and Total N BCO lesion severity. In exp 1, hock surface temperatures were higher for birds with high THN lesion severity compared to birds with low THN lesion severity (**Table 4.12**) regardless of if they were lame or sound. In exp 2 high FHN lesion severity caused hock and shank surface temperatures to be higher in sound and lame broilers (**Table 4.24**). The ischiadic artery is the major blood supply to the leg in avian species (Dzialowski and Dane, 2015). The ischiadic artery curves around the proximal femoral head, at the coxofemoral joint, and flows down to the knee-where a branch from an anastomosis with the femoral artery. The large proportion of time broilers spend sitting may create a pinch point at these two arterial locations, reducing the rate of blood supply to the legs (review: Wideman, 2016). Blood must travel the furthest distance to the foot, which may be

why foot surface temperatures were the lowest compared to hock and shank surface temperatures. These results are similar to the decreased surface temperatures of the footpad with increased footpad dermatitis scores (Jacob et al., 2016). The decrease in foot surface temperature could be related the increased tissue necrosis and decreased blood flow to the feet. The effect of increased heat on blood vessels to increase skin temperature may be a factor in the local regulation of blood flow to the skin (Richards, 1971). Perhaps there is early inflammation of the hock increases skin temperature while birds are going lame but once they are lame, blood flow reduces, and is exacerbated by bacterial proliferation and necrosis, to decrease leg surface temperature.

4.6 CONCLUSION

The results of these studies revealed no effects of light intensity on leg health, as measured by FHN and THN lesion scores attributed to BCO and minimal, transient effects on lameness incidence. Lameness incidence for broilers on wire flooring was over 3-fold higher in exp 1 than exp 2 and the measures in this study could not explain these differences. Due to the spontaneous nature of BCO lameness incidence, more research is warranted to elucidate predisposition factors leading BCO lameness. Compared to sound broilers, infrared thermography (IRT) surface temperatures of the eye, beak, hock, shank and foot body regions were lower for lame broilers. Therefore, IRT surface temperatures of broilers may be useful to detect lesions attributed to bacterial chondronecrosis with osteomyelitis (BCO) and predict the beginnings of necrosis to provide means of intervention to reduce lameness incidence.

4.7 REFERENCES

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Table 4.1. Effect of light intensity and flooring type on growth in broiler chickens. Data shown as mean body weight (BW) of chickens raised at 2, 5, or 10 lux light intensity[1] on litter or wire flooring[2] at 7, 9, 28, 30, 38, and 57-58 d of age.[3] Data in grams ± SEM (N=2 pens).

Floor (F)	**Litter**			**Wire**			**ANOVA p-value[4]**			
Light (L)	**2 lux**	**5 lux**	**10 lux**	**2 lux**	**5 lux**	**10 lux**	***All***	***L***	***F***	***L*F***
Age (d)										
7	170 ± 5.4	145 ± 21.8	188 ± 10.9	-	-	-	0.25	0.25	-	-
9	220 ± 12.0	212 ± 1.5	241 ± 11.0	-	-	-	0.23	0.23	-	-
28	1500 ± 30.0	1504 ± 26.0	1535 ± 7.0	-	-	-	0.57	0.57	-	-
30	1802 ± 2.7	1681 ± 13.6	1608 ± 46.3	1729 ± 20.0	1621 ± 50.8	1693 ± 69.9	0.11	0.05	0.25	0.18
38	2621 ± 11.0	2693 ± 93.0	2789 ± 9.0	2584 ± 54.0	2360 ± 104.0	2519 ± 55.0	0.04	0.23	0.70	0.14
57/58	4578 ± 72.0	4404 ± 94.0	4434 ± 32.0	3889 ± 143.0	3734 ± 12.0	4254 ± 252.0	0.02	0.18	0.01	0.16

[1]Light intensities in all pens were 20 lux from d 0 to 7 and 2, 5, or 10 lux from d 8 until the end of the trial.

[2]Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[3]Birds were euthanized and weighed after latency to lie (LTL) tests (described in Chapter 5) were conducted on d 57 and 58 of age.

[4]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 4.2. Effect of light intensity on surface temperatures (°C)[1] of the eye, beak, and the right and left hock, shank and foot. Broiler chickens were raised in pens with light intensities of 2, 5, or 10 lux.[2] Data shown are mean ± SEM (N=2 pens).

		Light intensity (lux)			ANOVA
		2	5	10	p-value
Age (d)	***Body region***				
7	Eye	34.8 ± 0.1[b]	34.7 ± 0.1[b]	35.1 ± 0.1[a]	0.04
	Beak	32.0 ± 0.1	31.5 ± 0.9	33.3 ± 0.6	0.24
	Hock	37.5 ± 0.0	36.4 ± 1.2	37.9 ± 0.3	0.32
	Shank	36.4 ± 0.0	35.2 ± 1.5	36.9 ± 0.5	0.50
	Foot	33.6 ± 0.1	32.7 ± 1.7	34.4 ± 0.9	0.60
9	Eye	34.1 ± 0.2	34.4 ± 0.2	34.7 ± 0.3	0.42
	Beak	29.7 ± 0.7	31.5 ± 1.3	31.5 ± 0.8	0.41
	Hock	36.6 ± 0.2	36.7 ± 0.2	37.1 ± 0.1	0.21
	Shank	35.4 ± 0.1	35.8 ± 0.2	36.0 ± 0.1	0.12
	Foot	29.8 ± 0.2	32.7 ± 0.5	31.9 ± 0.8	0.06
28	Eye	32.9 ± 0.1	32.7 ± 0.2	33.2 ± 0.1	0.15
	Beak	32.2 ± 0.9	33.2 ± 0.7	33.4 ± 0.4	0.51
	Hock	37.0 ± 0.0[c]	37.2 ± 0.0[b]	37.4 ± 0.0[a]	0.01
	Shank	36.5 ± 0.1	36.5 ± 0.2	37.1 ± 0.2	0.15
	Foot	32.6 ± 0.2	33.6 ± 0.4	33.7 ± 0.3	0.16

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial.

[abc] Means not sharing the same superscript letter across each row are different at $P \leq 0.05$.

Table 4.3. Effect of light intensity and flooring type on surface temperatures (°C)[1] of the eye, beak, and the left and right hock, shank, and foot of 30, 38, and 56 d old broiler raised in pens with light intensities of 2, 5, or 10 lux on litter or wire flooring.[2] Data are presented ± SEM (N = 2 pens).

Flooring (F)		**Litter**			**Wire**						
Light lux (L)		**2**	**5**	**10**	**2**	**5**	**10**	**ANOVA p-value[3]**			
Age (d)	***Body region***							***Main***	***L***	***F***	***L*F***
30	Eye	33.1 ± 0.3	33.6 ± 0.1	33.3 ± 0.2	33.0 ± 0.2	33.3 ± 0.2	32.9 ± 0.2	0.32	0.48	0.14	0.14
	Beak	33.5 ± 0.5	34.1 ± 0.4	33.4 ± 0.0	33.7 ± 1.0	33.9 ± 0.1	32.4 ± 0.5	0.41	0.50	0.48	0.20
	Hock	37.3 ± 0.2	37.9 ± 0.1	37.2 ± 0.1	37.6 ± 0.3	37.4 ± 0.3	37.6 ± 0.1	0.40	0.72	0.09	0.48
	Shank	36.4 ± 0.0	36.6 ± 0.1	36.9 ± 0.1	37.6 ± 0.4	37.3 ± 0.2	36.9 ± 0.2	0.04	0.93	0.01	0.08
	Foot	34.0 ± 0.5	34.0 ± 0.1	34.4 ± 0.3	36.4 ± 0.3	36.4 ± 0.2	35.3 ± 0.5	0.01	0.56	0.01	0.12
38	Eye	32.9 ± 0.1	33.0 ± 0.3	33.4 ± 0.2	33.4 ± 0.3	33.0 ± 0.2	32.6 ± 0.3	0.28	0.84	0.22	0.09
	Beak	33.7 ± 0.4	33.3 ± 0.7	34.0 ± 0.4	33.2 ± 0.5	33.2 ± 1.1	29.8 ± 1.4	0.10	0.24	0.64	0.11
	Hock	37.7 ± 0.0	36.5 ± 0.6	37.3 ± 0.4	37.9 ± 0.3	38.4 ± 0.1	37.5 ± 0.4	0.08	0.48	0.60	0.08
	Shank	36.8 ± 0.3	35.8 ± 0.6	36.9 ± 0.3	37.3 ± 0.3	37.9 ± 0.1	36.9 ± 0.6	0.13	0.87	0.37	0.11
	Foot	35.3 ± 0.6	33.2 ± 1.0	34.5 ± 1.0	36.0 ± 0.5	36.8 ± 0.4	33.8 ± 1.2	0.13	0.26	0.27	0.10
56	Eye	33.0 ± 0.2	33.3 ± 0.4	33.8 ± 0.3	31.7 ± 0.2	32.2 ± 0.1	32.5 ± 0.0	0.01	0.07	0.01	0.88
	Beak	35.2 ± 0.4[a]	35.0 ± 0.2[a]	35.5 ± 0.3[a]	32.7 ± 0.1[b]	30.1 ± 0.9[c]	33.3 ± 0.1[b]	0.01	0.01	0.01	0.04
	Hock	37.6 ± 0.0	37.5 ± 0.4	37.8 ± 0.2	37.6 ± 0.2	37.4 ± 0.1	37.3 ± 0.2	0.61	0.72	0.92	0.48
	Shank	37.3 ± 0.3	37.3 ± 0.6	37.6 ± 0.0	37.4 ± 0.2	36.4 ± 0.3	37.2 ± 0.1	0.30	0.28	0.75	0.34
	Foot	36.2 ± 0.3	36.5 ± 0.7	36.4 ± 0.2	34.3 ± 0.0	34.3 ± 0.8	36.2 ± 0.0	0.08	0.22	0.33	0.15

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.
[3]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).
[abc] Means not sharing the same superscript letter across each row are different.

Table 4.4. Effect of age on surface temperatures (°C).[1] Surface temperatures are for eye, beak, and the right and left hock, shank and foot at d 7, 9, 28, 30, 38 and 56 in broiler chickens. Birds were raised in pens with light intensities of 2, 5, or 10 lux and after d 29 were raised on litter or wire flooring.[2] Data Shown are mean ± SEM (N = 6 pens for d 7, 9 and 28; N = 12 pens for d 30, and 56).

	Age (d)						ANOVA p-value
	7	9	28	30	38	56	
Body region							
Eye	34.9 ± 0.1[a]	34.4 ± 0.2[a]	32.9 ± 0.1[b]	33.2 ± 0.1[bc]	33.1± 0.1[bc]	32.7 ± 0.2[c]	0.0001
Beak	32.3 ± 0.4[ab]	30.9 ± 0.6[b]	32.9 ± 0.4[a]	33.5 ± 0.2[a]	32.8 ± 0.5[a]	33.6 ± 0.6[a]	0.009
Hock	37.3 ± 0.4	36.8 ± 0.1	37.2 ± 0.1	37.5 ± 0.1	37.5 ± 0.2	37.5 ± 0.1	0.06
Shank	36.2 ± 0.5[bc]	35.7 ± 0.1[c]	36.7 ± 0.1[ab]	36.9 ± 0.1[a]	36.9± 0.2[a]	37.2 ± 0.1[a]	0.0006
Foot	33.6 ± 0.6[b]	31.4 ± 0.6[c]	33.3 ± 0.3[b]	35.1 ± 0.3[a]	34.9 ± 0.4[a]	35.8 ± 0.3[a]	0.0001

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.
[abc] Means not sharing the same superscript letter across each row are different.

Table 4.5. Effect of body region on surface temperatures. Body region surface temperatures (°C)[1] are shown for the eye, beak, and the right and left hock, shank and foot within 7, 9, 28, 30, 38 and 56 d of broiler chicken age. Birds were raised in pens with light intensities of 2, 5, or 10 lux and after d 29 on litter or wire flooring.[2] Data shown are mean ± SEM (N = 6 pens for d 7, 9 and 28; N = 12 pens for d 30, and 56).

Age (d)	**Body Region**					**ANOVA p-value**
	Eye	Beak	Hock	Shank	Foot	
7	34.9 ± 0.1^{b}	32.3 ± 0.4^{d}	37.3 ± 0.4^{a}	36.2 ± 0.5^{a}	33.6 ± 0.6^{c}	0.0001
9	34.4 ± 0.2^{b}	30.9 ± 0.6^{c}	36.8 ± 0.1^{a}	35.7 ± 0.1^{a}	31.4 ± 0.6^{c}	0.0001
28	32.9 ± 0.1^{b}	32.9 ± 0.4^{b}	37.2 ± 0.1^{a}	36.7 ± 0.1^{a}	33.3 ± 0.3^{b}	0.0001
30	33.2 ± 0.1^{c}	33.5 ± 0.2^{c}	37.5 ± 0.1^{a}	36.9 ± 0.1^{c}	35.1 ± 0.3^{b}	0.0001
38	33.1 ± 0.5^{c}	32.8 ± 0.5^{c}	37.5 ± 0.2^{a}	36.9 ± 0.2^{a}	34.9 ± 0.4^{b}	0.0001
56	32.7 ± 0.6^{c}	33.6 ± 0.6^{c}	37.5 ± 0.1^{a}	37.2 ± 0.1^{a}	35.8 ± 0.3^{b}	0.0001

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[abcd] Means not sharing the same superscript letter across each row are different at $P \leq 0.05$.

Table 4.6. Effect of light intensity and flooring type on lesion severities of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis [(Total N, (FHN+THN)][1] of 30, 38, and 56 d old broiler raised in pens with light intensities of 2, 5, or 10 lux on litter or wire flooring.[2] Data are presented ± SEM (N = 2 pens).

Flooring (F)		**Litter**			**Wire**						
Light lux (L)		**2**	**5**	**10**	**2**	**5**	**10**	**ANOVA p-value[3]**			
Age (d)	***Necrosis severity***							***Main***	***L***	***F***	***L*F***
30	FHN	0.5 ± 0.1	0.3 ± 0.3	0.9 ± 0.3	1.0 ± 0.4	1.2 ± 0.0	0.7 ± 0.3	0.31	0.98	0.24	0.20
	THN	1.2 ± 0.4	1.6 ± 0.4	1.4 ± 0.4	1.3 ± 0.5	1.7 ± 0.3	1.8 ± 0.2	0.84	0.55	0.86	0.90
	Total N	1.7 ± 0.5	1.9 ± 0.7	2.3 ± 0.7	2.3 ± 0.1	2.9 ± 0.3	2.5 ± 0.1	0.58	0.64	0.40	0.71
38	FHN	0.6 ± 0.0	0.5 ± 0.3	1.5 ± 0.3	1.0 ± 0.2	0.8 ± 0.0	1.0 ± 0.2	0.11	0.06	0.22	0.14
	THN	2.1 ± 0.1	1.7 ± 0.5	2.3 ± 0.1	2.1 ± 0.1	2.6 ± 0.0	1.9 ± 0.7	0.61	0.99	1.00	0.26
	Total N	2.7 ± 0.1	2.2 ± 0.8	3.8 ± 0.4	3.1 ± 0.3	3.4 ± 0.0	2.9 ± 0.5	0.29	0.46	0.54	0.13
57-58	FHN	3.1 ± 0.9	1.7 ± 0.1	2.9 ± 0.1	2.6 ± 1.2	1.5 ± 0.1	2.4 ± 0.6	0.52	0.21	0.61	0.97
	THN	2.8 ± 0.0	2.8 ± 0.4	3.5 ± 0.1	2.7 ± 0.1	3.3 ± 0.3	3.7 ± 0.3	0.11	0.03	0.78	0.51
	Total N	5.9 ± 0.9	4.5 ± 0.3	6.4 ± 0.2	5.3 ± 1.1	4.8 ± 0.2	6.1 ± 0.9	0.43	0.15	0.57	0.82

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

[2]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[3]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 4.7. Lesion severities of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis [(Total N, (FHN+THN)][1] for birds in each wire flooring pen (1 - 6) calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = # birds).

Pen		**1**	**2**	**3**	**4**	**5**	**6**
Necrosis	***Health Status***						
FHN							
	Sound	1.3 ± 0.4(15)[a]	0.9 ± 0.3(15)[a]	1.4 ± 0.5(15)[a]	2.1 ± 0.5(15)[a]	1.2 ± 0.3(15)[a]	1.3 ± 0.2(15)[a]
	Lame	4.1 ± 0.5(17)[b]	4.8 ± 0.3(25)[b]	4.6 ± 0.3(31)[b]	4.1 ± 0.3(20)[b]	4.1 ± 0.3(27)[b]	3.2 ± 0.3(24)[b]
THN							
	Sound	2.1 ± 0.2(15)[a]	2.2 ± 0.2(15)[a]	2.7 ± 0.3(15)[a]	1.9 ± 0.3(15)[a]	2.3 ± 0.3(15)[a]	2.7 ± 0.2(15)[a]
	Lame	3.4 ± 0.3(17)[b]	4.0 ± 0.1(25)[b]	3.6 ± 0.2(31)[b]	4.1 ± 0.2(20)[b]	3.7 ± 0.1(27)[b]	3.3 ± 0.2(24)[b]
Total N							
	Sound	3.4± 0.6(15)[a]	3.1 ± 0.4(15)[a]	4.3 ± 0.7(15)[a]	4.0 ± 0.7(15)[a]	3.5 ± 0.4(15)[a]	3.9 ± 0.6(15)[a]
	Lame	7.5 ± 0.6(17)[b]	8.8 ± 0.4(25)[b]	8.2 ± 0.4(31)[b]	8.2 ± 0.4(20)[b]	7.9 ± 0.4(27)[b]	6.5 ± 0.4(24)[b]

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[3]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.05$.

Table 4.8. Lesion severities of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis [(Total N, (FHN+THN)][1] for wire flooring pens of birds calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = 6 pens).

	Necrosis		
	FHN	THN	Total N
Health Status			
Sound	1.4 ± 0.2^{a}	2.3 ± 0.1^{a}	3.7 ± 0.2^{a}
Lame	4.2 ± 0.2^{b}	3.7 ± 0.1^{b}	7.9 ± 0.2^{b}

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[3]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.0001$

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Table 4.9. Surface temperatures (°C)[1] of the (mean right and left) hock, shank and foot for birds in each wire flooring pen (1 - 6) calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = # birds).

Pen		**1**	**2**	**3**	**4**	**5**	**6**
Leg region	***Health Status***						
Hock							
	Sound	36.9 ± 0.2(15)[a]	36.9 ± 0.4(15)[a]	37.2 ± 0.2(15)[a]	37.3 ± 0.5(15)[a]	37.1 ± 0.6(15)[a]	36.4 ± 0.6(15)[a]
	Lame	34.1 ± 0.5(17)[b]	33.7 ± 0.3(25)[b]	34.1 ± 0.4(31)[b]	33.4 ± 0.4(20)[b]	34.1 ± 0.4(27)[b]	34.1 ± 0.4(24)[b]
Shank							
	Sound	36.8 ± 0.2(15)[a]	37.1 ± 0.2(15)[a]	37.2 ± 0.1(15)[a]	37.7 ± 0.1(15)[a]	37.3 ± 0.4(15)[a]	37.1 ± 1.0(15)[a]
	Lame	34.4 ± 0.5(17)[b]	34.6 ± 0.3(25)[b]	34.4 ± 0.3(31)[b]	34.3 ± 0.4(20)[b]	34.7 ± 0.3(27)[b]	34.5 ± 0.5(24)[b]
Foot							
	Sound	34.9 ± 0.5(15)[a]	36.3 ± 0.4(15)[a]	36.1 ± 0.4(15)[a]	37.0 ± 0.6(15)[a]	36.8 ± 0.2(15)[a]	36.9 ± 0.2(15)[a]
	Lame	30.0 ± 0.8(17)[b]	30.2 ± 0.5(25)[b]	29.9 ± 0.4(31)[b]	29.6 ± 0.5(20)[b]	30.6 ± 0.6(27)[b]	30.1 ± 0.7(24)[b]

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.
[3]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.
[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.001$.

Table 4.10. Surface temperatures (°C)[1] of the (mean right and left) hock, shank and foot for wire flooring pens of birds calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = 6 pens).

	Leg region		
	Hock	Shank	Foot
Health Status			
Sound	37.0 ± 0.2[a]	37.2 ± 0.2[a]	36.3 ± 0.2[a]
Lame	34.0 ± 0.2[b]	34.5 ± 0.1[b]	30.1 ± 0.2[b]

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[3]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.0001$.

Table 4.11. Surface temperatures (°C)[1] of the (mean left and right) hock, shank and foot for broilers 29 – 57 d of age with either high or low proximal (mean left and right) femoral head necrosis (FHN)[2] lesion severity and/or lameness. The average surface temperatures (°C) were calculated for sound or cumulative lame[3] birds with or without severe FHN necrosis for each wire flooring pen. Data are presented as mean [combining data from pens, irrespective of the light intensity (2, 5 or 10 lux)[4] ± SEM (N = 6 pens].

Health status	Surface temperature		Status p value	FHN p value	Status*FHN p value
	Hock				
	Low Necrosis	***High Necrosis***			
Sound	37.0 ± 0.1[a]	37.1 ± 0.7[a]			
Lame	34.1 ± 0.3[b]	33.8 ± 0.1[b]			
			<0.0001	0.81	0.47
	Shank				
	Low Necrosis	***High Necrosis***			
Sound	37.2 ± 0.1[a]	37.1 ± 0.4[a]			
Lame	34.4 ± 0.2[b]	34.6 ± 0.1[b]			
			<0.0001	1.00	0.46
	Foot				
	Low Necrosis	***High Necrosis***			
Sound	36.3 ± 0.3[a]	35.7 ± 1.2[a]			
Lame	30.0 ± 0.3[b]	30.1 ± 0.2[b]			
			<0.0001	0.62	0.44

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6. Chickens with cumulative right and left proximal FHN scores ranging from 0-3 were categorized as having "low necrosis" and chickens with right and left proximal FHN scores ranging from 6-4 were categorized as having "high necrosis".
[3]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.
[4]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.
[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different.

Table 4.12. Surface temperatures (°C)[1] of the (mean left and right) hock, shank and foot for broilers 29 – 57 d of age with either high or low proximal (mean left and right) tibial head necrosis (THN)[2] lesion severity and/or lameness. The average surface temperatures (°C) were calculated for sound or cumulative lame[3] birds with or without severe FHN necrosis for each wire flooring pen. Data are presented as mean [combining data from pens, irrespective of the light intensity (2, 5 or 10 lux)[4] ± SEM (N = 6 pens].

Health status	Surface temperature		Status p value	THN p value	Status*THN p value
	Hock				
	Low Necrosis	***High Necrosis***			
Sound	37.0 ± 0.1[a]	36.6 ± 0.3[a]			
Lame	34.3 ± 0.4[b]	33.6 ± 0.1[b]			
			<0.0001	0.04	0.58
	Shank				
	Low Necrosis	***High Necrosis***			
Sound	37.2 ± 0.1[a]	37.1 ± 0.3[a]			
Lame	34.6 ± 0.2[b]	34.3 ± 0.1[b]			
			<0.0001	0.36	0.80
	Foot				
	Low Necrosis	***High Necrosis***			
Sound	36.2 ± 0.4[a]	36.6 ± 0.6[a]			
Lame	29.8 ± 0.6[b]	30.0 ± 0.2[b]			
			<0.0001	0.53	0.80

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]THN Tibial head necrosis. The right and left proximal tibial heads THN were scored on a 0-3 scale in the following categories: 0= no abnormalities of the proximal tibia (normal); 1= mild proximal tibial head necrosis (THN); 2= severe tibial head necrosis (THNS); and, 3= caseous THN (THNC). A quantitative THN category was created by summing the right (0-3) and left (0-3) proximal THN scores of increasing BCO lesion severity for a range of 0-6. Chickens with cumulative right and left proximal THN scores ranging from 0-3 were categorized as having "low necrosis" and chickens with right and left proximal THN scores ranging from 6-4 were categorized as having "high necrosis".

[3]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[4]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different.

Table 4.13. Effect of wire flooring on the surface temperatures (°C)[1] of the left and right hock, shank and foot on sound or cumulative lame[2] broilers 29 – 57 d of age with high or low Total N[3] lesion severity in pens with 2, 5 or 10 lux light intensity.[4] Data are presented ± SEM (N = 6 pens).

Health status	Surface temperature		Status p value	Total N p value	Status*Total N p value
	Hock				
	Low Necrosis	***High Necrosis***			
Sound	37.1 ± 0.1[a]	36.1 ± 0.6[a]			
Lame	33.9 ± 0.7[b]	33.8 ± 0.1[b]			
			<0.0001	0.26	0.36
	Shank				
	Low Necrosis	***High Necrosis***			
Sound	37.1 ± 0.1[a]	36.1 ± 0.3[a]			
Lame	33.9 ± 0.3[b]	33.8 ± 0.1[b]			
			<0.0001	0.60	0.26
	Foot				
	Low Necrosis	***High Necrosis***			
Sound	36.2 ± 0.4[a]	37.1 ± 0.4[a]			
Lame	29.7 ± 0.5[b]	30.0 ± 0.1[b]			
			<0.0001	0.11	0.47

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[3]Total N Total necrosis. A quantitative Total Necrosis (Total N) category was created by summing FHN and THN category scores of increasing BCO lesion severity for a range of 0-12. Chickens with a cumulative Total N score (left and right proximal FHN + left and right proximal THN) range of 0-5 categorized as having "low necrosis" were birds and "high necrosis" were birds with a cumulative Total N score range of 6-12.

[4]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different.

Table 4.14. Effect of light intensity and flooring type on growth in broiler chickens. Data shown as mean body weight (BW) [grams ± SEM (N=2 pens)] of chickens raised at 2, 5, or 10 lux light intensity[1] on litter or wire flooring[2] at 6, 8, 27, 29, 40, and 56-57 d of age.[3] Data in grams ± SEM (N=2 pens).

Floor (F)	**Litter**			**Wire**			**ANOVA p-value[4]**			
Light (L)	**2 lux**	**5 lux**	**10 lux**	**2 lux**	**5 lux**	**10 lux**	***All***	***L***	***F***	***L*F***
Age (d)										
6	151 ± 6.4	138 ± 1.8	148 ± 2.7	-	-	-	0.26	0.26	-	-
8	185 ± 7.0	178 ± 6.0	176 ± 10.0	-	-	-	0.72	0.72	-	-
27	1290 ± 84.0	1305 ± 87.0	1198 ± 98.0	-	-	-	0.69	0.69	-	-
29	1501 ± 131	1503 ± 51.0	1351 ± 9.0	1482 ± 58.0	1501 ± 7.0	1404 ± 78.0	0.56	0.22	0.85	0.87
40	2821 ± 11.0	2837 ± 133	2693 ± 94.0	2616 ± 167	2661 ± 67.0	2551 ± 161	0.53	0.33	0.48	0.90
56-57	4274 ± 194	4429 ± 69.0	4169 ± 261	3968 ± 24.0	4128 ± 64.0	3976 ± 70.0	0.30	0.38	0.18	0.90

[1]Light intensities in all pens were 20 lux from d 0 to 6 and 2, 5, or 10 lux from d 7 until the end of the trial.

[2]Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[3]Birds were euthanized and weighed after latency to lie (LTL) tests (described in Chapter 7) were conducted on d 56 and 57 of age.

[4]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 4.15. Effect of light intensity on core and body surface temperatures (°C)[1] of eye, beak, and the right and left hock, shank and foot of 6, 8, and 27 d old broilers. Broiler chickens were raised in pens with light intensities of 2, 5, or 10 lux.[2] Data shown are mean ± SEM (N=2 pens).

		Light intensity (lux)			ANOVA
		2	5	10	p-value
Age (d)	***Body region***				
6	Core	41.0 ± 0.1	41.1 ± 0.1	41.2 ± 0.0	0.48
	Eye	34.8 ± 0.4	35.1 ± 0.3	34.9 ± 0.2	0.83
	Beak	32.3 ± 0.5	33.2 ± 1.5	33.4 ± 1.2	0.80
	Hock	37.8 ± 0.1	37.8 ± 0.2	37.8 ± 0.4	0.99
	Shank	37.4 ± 0.3	37.3 ± 0.3	37.3 ± 0.5	0.96
	Foot	35.6 ± 0.5	35.3 ± 0.3	35.0 ± 0.9	0.83
8	Core	41.0 ± 0.1	41.0 ± 0.1	41.1 ± 0.1	0.53
	Eye	34.2 ± 0.6	34.1 ± 0.3	34.0 ± 0.4	0.91
	Beak	32.9 ± 1.0	32.3 ± 0.3	32.2 ± 0.0	0.72
	Hock	37.5 ± 0.5	37.1 ± 0.4	37.4 ± 0.3	0.73
	Shank	36.9 ± 0.6	36.9 ± 0.4	36.6 ± 0.2	0.86
	Foot	35.0 ± 1.3	34.7 ± 1.0	34.2 ± 0.5	0.86
27	Core	41.5 ± 0.0	41.4 ± 0.0	41.4 ± 0.1	0.34
	Eye	33.1 ± 0.4	32.8 ± 0.0	32.4 ± 0.9	0.67
	Beak	32.6 ± 0.1	32.2 ± 0.1	31.9 ± 2.1	0.93
	Hock	37.1 ± 0.1	36.9 ± 0.1	36.7 ± 0.4	0.50
	Shank	36.5 ± 0.0	36.2 ± 0.0	36.2 ± 0.6	0.78
	Foot	35.4 ± 0.1	35.4 ± 0.2	35.2 ± 0.9	0.89

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial.

Table 4.16. Effect of light intensity and flooring type on core and body surface temperatures (°C)[1] of the eye, beak, and the left and right hock, shank, and foot of 29, 40, and 55 d old broilers raised in pens with light intensities of 2, 5, or 10 lux on litter or wire flooring.[2] Data are presented ± SEM (N = 2 pens).

Flooring (F)		**Litter**			**Wire**						
Light lux (L)		**2**	**5**	**10**	**2**	**5**	**10**	**ANOVA p-value[3]**			
Age (d)	***Body region***							***Main***	***L***	***F***	***L*F***
29	Core	41.2 ± 0.2	41.5 ± 0.0	41.3 ± 0.0	41.5 ± 0.0	41.4 ± 0.1	41.4 ± 0.1	0.14	0.29	0.04	0.14
	Eye	33.8 ± 0.4	33.5 ± 0.2	33.9 ± 0.6	34.3 ± 0.5	33.8 ± 0.4	33.9 ± 0.3	0.84	0.56	0.47	0.84
	Beak	33.6 ± 0.2	32.9 ± 1.0	32.9 ± 0.8	34.1 ± 0.5	32.5 ± 1.2	34.4 ± 0.1	0.53	0.36	0.68	0.52
	Hock	37.6 ± 0.2	37.5 ± 0.2	37.2 ± 0.1	38.2 ± 0.1	37.7 ± 0.4	37.8 ± 0.2	0.14	0.20	0.09	0.60
	Shank	36.9 ± 0.2	36.8 ± 0.2	36.6 ± 0.2	37.5 ± 0.3	37.3 ± 0.6	37.6 ± 0.4	0.35	0.90	0.23	0.73
	Foot	34.4 ± 0.0	34.3 ± 0.8	34.0 ± 0.4	36.5 ± 0.3	36.4 ± 1.0	36.8 ± 0.6	0.06	0.97	0.06	0.81
40	Core	41.5 ± 0.0	41.5 ± 0.0	41.5 ± 0.1	41.6 ± 0.0	41.4 ± 0.0	41.3 ± 0.1	0.17	0.32	0.22	0.09
	Eye	34.2 ± 0.2	34.1 ± 0.2	33.9 ± 0.0	34.0 ± 0.2	33.9 ± 0.5	32.8 ± 0.4	0.13	0.11	0.79	0.31
	Beak	34.5 ± 0.3	34.3 ± 0.1	34.1 ± 0.5	34.3 ± 0.0	34.8 ± 0.2	32.9 ± 0.2	0.70	0.46	0.83	0.62
	Hock	37.5 ± 0.2	37.6 ± 0.3	37.4 ± 1.8	38.3 ± 0.3	38.3 ± 1.0	37.7 ± 1.0	0.18	0.34	0.07	0.58
	Shank	37.1 ± 0.5	37.2 ± 0.0	36.8 ± 0.8	38.0 ± 0.2	38.2 ± 0.5	37.3 ± 0.1	0.33	0.38	0.20	0.83
	Foot	35.0 ± 1.2	35.3 ± 0.0	34.9 ± 1.7	36.6 ± 0.7	37.0 ± 0.9	35.6 ± 0.5	0.62	0.66	0.31	0.85
55	Core	41.8 ± 0.3	42.0 ± 0.2	42.0 ± 0.2	41.6 ± 0.1	41.8 ± 0.3	42.1 ± 0.3	0.70	0.43	0.48	0.71
	Eye	35.4 ± 0.1	36.0 ± 0.5	35.8 ± 0.1	35.6 ± 0.1	35.4 ± 0.2	35.6 ± 0.0	0.51	0.58	0.46	0.30
	Beak	36.6 ± 0.7	37.3 ± 0.2	37.0 ± 0.2	36.4 ± 0.3	36.3 ± 0.4	37.0 ± 0.4	0.51	0.53	0.70	0.46
	Hock	38.6 ± 0.2	39.2 ± 0.1	39.0 ± 0.3	39.4 ± 0.1	39.3 ± 0.1	39.4 ± 0.4	0.30	0.51	0.06	0.42
	Shank	38.5 ± 0.4	38.8 ± 0.2	38.9 ± 0.1	38.9 ± 0.1	39.0 ± 0.1	39.4 ± 0.0	0.21	0.20	0.17	0.91
	Foot	37.9 ± 0.5	37.5 ± 0.7	38.1 ± 0.2	37.7 ± 0.0	38.1 ± 0.2	38.6 ± 0.2	0.50	0.35	0.72	0.53

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial. Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.
[3]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 4.17. Effect of age on surface temperatures (°C)[1] for the eye, beak, and the right and left hock, shank and foot of 6, 8, 27, 29, 40 and 55 d old broiler chickens. Birds were raised in pens with light intensities of 2, 5, or 10 lux and after d 28 were raised on litter or wire flooring.[2] Data Shown are mean ± SEM (N = 6 pens for d 6, 8 and 27; N = 12 pens for d 29, 40, and 55).

	Age (d) (#pens)						ANOVA p-value
	6	8	27	29	40	55	
Body region							
Core	41.1 ± 0.0^{c}	41.1 ± 0.0^{c}	41.4 ± 0.0^{b}	41.4 ± 0.0^{b}	41.5 ± 0.0^{b}	41.9 ± 0.1^{a}	0.0001
Eye	34.9 ± 0.1^{b}	34.1 ± 0.2^{c}	32.8 ± 0.3^{d}	33.9 ± 0.1^{c}	33.8 ± 0.2^{c}	35.6 ± 0.1^{a}	0.0001
Beak	33.6 ± 0.6^{b}	32.5 ± 0.3^{cd}	32.3 ± 0.6^{d}	33.4 ± 0.3^{bc}	34.1 ± 0.3^{b}	36.7 ± 0.2^{a}	0.0001
Hock	37.8 ± 0.1^{bc}	37.3 ± 0.2^{cd}	36.9 ± 0.1^{d}	37.7 ± 0.1^{bc}	37.8 ± 0.1^{b}	39.1 ± 0.1^{a}	0.0001
Shank	37.3 ± 0.2^{bc}	36.8 ± 0.2^{cd}	36.3 ± 0.2^{d}	37.1 ± 0.2^{bc}	37.4 ± 0.2^{b}	38.9 ± 0.1^{a}	0.0001
Foot	35.3 ± 0.3^{bc}	34.7 ± 0.5^{c}	32.7 ± 0.3^{d}	35.4 ± 0.4^{bc}	35.7 ± 0.4^{b}	38.0 ± 0.2^{a}	0.0001

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial. Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[abcd]Means not sharing the same superscript letter across each row are different.

Table 4.18. Effect of body region on surface temperatures (°C)[1] the of eye, beak, and the right and left hock, shank and foot within ages 6, 8, 27, 29, 40 and 55 d old broiler chickens. Birds were raised in pens with light intensities of 2, 5, or 10 lux and after d 28 on litter or wire flooring.[2] Data shown are mean ± SEM (N = 6 pens for d 6, 8 and 27; N = 12 pens for d 29, 40, and 55).

Age (d)	**Body Region**						**ANOVA p-value**
	Core	Eye	Beak	Hock	Shank	Foot	
6	41.1 ± 0.0^{a}	34.9 ± 0.1^{c}	33.6 ± 0.6^{d}	37.8 ± 0.1^{b}	37.3 ± 0.2^{b}	35.3 ± 0.3^{c}	0.0001
8	41.1 ± 0.0^{a}	34.1 ± 0.2^{c}	32.5 ± 0.3^{d}	37.3 ± 0.2^{b}	36.8 ± 0.2^{b}	34.7 ± 0.5^{c}	0.0001
27	41.4 ± 0.0^{a}	32.8 ± 0.3^{c}	32.3 ± 0.6^{c}	36.9 ± 0.1^{b}	36.3 ± 0.2^{b}	32.7 ± 0.3^{c}	0.0001
29	41.4 ± 0.0^{a}	33.9 ± 0.1^{d}	33.4 ± 0.3^{d}	37.7 ± 0.1^{b}	37.1 ± 0.2^{b}	35.4 ± 0.4^{c}	0.0001
40	41.5 ± 0.0^{a}	33.8 ± 0.2^{d}	34.1 ± 0.3^{d}	37.8 ± 0.1^{b}	37.4 ± 0.2^{b}	35.7 ± 0.4^{c}	0.0001
55	41.9 ± 0.1^{a}	35.6 ± 0.1^{e}	36.7 ± 0.2^{d}	39.1 ± 0.1^{b}	38.9 ± 0.1^{b}	37.9 ± 0.2^{c}	0.0001

[1]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial. Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.
[abcde] Means not sharing the same superscript letter across each row are different.

Table 4.19. Effect of light intensity and flooring type on lesion severities of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis [(Total N, FHN + THN)][1] of 29, 40, and 56-57 d old broiler raised in pens with light intensities of 2, 5, or 10 lux on litter or wire flooring.[2] Data are presented ± SEM (N = 2 pens).

Flooring (F)		**Litter**			**Wire**						
Light lux (L)		**2**	**5**	**10**	**2**	**5**	**10**	**ANOVA p-value[3]**			
Age (d)	***Necrosiss severity***							***Main***	***L***	***F***	***L*F***
29	FHN	0.4 ± 0.4	0.1 ± 0.5	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.68	0.30	1.00	0.89
	THN	1.8 ± 0.2	1.7 ± 0.5	1.7 ± 0.1	2.3 ± 0.5	2.1 ± 0.7	1.7 ± 0.7	0.92	0.79	0.51	0.87
	Total N	2.2 ± 0.6	1.8 ± 0.6	1.8 ± 0.0	2.7 ± 0.7	2.3 ± 0.5	1.7 ± 0.7	0.79	0.51	0.56	0.84
40	FHN	3.3 ± 0.3	1.3 ± 0.5	1.8 ± 0.0	2.7 ± 1.5	2.4 ± 1.2	1.4 ± 0.2	0.53	0.27	0.62	0.56
	THN	3.2 ± 0.2	3.0 ± 0.2	2.9 ± 0.1	3.0 ± 0.0	2.7 ± 0.1	2.7 ± 0.1	0.20	0.14	0.34	0.91
	Total N	6.5 ± 0.1	4.3 ± 0.3	4.7 ± 0.1	5.7 ± 1.5	5.1 ± 1.3	4.1 ± 0.1	0.40	0.17	0.52	0.60
56-57	FHN	2.8 ± 0.6	3.4 ± 0.8	2.8 ± 0.4	3.9 ± 0.1	3.0 ± 0.2	2.9 ± 0.1	0.52	0.56	0.14	0.31
	THN	2.7 ± 0.1	2.4 ± 0.0	2.7 ± 0.3	2.8 ± 0.2	2.4 ± 0.0	2.8 ± 0.2	0.43	0.14	0.70	0.95
	Total N	5.5 ± 0.7	5.8 ± 0.8	5.5 ± 0.1	6.7 ± 0.1	5.4 ± 0.2	5.7 ± 0.1	0.43	0.48	0.11	0.27

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6

[2]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial. Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[3]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 4.20 Lesion severities of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis [(Total N, (FHN+THN)][1] for birds in each wire flooring pen (1 - 6) calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = # birds).

Pen		**1**	**2**	**3**	**4**	**5**	**6**
Necrosis	***Health Status***						
FHN							
	Sound	1.5 ± 0.4(15)	1.3 ± 0.6(15)[a]	1.7 ± 0.6(15)	2.4 ± 0.6(15)	2.9 ± 0.7(15)	1.4 ± 0.6(15)
	Lame	2.8 ± 0.7(10)	4.1 ± 0.7(7)[b]	1.6 ± 0.6(7)	2.2 ± 0.2(5)	2.9 ± 0.7(6)	2.1 ± 0.8(8)
THN							
	Sound	2.2 ± 0.3(15)[a]	2.6 ± 0.2(15)	2.6 ± 0.3(15)	2.2 ± 0.2(15)	2.8 ± 0.2(15)	2.6 ± 0.3(15)
	Lame	3.2 ± 0.3(10)[b]	3.0 ± 0.2(7)	2.6 ± 0.4(7)	3.2 ± 0.6(5)	2.2 ± 0.3(6)	2.9 ± 0.4(8)
Total N							
	Sound	3.7 ± 0.6(15)[a]	3.9 ± 0.5(15)[a]	4.3 ± 0.7(15)	4.6 ± 0.7(15)	5.7 ± 0.7(15)	4.0 ± 0.7(15)
	Lame	6.0 ± 0.8(10)[b]	7.1 ± 0.9(7)[b]	4.1 ± 0.7(7)	5.4 ± 0.7(5)	5.5 ± 0.6(6)	5.0 ± 0.8(8)

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[3]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.05$.

Table 4.21 Lesion severities of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis [(Total N, (FHN+THN)][1] for wire flooring pens of birds calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = 6 pens).

	Necrosis		
	FHN	THN	Total N
Health Status			
Sound	1.9 ± 0.2[a]	2.5 ± 0.1[a]	4.4 ± 0.3[a]
Lame	2.7 ± 0.3[b]	2.9 ± 0.2[b]	5.6 ± 0.4[b]

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

[2]Sound birds were sampled on 29, 40 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.

[3]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial. Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.05$.

Table 4.22. Surface temperatures (°C)[1] of the eye, beak and (mean right and left) hock, shank and foot for birds in each wire flooring pen (1 - 6) calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = # birds).

Pen		**1**	**2**	**3**	**4**	**5**	**6**
Body region	***Health Status***						
Eye							
	Sound	33.9 ± 0.3(15)	34.7 ± 0.2(15)[a]	34.7 ± 0.3(15)[a]	34.0 ± 0.3(15)	34.6 ± 0.3(15)[a]	34.4 ± 0.3(15)[a]
	Lame	33.4 ± 0.4(10)	33.5 ± 0.3(7)[b]	33.6 ± 0.1(7)[b]	32.9 ± 0.7(5)	33.5 ± 0.3(6)[b]	33.5 ± 0.3(8)[b]
Beak							
	Sound	34.9 ± 0.4(15)[a]	35.4 ± 0.4(15)[a]	35.2 ± 0.4(15)[a]	33.7 ± 0.6(15)[a]	34.6 ± 0.5(15)	34.6 ± 0.7(15)
	Lame	32.4 ± 0.8(10)[b]	31.9 ± 1.3(7)[b]	31.6 ± 1.2(7)[b]	31.3 ± 0.9(5)[b]	33.3 ± 1.3(6)	32.8 ± 1.0(8)
Hock							
	Sound	38.0 ± 0.2(15)[a]	38.7 ± 0.2(15)[a]	38.7 ± 0.1(15)[a]	38.2 ± 0.2(15)[a]	38.3 ± 0.2(15)[a]	38.6 ± 0.3(15)[a]
	Lame	35.7 ± 0.4(10)[b]	36.3 ± 0.6(7)[b]	36.4 ± 0.3(7)[b]	35.5 ± 0.2(5)[b]	37.1 ± 0.5(6)[b]	36.5 ± 0.6(8)[b]
Shank							
	Sound	37.9 ± 0.3(15)[a]	38.5 ± 0.2(15)[a]	38.2 ± 0.2(15)[a]	37.8 ± 0.3(15)[a]	38.1 ± 0.3(15)	38.3 ± 0.3(15)[a]
	Lame	36.0 ± 0.4(10)[b]	36.5 ± 0.7(7)[b]	36.6 ± 0.5(7)[b]	36.2 ± 0.5(5)[b]	38.9 ± 0.6(6)	37.0 ± 0.5(8)[b]
Foot							
	Sound	37.0 ± 0.4(15)[a]	37.7 ± 0.2(15)[a]	36.8 ± 0.5(15)[a]	36.6 ± 0.5(15)[a]	37.0 ± 0.4(15)	37.0 ± 0.5(15)[a]
	Lame	33.2 ± 0.7(10)[b]	33.7 ± 1.0(7)[b]	33.9 ± 0.7(7)[b]	32.8 ± 1.0(5)[b]	35.0 ± 1.0(6)	34.7 ± 0.7(8)[b]

[1]Mean surface temperatures of the eye, beak and right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Sound birds were sampled on 29, 48 and 55 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 28-57 d of age.
[3]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.
[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P \leq 0.05$.

Table 4.23. Surface temperatures (°C)[1] of the (mean right and left) hock, shank and foot for wire flooring pens of birds calculated for sound or cumulative lame[2] broilers 29 – 57 d of age in pens with 2, 5 or 10 lux light intensity.[3] Data are presented ± SEM (N = 6 pens).

	Body region				
	Eye	Beak	Hock	Shank	Foot
Health Status					
Sound	34.4 ± 0.1[a]	34.7 ± 0.2[a]	38.5 ± 0.1[a]	38.1 ± 0.1[a]	37.0 ± 0.3[a]
Lame	33.4 ± 0.1[b]	32.3 ± 0.4[b]	36.2 ± 0.2[b]	36.5 ± 0.2[b]	33.9 ± 0.2[b]

[1]Mean surface temperatures of the eye, beak and right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]Sound birds were sampled on 29, 40 and 55 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 28-57 d of age.

[3]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.0001$.

Table 4.24. Surface temperatures (°C)[1] of the (mean left and right) hock, shank and foot for broilers 29 – 57 d of age with either high or low proximal (mean left and right) femoral head necrosis (FHN)[2] lesion severity and/or lameness. The average surface temperatures (°C) were calculated for sound or cumulative lame[3] birds with or without severe FHN necrosis for each wire flooring pen. Data are presented as mean [combining data from pens, irrespective of the light intensity (2, 5 or 10 lux)[4] ± SEM (N = 6 pens].

Health status	Surface temperature		Status p value	FHN p value	Status*FHN p value
	Hock				
	Low Necrosis	***High Necrosis***			
Sound	38.3 ± 0.1[a]	38.8 ± 0.2[a]			
Lame	36.1 ± 0.2[b]	36.6 ± 0.3[b]			
			<0.0001	0.02	0.82
	Shank				
	Low Necrosis	***High Necrosis***			
Sound	38.0 ± 0.1[a]	38.5 ± 0.2[a]			
Lame	36.3 ± 0.2[b]	36.8 ± 0.3[b]			
			<0.0001	0.03	0.93
	Foot				
	Low Necrosis	***High Necrosis***			
Sound	36.9 ± 0.2[a]	37.2 ± 0.4[a]			
Lame	33.7 ± 0.3[b]	34.3 ± 0.5[b]			
			<0.0001	0.24	0.58

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6. Chickens with cumulative right and left proximal FHN scores ranging from 0-3 were categorized as having "low necrosis" and chickens with right and left proximal FHN scores ranging from 6-4 were categorized as having "high necrosis".
[3]Sound birds were sampled on 29, 40 and 55 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 28-57 d of age.
[4]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.
[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.0001$.

Table 4.25. Surface temperatures (°C)[1] of the (mean left and right) hock, shank and foot for broilers 29 – 57 d of age with either high or low proximal (mean left and right) tibial head necrosis (THN)[2] lesion severity and/or lameness. The average surface temperatures (°C) were calculated for sound or cumulative lame[3] birds with or without severe FHN necrosis for each wire flooring pen. Data are presented as mean [combining data from pens, irrespective of the light intensity (2, 5 or 10 lux)[4] ± SEM (N = 6 pens].

Health status	Surface Temperature		Status p value	THN p value	Status*THN p value
	Hock				
	Low Necrosis	***High Necrosis***			
Sound	38.5 ± 0.3[a]	38.3 ± 0.2[a]			
Lame	36.4 ± 0.2[b]	35.8 ± 0.3[b]			
			<0.0001	0.06	0.35
	Shank				
	Low Necrosis	***High Necrosis***			
Sound	38.2 ± 0.1[a]	37.9 ± 0.3[a]			
Lame	36.5 ± 0.2[b]	36.3 ± 0.3[b]			
			<0.0001	0.30	0.88
	Foot				
	Low Necrosis	***High Necrosis***			
Sound	37.1 ± 0.2[a]	36.5 ± 0.5[a]			
Lame	33.9 ± 0.3[b]	33.7 ± 0.5[b]			
			<0.0001	0.28	0.55

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[2]THN Tibial head necrosis. The right and left proximal tibial heads THN were scored on a 0-3 scale in the following categories: 0= no abnormalities of the proximal tibia (normal); 1= mild proximal tibial head necrosis (THN); 2= severe tibial head necrosis (THNS); and, 3= caseous THN (THNC). A quantitative THN category was created by summing the right (0-3) and left (0-3) proximal THN scores of increasing BCO lesion severity for a range of 0-6. Chickens with cumulative right and left proximal THN scores ranging from 0-3 were categorized as having "low necrosis" and chickens with right and left proximal THN scores ranging from 6-4 were categorized as having "high necrosis".

[3]Sound birds were sampled on 29, 40 and 55 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 28-57 d of age.

[4]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.

[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.0001$.

Table 4.26. Effect of wire flooring on the surface temperatures (°C)[1] of the left and right hock, shank and foot on sound or cumulative lame[2] broilers 29 – 57 d of age with high or low Total N[3] lesion severity in pens with 2, 5 or 10 lux light intensity.[4] Data are presented ± SEM (N = 6 pens).

Health status	Surface Temperature		Status p value	Total N p value	Status*Total N p value
	Hock				
	Low Necrosis	***High Necrosis***			
Sound	38.3 ± 0.1[a]	38.8 ± 0.2[a]			
Lame	36.3 ± 0.2[b]	36.2 ± 0.2[b]			
			<0.0001	0.29	0.13
	Shank				
	Low Necrosis	***High Necrosis***			
Sound	38.0 ± 0.1[a]	38.5 ± 0.2[a]			
Lame	36.3 ± 0.2[b]	36.7 ± 0.2[b]			
			<0.0001	0.02	0.63
	Foot				
	Low Necrosis	***High Necrosis***			
Sound	36.9 ± 0.2[a]	37.3 ± 0.3[a]			
Lame	33.6 ± 0.4[b]	34.2 ± 0.4[b]			
			<0.0001	0.16	0.38

[1]Mean surface temperatures of the right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Sound birds were sampled on 29, 40 and 55 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.
[3]Total N Total necrosis. A quantitative Total Necrosis (Total N) category was created by summing FHN and THN category scores of increasing BCO lesion severity for a range of 0-12. Chickens with a cumulative Total N score (left and right proximal FHN + left and right proximal THN) range of 0-5 categorized as having "low necrosis" were birds and "high necrosis" were birds with a cumulative Total N score range of 6-12.
[4]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.
[ab] Means not sharing the same superscript letter within wire flooring pen columns for each leg region are different at $P < 0.0001$.

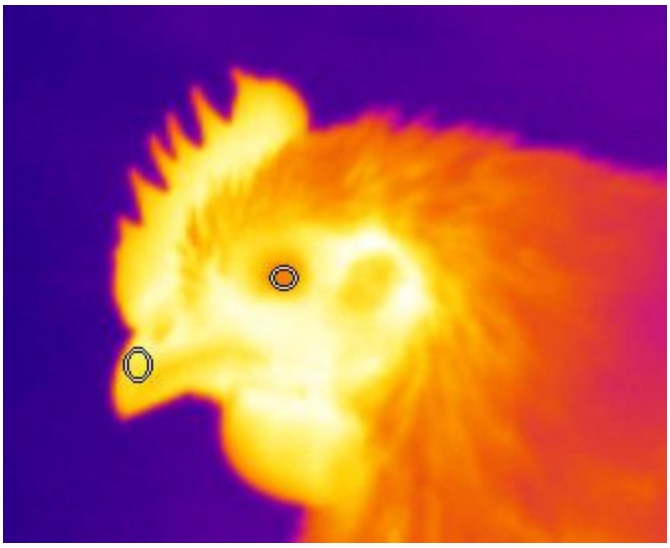


Figure 4.1. Pixels of the eye and beak were isolated (white circles) on the thermal image of the bird head for analysis.

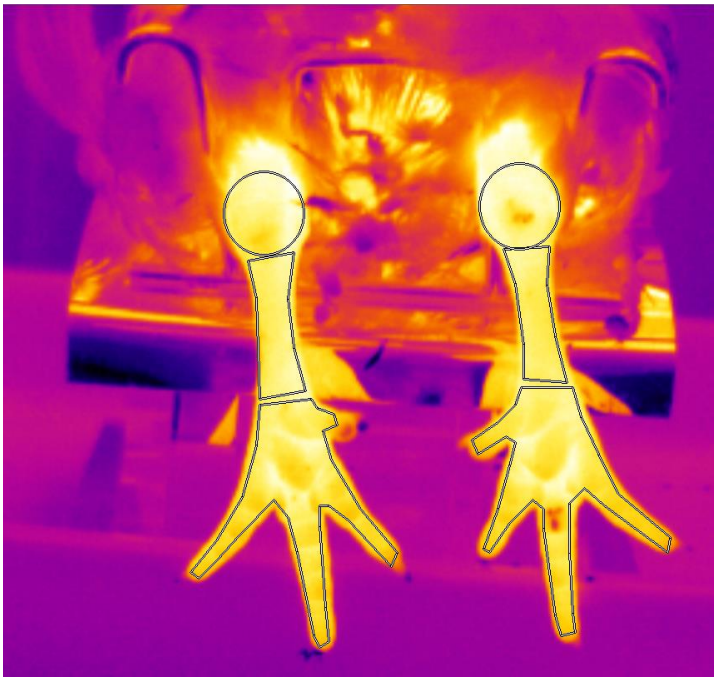


Figure 4.2. Pixels of the right and left hock joint, shank and foot were isolated (white shapes) on the thermal image for analysis.

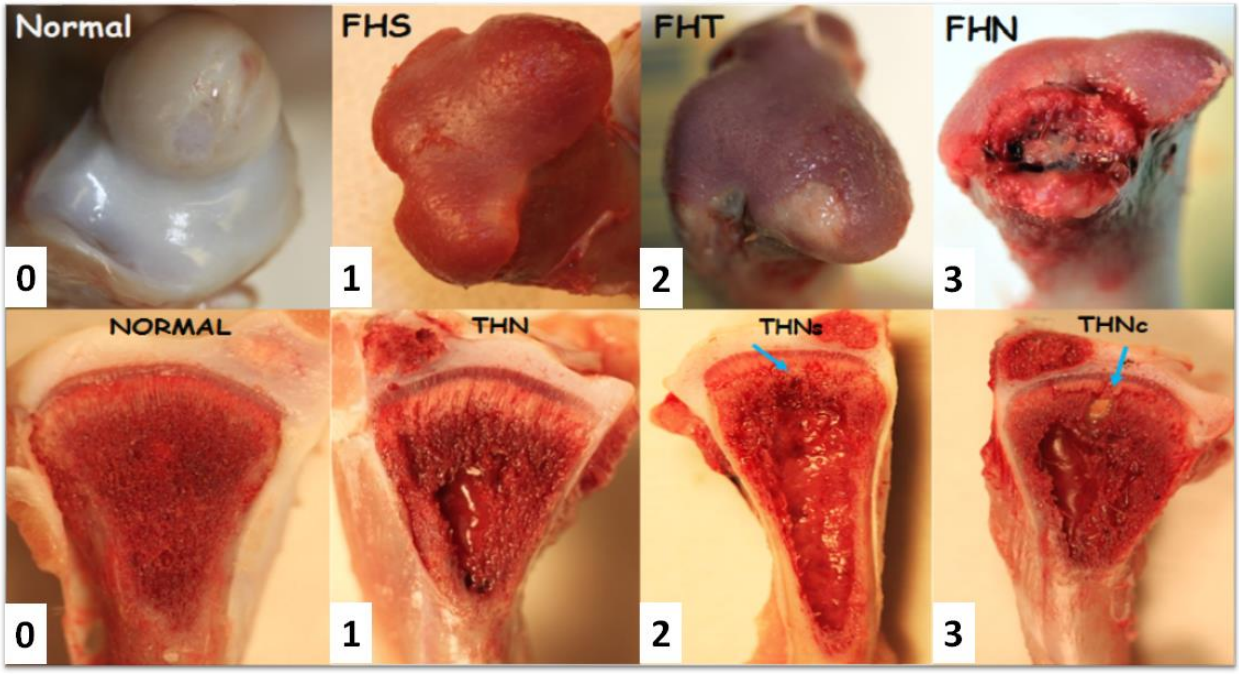


Figure 4.3.Stages of proximal femoral head necrosis (FHN) and proximal tibial head necrosis (THN) and scores (images from Wideman, 2014).

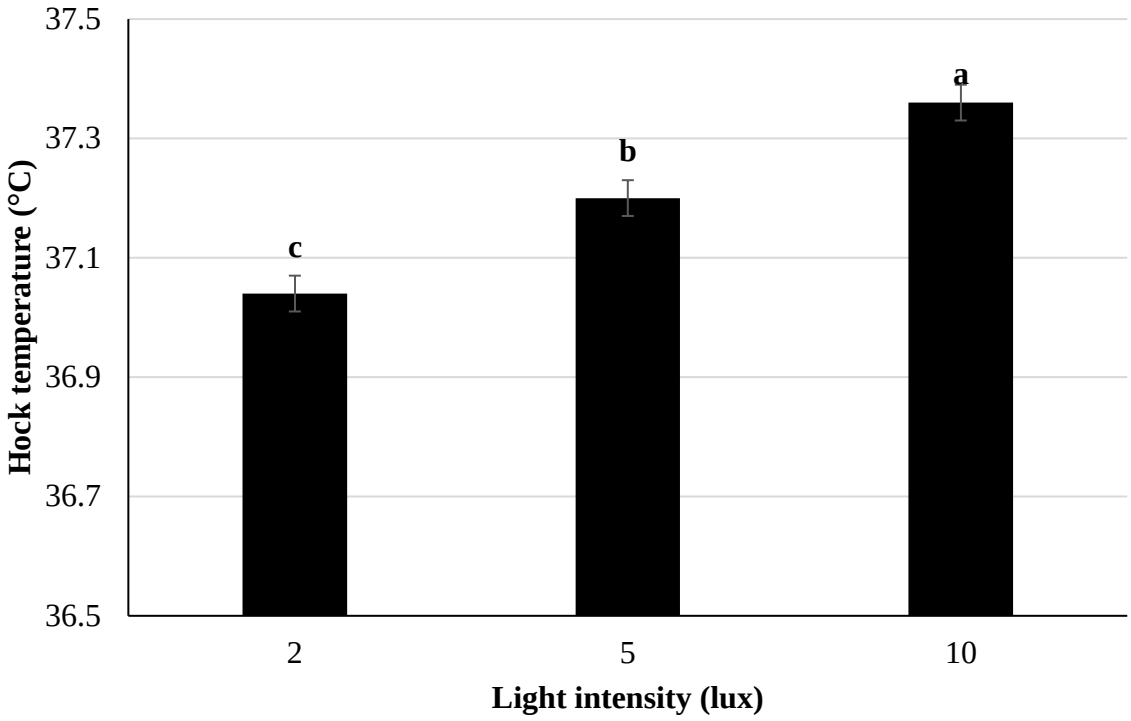


Figure 4.4. Effect of light intensity hock temperature (°C) of 28 d old broilers raised in pens at 2, 5, or 10 lux light intensity. Means with different superscripts differ significantly at $P \leq 0.05$.

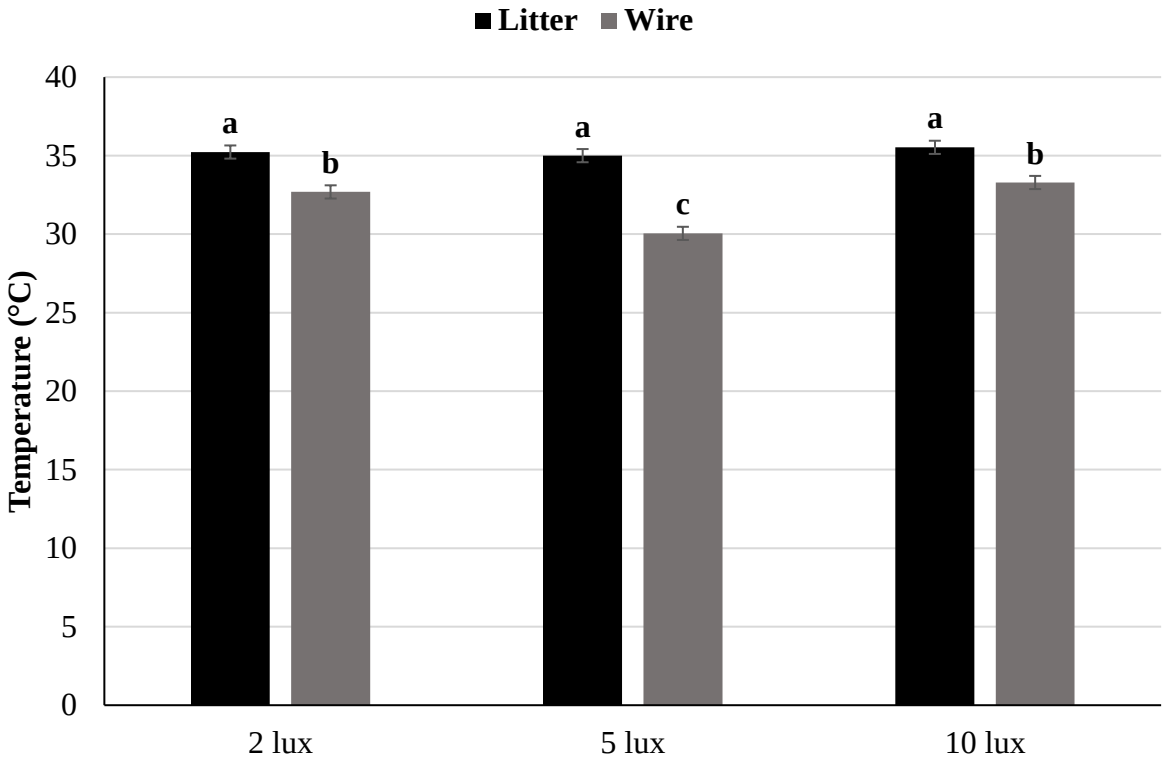
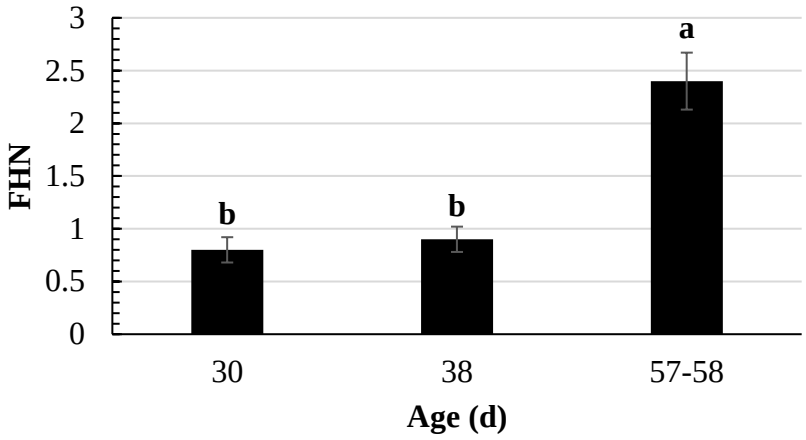
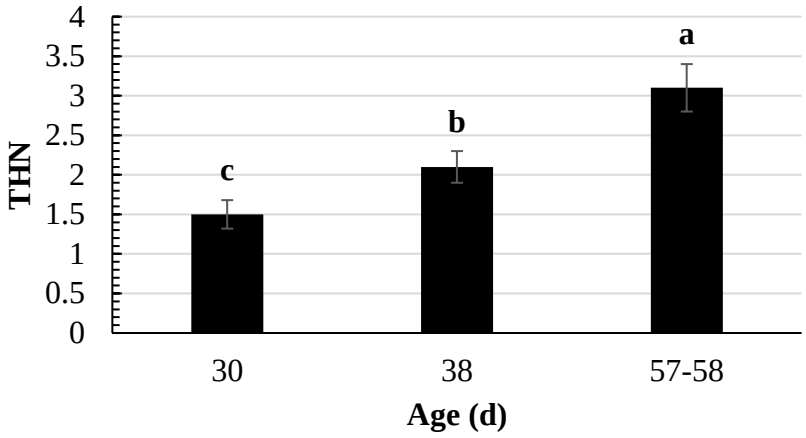


Figure 4.5. Effect of flooring type on beak surface temperatures (°C) of 56 d old broilers raised on litter or wire flooring and at 2 lux, 5 lux, or 10 lux light intensity. Means with different superscripts differ significantly at $P \leq 0.05$.

a)



b)



c)

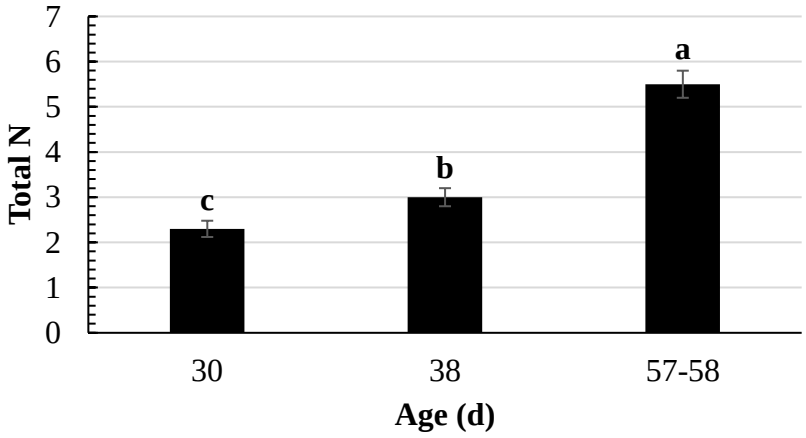


Figure 4.6. Lesion severity of a) femoral head necrosis (FHN), b) tibial head necrosis (THN) and c) total necrosis (FHN + THN, Total N) lesion severity scores for broilers 30, 38 and 57-58 d of age. (N = 12 pens).
[abc] Ages with different letters differ in lesion necrosis severity at $P \leq 0.05$.

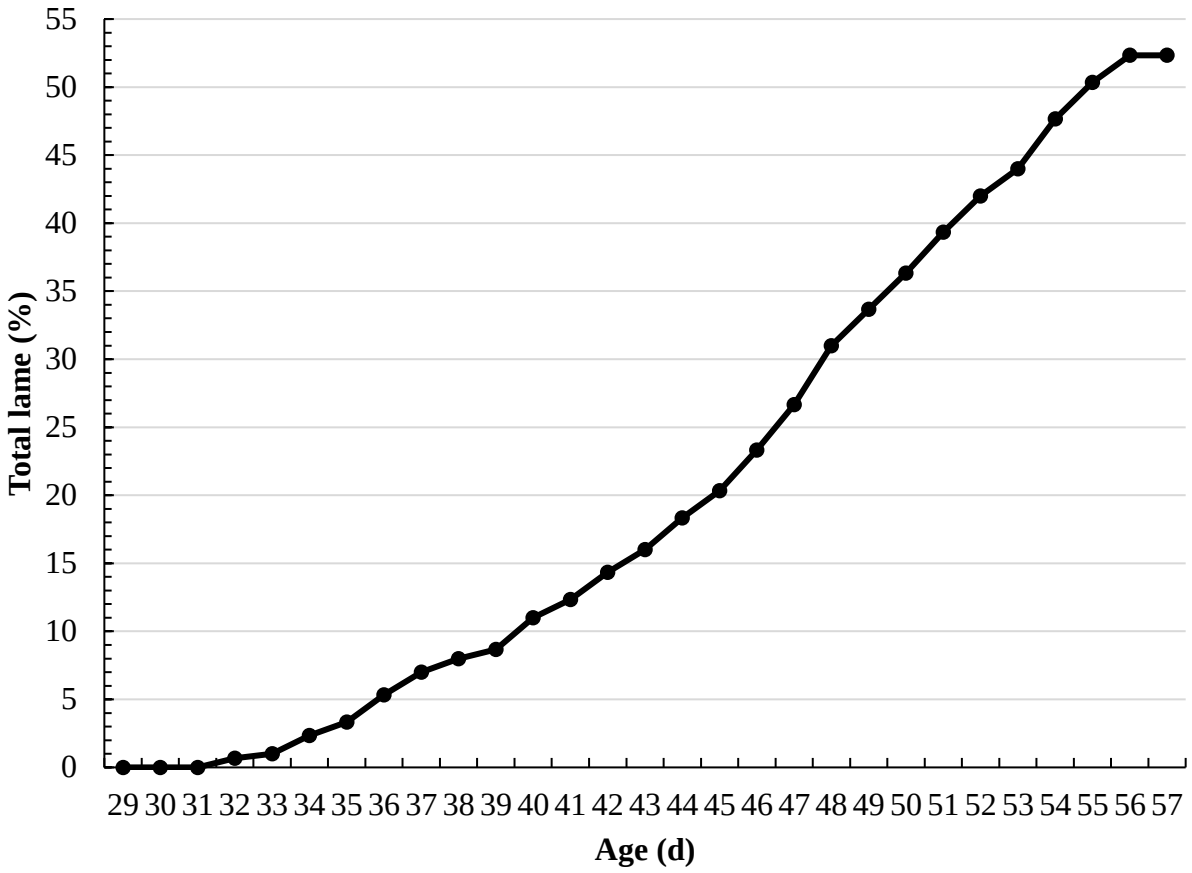


Figure 4.7. Total cumulative percentages broilers that developed lameness while raised on wire flooring from d 29 to 56 d of age.

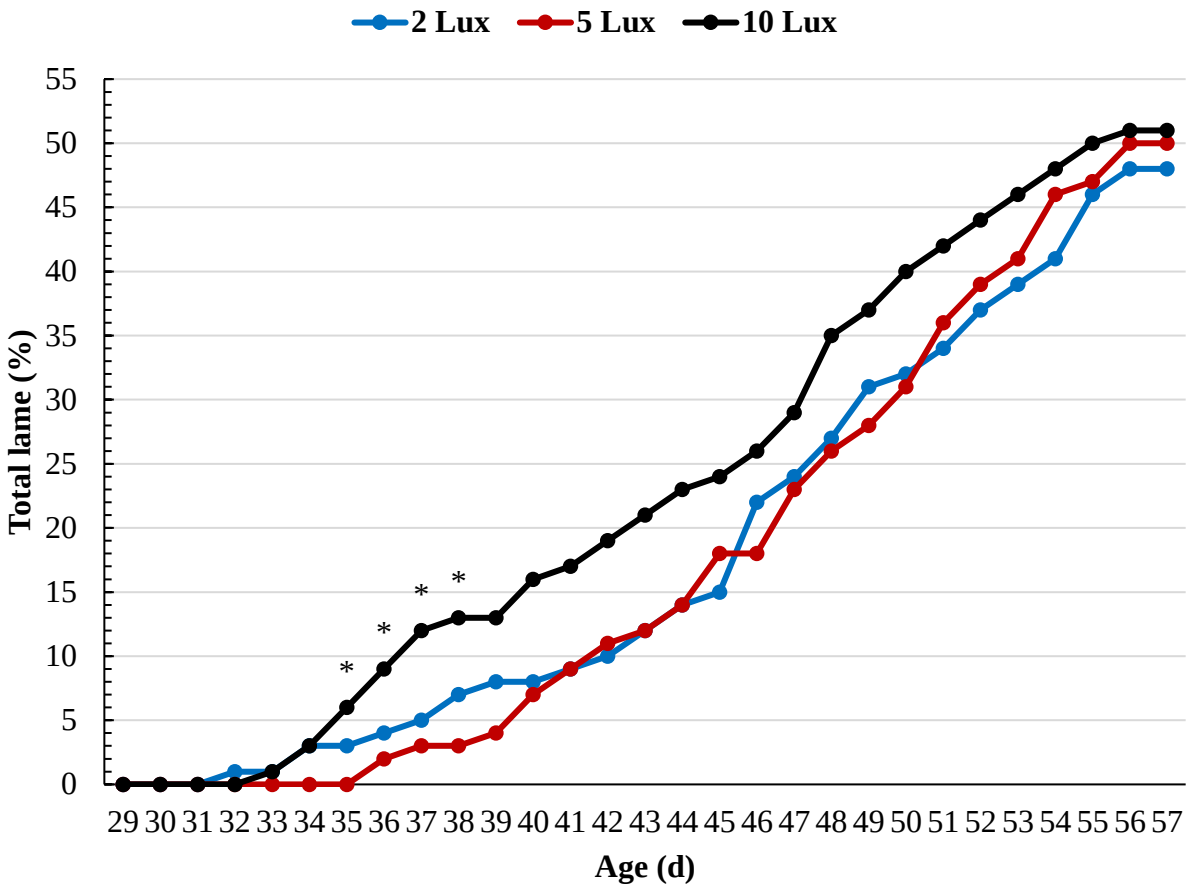


Figure 4.8. Cumulative percentages of lame broilers raised on wire flooring within each light intensity (2, 5 or 10 lux) from d 29 to do 56 of age. Asterisks indicate more birds raised at 10 lux went lame than birds raised at 2 or 5 lux at $P \leq 0.05$.

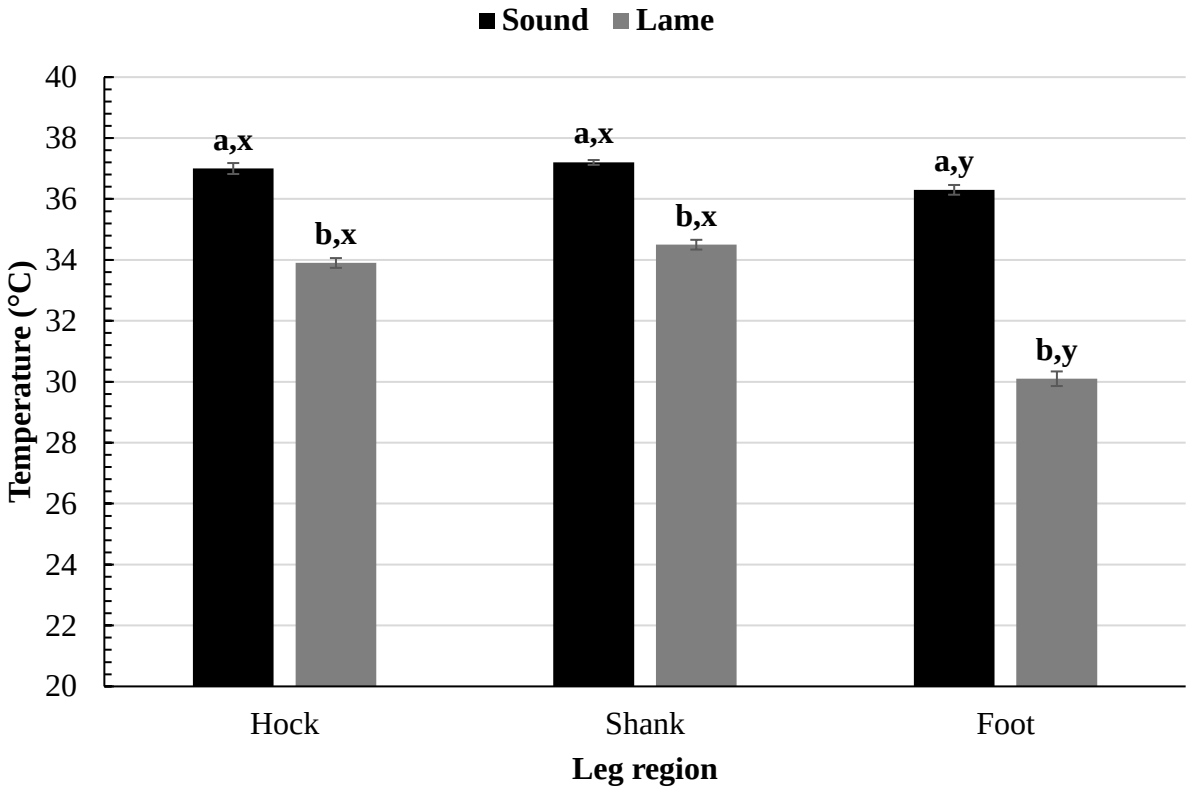
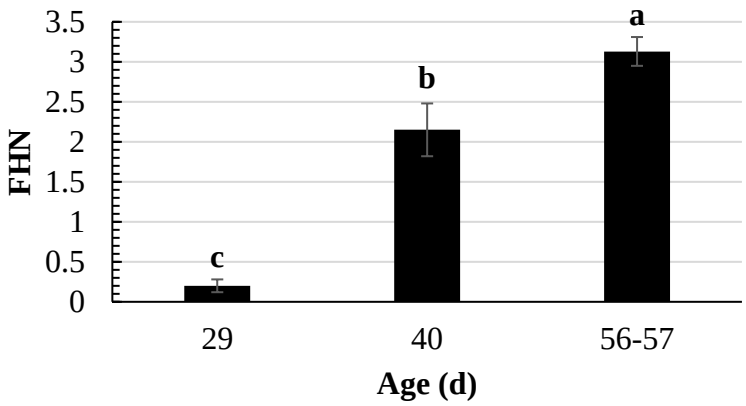


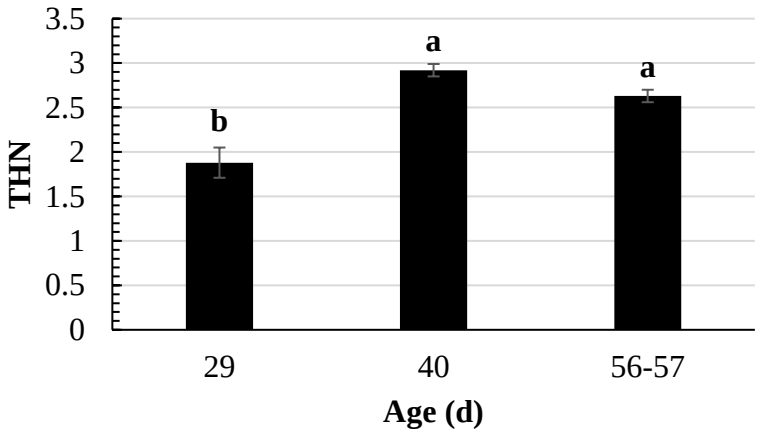
Figure 4.9. Effect of wire flooring on the surface temperatures (°C)1 of the right and left hock, shank and foot on sound or cumulative lame2 broiler chickens between 29 – 57 d of age. Birds were raised on wire flooring pens at 2, 5 or 10 lux light intensity.[3] The mean temperature (°C) for sound and lame birds was first calculated for each pen. Data are presented as a mean for each pen of sound and lame birds (N = 6 pens). Vertical bars indicate SEM.

[1]Leg hock, shank and foot surface temperatures were estimated by isolating pixels representing leg regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.
[3]There were no main effects of light intensity on the percentages of birds that went lame by the end of the trial or hock, shank and foot surface temperatures.
[ab] Differences between lame and sound are indicated by different superscript letters at $P < 0.0001$.
[xy] Differences between different leg regions (within sound or lame) are indicated by different superscript letters at $P \leq 0.05$.

a)



b)



c)

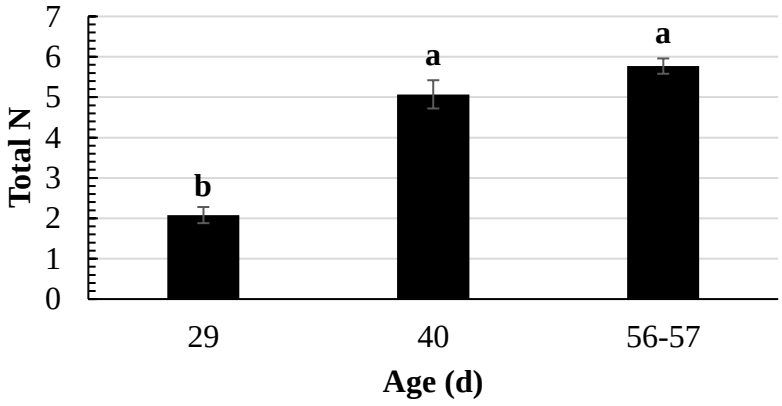


Figure 4.10. Lesion severity of a) femoral head necrosis (FHN), b) tibial head necrosis (THN) and c) total necrosis (FHN + THN, Total N) lesion severity scores for broilers 29, 40 and 56-57 d of age. (N = 12 pens). Ages with different letters differ in lesion necrosis severity at $P \leq 0.05$.

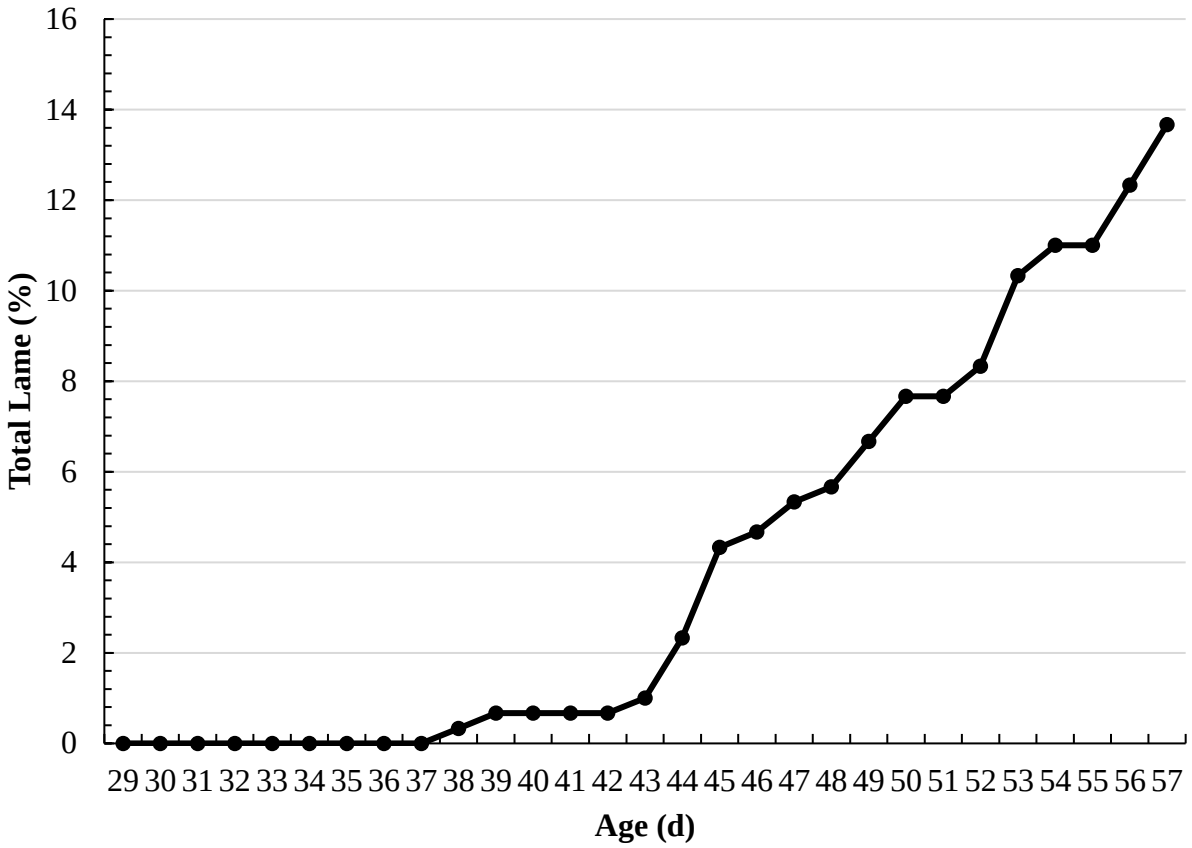


Figure 4.11. Total cumulative percentages of broilers that developed lameness while raised on wire flooring from d 29 to 57 d of age.

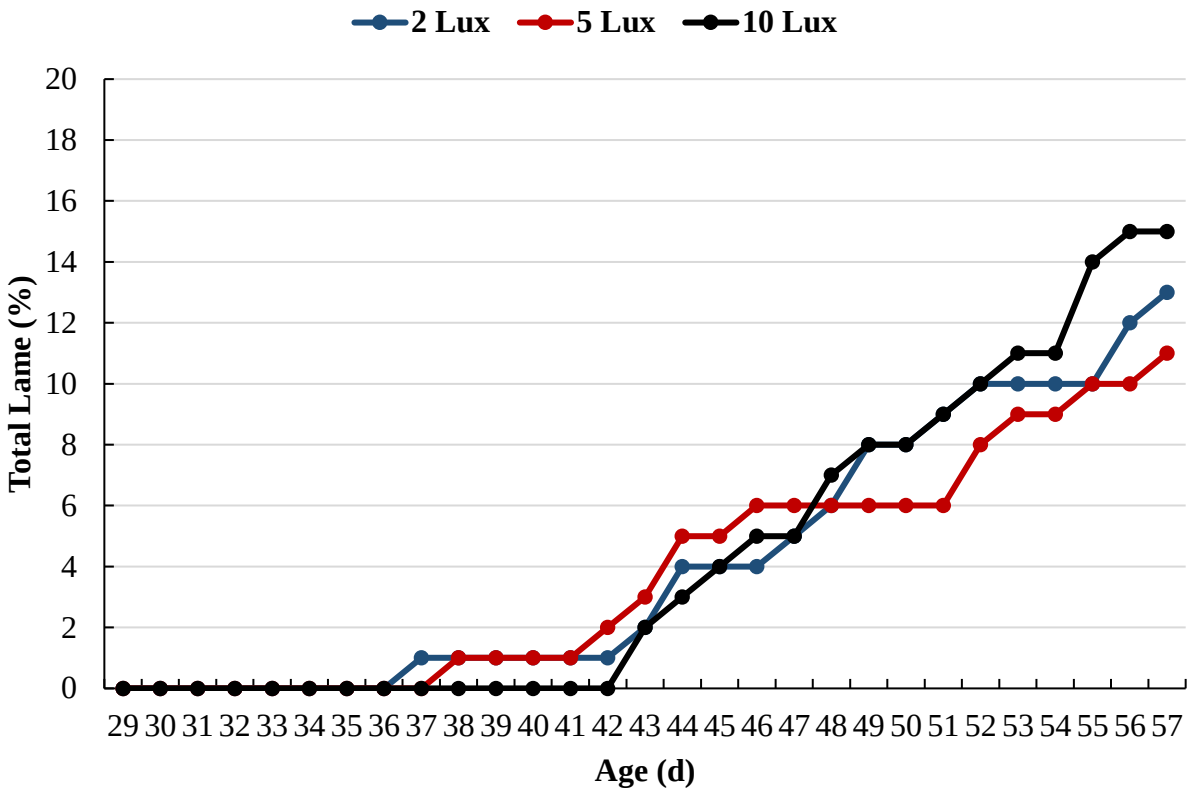


Figure 4.12. Cumulative percentages of lame broilers raised on wire flooring within each light intensity (2, 5 or 10 lux) from d 29 to do 57 of age.

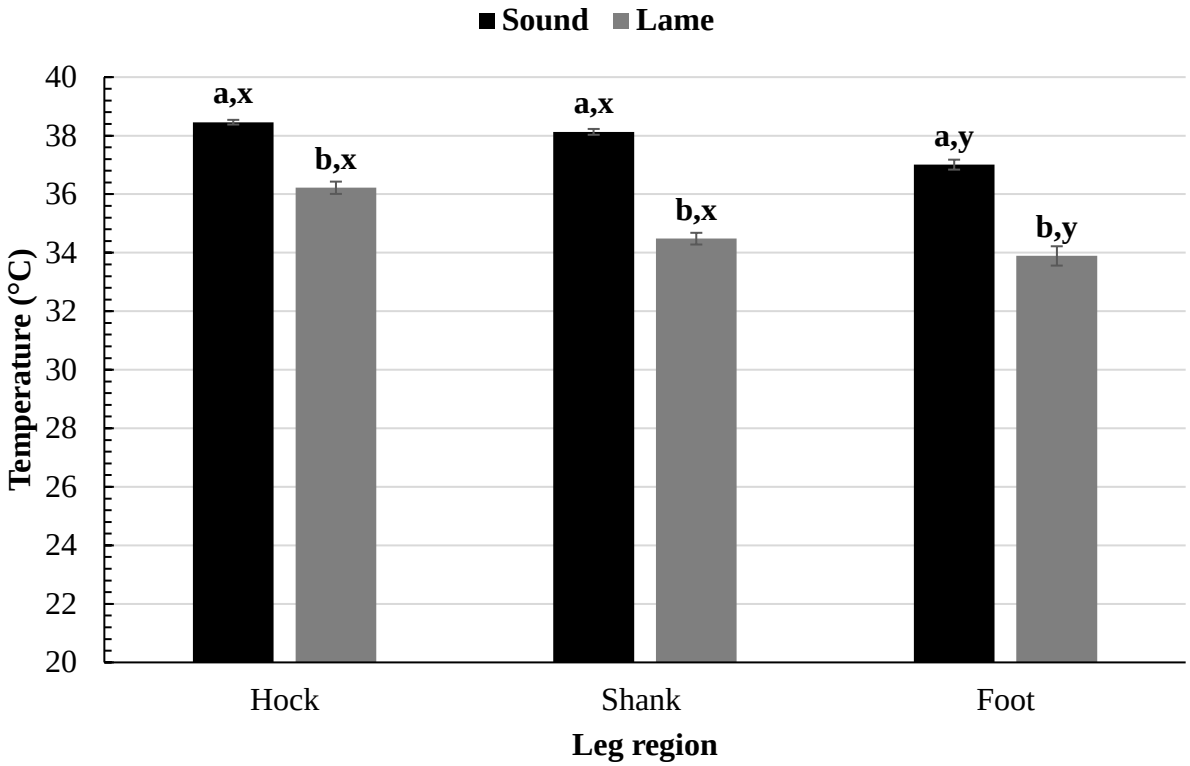


Figure 4.13. Effect of wire flooring on the surface temperatures (°C)[1] of the right and left hock, shank and foot on sound or cumulative lame[2] broiler chickens between 29 – 57 d of age. Birds were raised on wire flooring pens at 2, 5 or 10 lux light intensity.[3] The mean temperature (°C) for sound and lame birds was first calculated for each pen. Data are presented as a mean for each pen of sound and lame birds (N = 6 pens). Vertical bars indicate SEM.

[1]Leg hock, shank and foot surface temperatures were estimated by isolating pixels representing leg regions within infrared thermography (IRT) images taken with a thermal camera.
[2]Sound birds were sampled on 30, 38 and 56 d of age and were able to walk without difficulty. Lame birds were immobile upon gentle coaxing to walk and were sampled daily from 29-57 d of age.
[3]There were no main effects of light intensity on the percentages of birds that went lame or hock, shank and foot surface temperatures.
[ab] Differences between lame and sound are indicated by different superscript letters at $P < 0.0001$.
[xy] Differences between different leg regions (within sound or lame) are indicated by different superscript letters at $P \leq 0.05$.

CHAPTER 5 Effect of light intensity and flooring type on classical and noninvasive measures of stress and behaviors related to leg health of broilers

5.1 ABSTRACT

Stress is detrimental to broiler health, production, physiology and welfare. A classical method to evaluate stress is measuring circulating concentrations of corticosterone (CORT). However, the handling, restraint and invasive nature of blood sampling is stressful to the bird. This study examined the effects of light intensity (2 lux, 5 lux and 10 lux) and flooring (litter vs. wire) on: 1) "classical", commonly used methods for assessing level of stress (H:L ratio and serum corticosterone (sCORT) concentrations), 2) noninvasive measures of stress (feather corticosterone (fCORT) concentrations, eye surface temperatures and beak surface temperatures); and, 3) latency to lie (LTL) test behavioral differences in groups (LTLG) and individually (LTLI). Two studies (exp 1 and exp 2) were conducted on Cobb 500 byproduct males raised in pens within environmentally controlled chambers having one of two flooring types and one of the three light intensities. Light intensity treatments were applied to six pens at 1 wk of age and floor treatments were applied at 4 wk of age. On d 7, 9, 28, 30, 38, and 56-58 in exp 1 and d 6, 8, 27, 29, 40, and 55-57 in exp 2, birds had a blood sample taken for heterophil to lymphocyte (H:L) ratio, serum CORT (sCORT), and feathers for feather CORT (fCORT) determination. At 8 wk of age, broilers were subjected to a latency to lie (LTL) test individually (LTLI) and in groups of five (LTLG). There were minimal effects of light intensity or flooring type on stress and behavioral measures. While H:L ratios tended to increase ($P < 0.007$) with age, sCORT concentrations decreased with age ($P < 0.0001$). There were negative correlations between sCORT concentrations with LTLG and LTLI ($P < 0.05$) times in exp 1. However, these relationships were not seen in exp 2. There were marked correlations between H:L and sCORT with surface temperatures. Most apparent were the moderate the negative correlations of eye and, more distinct, beak surface temperatures with H:L and sCORT stress measures. The results of this study contribute to understanding and evaluating stress in broiler chickens.

5.2 INTRODUCTION

Stress can be defined as "*the biological response elicited when an individual perceives a threat to its homeostasis*" (review: Moberg, 2000). Stress is a broad term contextually used to describe stimuli challenging homeostasis, emergency responses and state of physiological imbalance (review: Siegel, 1995; Blas, 2015). Appropriate response to a stressor is essential for survival and malfunction of the stress response leads to decreased welfare (Blas, 2015). Selye referred to the stress response as the "*General Adaptation Syndrome*" where all major vital organs within an individual respond to stress by supplying energy to adapt to their environment (Selye, 1950). Chronic stress has a detrimental impact on broiler chicken production (review: Siegel, 1995; review: Virden and Kidd, 2009). Integrated methods of assessment are required to collect the data necessary for evaluating conditions that may be stressful for the animal. These assessments include measures of performance, behavior and physiological responses (review: Siegel, 1995; review: Moberg, 2000). Established measures of broiler performance include body weight gain, feed conversion ratio and growth rate. However, physiological and behavioral measures of broiler stress are not definitively established or repeatable for determining cause and effect relationships (Manning et al., 2007). Behavioral evaluations by visual examination can be biased and the large variation inherent to measures of physiological stress measures can be misleading (Post et al., 2003; Stewart et al., 2005).

Environmental stressors elicit a cascade of biochemical responses, resulting in the release of corticosterone (CORT; review: Siegel, 1995; Blas, 2015; Carsia, 2015). The hypothalamo-pituitary-adrenal (HPA) axis is the primary organ system mediating the stress response and CORT regulation (review: Siegel, 1995). As CORT concentrations increase, the proportions of heterophils to lymphocytes (H:L ratio) increase and circulating concentrations of cholesterol,

glucose also increase (Siegel, 1980; Puvadolpirod, 1997; Shini et al., 2009; review: Virden and Kidd, 2009). Blood plasma and/or sera are the most widely used samples to assay CORT in animal stress and welfare studies (review: Mormède et al., 2007). The physiological measures commonly employed to evaluate environmental effects on animals often include animal restraint and invasive techniques to collect blood and tissue samples. The additional stress of restraint and pain may affect stress measures and result in misleading interpretations of welfare status (review: Mormède et al., 2007; Chloupek et al., 2011).

Light intensity is an environmental factor that may affect broiler behavior, production and welfare (Newberry et al., 1988; review: Olanrewaju et al., 2006; Deep et al., 2010; Deep et al., 2013; Rault et al., 2017). Traditionally, lower light intensities have been shown to improve production performance, feed conversion (Lien et al., 2008) and body weight gain (Gross and Siegel, 1981; Lien et al., 2008) as well as behavioral benefits such as decreased aggression and cannibalism (Leeson and Walsh, 2004). However, there is no evidence that light intensity affects stress as measured by H:L ratio (Lien et al., 2007) or circulating concentrations of CORT (Olanrewaju et al., 2008; Olanrewaju et al., 2010; Olanrewaju et al., 2013; Olanrewaju et al., 2014; Rault et al., 2017).

Light intensity may also affect an individual's activity, time spent walking and leg health (Newberry et al., 1988; Alvino et al., 2009; Deep et al., 2012;). The latency to lie (LTL) test is method of measuring leg strength in broilers. The LTL test measures the amount of time it takes for a bird to sit after being placed in a standing position a pen of tepid water. Previous research has found relationships between gait score and LTL times (Weeks et al., 2002; Berg and Sanotra, 2003; Aydin et al., 2015) while others have not (Webster et al., 2008). The LTL quantitative time

measure is an advantage to gait scoring by eliminating inherent subjectivity gait scoring evaluations (Weeks et al., 2002). The LTL test is an indicator of leg strength, however the environment in which the LTL test is conducted may influence results. For instance, LTL times may be affected by the presence or absence of other birds during the test. Previous research has conducted the LTL test in groups (Weeks et al., 2002) and individually (Berg and Sanotra, 2003; Webster et al., 2008; Rault et al., 2017) yet no previous work has tested birds both individually and in groups to determine if other birds influence LTL times.

Concentrations of CORT in avian feathers (fCORT) have been utilized as a noninvasive sampling methodology (Bortolotti et al., 2008; Bortolotti et al., 2010; Fairhurst et al., 2011) to evaluate avian stress. Circulating CORT concentrations indicate a "snapshot" of acute stress at the time of collection whereas feather CORT concentrations are thought to indicate stress throughout the growth of the feather measured (Bortolotti et al., 2008). Given that the feather (largely keratin) becomes inert when fully developed, feathers can be painlessly clipped for sample collection (Bortolotti, 2010). However, it is unclear how quickly CORT is deposited in the feather and how well feather deposition is related to with circulating concentrations of CORT. Reports of CORT concentrations in feathers for wild birds (Bortolotti et al., 2008, Faihurst et al., 2011) and chickens (Carbajal et al., 2014) indicate this method may provide a noninvasive approach with potential for meeting the requirements for routine use. Chapters 2 and 3 examined the effect of CORT provided in the drinking water (20 mg/L) on two types of feather CORT concentrations on 4 wk old broiler chickens over a 72 h period. The results of these studies show marked increases in fCORT concentrations for CORT treated birds over time.

Broilers in this chapter were raised on wire flooring to experimentally induce bacterial chondronecrosis with osteomyelitis (BCO) and to compare stress and behavior measures to broilers raised on litter flooring. Raising broilers on wire flooring is reported to be a possible stressor in itself, yielding negative physiological and behavioral consequences of lameness (Wideman et al., 2012; Wideman et al., 2013; Wideman, 2016). Depriving broilers access to litter substrate can increase stress (El-Lethey et al., 2003) in an external manner, as opposed to endocrine interference (Puvadolpirod and Thaxton, 2000; El-Lethey et al., 2003). Stress-mediated immunosuppression has been reported to contribute to the pathogenesis of BCO of the proximal femoral and tibial heads (Wideman et al., 2013).

This study examined the effects of light intensity (2 lux, 5 lux and 10 lux) and flooring (litter versus wire) on the following: 1) "classical", commonly used methods for assessing level of stress (H:L ratio and serum corticosterone (sCORT) concentrations); 2) putative non-invasive measures of stress (feather corticosterone (fCORT) concentrations, eye surface temperatures (ET) and beak surface temperatures (BT) of broiler stress; and, 3) the behavioral differences in LTL when birds were tested in groups (LTLG) and individually (LTLI). In addition, measures of stress were correlated with performance and surface temperature indices reported in Chapter 4.

5.3 MATERIALS AND METHODS

5.3.1 Animals and Facility

Male Cobb 500 byproduct broiler chicks (N = 720) were obtained from a commercial hatchery (Cobb, Fayetteville, AR). They were randomly placed into six 1.5 m wide x 3 m long pens (120 per pen) within environmental chambers in the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm (IACUC # 16014). Birds had

access to *ad libitum* access to feed and water. Diets were formulated to meet minimum industry standards (NRC, 1994). A crumbled commercial starter diet was provided until d 28 when birds were switched to a pelleted commercial finisher diet. Water was provided via nipple drinkers. The photoperiod was 23L:1D for d 0-4; 20L:4D for d 5-14 and 18L:6D from d 15 through the end of trial. Room temperatures were measured and set at 33°C for d 0-3, 30°C for d 4-7, 26°C for d 8-14 and 24°C for d 15 through the end of trial. The accuracy of the set temperatures was determined by measuring ambient temperatures with a thermometer (TM99A Thermistor Temperature Instrument, Cooper Atkins, Middlefield, CT) and an infrared thermometer (IRT657, General Tools & Instruments LLC, Secaucus, NY).

5.3.2 Experiment 1

The trial was conducted between September - November 2015. On d 8, pens were assigned to one of three incandescent light intensity treatments (2 lux, 5 lux and 10 lux) (N = 2 pens per light intensity treatment). Light intensity prior to light intensity treatment was 20 lux. Light intensity of each pen was measured at bird level daily with a digital light meter (Model LT300, Extech, Nashua, NH). On d 14, bird density was reduced to 105 clinically healthy chicks per pen. Birds were removed prior to the implementation of the wire flooring if they walked with an awkward gait or were had visually smaller body size compared to their pen mates. The early culling protocol was instituted because previous studies revealed macroscopic evidence of BCO in runts and culls during the first 2 wk of age (Wideman et al., 2012).

Birds were raised in pens with wood shavings litter flooring until d 29. On d 29, fifty birds from each litter flooring pen (N = 6) were moved to one of six pens with wire flooring (N = 6) in separate chambers. The wire flooring pens had the same pen dimensions, bird density and

light intensity as the source litter pens. Thus, there were a total of 12 pens. Wire flooring panels consisted of hardwire cloth (1.3 x 2.54 cm mesh, 0.063-guage galvanized welded wire cloth; Direct Metals, Kennesaw, GA) attached to 5 cm x 5 cm lumber and were 1.5 m wide by 3 m long. The wire panels were elevated 30 cm high on masonry blocks. Litter flooring treatment birds were moved to pens with the same light intensity to control for the stress of handling and introduction to a novel pen.

Five randomly selected birds from each pen had a blood sample taken on d 7 (N = 6 pens), 9 (N = 6 pens), 28 (N = 6 pens), 30 (N = 12 pens), 38 (N = 12 pens) and 56 (N = 12 pens) of age. Blood samples were taken in the following manner. For d 7 and 9, blood samples were collected after rapid decapitation with scissors. For all subsequent blood sample collections, a 21 g X 1 ½ inch needle attached to a 3 mL syringe was used to draw blood from the brachial vein within 90 s of capture. Each blood sample was immediately injected into K_3 EDTA-coated blood tubes and serum separation tubes. Except on days 7 and 9, blood H:L was determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemocytometer. Serum separation tubes were centrifuged at 1500 x g to separate blood serum from whole blood. Serum was frozen at -20°C until assay. After blood draw, birds were humanely euthanized via cervical dislocation and third primary feathers were collected from the left wing and stored at -20°C in a freezer storage bag.

5.3.3 Corticosterone assay

CORT was extracted from the feathers using a methanol-based extraction method (Bortolotti et al., 2008), reconstituted in PBS (phosphate buffered saline; pH 7.4) and frozen at -20°C until assay. Serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) were

measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Inter- and intra-assay CVs were < 6%.

5.3.4 Latency to Lie (LTL) test

Birds from which blood samples were collected on d 56 were tagged with spray paint for individual identification. The LTL test was conducted in the hallway of the facility on days 57 and 58. The five birds sampled from each pen on d 56 were removed and placed in a rubber tub with litter. Each bird was subjected to the LTL test individually (LTLI) and in groups of five (LTLG). The LTL test order was randomized to control for possible bias from previous LTL test experience. The LTL test was a modified version of previously published methods (Weeks et al., 2002; Berg and Sanotra, 2003). The birds were placed in a 1.02 m long x 0.51 m wide plastic storage tub with four video cameras (GoPro Hero; San Mateo, CA) mounted to each side. A bucket of tepid (32°C) water was poured into the tub to a depth of 30 mm (**Figure 5.1**). A stopwatch was started when each bird was standing after the water was introduced. If a bird was not standing after water was introduced, the bird was gently coaxed to stand and the stopwatch was started. Birds that did not stand were considered lame and removed. The stopwatch was stopped when a bird sat for 3 s or longer and LTL time was recorded. If the bird did not sit after 900 s, the test ended and a LTL time of 900 s was recorded as the end-point. Video recordings of each LTL test were uploaded to a computer and observed to verify LTL times.

5.3.5 Experiment 2

Exp 2 followed the same design as exp 1 with the following minor exceptions. The trial duration was April – June 2016. On d 7, pens were assigned to one of three light intensity treatments (2 lux, 5 lux and 10 lux) (N = 2 pens per light intensity treatment). Birds were

sampled on d 6 (N = 6 pens), 8 (N = 6 pens), 27 (N = 6 pens), 29 (N = 12 pens), 40 (N = 12 pens) and 55 (N = 12 pens) of age. Final blood samples were collected on d 55. The LTLG and LTLI tests were conducted on d 56 and 57 of age.

Exp 1 and exp 2 body weight (BW) collection, eye, beak, hock, shank and foot surface temperatures (determined by infrared thermography) are described in Chapter 4.

5.3.6 Statistical Analysis

Data were statistically analyzed using JMP software (version 13, SAS Institute Inc, Cary, NC), with the pen of birds as the experimental unit. All continuous data were analyzed for distribution normality using JMP Pro 13 distribution histograms. H:L ratio, sCORT and fCORT concentrations had a lognormal distribution and the model used log transformed data for analysis. Means were back transformed to original values for results. Feather CORT concentrations were normalized by feather length to reflect CORT deposition over time throughout feather growth (Bortolotti et al., 2008). Linear regression models were used to evaluate the main effects of light intensity (2 lux, 5 lux and 10 lux), flooring type (litter versus wire) and their interaction for each age sampled in exp 1 for d 7, 9, 28, 30, 38, and 56-58 and exp 2 for d 6, 8, 27, 29, 40 and 55-57. Significant means were separated *post*-hoc with LSMeans. Pearson's pairwise correlations were analyzed for all untransformed individual bird data. Data were considered significant at $P \leq 0.05$. Data from the two experiments were analyzed together. However, there were experiment effects for each parameter and consequently combined data are not included.

Surface temperatures as determined by infrared thermography (IRT) together with body weight are reported in Chapter 4 and each individual measure was compared with Pearson's

pairwise correlations each indicator of stress (H:L, sCORT, fCORT) and leg health behavior (LTLG and LTLI) in this chapter. For completeness, all comparisons are included in this chapter.

5.4 RESULTS

5.4.1 Experiment 1

Tables 5.1 and 5.2 show data on H:L, sCORT, fCORT, LTLG and LTLI in broiler chickens on either wire or litter floor and three levels of light intensity. There were no effects of either light intensity (d 7, 9, 28, 30, 38 and 56-58) or flooring type (d 30, 38, and 56-58) effects on H:L, sCORT, fCORT, LTLG and LTLI (**Tables 5.1 and 5.2**).

There were age related shifts in some stress indices. H:L ratios increased ($P < 0.05$) with age between d 28 or d 38 and d 56-58 (**Table 5.3**). In contrast, sCORT concentrations were lower ($P < 0.0001$) on d 38 and 56 of age than on d 7 or d 9 or d 28 or d 30 (**Table 5.3**). fCORT concentrations at 30 and 38 d of age were lower ($P < 0.0001$) compared to fCORT concentrations at 9 and 57 -58 d of age (**Table 5.3**).

Table 5.4 shows the correlations between BW, H:L, sCORT, fCORT, and eye and beak surface temperatures on broiler chickens at d 7, 9, and 28. On d 7, there were positive correlations between BW with eye surface temperature ($r = 0.41$; $P = 0.04$); between sCORT with fCORT ($r = 0.57$; $P = 0.002$) and between eye surface temperature with beak surface temperature ($r = 0.58$; $P = 0.003$). On d 9, there was a negative correlation between sCORT with beak surface temperature ($r = -0.39$; $P = 0.04$) and a positive correlation between eye surface temperature and beak surface temperature ($r = 0.53$; $P = 0.002$). On d 28, there were positive correlations between H:L and fCORT concentrations ($r = 0.44$; $P = 0.03$) and between eye and beak surface temperatures ($r = 0.38$; $P = 0.04$).

Table 5.5 shows the correlations between BW, H:L, sCORT, fCORT and eye, beak, hock, shank and foot surface temperatures on d 30 and 38. On d 30, there were positive correlations ($P < 0.05$) between eye, beak, hock, shank and foot surface temperatures. However on d 30, there were no correlations between any stress measures with body region surface temperatures. On d 38, there were negative correlations between BW with sCORT concentrations ($r = -0.36$; $P = 0.006$) and hock surface temperatures ($r = -0.28$; $P = 0.04$). There were negative correlations between H:L with beak surface temperature ($r = -0.41$; $P = 0.002$) and sCORT with hock ($r = -0.34$; $P = 0.009$), shank ($r = -0.38$; $P = 0.004$) and foot ($r = -0.28$; $P = 0.03$) surface temperatures. There were positive correlations between eye surface temperature and beak surface temperature ($r = 0.31$; $P = 0.02$) and beak surface temperature with shank ($r = 0.29$; $P = 0.03$) and foot ($r = 0.40$; $P = 0.002$) surface temperatures. There were positive correlations with all pairs of hock, shank and foot surface temperatures ($P < 0.0001$).

Table 5.6 shows the correlations between BW, H:L, sCORT, fCORT and eye, beak, hock, shank and foot surface temperatures as well as LTLG and LTLG for 56 – 58 d old broilers. There was a negative correlation between BW with H:L ($r = -0.36$; $P = 0.009$) and sCORT ($r = -0.49$; $P = 0.0002$) and positive correlations between BW with eye ($r = 0.36$; $P = 0.007$), beak ($r = 0.64$; $P < 0.0001$), and shank ($r = 0.34$; $P = 0.01$) and foot ($r = 0.39$; $P = 0.004$) surface temperatures. There was a positive correlation between H:L with sCORT ($r = 0.39$; $P = 0.004$) and negative correlations between H:L with beak ($r = -0.43$; $P = 0.001$), shank ($r = -0.40$; $P = 0.003$), and foot ($r = -0.40$; $P = 0.003$) surface temperatures. There were negative correlations between sCORT with eye ($r = -0.36$; $P = 0.008$), beak ($r = -0.61$; $P < 0.0001$), shank ($r = -0.39$; $P = 0.004$) and foot ($r = -0.45$; $P = 0.0007$) surface temperatures. There were positive correlations between all body region surface temperatures ($P < 0.05$).

There were positive correlations between LTLG with sCORT (r = 0.29; P = 0.04) and with fCORT (r = 0.37; P = 0.007). There was a negative correlation between LTLI with BW (r = -0.30; P = 0.03) and positive correlations between LTLI with H:L (r = 0.32; P = 0.02) and sCORT (r = 0.44; P = 0.001). There was a positive correlation between LTLG and LTLI (r = 0.43; P = 0.001) (**Table 5.6**).

Table 5.7 shows correlations between FHN, THN, and Total N BCO lesion severity scores with BW, H:L, sCORT, fCORT, and eye, beak, hock, shank and foot surface temperatures of 30, 38 and 56-58 d old broilers. There were positive correlations between THN with hock (r = 0.44, P < 0.0004), shank (r = 0.43, P < 0.0005) and foot (r = 0.46, P < 0.0002) surface temperatures at d 30. On d 56-58 there were positive correlations between shank surface temperatures with FHN (r = 0.29, P = 0.02) and Total N BCO lesion severity scores (r = 0.31, P = 0.02) with shank surface temperatures.

5.4.2 Experiment 2

Tables 5.8 and 5.9 show data on H:L, sCORT, fCORT, LTLG and LTLI in broiler chickens on either wire or litter floor and three levels of light intensity. There were no light intensity (d 6, 8, 27, 29, 40 and 55-57) or flooring type (d 29, 40, and 55-57) effects (P > 0.05) on sCORT, LTLG and LTLI (**Tables 5.8 and 5.9**). H:L ratios were higher on d 6 for pens that were to receive the light intensity treatment of 10 lux compared to 2 and 5 lux (**Table 5.8**). On d 8, fCORT concentrations were higher for birds in pens at 5 lux light intensity compared to 2 lux and 10 lux (**Table 5.8**). H:L ratios were higher (P = 0.04) for 55 d old broilers on litter compared to wire flooring (**Table 5.9**). H:L ratio increased (P = 0.006) with age (**Table 5.10**). sCORT

concentrations were lower ($P < 0.0001$) d 29, 40 and 55 (**Table 5.10**) compared to d 6, 8 and 27. fCORT concentrations were the highest ($P < 0.0001$) on d 40 (**Table 5.10**).

Table 5.11 shows the correlations between BW, H:L, sCORT, fCORT, and eye and beak surface temperatures on broilers d 6, 8, and 27. On d 6 of age there was a negative correlation between fCORT and eye surface temperature ($r = -0.60$; $P = 0.04$). There was a negative correlation between sCORT with beak surface temperature ($r = -0.47$; $P = 0.008$). There was a positive correlation between eye surface temperature with beak surface temperature ($r = 0.50$; $P = 0.005$). On d 8, there was a positive correlation between BW with beak surface temperature ($r = 0.38$; $P = 0.04$), a negative correlation between fCORT and eye surface temperature ($r = -0.62$; $P = 0.01$), a positive correlation between core body temperature and eye surface temperature ($r = 0.45$; $P = 0.01$), and a positive correlation between eye surface temperature and beak surface temperature ($r = 0.52$; $P = 0.004$). On d 27 there was a negative correlation between BW and sCORT concentrations ($r = -0.41$; $P = 0.03$); a negative correlation between core body temperature and beak surface temperature ($r = -0.36$; $P = 0.05$), and a positive correlation between eye and beak surface temperatures ($r = 0.44$; $P = 0.01$).

Table 5.12 shows the correlations between BW, H:L, sCORT, fCORT and eye, beak, hock, shank and foot surface temperatures on d 29 and 40. On d 29 there were positive correlations ($P < 0.001$) between eye, beak, hock, shank and foot surface temperatures. However on d 29 there were no correlations ($P > 0.05$) between any stress measures with body region surface temperatures. On d 40, there was a negative correlation between H:L with beak surface temperature ($r = -0.27$; $P = 0.04$) and a positive correlations between body temperature with eye,

hock, shank and foot surface temperatures ($P < 0.05$). There were positive correlations with all pairs of hock, shank and foot surface temperatures ($P < 0.05$).

Table 5.13 shows the correlations between BW, H:L, sCORT, fCORT and eye, beak, hock, shank and foot surface temperatures as well as LTLG and LTLG for 55 – 57 d old broilers. There was a negative correlation between BW with hock surface temperatures ($r = -0.30$; $P = 0.03$) and between sCORT and beak surface temperatures ($r = -0.33$; $P = 0.02$). There were positive correlations between core body temperature with shank ($r = 0.32$; $P = 0.02$) and foot ($r = 0.27$; $P = 0.05$) surface temperatures as well as positive correlations between beak surface temperature with hock, shank and foot surface temperatures ($P < 0.05$).

There were positive correlations between LTLI with fCORT ($r = 0.38$; $P = 0.003$). There were negative correlations ($P < 0.05$) between LTLG and LTLI times with shank and foot surface temperatures (**Table 5.13**). There was not a correlation ($P > 0.05$) between LTLG and LTL times (**Table 5.13**).

Table 5.14 shows correlations between FHN, THN, and Total N BCO lesion severity scores with BW, H:L, sCORT, fCORT, core body temperature, and eye, beak, hock, shank and foot surface temperatures of 29, 40 and 55-57 d old broilers. On d 29 THN was negatively correlated with sCORT ($r = -0.28$, $P = 0.03$) and positively correlated with fCORT ($r = 0.32$, $P = 0.01$). Total N was also positively correlated with fCORT ($r = 0.38$, $P = 0.003$) on d 28. On d 40 BW was positively correlated with Total N ($r = 0.30$, $P = 0.02$). On d 55-57, FHN and Total N were negatively correlated with foot surface temperature ($r = -0.32$, $P = 0.03$; $r = -0.38$, $P = 0.008$, respectively), THN was positively correlated with sCORT ($r = 0.35$, $P = 0.02$) and LTLG

(r = 0.33, P = 0.02). Also on d 55-57 THN and Total N were negatively correlated with beak surface temperature (r = -0.37, P = 0.01; r = -0.39, P = 0.007, respectively).

5.5 DISCUSSION

Robust measures of stress in broiler chickens remain enigmatic. This, in part, is due differences in genetics, nutrition, environment and management (Bessei, 2006). However, the focus of this chapter is on the effects of environment on broiler stress and leg health. Environmental management is the key factor to overall broiler flock welfare (Dawkins et al., 2004). The two experimentally controlled environmental factors in this chapter were light intensity (2, 5 and 10 lux) and flooring type (litter vs. wire) and their effect on male broiler stress and behavior at 1, 4, 5 and 8 wk. There were no effects of light intensity (**Table 5.1 and 5.8**) or flooring type (**Tables 5.2 and 5.9**) on H:L, sCORT, LTLG or LTLI [in exp 1 or exp 2]. This is surprising given the increased in stress predicted in the birds on wire flooring.

Raising broilers on wire flooring is reported to be a stressor in itself, yielding negative health and behavioral consequences of lameness (Wideman et al., 2013). Stress-mediated immunosuppression has been hypothesized to contribute to the pathogenesis of BCO of the proximal femoral and tibial head growth plates in broilers (Wideman et al., 2013; review: Wideman, 2015, 2016). Briefly, it is envisioned that stress may increase permeability of intestinal epithelia, allowing bacteria to translocate into systemic circulation (Wideman and Prisby, 2013). Once in circulation, bacteria can migrate to growth plates of leg bones to induce BCO. Indeed, studies inducing physiological stress (dexamethasone injection and in feed) have found increased gut epithelial leakage, H:L ratios, bacterial translocation to the liver (Vicuña et al., 2015) and increased THN lesion severity scores (Wideman and Pevzner, 2012). However,

there have been no reports on the effects of wire flooring on H:L, sCORT or fCORT in broilers. El-Lethey and colleagues (2003) evaluated the effects of housing laying hens on slatted versus litter flooring. While birds slatted flooring had increased H:L ratios, there was no difference in sCORT concentrations compared to litter raised laying hens. This results of this chapter follow these outcomes with no differences in sCORT concentrations yet contrast these outcomes as there were also no flooring type effects on H:L ratios in both exp 1 (**Table 5.2**) or exp 2 (**Table 5.9**).

There were age effects on H:L and sCORT indices of stress with H:L ratio increasing and sCORT concentrations decreasing with age (exp 1 **Table 5.3** and exp 2 **Table 5.10**). In contrast, fCORT concentrations showed no pattern with age. It is frequently assumed that circulating CORT and H:L ratio measures are tightly linked and both are indicative of an increased stress response (Gross and Siegel, 1983; Post et al., 2003; Shini et al., 2008). The increases in H:L ratio is thought to be an accepted valid indicator of chronic stress effects on the immune system of broilers (Gross and Siegel, 1983; Shini et al., 2008, Shini et al., 2009). However, sCORT concentrations did not follow the same pattern as H:L ratios. H:L ratios may be shifted in a glucocorticoid independent manner (Scanes, 2016), which the results of this chapter agree with. CORT concentrations are the highest during early development and decline thereafter (Carsia, 2015). The physiological effects of CORT may be secondary to changes in other hormones (Scanes, 2009). Growth hormone and thyroid hormone also decrease with age (review: Kim, 2010). The shifts in H:L ratio and sCORT concentrations in this chapter are novel, to date, as we have been unable to find any research comparing circulating CORT concentrations throughout the grow out period.

Light intensity effects were minimal on fCORT concentrations. D 8 fCORT concentrations in exp 2 were higher for birds raised at 10 lux than birds raised at 2 or 5 lux (**Table 5.2**). There were no effects of flooring type on fCORT concentrations. On d 7 fCORT concentrations had a positive correlation with sCORT concentrations (**Table 5.4**) and LTLG on d 56-58 in exp 1(**Table 5.6**). fCORT concentrations had a negative correlation with eye surface temperature on d 6 and 8 (**Table 5.11**) and a positive correlation with LTLI on d 56 – 57 in exp 2 (**Table 5.13**). The method of extracting CORT from feathers is primarily used in avian wildlife research and may not be applicable in domestic avian research. Growing feather cells are highly vascularized and receive CORT is deposited in the keratin structure (Bortolotti et al., 2008), However the factors affecting the rate of CORT deposition are nebulous. There is conflicting evidence of feather CORT concentrations being positively or negatively related to other measures of stress and fitness in this chapter. This suggest the current understanding of CORT concentrations from feathers may be inadequate to allow using feather CORT concentrations as a biomarker of stress (Harris et al., 2017).

While there is limited research on the environmental stress effects of BCO lameness, abnormal behaviors have been reported. Broilers with BCO have been observed to have limping gaits and use their wing tips to for support while standing and walking (review: McNamee and Smyth, 2000; review: Wideman, 2016). It was our intention to apply the LTL test to 1) provide a quantitative determinant of the stress and pain experienced by broilers on wire flooring and/or with BCO and 2) to determine if there were differences between LTLG and LTLI. A presumption of the LTL test is that birds who sit sooner are experiencing more leg pain than those who stand longer. This was not the case as the wire flooring had no effect on LTLG or LTLI (exp 1 **Table 5.2** or exp 2 **Table 5.9**). Additionally, THN was positively correlated with

LTLG times in exp 2 (**Table 5.14**). Other measures may give indications as to why no differences were seen. H:L ratios and fCORT concentrations of d 56-58 old broilers were negatively correlated with LTLI in exp 1 (**Table 5.6**). Additionally, sCORT concentrations were negatively correlated with both LTLI and LTLG in exp 1(**Table 5.6**).

Previous research has conducted the LTL test in groups (Weeks et al., 2002) and individually (Berg and Sanotra, 2003; Webster et al., 2008; Rault et al., 2017). No previous work has tested birds both individually and in groups. A hypothesis of this chapter was LTL times would be different when birds were tested individually versus in groups of five. We must reject this hypothesis as there were no differences in LTL times when the same bird was tested in a group or individually. However, results of the LTLI test may have been affected by isolation stress. Preliminary observations of individual birds displaying behavioral signs of stress (panting, vocalizations, attempts to jump out of the test pen) during the LTLI test were noted. These behavioral observations may indicate a link as to the stress experienced by the bird. Gentle (2001) explains the concept of stress-induced analgesia in chickens, where behavioral modifications or "attentional shifts" to novelty may reduce pain intensity. In a 1995 study by Gentle and Corr, ankles of birds were injected with sodium urate. The authors reported birds placed into individual cages displayed more pain-related behaviors than birds placed into pens with conspecifics. This suggests the chickens placed in pens may have been motivated to suppress pain-related behavior for social hierarchy reasons. Stress and LTL relationships in exp 1 were not seen in exp 2 (**Table 5.12**). Relatedly, there was a moderate positive correlation between the two behavioral tests, LTLG and LTLI in exp 1 (**Table 5.6**) but not exp 2 (**Table 5.13**). These experimental differences may have been due to the inherent differences in bird genetics or season the experiment was conducted.

The correlations between stress measures and body region surface temperatures showed inconsistent patterns. Giloh and colleagues (2012) also found weak relationships between plasma CORT concentrations and facial surface temperatures. Handling stress may have influenced physiological stress measures differently than body region surface temperature measures. Thermoregulatory control is mediated by the hypothalamus (Esmay, 1978). The peripheral vasoconstriction due to stress from handling and/or being taken out of their pen may have affected body surface temperatures. Cabanac and Aizawa (2000) found that gentle handling caused increases in core body temperature and heart rate but decreases in skin temperatures. Peripheral vasoconstriction, as measured by decreased skin temperatures, only lasted about 6 min, which was the time it took core body temperature to rise (Cabanac and Aizawa, 2000). In a 1999 study, Gentle and Tilston found pain-related behavior of birds (induced by sodium urate injection into the ankle) decreased after they were placed in a novel environment. Furthermore, when surface skin temperature of the injected ankle joint was measured, peripheral inflammation decreased when broilers were moved from their individual cages and placed in a novel pen. Another study reported increases in head region surface temperatures and decreases in footpad surface temperatures after handling stress (Moe et al., 2017).

The relationship between core body temperature and surface body temperatures was examined in exp 2. There were positive correlations between core body temperature and body region surface temperatures on d 29 and 40 (**Table 5.12**) but not on d 55-57 of age (**Table 5.13**). This was unexpected as previous research has found high positive correlations between core body temperatures and body region surface temperatures (Nääs et al. 2010; Giloh et al., 2012; Yahav and Giloh; 2012).

There were some strong correlations between body region surface temperatures, with that between beak and eye surface temperature being the most dramatic. At each age and in both studies, there was a positive correlation ($P < 0.05$) between eye and beak surface temperatures (except d 55 - 57 in exp 2 where there was a trending positive correlation between eye and beak surface temperatures ($r = 0.25$, $P = 0.07$). This would suggest that both are under similar physiological control. Under acute stress, peripheral blood is sequestered to increase core temperature, dilating surface blood vessels to result in decreases in skin temperature (review: Siegel, 1995). Vasoconstriction of the skin on the head and nasal mucosa decreases the flow and heat exchange of blood traveling to the hypothalamus. These blood flow changes may be a reflex response to unidentified stimuli (Richards, 1971). In an IRT study evaluating handling stress, Edgar and colleagues (2013) found decreases in eye and comb temperatures initially decreased, then increased steadily back to baseline temperatures during the 20 minute period. Similarly, Herborn and colleagues (2015) found layer pullet eye temperatures decreased with handling but did not exhibit proportionally with stressor intensity. It is suggested that eye surface temperatures may be more of an indicator of thermoregulation than the stress response (Herborn et al., 2015).

The beak region isolated on the thermal image was on the culmen (medial, curved dorsal ridge) anterior to the nasal opening (Kuenzel, 2007). The upper beak receives sensory signals from the opthalamic branch of the trigeminal nerve as well as sympathetic and parasympathetic innervation from the facial nerve (Lunam, 2005; Kuenzel, 2007). A specific structure which is also included in the isolated beak regions on the thermal image are Herbst corpuscles (Lunam, 2005; Kuenzel, 2007). Herbst corpuscles are mechanoreceptors innervated by the trigeminal nerve and may have influenced beak temperature when the thermal image was taken. The mechanoreceptors may have received pain signals from the wire flooring or touch signals from

handling for image capture. We speculate that beak temperature measures may capture this reflex response linked to the hypothalamus, thus making it an ideal IRT location to measure stress.

5.6 CONCLUSION

In conclusion, the environmental effects of light intensity had minimal impact on stress and behavior parameters measured. This may reflect the low number of replicates or that the differences in light intensity were not extreme enough to elicit response differences. Unexpectedly, there were no differences in the indices of stress between birds on wire or litter flooring. The birds sampled for stress measures from wire flooring appeared clinically healthy, and based on H:L ratios and sCORT concentrations, were not more stressed than birds on litter. H:L ratios increased with age while sCORT concentrations decreased with age. IRT beak temperatures were moderately related to classical physiological measures and stress measures. Eye temperature followed the same pattern as beak temperature but to a lesser extent. Although sCORT concentrations and H:L ratios were not elevated in birds raised on wire flooring, beak temperatures were lower (Chapter 4) indicating that beak surface temperatures may be a more sensitive noninvasive indicator of stress. This present study shows that there were no effects of light intensity or flooring type treatments on stress indices. In contrast, there are treatment differences with IRT (Chapter 4). This would suggest that IRT to be a potentially promising noninvasive tool more reliably measure broiler stress and lameness than "classical" measures. While there were correlations between the various indices of stress, these were not consistently observed between the two studies.

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Table 5.1. Effect of light intensity on heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT; ng/mL) and feather corticosterone concentrations (fCORT, pg/mm)[1] of 7, 9, and 28 d old broiler chickens. Broilers were raised in pens with light intensities of 2, 5, or 10 lux.[2] Data shown are mean ± SEM (N = 2 pens).

		Light intensity (lux)			ANOVA
		2	**5**	**10**	**p-value**
Age (d)	***Stress index***				
7	sCORT	11.67 ± 0.29	13.39 ± 5.38	8.77 ± 1.48	0.54
	fCORT	4.51 ± 0.07	1.61 ± 0.62	3.33 ± 0.02	0.09
9	sCORT	11.32 ± 0.68	7.67 ± 1.78	8.85 ± 0.14	0.27
	fCORT	4.54 ± 0.99	4.90 ± 0.79	4.58 ± 0.11	0.83
28	H:L	0.32 ± 0.21	0.19 ± 0.05	0.32 ± 0.07	0.60
	sCORT	15.89 ± 0.05	11.04 ± 0.45	8.46 ± 4.11	0.26
	fCORT	2.95 ± 0.80	2.74 ± 0.02	2.65 ± 0.17	0.89

[1]D 7 and 9 H:L ratios were not run. H:L ratios were determined from a Cell-Dyn 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) concentrations were measured by DetectX Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).

[2]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial.

Table 5.2. Effect of light intensity and flooring type on heterophil to lymphocyte ratio (H:L), serum corticosterone (sCORT, ng/mL), and feather corticosterone (fCORT, pg/mm)[1] concentrations of 30, 38, and 56-58 d old broiler chickens. On d 57-58 of age, broilers were subjected to a latency to lie test (LTL, seconds) in groups of five (LTLG) and individually (LTLI).[2] Birds were raised in pens with light intensities of 2, 5, or 10 lux on litter or wire flooring.[3] Data are presented ± SEM (N = 2 pens).

Flooring (F)		**Litter**			**Wire**						
Light lux (L)		**2**	**5**	**10**	**2**	**5**	**10**	**ANOVA p-value[4]**			
Age (d)	***Stress index***							***All***	***L***	***F***	***L*F***
30	H:L	0.45 ± 0.20	0.50 ± 0.53	0.38 ± 0.25	0.30 ± 0.11	0.30 ± 0.00	0.40 ±0.17	0.97	0.99	0.62	0.86
	sCORT	8.25 ± 1.77	12.75 ± 0.46	10.71 ± 0.44	10.86 ± 1.52	9.73 ± 0.62	13.2 ± 0.79	0.16	0.20	0.14	0.10
	fCORT	1.56 ± 0.10	2.05 ± 0.66	1.50 ± 0.19	2.36 ± 0.04	1.79 ± 0.26	1.51 ± 0.16	0.36	0.30	0.12	0.28
38	H:L	0.16 ± 0.01	0.21 ± 0.04	0.14 ± 0.00	0.30 ± 0.11	0.39 ± 0.12	0.47 ±0.20	0.09	0.67	0.16	0.50
	sCORT	5.77 ± 0.21	6.98 ± 1.37	5.28 ± 0.56	5.39 ± 0.52	5.78 ± 0.36	5.59 ± 0.01	0.52	0.34	0.66	0.53
	fCORT	2.64 ± 0.16	1.82 ± 0.10	1.91 ± 0.29	1.99 ± 0.48	3.45 ± 2.35	1.43 ± 0.25	0.45	0.40	0.53	0.27
56 - 58	H:L	0.43 ± 0.38	0.32 ± 0.03	0.47 ± 0.09	0.70 ± 0.27	1.18 ± 0.16	0.69 ±0.22	0.35	0.96	0.42	0.48
	sCORT	5.95 ± 0.04	5.61 ± 0.16	5.70 ± 0.35	6.98 ± 0.44	7.39 ± 0.13	6.66 ± 0.87	0.10	0.75	0.14	0.61
	fCORT	3.65 ± 2.79	4.74 ± 0.69	3.64 ± 1.41	2.77 ± 0.63	5.64 ± 2.68	3.18 ± 0.07	0.81	0.45	0.64	0.85
	LTL										
	LTLG	157 ± 80	94 ± 64	47 ± 8	60 ± 18	169 ± 67	59 ± 32	0.48	0.37	0.24	0.32
	LTLI	130 ± 46	53 ± 11	83 ± 39	101 ± 7	232 ± 165	135 ± 70	0.68	0.90	0.80	0.45

[1]H:L ratios were determined from a Cell-Dyn 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) concentrations were measured by DetectX Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).

[2]Blood was drawn on d 56 for H:L and sCORT concentrations and feathers were collected after the LTLG and LTLI tests were conducted on d 57 and 58. Birds were subjected to the LTL test individually (LTLI) and in groups of five (LTLG) in a random order. The LTL test was a modified version of the method published by Weeks and colleagues (2002).

[3]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial.

[4]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 5.3. Effect of age on heterophil to lymphocyte (H:L) ratio, serum corticosterone (sCORT, ng/mL), and feather corticosterone (fCORT, pg/mm)[1] concentrations of 7, 9, 28, 30, 38 and 56-58 d old broiler chickens.[2] Birds were raised in pens with light intensities of 2, 5, or 10 lux and after d 29 were raised on litter or wire flooring.[3] Data Shown are mean ± SEM (N = 6 pens on d 7, 9 and 28; N = 12 pens on d 30, 38 and 56-58).

	Age (d)						
	7	**9**	**28**	**30**	**38**	**56-58**	**ANOVA p-value**
Stress index							
H:L	-	-	0.27 ± 0.07^{b}	0.38 ± 0.09^{ab}	0.25 ± 0.05^{b}	0.58 ± 0.11^{a}	0.007
sCORT	11.11 ± 1.76^{a}	9.16 ± 0.82^{a}	11.41 ± 1.73^{a}	10.78 ± 0.59^{a}	5.78 ± 0.27^{b}	6.35 ± 0.24^{b}	0.0001
fCORT	2.89 ± 0.53^{bc}	4.67 ± 0.23^{a}	2.78 ± 0.22^{bc}	1.77 ± 0.14^{d}	2.12 ± 0.40^{cd}	3.82 ± 0.62^{ab}	0.0001

[1]D 7 and 9 H:L ratios were not run. H:L ratios were determined from a Cell-Dyn 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) concentrations were measured by DetectX Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).

[2]Blood samples were collected on d 56 of age and feather samples were collected on d 57 and 58 of age.

[3]Light intensities in all pens were 20 lux from d 0 to 7 and changed to 2, 5, or 10 lux from d 8 until the end of the trial. Flooring treatments were all litter until d 29 when half of the birds from each litter pen were moved to a wire-flooring pen with the same light intensity as the corresponding source litter pen.

[abcd] Means not sharing the same superscript letter across each row are different.

Table 5.4. Correlations (r) of body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), eye surface temperatures (ET, °C), and beak surface temperatures (BT, °C)[1] of broilers 7, 9 and 28 d of age (N = 30 birds at each age).

		Correlations (r)					
Age (d)		BW	H:L	sCORT	fCORT	ET	BT
7	BW	1.00	-	-0.12	-0.27	0.41*	0.36
	sCORT			1.00	0.57**	-0.11	-0.22
	fCORT				1.00	0.05	0.25
	ET					1.00	0.58**
	BT						1.00
9	BW	1.00	-	0.05	-0.33	0.28	0.10
	sCORT			1.00	-0.06	-0.20	-0.39*
	fCORT				1.00	0.00	0.19
	ET					1.00	0.53**
	BT						1.00
28	BW	1.00	0.29	0.09	0.24	0.33	0.00
	H:L		1.00	0.11	0.44*	0.26	-0.10
	sCORT			1.00	0.09	0.08	-0.26
	fCORT				1.00	0.16	-0.06
	ET					1.00	0.38*
	BT						1.00

[1]D 7 and 9 H:L ratios were not run, d 28 H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4.

Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 5.5. Correlations (r) of body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), and foot surface temperatures (FT, °C)[1] of 30 and 38 d old broilers (N = 60 birds at each age).

		Correlations (r)								
Age (d)		BW	H:L	sCORT	fCORT	ET	BT	HT	ST	FT
30	BW	1.00	0.19	-0.14	0.01	0.12	-0.01	0.06	0.05	0.08
	H:L		1.00	0.16	-0.04	0.10	-0.21	-0.10	-0.07	-0.12
	sCORT			1.00	-0.01	-0.07	-0.12	-0.13	-0.02	-0.06
	fCORT				1.00	-0.17	0.09	0.01	-0.03	0.00
	ET					1.00	0.33**	0.42**	0.47**	0.36**
	BT						1.00	0.28*	0.40**	0.38**
	HT							1.00	0.87**	0.70**
	ST								1.00	0.86**
	FT									1.00
38	BW	1.00	-0.14	-0.36**	-0.10	-0.09	0.10	-0.28*	-0.23	-0.15
	H:L		1.00	-0.11	-0.07	-0.24	-0.41**	0.14	0.07	-0.01
	sCORT			1.00	-0.06	0.01	-0.06	-0.34**	-0.38**	-0.28*
	fCORT				1.00	-0.01	0.08	0.10	0.14	0.17
	ET					1.00	0.31*	0.01	0.14	0.19
	BT						1.00	0.15	0.29*	0.40**
	HT							1.00	0.85**	0.70**
	ST								1.00	0.85**
	FT									1.00

[1]H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4. Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 5.6. Correlations (r) of 56-58 d old broiler body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), foot surface temperatures (FT, °C)[1] and latency to lie test (LTL, seconds) times in groups (LTLG) and individually (LTLI)[2] (N = 60 birds).

	Correlations (r)										
	BW	H:L	sCORT	fCORT	ET	BT	HT	ST	FT	LTLG	LTLI
BW	1.00	-0.36**	-0.49**	-0.10	0.36**	0.64**	0.17	0.34*	0.39**	-0.26	-0.30*
H:L		1.00	0.39**	-0.05	-0.19	-0.43**	-0.09	-0.40**	-0.40**	0.03	0.32*
sCORT			1.00	0.21	-0.36**	-0.61**	-0.15	-0.39**	-0.45**	0.29*	0.44**
fCORT				1.00	-0.12	-0.12	-0.05	-0.04	-0.04	0.37**	0.11
ET					1.00	0.51**	0.28*	0.35*	0.42**	-0.23	-0.27
BT						1.00	0.32*	0.66**	0.74**	-0.06	-0.14
HT							1.00	0.48**	0.33*	-0.06	-0.14
ST								1.00	0.83**	-0.18	-0.21
FT									1.00	-0.26	-0.19
LTLG										1.00	0.43**
LTLI											1.00

[1]Blood samples were collected on d 56 of age. BW and feather samples were collected on d 57 and 58 of age. H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4.

[2]The LTL test was conducted in the hallway of the facility on days 57 and 58. The five birds on d 56 were subjected to the LTL test individually (LTLI) and in groups of five (LTLG). The LTL test order was randomized to control for possible bias from previous LTL test experience. The LTL test was a modified version of the method published by Weeks and colleagues (2002).

Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 5.7. Correlations (r) of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis (FHN+THN);Total N) with body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), and foot surface temperatures (FT, °C)[1] and latency to lie test (LTL, seconds)[2] times in groups (LTLG) and individually (LTLI)of 30, 38 and 56-58 d old broilers (N = 60 birds at each age).

		Correlations (r)									
Age (d)		BW	H:L	sCORT	fCORT	ET	BT	HT	ST	FT	LTLG	LTLI
30	FHN	-0.08	-0.16	-0.07	-0.05	0.21	0.14	0.20	0.21	0.24		
	THN	-0.19	0.12	0.14	0.16	-0.15	-0.19	-0.19	-0.16	-0.05		
	Total N	-0.21	-0.03	0.06	0.09	0.04	-0.04	0.00	0.03	0.14		
38	FHN	0.10	0.05	-0.12	-0.13	-0.03	-0.08	0.03	0.03	0.03		
	THN	-0.16	0.05	-0.16	0.10	0.21	0.20	0.44**	0.43**	0.46**		
	Total N	0.02	0.07	-0.17	-0.07	0.06	0.02	0.21	0.21	0.22		
56-58	FHN	0.06	-0.08	0.08	0.10	-0.03	0.21	0.17	0.29*	0.21	0.06	0.00
	THN	0.00	0.22	-0.06	-0.12	0.16	-0.03	0.01	0.13	0.12	0.03	0.11
	Total N	0.05	0.04	0.04	0.04	0.06	0.16	0.15	0.31*	0.24	0.06	0.06

[1]H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4.
[2]Blood was drawn on d 56 for H:L and sCORT concentrations. BW and feathers were collected after the LTLG and LTLI tests were conducted on d 57 and 58. Birds were subjected to the LTL test individually (LTLI) and in groups of five (LTLG) in a random order. The LTL test was a modified version of the method published by Weeks and colleagues (2002).

Table 5.8. Effect of light intensity on heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT; ng/mL) and feather corticosterone concentrations (fCORT, pg/mm)1 for broilers at 6, 8 and 27 d of age.[1] Broiler chickens were raised in pens with light intensities of 2, 5, or 10 lux.[2] Data shown are mean ± SEM (N = 2 pens).

		Light intensity (lux)			ANOVA
		2	**5**	**10**	**p-value**
Age (d)	***Stress index***				
6	H:L	0.07 ± 0.02[b]	0.04 ± 0.00[b]	0.38 ± 0.13[a]	0.01
	sCORT	13.9 ± 0.74	11.7 ± 0.39	15.2 ± 2.22	0.27
	fCORT	0.92 ± 0.04	0.82 ± 0.10	0.77 ± 0.32	0.87
8	H:L	0.19 ± 0.01	0.14 ± 0.05	0.15 ± 0.00	0.62
	sCORT	13.5 ± 0.12	12.4 ± 0.02	11.6 ± 1.64	0.51
	fCORT	0.39 ± 0.27[b]	0.87 ± 0.49[a]	0.04 ± 0.01[b]	0.05
27	H:L	0.20 ± 0.03	0.16 ± 0.01	0.22 ± 0.01	0.17
	sCORT	7.71 ± 0.35	8.82 ± 0.87	9.82 ± 0.56	0.20
	fCORT	0.50 ± 0.16	0.68 ± 0.05	0.62 ± 0.00	0.57

[1]H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).

[2]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial.

[ab] Means not sharing the same superscript letter across each row are different

Table 5.9. Effect of light intensity and flooring type on heterophil to lymphocyte ratio (H:L), serum corticosterone (sCORT, ng/mL), and feather corticosterone (fCORT, pg/mm)[1] concentrations of 29, 40, and 55-57 d old broiler chickens. On d 56-57 of age, broilers were subjected to a latency to lie test (LTL, seconds) in groups of five (LTLG) and individually (LTLI).[2] Birds were raised in pens with light intensities of 2, 5, or 10 lux on litter or wire flooring.[3] Data are presented ± SEM (N = 2 pens).

Flooring (F)		**Litter**			**Wire**						
Light lux (L)		**2**	**5**	**10**	**2**	**5**	**10**	**ANOVA p-value[4]**			
Age (d)	***Stress index***							***All***	***L***	***F***	***L*F***
29	H:L	0.22 ± 0.01	0.18 ± 0.00	0.25 ± 0.03	0.20 ± 0.03	0.20 ± 0.01	0.19 ± 0.05	0.70	0.66	0.68	0.47
	sCORT	6.83 ± 1.03	6.98 ± 0.01	7.36 ± 2.03	6.45 ± 0.94	6.66 ± 0.37	6.83 ± 1.52	0.99	0.93	0.81	1.00
	fCORT	0.72 ± 0.14	0.56 ± 0.24	0.69 ± 0.10	0.68 ± 0.11	0.63 ± 0.24	0.64 ± 0.04	0.98	0.83	0.89	0.91
40	H:L	0.44 ± 0.40	0.18 ± 0.02	0.20 ± 0.01	0.78 ± 0.56	0.33 ± 0.06	0.49 ± 0.52	0.53	0.37	0.51	0.95
	sCORT	6.59 ± 1.05	7.07 ± 0.44	6.25 ± 0.71	7.30 ± 1.66	8.12 ± 0.84	7.10 ± 1.14	0.85	0.69	0.64	0.99
	fCORT	1.92 ± 0.28	1.86 ± 0.28	1.56 ± 0.05	1.60 ± 0.04	1.59 ± 0.20	1.41 ± 0.01	0.38	0.28	0.26	0.92
55 - 57	H:L	0.28 ± 0.05	0.34 ± 0.06	0.84 ± 0.05	0.51 ± 0.07	0.50 ± 0.13	0.45 ± 0.04	0.02	0.03	0.06	0.02
	sCORT	7.20 ± 0.23	7.28 ± 0.39	6.43 ± 0.40	7.71 ± 0.17	7.59 ± 0.08	7.14 ± 0.61	0.30	0.18	0.38	0.83
	fCORT	1.51 ± 0.16	1.66 ± 0.09	1.21 ± 0.20	1.47 ± 0.43	0.74 ± 0.27	1.63 ± 0.37	0.11	0.29	0.92	0.06
	LTL										
	LTLG	71 ± 22	44 ± 16	100 ± 17	149 ± 70	45 ± 0.0	88 ± 39	0.53	0.37	0.21	0.51
	LTLI	133 ± 78	117 ± 22	127 ± 47	192 ± 75	96 ± 0.0	86 ± 10	0.72	0.47	0.43	0.59

[1]Blood samples were collected on d 56 of age. BW and feather samples were collected on d 57 and 58 of age. H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).

[2]The LTL test was conducted in the hallway of the facility on d 56 and 57. The five birds sampled on d 55 were subjected to the LTL test individually (LTLI) and in groups of five (LTLG). The LTL test order was randomized to control for possible bias from previous LTL test experience. The LTL test was a modified version of the method published by Weeks and colleagues (2002).

[3]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial.

[4]ANOVA p-values represent the overall effects, the main effects of light intensity (L) and flooring type (F) and the interaction of light intensity and flooring type (L*F).

Table 5.10. Effect of age on heterophil to lymphocyte ratio (H:L), serum corticosterone (sCORT, ng/mL), and feather corticosterone (fCORT, pg/mm)[1] concentrations of 6, 8, 27, 29, 40 and 55-57 d old broiler chickens.[2] Birds were raised in pens with light intensities of 2, 5, or 10 lux and after d 28 were raised on litter or wire flooring.[3] Data Shown are mean ± SEM (N = 6 pens for d 6, 8 and 27; N = 12 pens for d 29, 40 and 55-57).

	Age (d)						
	6	**8**	**27**	**29**	**40**	**55-57**	**ANOVA p-value**
Stress index							
H:L	0.17 ± 0.08^{b}	0.16 ± 0.02^{b}	0.19 ± 0.02^{b}	0.21 ± 0.01^{b}	0.50 ± 0.14^{a}	0.49 ± 0.06^{a}	0.006
sCORT	13.69 ± 0.91^{a}	12.56 ± 0.53^{a}	8.81 ± 0.48^{b}	6.95 ± 0.37^{c}	7.15 ± 0.36^{c}	7.23 ± 0.16^{c}	0.0001
fCORT	0.86 ± 0.09^{c}	0.51 ± 0.23^{c}	0.61 ± 0.05^{c}	0.67 ± 0.05^{c}	1.67 ± 0.08^{a}	1.39 ± 0.12^{b}	0.0001

[1]D 7 and 9 H:L ratios were not run. H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4.
[2]Blood samples were collected on d 55 of age and feather samples were collected on d 56 and 57 of age.
[3]Light intensities in all pens were 20 lux from d 0 to 6 and changed to 2, 5, or 10 lux from d 7 until the end of the trial. Flooring treatments were all litter until d 28 when half of the birds from each litter pen were moved to a wire flooring pen with the same light intensity as the corresponding source litter pen.
[abc] Means not sharing the same superscript letter across each row are different.

Table 5.11. Correlations (r) of body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), core body temperatures (CT, °C), eye surface temperatures (ET, °C), and beak surface temperatures (BT, °C)[1] of broilers 7, 9 and 28 d of age (N = 30 birds per age).

		Correlations (r)						
Age (d)		BW	H:L	sCORT	fCORT	CT	ET	BT
6	BW	1.00	-0.10	0.16	-0.41	0.32	-0.04	0.23
	H:L		1.00	0.25	-0.35	0.14	-0.01	-0.33
	sCORT			1.00	0.12	0.05	-0.29	-0.47**
	fCORT				1.00	-0.54	-0.60*	-0.33
	CT					1.00	0.31	-0.07
	ET						1.00	0.50**
	BT							1.00
8	BW	1.00	0.33	-0.14	0.01	-0.06	0.13	0.38*
	H:L		1.00	0.37	0.01	-0.23	0.02	0.02
	sCORT			1.00	0.24	-0.11	-0.06	-0.07
	fCORT				1.00	-0.19	-0.62*	-0.06
	CT					1.00	0.45*	0.20
	ET						1.00	0.52**
	BT							1.00
27	BW	1.00	-0.36	-0.41*	-0.16	-0.09	0.21	0.29
	H:L		1.00	0.03	0.19	0.20	-0.10	0.07
	sCORT			1.00	-0.09	0.12	-0.35	-0.32
	fCORT				1.00	-0.06	-0.08	0.16
	CT					1.00	-0.15	-0.36*
	ET						1.00	0.44*
	BT							1.00

[1]H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4. Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 5.12. Correlations (r) of body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), core body temperatures (CT, °C), eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), foot surface temperatures (FT, °C)[1] of 29 and 40 d old broilers (N = 60 birds per age).

		Correlations (r)									
Age (d)		BW	H:L	sCORT	fCORT	CT	ET	BT	HT	ST	FT
29											
	BW	1.00	0.07	-0.13	0.04	0.04	-0.14	0.08	0.23	0.15	0.18
	H:L	0.07	1.00	-0.24	-0.02	-0.23	0.18	0.09	0.06	0.07	-0.01
	sCORT	-0.13	-0.24	1.00	-0.20	0.13	-0.13	-0.05	-0.22	-0.15	-0.11
	fCORT	0.04	-0.02	-0.20	1.00	-0.04	-0.19	-0.07	-0.14	-0.11	-0.15
	CT					1.00	0.24	0.30*	0.32*	0.38**	0.40**
	ET						1.00	0.46**	0.44**	0.54**	0.52**
	BT							1.00	0.53**	0.62**	0.69**
	HT								1.00	0.87**	0.78**
	ST									1.00	0.91**
	FT										1.00
40	BW	1.00	0.08	0.01	-0.15	-0.04	0.10	-0.04	-0.10	-0.09	-0.16
	H:L		1.00	0.05	-0.02	0.15	0.08	-0.27*	0.13	0.08	0.01
	sCORT			1.00	0.01	0.02	-0.09	0.18	0.18	0.20	0.19
	fCORT				1.00	0.03	0.14	0.02	-0.05	0.01	0.08
	CT					1.00	0.27*	0.13	0.34**	0.31*	0.27*
	ET						1.00	0.28*	0.24	0.31*	0.29*
	BT							1.00	0.22	0.39**	0.44**
	HT								1.00	0.80**	0.71**
	ST									1.00	0.87**
	FT										1.00

[1]H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4. Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ ** $P \leq 0.01$.

Table 5.13. Correlations (r) of 55-57 d old broiler body weight (BW,g) heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), core body temperature (CT, °C), eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), foot surface temperatures (FT, °C)[1] and latency to lie test (LTL) times in groups (LTLG) and individually (LTLI)[2] (N = 60 birds).

	Correlations (r)											
	BW	H:L	sCORT	fCORT	CT	ET	BT	HT	ST	FT	LTLG	LTLI
BW	1.00	-0.13	0.02	-0.08	0.25	0.16	0.08	-0.30	-0.05	-0.03	-0.18	0.20
H:L		1.00	0.03	-0.15	-0.09	-0.16	0.04	-0.07	-0.08	-0.13	0.14	0.00
sCORT			1.00	-0.01	-0.06	-0.01	-0.33*	-0.14	-0.19	-0.22	0.04	-0.07
fCORT				1.00	-0.05	-0.03	0.14	0.03	-0.15	-0.23	-0.16	0.38**
CT					1.00	0.18	0.11	0.01	0.32	0.27	-0.22	-0.13
ET						1.00	0.25	0.10	0.14	0.08	0.00	-0.03
BT							1.00	0.44**	0.51**	0.41**	-0.25	-0.17
HT								1.00	0.51**	0.30*	0.13	-0.14
ST									1.00	0.78**	-0.28*	-0.28*
FT										1.00	-0.43**	-0.43**
LTLG											1.00	0.10
LTLI												1.00

[1]Blood samples and body region surface temperatures (see Chapter 4) were collected on d 55 of age and feather samples were collected on d 56 and 57 of age. H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4.
[2] The LTL test was conducted in the hallway of the facility on days 56 and 57. The five birds sampled on d 55 were subjected to the LTL test individually (LTLI) and in groups of five (LTLG). The LTL test order was randomized to control for possible bias from previous LTL test experience. The LTL test was a modified version of the method published by Weeks and colleagues (2002).
Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 5.14. Correlations (r) of right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis (FHN+THN);Total N) with body weight (BW,g), heterophil to lymphocyte ratio (H:L), serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm), eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), and foot surface temperatures (FT, °C)[1] and latency to lie test (LTL, seconds)[2] times in groups (LTLG) and individually (LTLI)of 29, 40 and 55-57 d old broilers (N = 60 birds at each age).

		Correlations (r)											
Age (d)		BW	H:L	sCORT	fCORT	CT	ET	BT	HT	ST	FT	LTLG	LTLI
29	FHN	-0.01	-0.12	0.06	0.24	-0.23	-0.07	-0.16	0.04	-0.07	-0.15		
	THN	0.20	-0.02	-0.28*	0.32*	0.02	0.05	0.11	0.10	0.06	0.09		
	Total N	0.17	-0.07	-0.22	0.38*	-0.08	0.02	0.03	0.11	0.02	0.01		
40	FHN	0.24	0.05	-0.23	0.01	0.01	0.09	-0.11	0.06	0.20	0.12		
	THN	0.21	0.07	0.01	0.08	-0.08	0.14	0.00	-0.11	-0.10	-0.22		
	Total N	0.30*	0.07	-0.22	0.03	-0.01	0.13	-0.11	0.03	0.16	0.05		
55-57	FHN	-0.01	0.04	0.03	-0.02	-0.12	-0.20	-0.28	-0.12	-0.27	-0.32*	0.05	0.07
	THN	-0.01	0.00	0.35*	-0.07	0.08	-0.16	-0.37**	-0.20	-0.08	-0.25	0.33*	0.01
	Total N	-0.01	0.04	0.16	-0.05	-0.08	-0.24	-0.39**	-0.18	-0.27	-0.38**	0.17	0.07

[1]Blood samples and body region surface temperatures (see Chapter 4) were collected on d 55 of age and feather samples were collected on d 56 and 57 of age. H:L ratios were determined from a Cell-Dyn® 3700 (Abbott Diagnostics, Lake Forest, IL) automated hemacytometer and serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Surface temperatures were collected as described in Chapter 4.
[2]The LTL test was conducted in the hallway of the facility on days 56 and 57. The five birds sampled on d 55 were subjected to the LTL test individually (LTLI) and in groups of five (LTLG). The LTL test order was randomized to control for possible bias from previous LTL test experience. The LTL test was a modified version of the method published by Weeks and colleagues (2002).
Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

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a)

b)

Figure 5.1. The LTL test a) individually (LTLI) and b) in groups of five (LTLG).

CHAPTER 6 Comparison of health, physiology and stress measures of clinically healthy and lame broilers

6.1 ABSTRACT

Lameness compromises the welfare of broilers and is assumed to be stressful. Infrared thermography (IRT) may detect external changes in skin temperature related to stress and/or inflammation related to lameness. Our first objective was to compare stress, leg surface temperatures and leg bone necrosis severity in clinically healthy vs. lame broilers. Our second objective was to explore possible relationships between leg blood oxygen saturation, leg temperatures and leg bone necrosis severity. Broilers were raised in twelve pens with either litter or wire flooring. Wire flooring is thought to impose additional mechanical stress on the legs, which in turn induces lameness attributable to bacterial chondronecrosis with osteomyelitis (BCO). The study evaluated possible differences lame birds on wire flooring (Lame) and clinically healthy birds (Sound) between 25 and 56 days of age. The following parameters were determined: serum corticosterone concentrations (sCORT), surface temperatures of the head and legs, core body temperatures, blood oxygen saturation in the left and right legs (leg O_2), body weights, relative bursa weights and femoral head necrosis (FHN) and tibial head necrosis (THN) lesion severity. Lame birds had higher ($P < 0.05$) FHN and THN lesion severities, core body temperature, and sCORT concentrations and lower body weights, relative bursa weights, leg O_2, and beak, hock, shank, and foot surface temperatures compared to sound birds. There were linear relationships ($P < 0.05$) of leg O_2, and temperatures of the hocks, shanks and feet and with FHN, THN and Total N lesion severity. There were negative correlations ($P < 0.001$) between sCORT with hock, shank and feet temperatures. Finally, to predict lameness, a logistic regression model for the binary response of Lame vs. Sound was fit to the data using core body temperature, sCORT, leg O_2 and surface temperatures of the beak, shanks and feet. These results are consistent with lame birds being stressed and having reduced oxygen saturation of blood in the

legs. Additionally, these results indicate the utility of IRT as a noninvasive measure of broiler lameness and stress.

6.2 INTRODUCTION

Lameness is a major welfare issue affecting broiler production (review: Bradshaw et al., 2002; review: Gentle, 2011). Moreover, lameness presents a production issue if the bird cannot reach feed and water (review: McNamee and Smyth, 2000; review: Bradshaw et al., 2002; Weeks et al., 2002). Lameness is logically assumed to be painful (McGeown et al., 1999; Danbury et al., 2000; Weeks et al., 2000; Nääs et al., 2009) and consequently stressful. Broilers can become lame from infectious and/or noninfectious origins (review: Gentle, 2011). Gait scoring provides a subjective index of overall flock health (NCC, 2014) but cannot delineate what causes the changes in the individual broiler's gait (review: Gentle, 2011). However, there are no studies examining the effects of lameness on parameters related to environmental stress in broilers.

Bacterial chondronecrosis with osteomyelitis (BCO) is an infectious type of lameness. Broilers succumb to bacterial infiltration and subsequent infection of the leg bone growth plate, leading to necrosis and eventually lameness (review: Wideman, 2015; 2016). The mechanical stress of walking may create micro fractures in the growth plates of the femora and tibiae, thereby increasing opportunities for bacteria to colonize in the damaged cartilage (Wideman et al., 2012, review: Wideman, 2015). Vascular occlusion and reduced blood flow have been hypothesized as contributing factors to the pathogenesis of BCO lameness (review: Wideman and Prisby, 2013; review: Wideman 2015, 2016).

Previous experiments (Chapter 4) revealed that, when compared with sound birds raised on wire flooring, lame birds have lower hock, shank and foot surface temperatures together with higher femoral head necrosis (FHN), tibial head necrosis (THN) and aggregate necrosis (Total N,

FHN + THN) BCO lesion severity scores. It is assumed that lame birds have higher levels of physiological stress. Yet there are no studies confirming this assumption to date. However, only thermal images and BCO lesion scores were collected from lame birds evaluated in previous experiments (Chapter 4) and resultantly no stress measures were available for comparison (Chapter 5). Therefore, the present study was conducted to further investigate body region surface temperatures in association with stress and leg health measures.

This study evaluated stress and lameness measures on lame and clinically healthy (sound) broiler chickens. Stress measures included: 1) physiological measures previously identified as indicators of stress [body weight (BW), core body temperature (CT) serum corticosterone concentrations (sCORT), and relative bursa weights (bursa%)]; and, 2) putative noninvasive measures of stress [feather corticosterone concentrations (fCORT), eye surface temperatures (ET) and beak surface temperatures (BT)]. Lameness measures included: 1) *pre-mortem* noninvasive measures of leg health [blood oxygen saturation ($legO_2$), hock, shank and foot surface temperatures]; and, 2) *post-mortem* clinical scores of BCO [proximal femoral head necrosis (FHN), proximal tibial head necrosis (THN) and aggregate (FHN + THN) lesion severity scores]. Additionally, simple linear models were used to analyze: 1) the relationships of $legO_2$ with leg bone necrosis lesion severity scores and leg region surface temperatures; and, 2) the relationship of leg bone necrosis lesion severity scores with leg region surface temperatures. All data were subjected to stepwise regression for selection and inclusion into a logistic multiple regression model to predict lameness.

6.3 MATERIALS AND METHODS

6.3.1 Animals and Facility

The study employed male Cobb 500 byproduct broiler chicks (N = 720 chicks) from a commercial hatchery (Cobb, Fayetteville, AR). These were divided randomly and placed into 1.5 m wide x 3 m long pens (N = 60 chicks per pen) within 12 environmental chambers (3.7 m long x 2.5 m wide x 2.5 m high) in the Poultry Environmental Research Laboratory at the University of Arkansas Poultry Research Farm (IACUC # 16014). Pens had either wood shavings litter flooring (N = 6 pens) or wire flooring (N = 6 pens). Wire flooring consisted of raised wire panels constructed from 5 x 5 cm lumber and were 1.5 m wide by 3 m long. The wire panels were made of hardwire cloth (1.3 x 2.54 cm mesh, 0.063-guage galvanized welded wire cloth; Direct Metals, Kennesaw, GA) and were elevated 30 cm high on masonry blocks. 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. Water was provided via nipple drinkers.

The light intensity in each chamber was 20 lux and the photoperiod was: 23L:1D for d 0-4; 20L:4D for d 5-14; and 18L:6D from d 15 through the end of trial. Chamber temperatures were measured daily and set to 33°C for d 0-3; 30°C for d 4-7; 26°C for d 8-14; and 24°C for d 15 through the end of the trial. The accuracy of the set temperatures was determined by measuring ambient temperatures with a thermometer (TM99A Thermistor Temperature Instrument, Cooper Atkins, Middlefield, CT) and 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 per chamber. Cull birds were selected if they walked with an awkward gait or were had visually

smaller body size compared to their pen mates. The early culling protocol was instituted to eliminate runts and unhealthy individuals during the first 2 wk (Wideman et al., 2012). The trial was conducted October – November 2016.

6.3.2 Pre-mortem samples

Starting on d 25 (when the first bird raised on wire flooring became clinically lame) until d 56, birds identified during daily observations as being lame were removed from their pen. No birds raised on litter flooring became lame over the course of this experiment. For every lame bird (Lame) in a wire flooring pen, a clinically healthy bird (Sound) was randomly selected and removed from the adjacent litter flooring pen. Birds were considered lame if they would not step to walk when gently coaxed. Lame birds often used their wing tips for support to stand. The rationale for pairing lame and sound birds from adjacent pens was to control for pen stocking density. Once removed from their pen, each bird had a thermal image taken of their head and legs, blood was collected, core body temperature was recorded and blood oxygen saturation was measured with a pulse oximeter.

Thermal images were captured with a thermal imaging camera (Fluke Ti400, Fluke®, Everett, WA). The camera background temperature was 22°C, emissivity was 0.95 and with 100% transmission. The focal distance of the camera from the bird was 3.3 to 3.6 m. Images were uploaded to a computer and analyzed using SmartView® (v 2.8) software. Each individual pixel (76, 800 total) within the thermal image had an associated temperature recorded. Within each head image, shapes were made to isolate pixels of the eye pupil and the beak region anterior to the nostril (**Figure 4.1**). Within each leg image, shapes were made to isolate pixels of the right and left hock joints (intertarsal joint: distal tibiotarsus, proximal tarsometatarsus), shanks

(tarsometatarsus) and feet (metatarsus and phalanges; **Figure 4.2**). The averages of the pixel temperatures within each shape of bird body regions were recorded and used in all subsequent temperature calculations. The pixel averages within thermal images of each bird's legs representing the right and left hock joint, shanks and feet were averaged [ex: (right shank pixel average + left shank pixel average)/2)] for data analysis.

Blood samples were taken in the following manner. A 21 g X 1 ½ inch needle attached to a 3 mL syringe was used to draw blood from the brachial vein within 60 s of capture. Each blood sample was immediately injected into serum separation tubes. Serum separation tubes were centrifuged at 1500 x g to separate serum from whole blood. Serum was frozen at -20°C until assay. Core body temperatures were measured with a thermometer (TM99A Thermistor Temperature Instrument, Cooper Atkins, Middlefield, CT) inserted 5 cm into the cloaca. Blood oxygen saturation (%) of the right and left leg (leg O_2) was measured with a veterinary pulse oximeter (Model 2500A, Nonin Medical, Inc., Plymouth, MN). Pulse oximetry is a noninvasive indicator of arterial blood oxygen saturation. The flexible sensor was wrapped around the distal right and left shank and readings were recorded after 3 continuous seconds of monitor display. The average of the left and right blood oxygen saturation percentages (leg O_2) were used for analysis.

6.3.3 Post-mortem samples

Birds were euthanized via rapid cervical dislocation, body weight (BW,g) was recorded, bursas were removed, feathers were collected and right and left proximal femur and proximal tibiotarsus (tibia) bones were scored for BCO lesion severity. The third primary feathers were collected from the left wing. Bursas were collected and immediately placed into a 10% formalin

solution. Bursas were removed from the formalin solution, weighed (g) and normalized for body weight (BW/bursa weight) to calculate relative bursa weight for analysis.

BCO lesion severity was macroscopically diagnosed for proximal femoral head necrosis (FHN) and proximal tibiotarsus (referred to as tibia) head necrosis (THN) lesion severity. The scoring scheme was a modified version of the methods described in previous research (Wideman et al., 2012, Wideman and Prisby, 2013;Wideman et al., 2013; 2014; Gilley et al., 2014; Wideman, 2014). FHN lesion severity was scored on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). THN lesion severity was also scored on a 0-3 scale in the following categories: 0- no abnormalities of the proximal tibia (normal); 1- mild proximal tibial head necrosis (THN); 2- severe tibial head necrosis (THNS); and 3- caseous THN (THNC; **Figure 4.3**).

A quantitative FHN index was created by summing the right (0-3) and left (0-3) proximal FHN lesion severity scores for a range of 0-6. A quantitative THN index was created by summing the right (0-3) and left (0-3) proximal THN lesion severity scores for a range of 0-6. A quantitative Total Necrosis (Total N) index was created by summing FHN and THN category scores for a range of 0-12.

6.3.4 Serum and Feather CORT Assay

CORT was extracted from the feathers using a methanol-based extraction method (Bortolotti et al., 2008), reconstituted in the ELISA kit assay buffer, and frozen at -20°C until assay. Serum CORT (sCORT; ng/mL) and feather CORT (fCORT; pg/mm) were measured by

DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI). Inter- and intra-assay CVs were < 7%.

6.3.5 Statistical Analysis

The experimental design was a matched pair design, where each lame bird from a wire flooring pen was matched to a bird from a designated adjacent litter flooring pen. The pen of birds was the experimental unit. All data were analyzed in JMP (version 13, SAS Institute Inc, Cary, NC). To analyze the effect of health status (lame vs. sound) data were subjected to an ANOVA with the matched pair included as a random blocking factor. Simple linear regressions were performed to analyze the relationship between legO_2, BCO necrosis lesion severity scores (FHN, THN and Total N) and leg region (hock, shank and foot) surface temperatures. The forward stepwise regression technique was used for variable selection and inclusion in a logistic regression model to predict the binary health status response (lame vs. sound). Data were considered significant at $P < 0.05$.

6.4 RESULTS AND DISCUSSION

This study examined physiological, health and stress differences between lame and sound male broiler chickens. Lame broilers were hypothesized to be experiencing greater physiological stress than sound broilers. As expected, lame broiler BW was lower ($P < 0.0001$) by 32% than sound birds (**Figure 6.1**). Previous studies reported reduced body weights of clinically healthy broilers at 8 wk of age raised on wire flooring compared to clinically health birds raised on litter (Gilley et al., 2014). Core body temperatures were higher ($P < 0.0001$) for lame birds compared to sound birds (**Figure 6.2**). Results from a small study reported increased core body temperature from handling stress on chickens (Cabanac and Aizawa, 2000). During the stress response peripheral blood is sequestered to the core to increase core body temperature. Elevated core body

temperatures of lame birds may have been reflective cytokines involved in the febrile response to stress (Liu et al., 2015). Further evidence to support this report is that lame birds had 20% lower ($P < 0.0001$) relative bursa weights than sound birds (**Figure 6.3**). sCORT concentrations of lame birds were 86% higher ($P < 0.0001$) than sound birds (**Figure 6.4**). No research to date has measured circulating CORT concentrations in lame broiler chickens, nor compared them to sound broilers in the same study. These novel results indicate lame broilers are indeed more stressed than sound broilers. 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). Decreased relative bursa weights may reflect an inhibitory effect of CORT on immune function (Gross et al., 1980; Yang et al., 2015) and/or immunological responses to the presumed bacterial infection in lame birds (review: Wideman, 2015, 2016). Therefore, lame birds may have been experiencing immunological stress. *Staphlococcus aureus* has been reported to be the cause of BCO in broiler chickens (review: McNamee and Smyth, 2000). However, *Staphylococcus agnetis* has been reported to be the resident pathogen at the University of Arkansas Poultry Research Farm and may have biofilmed in the water lines to contribute to BCO in the present study (Al-Rubaye et al., 2015; 2017). Previous research has reported the relationships between increased stress as measured increases in sCORT and decreases in body weight and bursa% (Puvadolpirod and Thaxton, 2000; review: Kidd, 2004; review: Laura et al, 2013).

There were no differences ($P > 0.05$) comparing lame and sound bird fCORT concentrations (**Figure 6.5**). Circulating CORT measures indicate a "snapshot" of acute stress at the time of collection whereas feather CORT is thought to indicate an integral measure of CORT through the growth of the feather measured (Bortolotti et al., 2008). Many factors such as

molting, keratin deposition, nutrition and genetics could effect CORT deposition into growing feathers (Harris et al., 2017). Given the assumption that the pathology of BCO progressively leads to lameness, we hypothesized fCORT concentrations would capture the additive stress of lame birds becoming lame. However, this was not the case. The present results suggest fCORT is not a useful retrospective index of stress in commercial broiler chickens.

The eye surface temperatures and beak surface temperatures were proposed noninvasive indicators of stress. There were no differences ($P > 0.05$) in eye surface temperatures between lame and sound broiler chickens (**Figure 6.6**). Some studies have found handling stress to decrease eye surface temperatures (Edgar et al., 2013; Herborn et al., 2015), while others have found increases in eye surface temperatures in chickens (Moe et al., 2017). However, delta beak surface temperatures of lame broilers were lower ($P < 0.0001$) by 3.1°C when compared to sound broilers (**Figure 6.6**). These results are novel and interesting as eyes are anatomically in close proximity to the beak. The handling stress for thermal image capture may have affected eye temperatures for both lame and sound birds, thus making surface temperatures more similar. However, no studies have evaluated beak surface temperatures as a noninvasive measure of stress. Given the contradictory literature on eye temperatures as a noninvasive stress measure, the results of this study indicate beak surface temperatures may serve as a more accurate indicator of stress.

Pre-mortem leg health measures recorded included blood oxygen saturation percentages and hock, shank and foot surface temperatures of each bird's right and left leg. Lame broilers had 4.3% lower ($P = 0.0009$) blood oxygen saturation in their legs compared to sound broilers (**Figure 6.7**). Recent studies using IRT to evaluate footpad dermatitis have found broilers with lower footpad surface temperatures had more severe footpad dermatitis scores (Jacob et al.,

2016). The decrease in footpad surface temperature was attributed to the increased tissue necrosis and decreased 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. For the first time, the results of this study provide evidence that this hypothesis may be true.

The data presented in Chapter 4 revealed lame birds had lower hock, shank and foot surface temperatures when compared to sound birds raised on wire flooring. Similar results were found in the present chapter for comparisons of lame birds raised on wire flooring to sound birds raised on litter flooring. Hock, shank and foot surface temperatures were lower ($P < 0.0003$) for lame birds compared to sound birds (**Figure 6.8**). Deltas for the reduced lame bird hock, shank and foot surface temperatures were 0.5°C, 0.8°C, and 2.6°C compared to sound birds, respectively. The combination of results from this study and the studies in Chapter 4 suggest the foot surface temperatures serve as reproducible noninvasive IRT indicator of leg health. Delta foot surface temperatures were the lowest ($P < 0.0001$) compared to hock and shank surface temperatures for sound and lame birds by 1.3°C and 3.3°C, respectively (**Figure 6.8**).

Post-mortem clinical diagnosis of BCO was confirmed after lame birds were identified visually on wire flooring pens. Lame broilers had more severe FHN, THN and Total N lesion severity scores than sound broilers (**Figure 6.9**). Percentage differences for the increased lame bird FHN, THN and Total N were 118%, 36%, and 72% compared to sound birds, respectively. It is interesting that sound, clinically healthy birds raised on litter also exhibited macroscopic evidence of BCO lesions. This may be due to factors such as genetic predisposition to fast growth (review: Bessei, 2006). Zhang and colleagues (2017) injected the glucocorticoid methylprednisolone (20mg/kg body weight) 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 to control birds injected with saline (Zhang et al., 2017). Lame birds in the present study had higher concentrations of sCORT and lower leg O_2. Stress due to dexamethasone injections leading to immunosuppression are implied contributors to the pathogenesis of BCO and turkey osteomyelitis complex (TOC) (Wideman and Pevzner, 2012; Huff et al., 1998). The combination of high circulating CORT and possible reduced blood flow to the leg (as measured by blood oxygen saturation) which may have exacerbated the proliferation of bacteria in the proximal femoral and tibial growth plates.

There were significant linear relationships between leg O_2, surface temperature and necrosis data. **Table 6.1** shows the linear regression statistics between leg O_2 with leg region surface temperatures and leg bone necrosis lesion severities. There were positive linear relationships between leg O_2 with shank ($P = 0.0012$) and foot ($P < 0.0001$) surface temperatures (**Table 6.1**). This indicates lower leg temperatures were indicative of lower blood oxygen saturation. There were negative linear relationships between leg O_2 with FHN ($P = 0.0005$), THN ($P < 0.0001$) and Total N ($P < 0.0001$) (**Table 6.1**). This indicates birds with more severe proximal femoral and tibial head necrosis lesions had lower leg blood oxygen saturation. Vascular occlusion by bacteria has been reported to be a component of BCO pathogenesis (review: McNamee and Smyth, 2000; Wideman, 2016) and this may be a factor in blood oxygen saturation. There were significant linear relationships between temperatures and necrosis data. **Table 6.2** shows the linear regression model statistics between surface temperature and necrosis data. Hock, shank and foot surface temperatures were lower ($P < 0.02$) for birds with higher FHN, THN and Total N lesion severities (**Table 6.2**).

Additionally, there were significant correlations between stress and lameness measures. **Table 6.3** shows the correlations of BW, CT, bursa%, sCORT, fCORT, ET, BT, leg O_2, HT, ST and FT. BW was negatively correlated with sCORT concentrations ($r = -0.41$, $P < 0.0001$) and positively correlated with leg O_2 ($r = 0.19$, $P < 0.0015$). sCORT concentrations were negatively correlated ($P < 0.01$) with leg O_2 and all body region surface temperatures (**Table 6.3**). There were positive ($P < 0.05$) correlations between all body region surface temperatures (**Table 6.3**). **Table 6.4** shows the correlations between FHN, THN, and Total N lesion severities with measures from **Table 6.3**. Bursa% and BT were negatively ($P < 0.05$) correlated with FHN, THN and Total N. sCORT concentrations were positively correlated ($P < 0.0001$) with FHN ($r = 0.34$), THN ($r = 0.24$) and Total N ($r = 0.35$) (**Table 6.4)**.

Using the forward stepwise regression technique, all measures were input for a multiple regression model to predict lameness. The significant selected measures were fit in the final logistic model to predict lameness, which were sCORT, core body temperature, leg O_2, and beak, shank and foot surface temperatures (**Table 6.5**). This model was very robust, as the area under the curve (AUC) was 0.98, the misclassification rate was 6% (**Appendix 1.1**). In studies evaluating BCO lameness, we suggest to include stress and leg health measures.

6.5 CONCLUSION

The results of this study provide evidence that lame broilers are more stressed than sound broilers. The correlations between sCORT concentrations, bursa% and core body temperature with beak surface temperatures advocate beak temperatures may be a more robust noninvasive measure of stress than eye temperatures in broilers. Additionally, the results from multiple experiments in this dissertation are novel and these are the first reports of beak surface temperatures as a noninvasive measure of stress in broilers. In combination with the results from

Chapter 4, IRT surface temperatures of hock, shank and foot temperatures are promising noninvasive measures, and possible predictors, of BCO lameness in broiler chickens. Linear regression relationships between leg O_2, surface temperatures and necrosis add additional merit to the utility of IRT in lameness evaluation. Moreover, there is reduced blood oxygen saturation in lame broiler legs compared to sound, which may be indicative of reduced blood flow. These results indicate the utility of IRT as a noninvasive measure of broiler stress and lameness.

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Table 6.1. Simple linear regressions of mean left and right leg oxygen saturation ($LegO_2$)[1] with hock surface temperature (HT, °C), shank surface temperature (ST, °C), and foot surface temperature (FT, °C)[2] and femoral head necrosis (FHN), tibial head necrosis (THN) and total necrosis (Total N)[3] lesion severity scores. (N = 268 birds).

		ANOVA		
Variable	Slope	R^2	F ratio	P-value
Leg region				
HT	0.03	0.012	3.23	0.0732
ST	0.06	0.039	10.77	0.0012
FT	0.17	0.167	25.43	< 0.0001
Necrosis				
FHN	-0.09	0.044	12.40	0.0005
THN	-0.06	0.097	28.45	< 0.0001
Total N	-0.15	0.074	21.41	< 0.0001

[1]Leg O_2 of the left and right leg blood oxygen saturation was measured with a flexible pulse oximeter sensor.

[2]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

[3]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

Table 6.2. Simple linear regressions of femoral head necrosis (FHN), tibial head necrosis (THN) and total necrosis (Total N)[1] lesion severity scores[1] with hock surface temperature (HT, °C), shank surface temperature (ST, °C)[2] (N = 268 birds).

			ANOVA		
Necrosis	Surface Temperature	Slope	R2	F ratio	P-value
FHN	HT	-0.27	0.029	7.84	0.0055
	ST	-0.21	0.022	6.07	0.0145
	FT	-0.18	0.061	17.17	< 0.0001
THN	HT	-0.12	0.029	7.99	0.0051
	ST	-0.13	0.041	11.27	0.0009
	FT	-0.10	0.082	23.80	< 0.0001
Total N	HT	-0.39	0.037	10.02	0.0017
	ST	-0.34	0.035	9.57	0.0022
	FT	-0.28	0.085	24.64	< 0.0001

[1]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.
[2]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

Table 6.3. Correlations (r) of lame and sound[1] 25-56 d old broiler body weight (BW,g), core body temperature (CT, °C), relative bursa weight (Bursa%, % BW) serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm)[2], eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), leg oxygen saturation ($LegO_2$, %)[3], hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), foot surface temperatures (FT, °C)[4] (N = 268 birds).

	Correlations (r)										
	BW	CT	Bursa%	sCORT	fCORT	ET	BT	$LegO_2$	HT	ST	FT
BW	1.00	-0.14*	-0.16*	-0.41**	-0.15*	-0.07	0.26**	0.19**	-0.13*	0.01	0.25**
CT		1.00	-0.23**	0.11	0.00	0.29**	0.19**	-0.10	0.35**	0.40**	0.19**
Bursa%			1.00	-0.29**	-0.05	0.11	0.17**	0.01	0.24**	0.17**	0.16**
sCORT				1.00	0.28**	-0.12	-0.42**	-0.21**	-0.29**	-0.31**	-0.40**
fCORT					1.00	-0.07	0.01	0.06	-0.09	-0.02	0.00
ET						1.00	0.51**	0.02	0.60**	0.57**	0.39**
BT							1.00	0.29**	0.57**	0.68**	0.68**
$LegO_2$								1.00	0.11	0.20**	0.30**
HT									1.00	0.83**	0.62**
ST										1.00	0.85**
FT											1.00

[1]Birds were considered lame if they would not step to walk when gently coaxed and often used wing tips for support to stand. All birds in litter pens were considered healthy.
[2]Blood samples were drawn from the brachial vein within 60 s of bird capture. Serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).
[3]Left and right leg blood oxygen saturation was measured with a flexible pulse oximeter sensor.
[4]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.
Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$

Table 6.4. Correlations (r) of lame and sound[1] 25-56 d old broiler right and left femoral head necrosis (FHN), right and left tibial head necrosis (THN) and total necrosis (FHN+THN);Total N)[2] with body weight (BW,g), core body temperature (CT, °C), relative bursa weight (RelB, % BW) serum corticosterone concentrations (sCORT, ng/mL), feather corticosterone concentrations (fCORT, pg/mm)[3], eye surface temperatures (ET, °C), beak surface temperatures (BT, °C), leg oxygen saturation ($LegO_2$, %)[4], hock surface temperatures (HT, °C), shank surface temperatures (ST, °C), foot surface temperatures (FT, °C)[5] (N = 268 birds).

	Correlations (r)										
	BW	CT	Bursa%	sCORT	fCORT	ET	BT	$LegO_2$	HT	ST	FT
FHN	-0.11	0.26**	-0.39**	0.34**	-0.05	0.00	-0.23**	-0.21**	-0.17**	-0.15*	-0.25**
THN	-0.18**	0.17**	-0.25**	0.24**	-0.01	-0.03	-0.22**	-0.31**	-0.17**	-0.20**	-0.29**
Total N	-0.15*	0.26**	-0.39**	0.35**	-0.05	-0.01	-0.25**	-0.27**	-0.19**	-0.19**	-0.29**

[1]Birds were considered lame if they would not step to walk when gently coaxed and often used wing tips for support to stand. All birds in litter pens were considered healthy.

[2]FHN Femoral head necrosis. The right and left proximal femoral heads were scored for FHN lesion severity on a 0-3 scale in the following categories: 0- no macroscopic abnormalities of the proximal femoral head (Normal); 1- proximal femoral head separation (FHS; epiphyseolysis); 2- proximal femoral head transitional degeneration (FHT); and 3- proximal femoral head necrosis (FHN). A quantitative FHN category was created by summing the right (0-3) and left (0-3) proximal FHN scores of increasing BCO lesion severity for a range of 0-6.

[3]Blood samples were drawn from the brachial vein within 60 s of bird capture. Serum corticosterone (sCORT; ng/mL) and feather corticosterone (fCORT; pg/mm) concentrations were measured by DetectX® Corticosterone EIA kits (Arbor Assays, Ann Arbor, MI).

[4]Left and right leg blood oxygen saturation (%) was measured with a pulse oximeter.

[5]Mean surface temperatures of the eye, beak, right and left hock, right and left shank and right and left foot were estimated by isolating pixels representing body regions within infrared thermography (IRT) images taken with a thermal camera.

Means with asterisks indicate significant pairwise correlations at * $P \leq 0.05$ **$P \leq 0.01$.

Table 6.5. Significant predictor variables (selected by forward stepwise regression) for a nominal logistic regression model to predict the binary response of lame vs. sound health status in broiler chickens.

Predictor variable[1]	**ChiSquare value**	**P-value**
sCORT	29.61	< 0.0001
CT	22.45	< 0.0001
FT	18.18	< 0.0001
ST	7.50	0.006
$LegO_2$	7.09	0.006
BT	6.70	0.01

[1]sCORT = serum corticosterone (ng/mL), CT = core body temperature (°C), FT = foot surface temperature (°C), ST = shank surface temperature (°C), $LegO_2$= mean right and left leg oxygen saturation (°C), BT = beak surface temperature (°C).

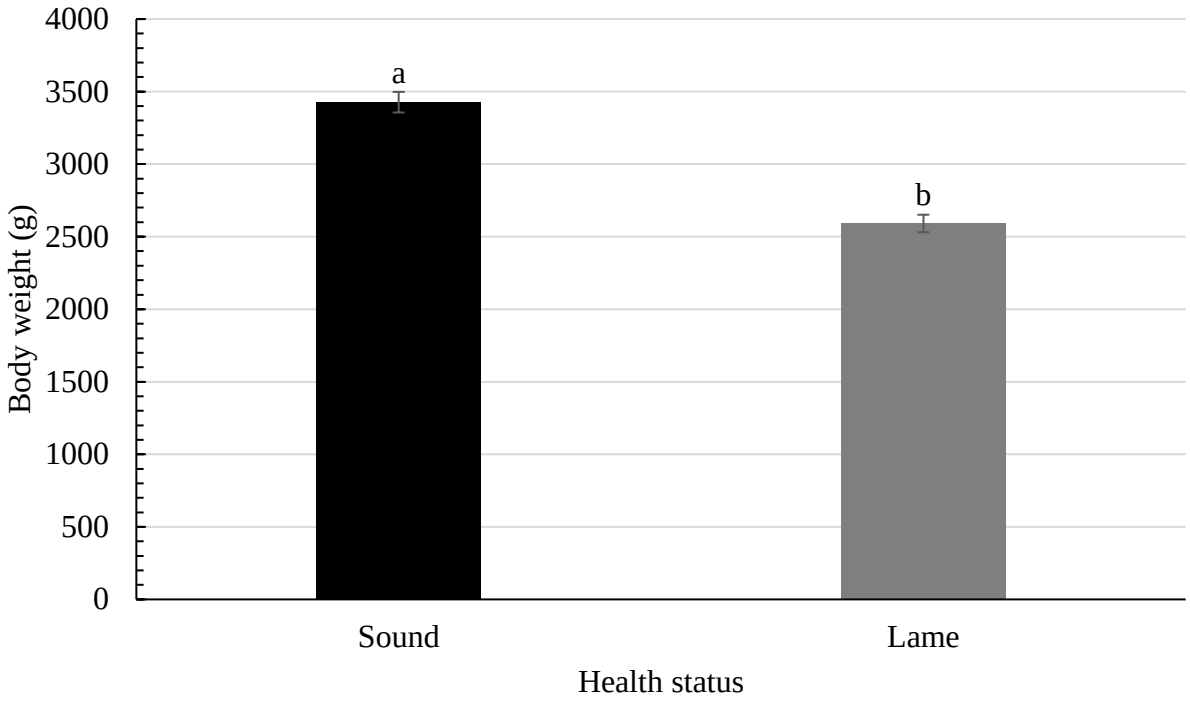


Figure 6.1. Body weight (BW, g) of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at $P < 0.0001$.

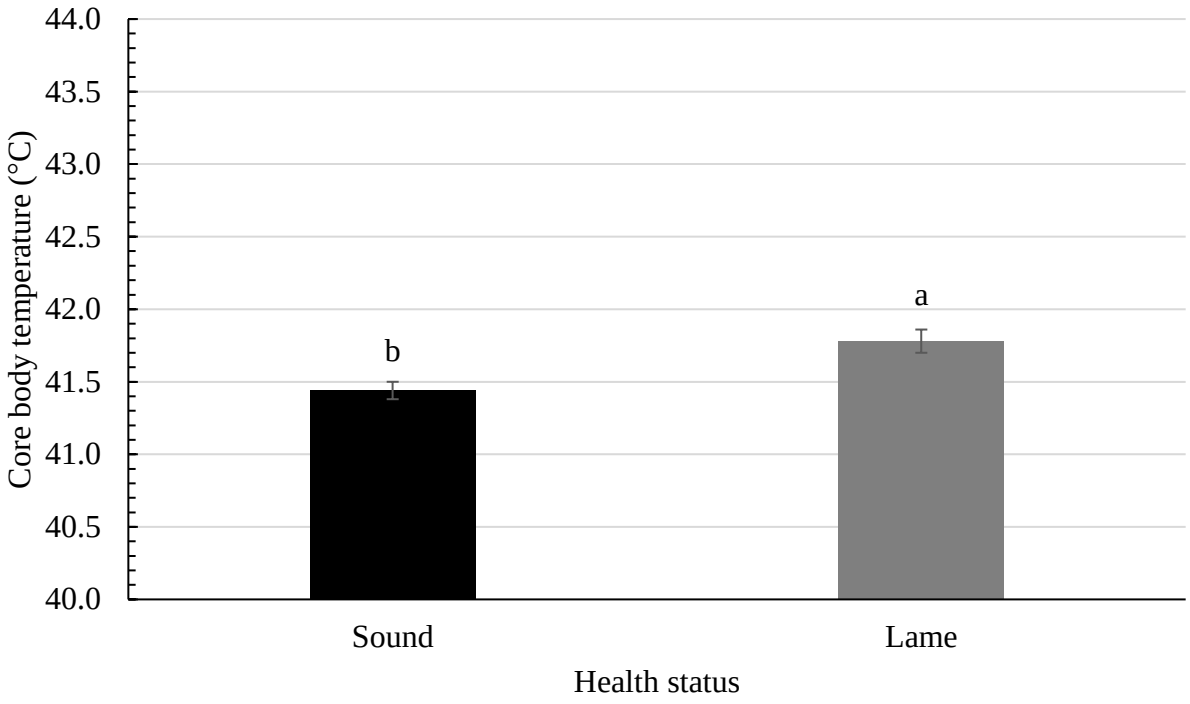


Figure 6.2. Core body temperature (°C) of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at $P < 0.0001$. Note the scale does not start at 0

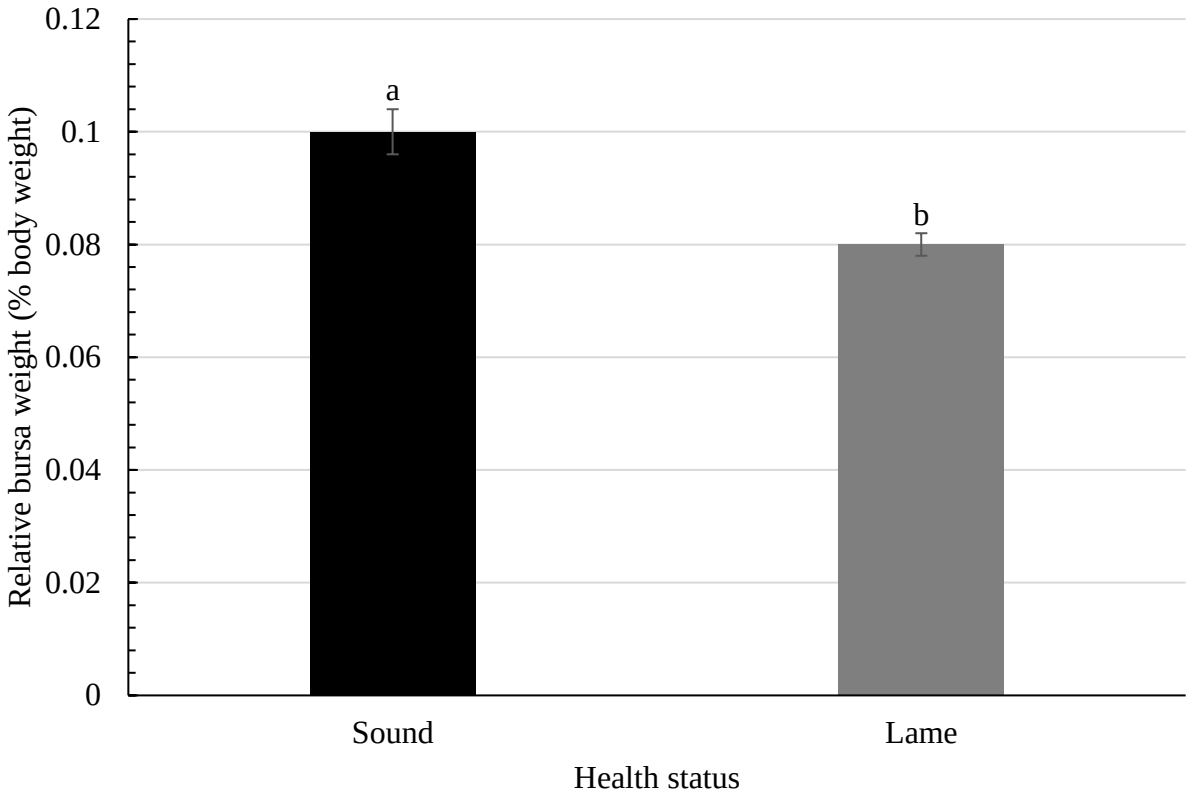


Figure 6.3. Relative bursa weight (% BW) of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at $P < 0.0001$.

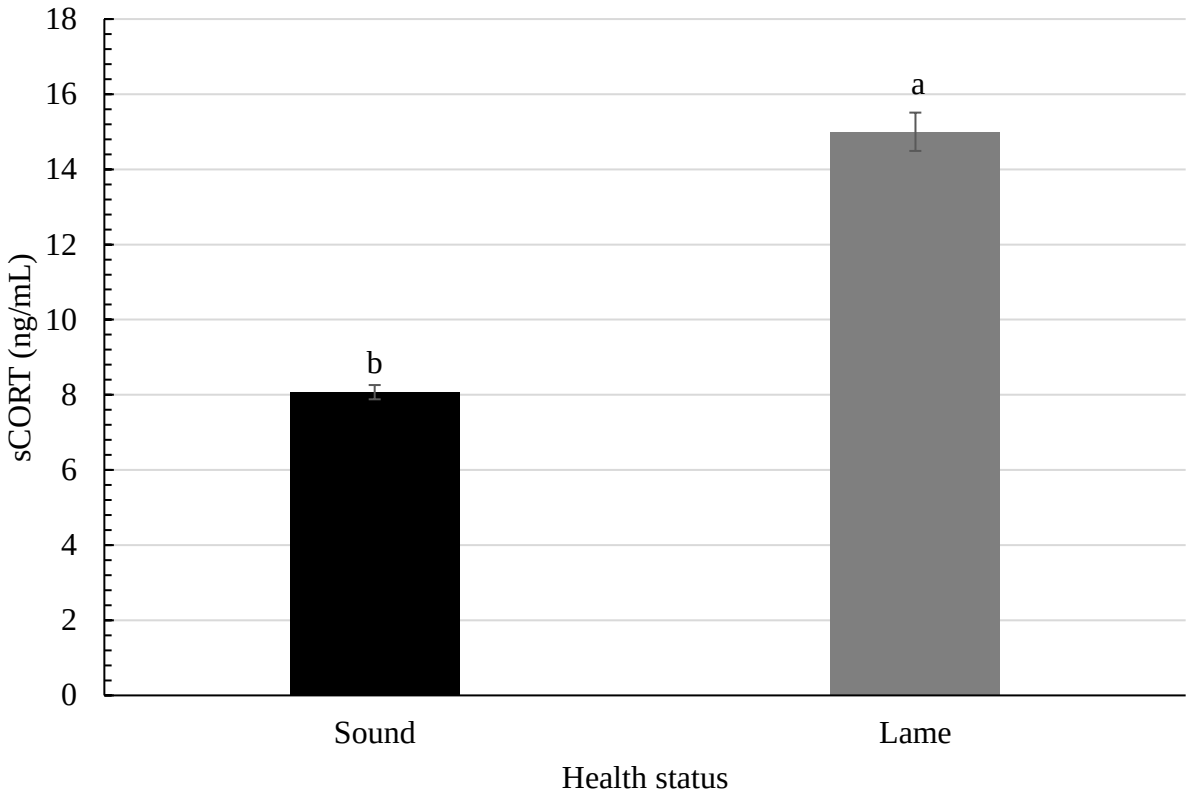


Figure 6.4. Serum corticosterone (sCORT, ng/mL) concentrations of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at $P < 0.0001$.

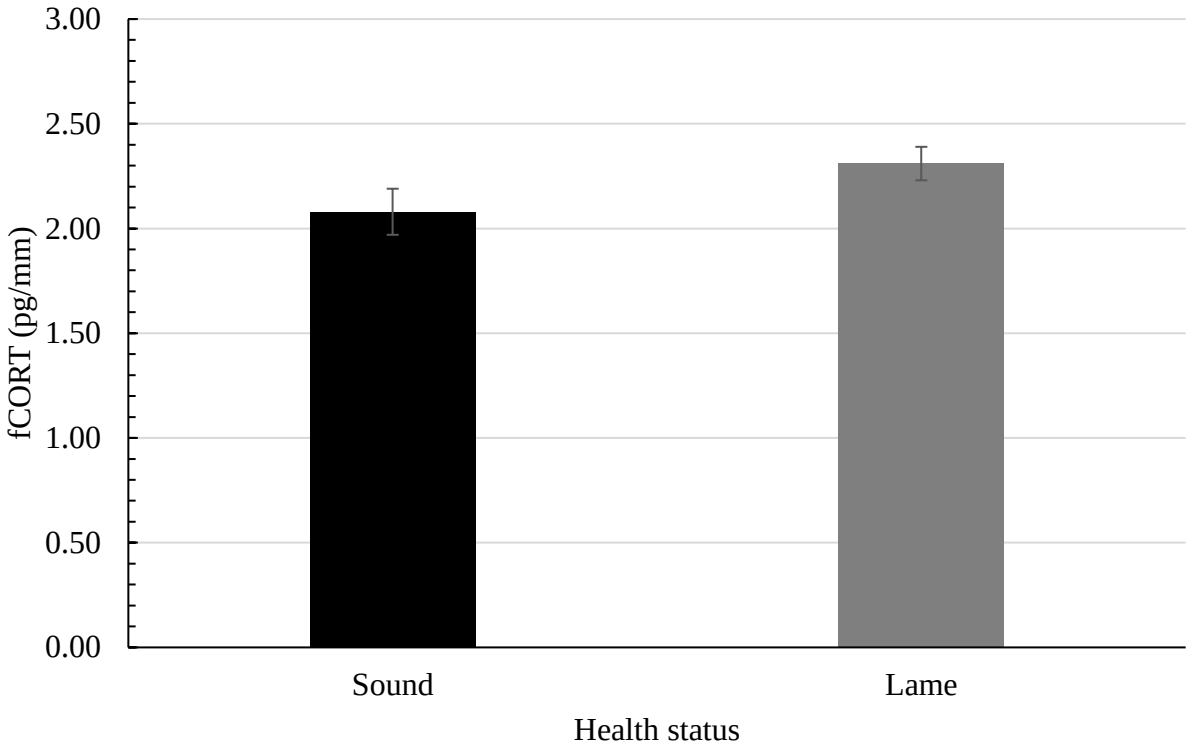


Figure 6.5. Feather corticosterone (fCORT, pg/mm) concentrations of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens).

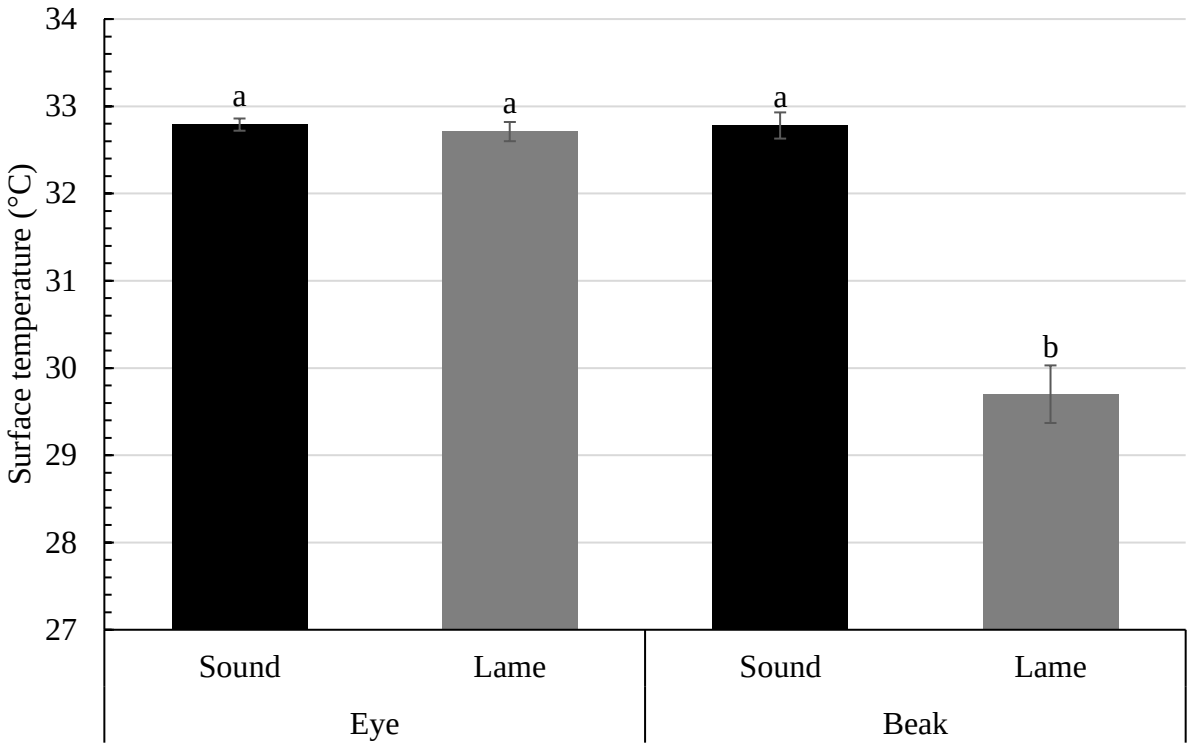


Figure 6.6. Eye and beak surface temperatures (°C) of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at ($P < 0.0001$). Note the scale does not start at 0.

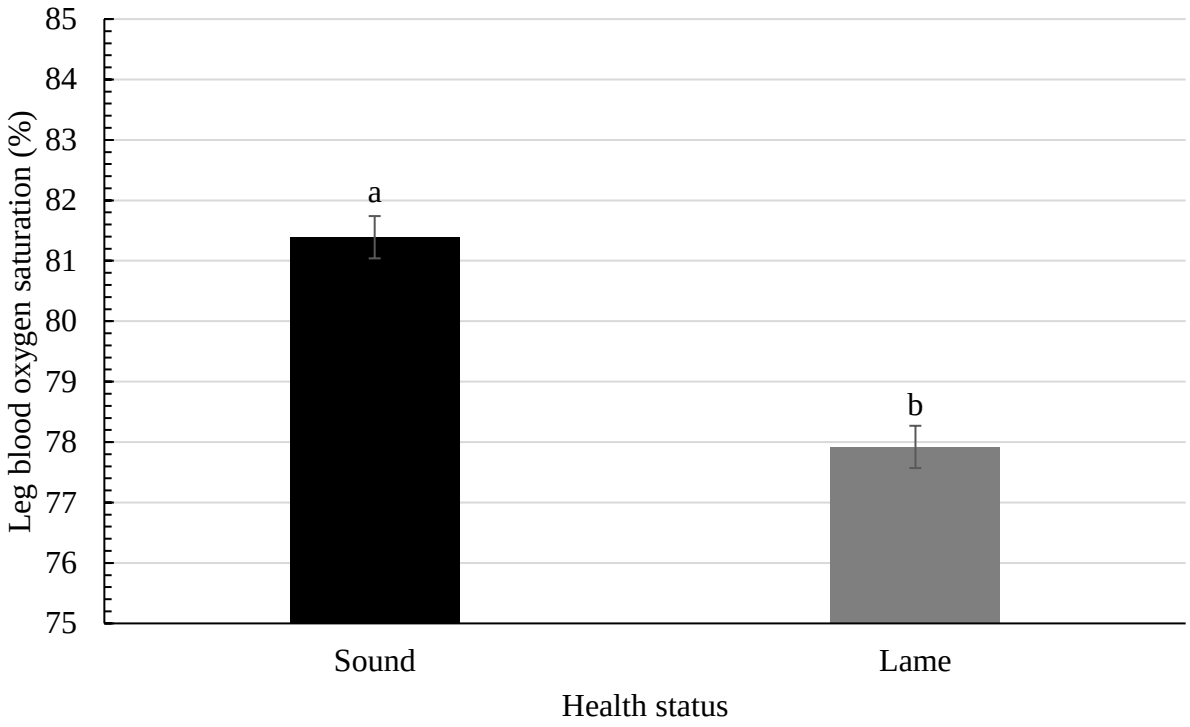


Figure 6.7. Leg blood oxygen saturation (leg O_2, %) of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at $P < 0.0009$. Note the scale does not start at 0.

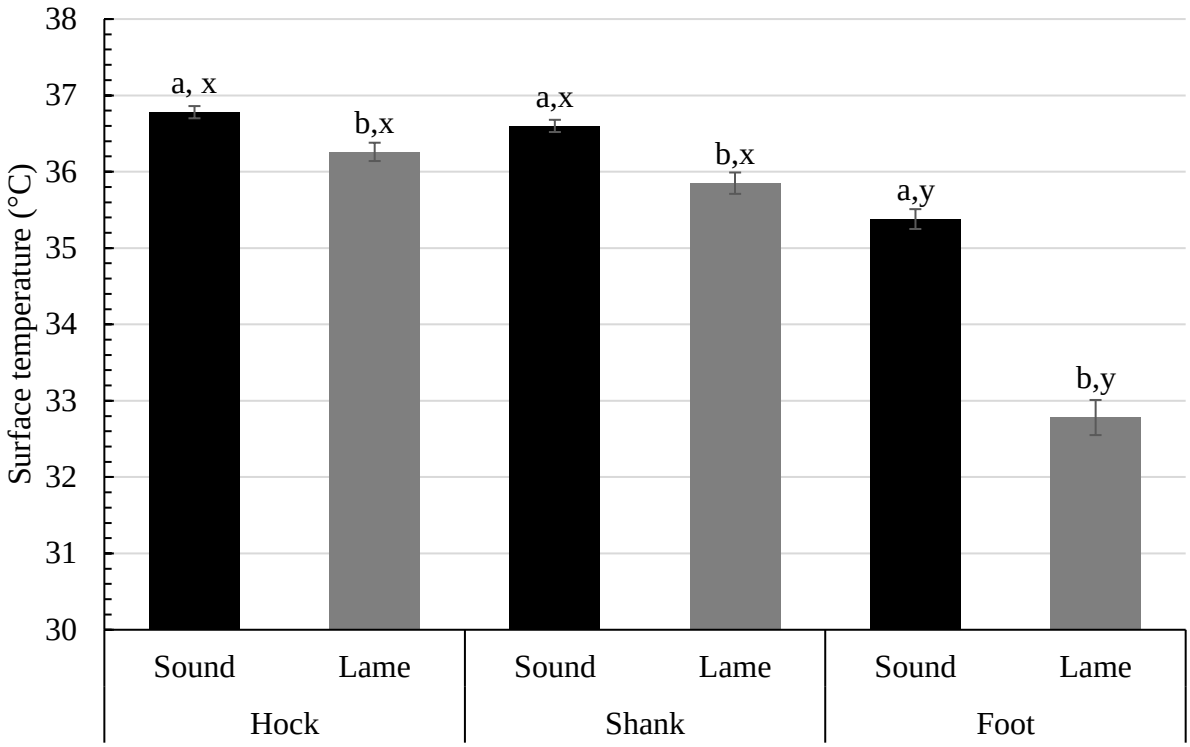


Figure 6.8. Hock, shank and foot surface temperatures (°C) of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens).
[ab] Differences between lame and sound are indicated by different superscript letters at $P < 0.0003$.
[xy] Differences between different leg regions (within sound or lame) are indicated by different superscript letters at $P \leq 0.05$.
Note the scale does not start at 0

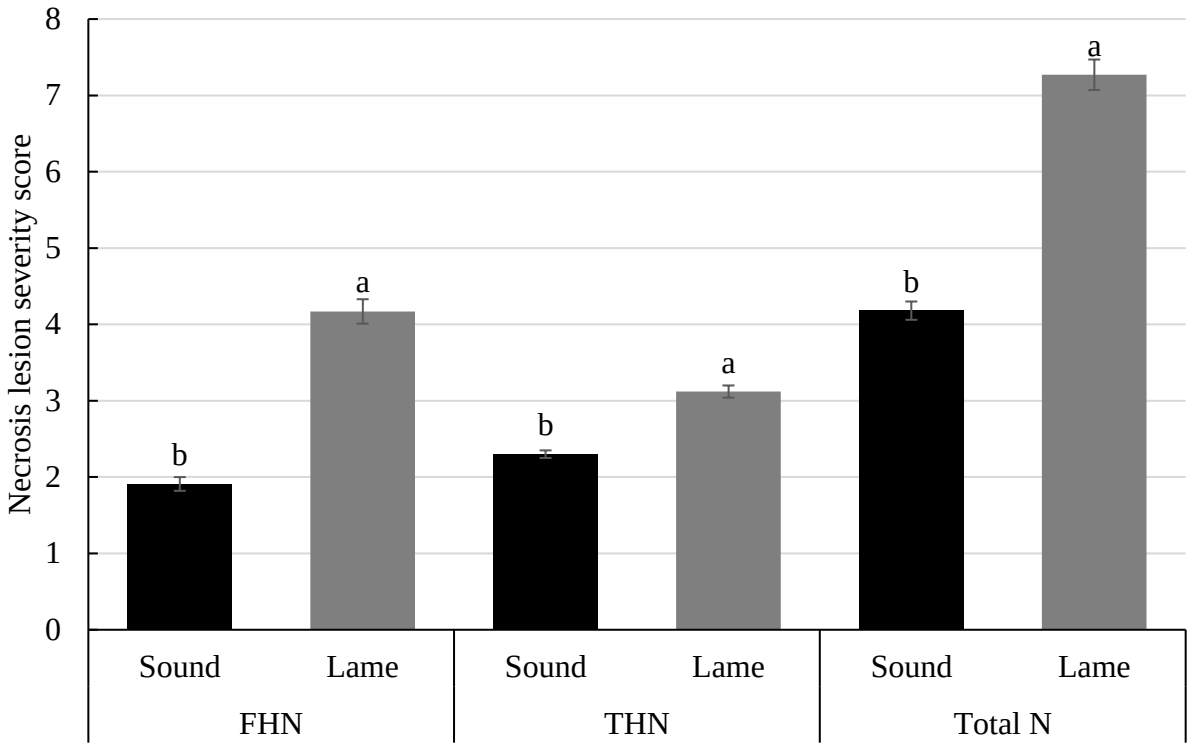


Figure 6.9. Left and right proximal femoral head necrosis (FHN), tibial head necrosis (THN) and aggregate [Total N, (FHN + THN)] lesion severity scores of cumulative 25 – 56 d old sound and lame broilers. Data presented as mean ± SEM (N = 6 pens). Columns not sharing the same letter are different at ($P < 0.0001$).

CHAPTER 7 Conclusion

The main objective of the series of studies included in this dissertation was to investigate noninvasive measures of broiler stress and lameness. The two primary noninvasive methods were extraction of CORT from feathers to evaluate stress and infrared thermography (IRT) to evaluate stress and lameness attributed to bacterial chondronecrosis with osteomyelitis (BCO).

Corticosterone (CORT) and CORT Assay

The first step was to evaluate the ELISA assay of CORT from serum, cecal contents, excreta and two feather types. To evaluate this, CORT was administered to broilerss (CS) in the drinking water over a 72 h period (Chapters 2 and 3). The results are novel as no research has evaluated the timeline of how quickly CORT administration affected body weight, heterophil to lymphocyte (H:L) ratio, and serum, cecal content, excreta, body and primary feather CORT concentrations. Serum CORT concentrations increased at 6 h and sustained elevated concentrations until 72 h.

The validity of serum CORT concentrations, as determined by ELISA, is supported by serum CORT concentrations having the lowest coefficients of variation compared to feather, cecal and excreta CORT concentrations (Chapters 2 and 3). CS broiler excreta CORT concentrations increased more consistently with time than cecal content CORT concentrations and both cecal contents and excreta CORT had similar coefficients of variation. There were moderate correlations between H:L and CORT concentrations. There was an unexpected and unexplained increase in CORT in feathers from control broilers (Chapter 2 body and primary feather; Chapter 3, primary feathers). Body and primary feather CORT concentrations were moderately positively correlated at approximately 40 percent. These data allude to unknown interfering factors that may indicate feather CORT concentrations are not consistently useful to measure stress in broilers. Both H:L ratios and core body temperatures increased with age and

serum CORT concentrations decreased with age (Chapter 5). These findings with H:L ratios and serum CORT concentrations are novel.

Lameness

Broilers were raised on wire flooring to experimentally induce higher incidences of lameness than seen in commercial conditions. Broilers were also raised on litter flooring for comparison. Three light intensities (2, 5 and 10 lux) were added to the study design to explore possible effects on lameness. Light intensity had no effect on the incidence of lameness, proximal femoral head necrosis (FHN), proximal tibial head necrosis (THN) lesion severity scores, or IRT hock, shank and foot leg region surface temperatures. As expected, virtually no broilers went lame while being raised on litter flooring. There were markedly increased numbers of lame broilers when raised on wire. Lameness incidence of broilers raised on wire flooring was over three-fold higher in exp 1 than exp 2. This is difficult to explain as the experimental design was replicated with chicks from the same source for both studies. A particularly interesting result of Chapter 4 is that clinically healthy broilers raised on litter had the same degree of proximal femoral head necrosis (FHN) and tibial head necrosis (THN) as clinically healthy broilers raised on wire flooring. These results provide further evidence that visually clinically healthy broilers may have subclinical degrees of necrosis in their leg bone growth plates. This could possibly be due to high growth rates and heavy body weights that are insufficiently supported by the skeletal system in today's commercial broiler.

Latency to lie (LTL)

In Chapter 5, birds 8 wk of age were subjected to the LTL test in groups and individually. LTL is reported to be a behavioral measure of leg health. There were no light intensity or flooring type effects on LTL, nor were there group versus individual differences in LTL times.

The comparison of LTL behavioral differences in groups and individually is novel and has not been evaluated previously.

Hock, shank and foot surface temperatures

The evaluation of leg region hock, shank and foot surface temperatures, as measured by IRT, are a highlight of this dissertation. In Chapter 4, we hypothesized IRT leg region surface temperatures would be indicative of BCO necrosis lesion severity in femora and tibiae. We also hypothesized that sound broilers raised on wire flooring would have more severe BCO necrosis lesion severities than broilers raised on litter. However, there were no differences in hock, shank and foot surface temperatures in the comparison of clinically healthy broilers raised on litter versus wire flooring. This is was unexpected. In Chapters 4 and 6 lame broiler surface temperatures of the hock, shank and foot leg regions were lower than sound broilers. In chapter 6, linear relationships were found between leg region surface temperatures with both FHN and THN lesion severities. As expected, FHN and THN lesion severities for lame broilers were more severe than for sound broilers.

Eye and beak surface temperatures

Eye and beak surface temperatures were evaluated as determined by IRT in Chapters 3, 5 and 6 as a noninvasive measure of stress. There were no effects of wire flooring on beak or eye surface temperatures. While there were correlations between the various indices of stress, these were not consistently observed between the two studies in Chapter 5. There were moderate correlations between serum CORT concentrations with eye and beak surface temperatures in Chapters 5 and 6. Surprisingly, neither eye nor beak temperatures were influenced by CORT treatment (Chapter 3). However, in Chapters 6, lame bird eye and beak surface temperatures were lower than sound broilers.

Lameness and stress

The final study on lameness and stress is the highlight of this dissertation (Chapter 6). The experiment was designed to further investigate the stress and lameness measures used in previous chapters. As expected, lame broilers weighed less than sound broilers. Also as expected, lame broilers had more severe FHN and THN lesions than sound broilers. Both serum CORT concentrations and core body temperatures in lame broilers were higher than sound broilers. Beak temperatures were lower for lame broilers than sound broilers. In combination with the results from Chapter 5, beak surface temperatures show considerable utility as a noninvasive measure of stress in broilers. Leg blood oxygenation (leg O_2) was measured with a pulse oximeter and results show lame broilers had lower leg O_2. A robust logistic regression model was built to statistically predict lameness shown in Appendix 1. This model included serum CORT concentrations, leg O_2, core body temperature, shank surface temperature, foot surface temperature and beak surface temperature.

Final Conclusions

Blood serum CORT concentrations were a reliable measure of broiler stress. They were increased in lame broilers and following CORT administration. Although the provision of CORT in the drinking water increased body and primary feather CORT concentrations over time, environmental stressors did not affect feather CORT concentrations. The understanding of mechanisms of internal and external interference affecting CORT deposition into feathers is still nebulous. Feather CORT concentrations were inconsistent and did not reflect stress status of broilers in the experiments conducted for this dissertation. Beak surface temperatures were the most robust noninvasive measure of broiler stress.

The present data on IRT were novel and support the use of thermography as a useful tool for physiological investigation. Lameness was associated with depressed surface temperatures of the beak, hock, shank and foot but not the eye. This research gives insight into IRT as a noninvasive tool to evaluate broiler health and welfare. There is still much work to be done to further understand the physiological factors that contribute to individual IRT measures such as metabolism, thermoregulation, and blood flow.

The present studies reveal the importance of collecting multiple parameters to measure stress and lameness as well as applying multiple analyses to predict lameness in broilers.

APPENDIX

1.1. JMP logistic regression model to predict the binary response of lame vs. sound broiler chickens statistical output.

Nominal Logistic Fit for Status

Effect Summary

Source	LogWorth	PValue
SCORT	16.182	0.00000
C Temp	8.439	0.00000
Foot Avg	6.089	0.00000
Leg O2	2.440	0.00363
Shank Avg	2.412	0.00387
Bavg	2.210	0.00616

Converged in Gradient, 8 iterations

Iterations

Iter	Objective	Relative Gradient	Norm Gradient
0	185.07029721	13.199537947	574.55691217
1	83.837044735	6.0575237294	576.07023192
2	60.432228063	3.8285242722	328.92085735
3	51.280758418	2.0693266979	128.75155882
4	48.726279605	0.7875519945	37.107763401
5	48.385908431	0.1303240344	4.6684758942
6	48.377258761	0.0037124173	0.0987442436
7	48.377251867	3.0594097e-6	0.0000654069
8	48.377251866	2.064938e-12	9.311778e-11

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	136.69117	6	273.3823	<.0001*
Full	48.37725			
Reduced	185.06842			

RSquare (U)	0.7386
AICc	111.187
BIC	135.865
Observations (or Sum Wgts)	267

1.1. JMP logistic regression model to predict the binary response of lame vs. sound broiler chickens statistical output (Cont).

Fit Details

Measure	Training	Definition
Entropy RSquare	0.7386	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.8544	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.1812	∑ -Log(ρ[j])/n
RMSE	0.2269	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.1060	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.0599	∑ (ρ[j]≠ρMax)/n
N	267	n

Lack Of Fit

Source	DF	-LogLikelihood	ChiSquare
Lack Of Fit	260	48.377252	96.7545
Saturated	266	0.000000	**Prob>ChiSq**
Fitted	6	48.377252	1.0000

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	-222.35895	45.727189	23.65	<.0001*
C Temp	2.17772764	0.4596526	22.45	<.0001*
SCORT	0.61151162	0.1123846	29.61	<.0001*
Bavg	-0.3975949	0.1536484	6.70	0.0097*
Leg O2	-0.2003128	0.0752179	7.09	0.0077*
Shank Avg	1.26879623	0.4634469	7.50	0.0062*
Foot Avg	-1.0002834	0.2346175	18.18	<.0001*

For log odds of Lame/Normal

Effect Wald Tests

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
C Temp	1	1	22.4464477	<.0001*
SCORT	1	1	29.6070754	<.0001*
Bavg	1	1	6.69616023	0.0097*
Leg O2	1	1	7.09209616	0.0077*
Shank Avg	1	1	7.49520964	0.0062*
Foot Avg	1	1	18.1771215	<.0001*

1.1. JMP logistic regression model to predict the binary response of lame vs. sound broiler chickens statistical output (Cont).

Effect Likelihood Ratio Tests

Source	Nparm	DF	L-R ChiSquare	Prob>ChiSq
C Temp	1	1	34.8079424	<.0001*
SCORT	1	1	69.7944182	<.0001*
Bavg	1	1	7.50200646	0.0062*
Leg O2	1	1	8.4613561	0.0036*
Shank Avg	1	1	8.34236214	0.0039*
Foot Avg	1	1	24.3240424	<.0001*

Odds Ratios

For Status odds of Lame versus Normal

Unit Odds Ratios

Per unit change in regressor

Term	Odds Ratio	Lower 95%	Upper 95%	Reciprocal
C Temp	8.826227	3.585239	21.72862	0.1132987
SCORT	1.843216	1.478816	2.297408	0.5425301
Bavg	0.671934	0.497211	0.908056	1.4882411
Leg O2	0.818475	0.706284	0.948486	1.2217849
Shank Avg	3.556569	1.433985	8.821002	0.2811699
Foot Avg	0.367775	0.232207	0.582491	2.7190522

Range Odds Ratios

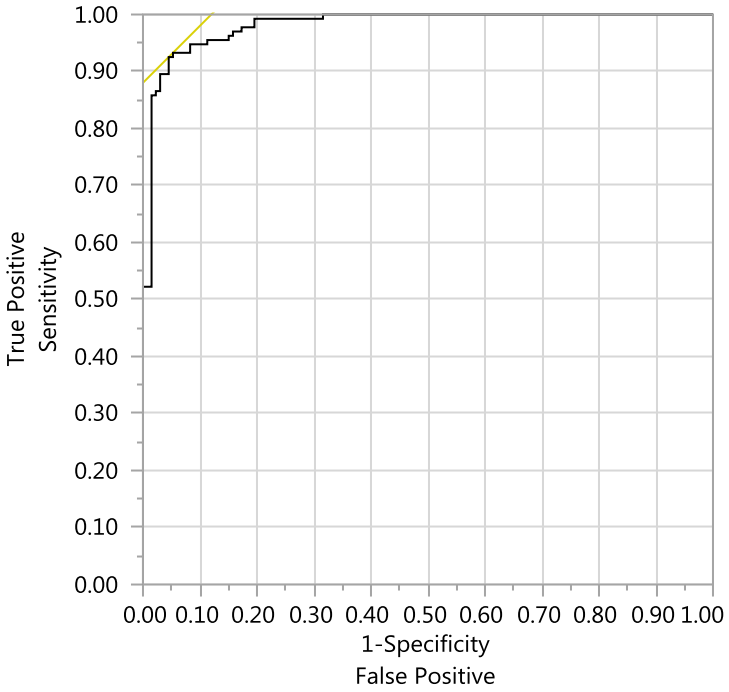
Per change in regressor over entire range

Term	Odds Ratio	Lower 95%	Upper 95%	Reciprocal
C Temp	53564.31	592.3663	4843515	1.8669e-5
SCORT	3.436e+8	289260.4	4.08e+11	2.9107e-9
Bavg	0.001945	1.721e-5	0.219971	514.00878
Leg O2	0.000547	2.171e-6	0.137612	1829.3782
Shank Avg	58400.95	22.60007	1.509e+8	1.7123e-5
Foot Avg	2.489e-6	6.604e-9	0.000938	401778.12

Tests and confidence intervals on odds ratios are Wald based.

1.1. JMP logistic regression model to predict the binary response of lame vs. sound broiler chickens statistical output (Cont).

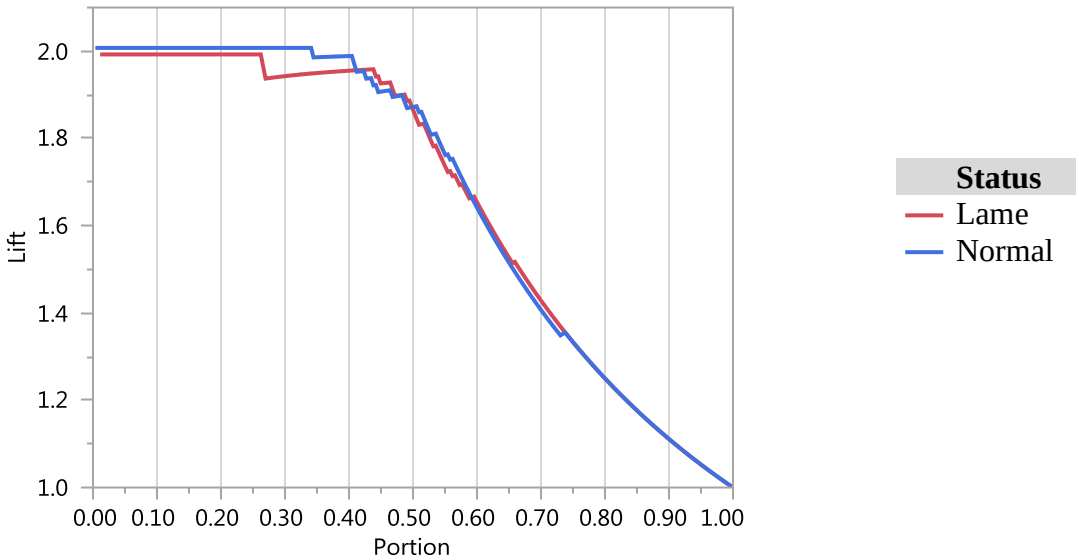
Receiver Operating Characteristic



Using Status='Lame' to be the positive level

AUC
0.98120

Lift Curve



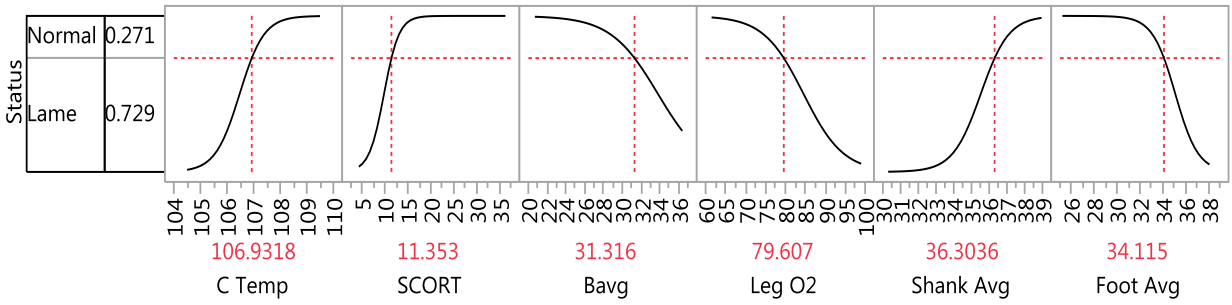
1.1. JMP logistic regression model to predict the binary response of lame vs. sound broiler chickens statistical output (Cont).

Confusion Matrix

Training

Actual	Predicted Count	
Status	**Lame**	**Normal**
Lame	125	9
Normal	7	126

Prediction Profiler



1.2 IACUC Approval #140005

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Dr. Robert Wideman

FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee (IACUC)

DATE: August 29, 2014

SUBJECT: IACUC APPROVAL
Expiration date: September 9, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your modification to add a students to protocol 140005: *'Bacterial causes of lameness in chickens'*
In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing(via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond September 9, 2015 you must submit a modification for an extension or a new protocol prior to that date. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
Fax: 479-575-3846 • http://vpred.uark.edu/199

1.3 IACUC Approval #16014

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Karen Christensen

FROM: Craig N. Coon, Chairman

DATE: 9/14/15

SUBJECT: IACUC Approval

Expiration Date: Sep 13, 2016

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 16014 : "Effect of Light Intensity on BCO Lameness in Commercial Broiler Chickens", you may begin immediately.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond Sep 13, 2016 you must submit a modification or new protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
Fax: 479-575-3846 • http://vpred.uark.edu/199

1.4 IACUC Approval #16014 extension

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

To: Karen Christensen

From: Craig Coon, IACUC Chair

Date: August 30, 2016

Subject: IACUC Approval

Expiration Date: September 13, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your the extension of protocol # 16014 "Effect of Light Intensity on BCO Lameness in Commercial Broiler Chickens" for an additional year.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond September 13, 2017 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem
cc: Animal Welfare Veterinarian

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Fax: 479-575-3846 • http://vpred.uark.edu/199