

## ABSTRACT

Title of Thesis: THE IMPACT OF PLATFORM AND SPOTLIGHT ENRICHMENTS ON CONVENTIONAL BROILER PERFORMANCE, TIBIA MORPHOLOGY, AND WELFARE

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Master of Science, 2021*

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Welfare is an important consideration in proper management of broilers. Providing broilers with environmental enrichments may help improve welfare on many levels, and evaluation of potential enrichments is important to ensure they improve broiler welfare. The objective of this study was to evaluate the effect of no enrichments (C), platform (P), spotlight (S), and a combination of spotlight and platform (S&P) enrichments on broiler production, welfare, stress, tibia morphology, tibia ash, and fear behavior. Production, corticosterone, and tibia ash measures were unaffected by enrichments. Compared to other treatments, P broilers had wider ( $P \leq 0.02$ ) tibias at 90%, 75%, and 25% length locations and S broilers had narrower ( $P \leq 0.04$ ) tibias at 90% and 75% length locations. Fear decreased from week 3 to week 5 ( $P \leq 0.05$ ). The results indicate that both platform and spotlight enrichments can influence tibial morphology without decreasing production or animal-based measures of welfare.

THE IMPACT OF PLATFORM AND SPOTLIGHT ENRICHMENTS ON  
CONVENTIONAL BROILER PERFORMANCE, TIBIA MORPHOLOGY, AND  
WELFARE

by

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Thesis submitted to the Faculty of the Graduate School of the  
University of Maryland, College Park, in partial fulfillment  
of the requirements for the degree of  
Master of Science  
2021

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## **Acknowledgements**

First, I would like to thank my advisor, Dr. Shawna Weimer for her dedication, mentorship, and guidance during my time in her lab. I am very thankful to her for the opportunity to study animal behavior and well-being under her guidance. Her support has been an integral part of my graduate experience.

I would also like to thank my advisory committee members Dr. Roselina Angel, Dr. Gregory Ball, and Dr. Rachel Dennis for all their guidance and expert advice throughout all parts of this project.

I want to thank my friends and family for all their support. I am so glad to know that you all would take the time out your busy lives to make sure I knew I was supported no matter what I went through. Not only in graduate school, but in life as well.

Finally, I would like to thank my parents, Dino and Michelle Magnaterra Jr., for all the love, and guidance they gave me during their lifetimes. You are two of the strongest and most tenacious people I have ever met and you continue to be an inspiration in my life every day. I am forever grateful to you both for encouraging me to further my education.

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## **Chapter 1: LITERATURE REVIEW**

## **1.1 INTRODUCTION**

Poultry products, specifically chicken meat, are a popular protein source throughout much of the world. As explained in a review paper written by Phibbs et al. (2021) the low feed conversion ratio and quick growth rate of broiler chickens has led poultry meat production to be lower cost compared to other agricultural animal industries. In addition to the low cost to farmers, the meat is lower cost to consumers and considered to be a healthier animal protein option than red meats. These factors are what has allowed chicken meat to become so popular in many countries (Phibbs et al., 2021).

Common concerns surrounding the welfare of chickens include: the use of battery cages for laying hens, leg health in broiler chickens, and the ability to express natural behaviors in both laying hens and broilers (Lay et a., 2011; Phibbs et al., 2021). There is not a single consensus on how to best enrich broilers' lives to promote their expression of natural behaviors (Souza Da Silva and de Jong, 2019). Often the addition of complexity in the environment is added through structural, edible, or transient enrichments. Enrichments provided should add to the bird's welfare by providing them the opportunity to thoroughly express a natural behavior or redirect negative behaviors (Riber et al., 2018). In addition to the expression of natural behavior, housing system research has been conducted on both laying hens and broilers to determine if enrichments increase the welfare of the birds in other ways, such broiler foot and leg health (Riber et al., 2018; Campbell et al., 2019). In broilers, there is focus on if the environmental enrichments can help to improve leg health and change behavior, particularly to decrease time spent sitting (Riber et al., 2018). In laying hens, the use of aviary systems and enriched colony caging systems has been the focus of research concerning injuries and social interactions within the group, for example

feather pecking is a common issue which needs to be limited for all birds in the system to have fewer negative experiences (Campbell et al., 2019).

Growing concern of animal welfare in the eyes of the public has led to the passing of legislation affecting the poultry industry. Federal legislature is present in the United States and individual states have also passed legislation surrounding the topic of animal welfare (Hirsch, 2003). In the United States the major federal law, the Animal Welfare Act, protecting animals by setting standards of care excludes farm animals (Animal Welfare Act, 1966). Every state in the United States has state-level legislation to protect animals from cruelty, but some states exclude farm animals except in cases of severe neglect (Hirsch, 2003). The details of the legislation will vary from state to state (Hirsch, 2003). An example of state-level legislature affecting the poultry industry is the current or future banning of caged systems for layer hens in California, Colorado, Massachusetts, Michigan, Nevada, Oregon, Rhode Island, Utah, and Washington (ASPCA, 2021).

There are several definitions of animal welfare. This is because one's ethical views will influence how they believe animals should be cared for (Held and Spinka, 2011). The OIE World Organization for Animal Health (2021) has broadly defined animal welfare as "the physical and mental state of an animal in relation to the conditions in which it lives and dies." Some noticeable aspects of this definition are that there is concern for both animals' physical biological and subjective mental welfare. This definition is also concerned with the animals' welfare both throughout their life, but also at the time of death. Other definitions of animal welfare may also have a focus on the ability to express natural behaviors (Held and Spinka, 2011). To best understand approaches to animal welfare, we should also understand that formal guidance on animal welfare did not exist before 1979.



The first document containing guidance on animal welfare was the 1965 Brambell Report in the United Kingdom (Brambell, 1965). From the guidance contained in the Brambell Report, a necessity for further breakdown of basic animal welfare standards to create formal guidance was seen, and the Five Freedoms were defined by the Farm Animal Welfare Council in 1979 (FAWC, 1979). The five freedoms are: (1) freedom from hunger or thirst; (2) freedom from discomfort; (3) freedom from pain, injury or disease; (4) freedom to express normal behavior; and (5) freedom from fear and distress (Farm Animal Welfare Council, 2009). In addition to the Five Freedoms, another model called the Five Domains was introduced more recently state (Mellor and Beausoleil, 2015). In 2015, Mellor and Beausoleil expanded the definition of the Five-Domain model to focus on creating a positive affective state for the animal, instead of simply avoiding a negative state (Mellor and Beausoleil, 2015). The Five Domains are nutrition, environment, health, behavior, and mental state. These domains all ultimately impact the welfare of animals (Phibbs et al., 2021). Affective states are the underlying emotional state of an animal. Affective states are not the quick response to environmental stimuli, but rather longer-term emotional state, which are the resulting amalgamation of many experiences (Campbell et al., 2019).

The field of animal welfare science is a growing body of research. Ensuring a quality of life for animals involves researching and understanding complex physiological and behavioral indicators of overall health and welfare. Physiological measures may include production measures, stress, illness, metabolic function, and many other measures (Scanes, 2016). Stress is a challenge to homeostasis in the animal, and stress response is paramount to survival of the animal (Moberg, 2009; Weimer et al., 2018). To support

homeostasis and help animals cope with changes in their environment or life stages. Allostasis works by modifying physiological “set points” (McEwen and Wingfield, 2003). Allostasis is the change of physiological “set points” in response to a stressor in order to maintain homeostatic stability of physiological systems in the body (McEwen and Wingfield, 2003). There are many mediators of allostasis in the body, and glucocorticoids (such as corticosterone) are considered the primary mediators of allostasis (McEwen and Wingfield, 2003). Behavior indicators of welfare may include positive behaviors such as play behaviors, grooming, and exploration of the environment (Held and Spinka, 2011), and more negative behaviors such as displays of aggression (Ventura et al., 2012).

## **1.2 WELFARE ASSESSMENTS**

There are multiple kinds of voluntary animal welfare assessment guidelines available, which include industry standards from national producer organizations and product differentiation and labeling certifications (Weimer et al., 2018).

There are two major audit and assessment approaches taken to measure animal welfare on-farm: resource-based and animal-based measures (Weimer et al., 2018). Resource-based measures consider the animal’s environment and resources provided to the animal in the environment, while animal-based measures focus on the physical state of the animal. Resource-based measures of animal welfare can be easier to audit than animal-based measures (Mench et al., 2003). There are, however, potential shortcomings of using resource-based measures in audits. By focusing on the resources provided or not provided to the animals, we are only looking at management practices, but not the state of the animals themselves. This discrepancy is what has led to the development of animal-based measures of animal welfare. The Welfare Quality® project has developed animal-based welfare

assurance protocols for a variety of farm animal species both on-farm and at slaughter and has been utilized by many farms in Europe (Rushen et al., 2011). Animal-based measures may be more difficult to evaluate on farm and may allow for greater subjectivity of the measures related to affective state. Despite these drawbacks, animal-based measures of welfare are growing in popularity.

The National Chicken Council is the broiler industry trade association based in the U.S. and also provides welfare guidelines for certification in broiler and broiler breeder welfare. The certification includes both animal-based and resource-based measures (National Chicken Council, 2020). **Table 1.1** provides a comparison of resource-based measures of broiler welfare in NCC guidelines (National Chicken Council, 2020) and Welfare Quality® Assessment for Poultry protocol (2009), while **Table 1.2** and **Table 1.3** provide a comparison of animal-based measures of broiler welfare in NCC guidelines and Welfare Quality® protocols.

#### **1.4 STRESS**

Stress is a response to an internal or external stressor (Blas, 2015). In the short-term, stress responses may or may not be beneficial for the animal, but typically prolonged chronic stress is harmful to the animal (Romero et al., 2015). Stress is a very complicated research topic as genetics, cells, tissues, individuals, groups, and populations can affect the biological response. Currently no model of stress can address all levels of this biological response scale (Romero et al., 2015). The stress response is essential to the survival of an animal, but maladaptive stress responses can decrease welfare (Blas, 2015). Measures of stress in the lab can be physiological or behavioral.

Currently, measure the levels of glucocorticoids are a common method to stress levels of an individual animal. The primary glucocorticoid in avian species is corticosterone (CORT) (Scanes, 2016). CORT is produced in the cortex of the adrenal gland and increases in response to levels of adrenocorticotrophic hormone (ACTH) secreted by the anterior pituitary gland. Examples of stressors which may increase CORT levels include cold, heat, stocking density, immobilization, restraint, handling, fasting, and feed restriction (Scanes, 2016). Effects of stress on the body include decreased gastrointestinal functions, decreased growth, changes in metabolism, namely protein and lipid metabolism, increased fear behaviors and tonic immobility, depression of immune function (Blas, 2015; Scanes, 2016; Riber et al., 2018).

### **1.5 FEAR RESPONSE TESTS**

Fear is an example of a negative affective state that can be measured with behavioral tests. Fear is described as an “emotional state in response to real or perceived danger” (Forkman et al., 2007). It is worth noting that fear and anxiety in avian behavior studies are often not differentiated because the behavioral response to fear and anxiety are similar (Forkman et al., 2007). Fear and anxiety are evaluated using the same behavioral tests and both suggest a negative affective state (Campbell, 2019). Overall tests of fearfulness/anxiety are useful to evaluate the welfare and emotional state of animals. Some tests used to measure fearfulness in chickens include Tonic Immobility, Open Field Tests, Novel Object Tests, Avoidance Distance Tests, and Touch Tests.

Tonic Immobility and Open Field Tests are the most common fear tests in poultry (Forkman et al., 2007). However, the Open Field Test is typically performed on laying hens (Forkman et al., 2007). Other fear tests include Novel Object, Avoidance Distance, and

Emergence Tests. All of these tests are considered to be validated in laying hens (Forkman et al., 2007). According to the 2009 Welfare Quality<sup>®</sup> Assessment for Poultry protocol, the only fully validated fear test in broilers is the Avoidance Distance Test. Although the Novel Object Test is not included in the Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009) as a test for broilers, it is not uncommon to use the Novel Object Test in research experiments measuring fear in broilers (Pichova et al., 2016; Giersberg et al., 2020).

The Open Field Test is performed by placing individual birds into a novel arena and measuring behaviors such as latency to move or latency to vocalize (Forkman et al., 2007). Locomotor and vocalization behavior seen in the Open Field Test are believed to stem from two competing desires. Vocalization comes from the desire to return to the flock, while locomotor behaviors often decrease in an attempt to remain unseen by possible predators (Forkman et al., 2007). The decrease in locomotion exemplifies a fear response to the novel environment (Forkman et al., 2007). The steps taken and areas entered within the arena are good measures of ambulation (walking) and are highly correlated. It is worth noting that there is a strong genetic component to the test behaviors, for both flying and ambulation (Rodenburg et al., 2004). This test can be more difficult to interpret than other tests because of two competing factors, fear and desire to return to flock. It is possible that vocalization is more related to social desire and the level of movement is more related to the fear, or lack thereof (Forkman et al., 2007). The Open Field Test (also called the Novel Arena Test) is a common fear test validated in laying hens (Forkman et al., 2007). In one study by de Jong and colleagues (2003), broiler breeders were used in Open Field Tests, at 6 weeks of age. Tests took place over 3 days from 10:00 to 14:00. The novel arena was 2 x 2 m with 1.5 m tall walls covered with brown paper. The research team analyzed

vocalizations, length of time sitting and standing, and found broilers breeders with feed restriction and spent time less walking during the Open Field Test indicating a potential increase in fear (de Jong et al., 2003). Other literature on the use of broilers in Open Field Tests is limited. Open Field or Novel Arena Tests may be used on other farm species including horses, quail, sheep, goats, pigs, and cattle (Forkman et al., 2007).

In the Tonic Immobility Test, the researcher represents a predator and the bird is exhibiting a response seen in prey species and essentially the bird is “playing dead”. There are six positions for inducing tonic immobility: table, table with head hanging, in cloth, in cloth with head hanging, cradle, and cradle with cloth (Forkman et al., 2007). There is not a clear consensus on the effect of the presence of an observer on the results of the Tonic Immobility Test (Forkman et al., 2007). The duration of tonic immobility can be influenced by the presence of conspecifics. Researchers have seen a shorter length of tonic immobility when the birds have conspecifics nearby and this is why the Tonic Immobility Test is typically performed on chickens isolated from conspecifics. In addition, the frequency of handling the birds regularly receives has an influence on the duration of tonic immobility (Forkman et al., 2007).

The Novel Object Test is a fear test which can minimize the effects of handling on the fear response. In a Novel Object Test, a novel (unknown) object is placed in the testing environment (Forkman et al., 2007). For layer hens, the object is typically placed in front of their home cage (Welfare Quality<sup>®</sup>, 2009). Other testing environments include a novel arena or placing the novel object in the home pen or house for floor raised birds (de Jong et al., 2019). Typically, birds with enriched environments approach the novel object quicker than the birds with a barren environment (Jones and Waddington, 1992). Since the

effect of handling can be minimized by placing the novel object in the home environment, the Novel Object Test may be a better test for general fearfulness compared to the Tonic Immobility Test (Forkman et al., 2007). Other farm species in which the novel object test may be used to measure fear include horses, quail, sheep, goats, and pigs.

In the Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009), there are detailed procedures for the Novel Object Test in layers. A 50 cm colorful stick is placed in the housing environment. If the birds are floor-raised, then the stick is placed on the litter and the observer steps back 1.5 m to record the number of hens within one bird's length distance from the object every 10 seconds for a 120-second duration. Although this test is not included in the Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009) for broilers, it is a common test performed in experimental settings. In addition to the procedure described in the 2009 Welfare Quality<sup>®</sup> Assessment for Poultry protocol, researchers have measured the latency to first approach the object (de Haas et al., 2014; Pichova et al., 2016; Bailie et al., 2019; de Jong et al., 2019; Giersburg et al., 2021; Jessen et al., 2021; van der Oever 2021) latency of the first peck at the novel object (de Jong et al., 2019; Bailie et al., 2019), and the number of times contact is made (i.e. pecks) with the novel object (Bailie et al., 2019). Researchers have also modified the distance of approach from one bird length to either 25 cm (de Haas et al., 2014; Giersburg et al., 2021; Jessen et al., 2021) or 50 cm (de Jong et al., 2018 Bailie et al., 2019) from the novel object.

The Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009) provides detailed procedures for the Avoidance Distance Test in broilers and the procedures are described in **Table 1.2**. In the Avoidance Distance Test, the observer enters the housing environment and assumes a crouching position. The number of birds within an arm's distance of the

observer can then be measured after 10 or 20 seconds of crouching (Welfare Quality<sup>®</sup>, 2009). Sometimes this method is also referred to as the Touch Test (Graml et al., 2008). The procedure in the Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009) includes repeating this method 21 times throughout the broiler house. Another method used in research, which has also been referred to as the Avoidance Distance Test, measures the distance of an individual bird from the observer's arm's length. In this test, the observer approaches a bird with one arm extended at a slow pace, until the bird withdraws. Then the distance from the observer's extended arm to where the bird's feet were located before the bird withdrew is measured (Graml et al., 2008). The Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009) suggests that this procedure should be performed when evaluating laying hens. The observer begins 1.5 meters from the bird. Although the second method of avoidance distance was initially validated for laying hens it has since been used as a measure of fear in broilers (Forkman et al., 2007). In one study, the distance the observer first began walking towards the bird was 5 meters and this distance was selected because the researchers wanted to ensure that the birds selected were not accidentally selected because the broilers were unable to walk properly (Baxter et al., 2019). Interestingly, when a Touch Test was performed on different flocks on various farms, it was found that there are considerable differences in the flocks' response to the presence of a human (Vasal et al., 2017, Muri et al., 2019). It is thought that these differences may be a result of daily farmer interactions with the birds (Muri et al., 2019). Avoidance Distance Tests can also be performed on cattle and pigs (Forkman et al., 2007).



### **1.3 SKELETAL DEVELOPMENT AND LEG MORPHOLOGY**

Broiler chickens have quickly increased in body weight and growth rate over the past 60 years since the start of specific broiler chicken lines by breeder companies roughly 60 years ago (Havenstein, et al., 2003; Shim et al., 2012). There is concern that skeletal growth may not occur at the same speed as the overall growth rate of the broiler, and it has been observed that the fast-growing broilers have poorer quality bones than slower growing broilers (Shim et al., 2012). However, a study that decreased the growth rate of broilers through a low energy diet did not improve cortical bone porosity of the tibia, indicating that slowing broiler growth rate through nutrition is not an optimal way to improve skeletal health in broilers (Leterrier et al., 1998). During the first two weeks of life, there is a rapid growth of bone tissue, but the mineralization rate does not allow the organic matrix to become fully mineralized at this stage in growth, and it has been proposed that this low level of mineralization and high porosity may contribute to leg deformities seen in broilers (Sanchez-Rodriguez et al., 2019). Mineralization is important because the inorganic material provides hardness and compression strength to the bone, and to measure the degree of mineralization percent bone ash of the bone weight can be used (Bonser and Casinos, 2003). To measure bone mineralization in the lab, bone ash can be used to indicate overall mineralization (Dilworth and Day, 1965).

Leg deformities seen in broilers can include varus valgus deformities, tibial dyschondroplasia (TD), epiphyseal separation, ruptured gastrocnemius tendon, and rickets (Julian, 1998). Rickets occurs when Vitamin D requirements are not met, but other leg deformities can occur regardless of nutritional status (Julian, 1998). In a commercial setting, a rupture to the gastrocnemius tendon is not common in broilers because of their

young age, but may be seen in breeder populations (Julian, 1998). The epiphyseal plate separation of the leg bones is indicative of TD or inflammation of the bone (osteomyelitis), TD occurs when the vascular penetration at the growth plate is insufficient to allow for proper formation of the bone (Julian, 1998).

Leg deformities may cause changes to the morphology of the tibia. Birds with varus valgus deformities have a wider tibia at the mid diaphysis and deeper intercondylar depth at the distal head (Cruickshank and Sim, 1986). Birds with varus valgus deformities also tend to have longer, heavier tibias with a wider proximal head (Guo et al., 2019). Shim et al. (2012) evaluated the length, width at the mid diaphysis, weight, breaking strength, and ash content of fast-growing and slow-growing broilers and found that at 6 weeks of age all the measures were greater for fast-growing broilers, except ash on a percent bone weight basis; however, on a per unit BW basis the breaking strength and ash content were greater for the slow-growing broilers. This shows that the development of broiler tibias can vary based on genotype and fast-growing compared to slow-growing broilers may not have the same optimal housing environment because of phenotype differences.

## **1.6 ENRICHMENTS**

Environmental enrichments are stimuli added to an animal's environment which provide complexity to the housing environment, the goal of environmental enrichment is to improve animal welfare (de Azevedo et al., 2007). Notably, the housing systems of broiler chickens are considered barren and a large number of birds are kept together (Bessei et al., 2006). Common enrichments provided to broilers in an enriched broiler house include perches, platforms, panels, barriers, bale(s) of substrate, and range access (Riber et al., 2018).

The activity levels of broilers decreases with age and it is thought that increasing activity levels may help to reduce leg health issues (Kestin et al., 1992; Weeks et al., 2000). To test this theory, researchers have provided broilers with enrichments within their housing environment. Pedersen et al. (2020) observed that providing vertical platforms increased the width of the leg muscles. It was also found that increasing the distance between the feed and water increased the tibial bone diameter at the distal head and it is possible that this increase in diameter shows improved leg health (Pedersen et al., 2020). Platforms have also been used to improve walking ability (gait score) and reduce tibial dyschondroplasia in broilers (Kaukonen et al., 2017). The comparison of fast-growing broilers to chickens from a dual-purpose breed showed that the rigidity of the tibia was greater for the dual-purpose breed, but cortical bone (at 15% of the bone length from the growth plate), mineral density, and bone volume was similar between the breeds, while broilers had longer, wider, and heavier tibias compared with birds from the dual-purpose breed (Harash et al., 2020).

Enrichments can be used to improve the spatial distribution of birds in their environment. It has been observed that birds prefer to congregate at the perimeter of the pen or closer to the wall of the house (Riber et al., 2018). One welfare issue that may arise from the high number of birds in a small area is increased incidence of contact dermatitis, primarily due to decreased quality of the litter (Riber et al., 2018). One theory as to why broilers prefer to congregate near the walls, as opposed to the center, of the enclosure is that the birds may be less disturbed (Cornetto et al., 2002). However, a 2002 study conducted by Cornetto et. al found that there were more disturbances along the wall than the middle of the house. The second theory is that, as a prey species, the birds may be trying

to seek shelter and the environment is lacking enough complexity to provide the birds with that shelter (Newberry and Shackleton, 1997).

Since perching or resting on raised surfaces is a common natural behavior for Jungle Fowl, the common ancestor of domesticated chickens, perching surfaces are common environmental enrichments for domesticated chickens (McBride et al., 1969). Compared with laying hens, broiler chickens, particularly fast-growing broilers, do not use the perches provided as frequently (Groves and Muir, 2014; Ventura et al., 2012; Heckert et al., 2002). Researchers have found that fast-growing broilers use perches beginning around 7 days of age, but after 3-4 weeks of age, they rarely use the perches for the remaining duration of their life (LeVan et al., 2000; Pettit-Riley and Estevez 2001; Ventura et al., 2012; Bailie and O'Connell 2015). In one study, slower growing strains of chickens were reported to continue perching until 10 weeks of age (Bokkers and Koene, 2003).

In addition to age and breed of the birds, another factor which appears to impact perch utilization is the provision of ramp access to the perches. Broilers will more frequently utilize perches that can be accessed from the ground more than the perches that would only be accessed via a ramp (LeVan et al., 2000). It should also be noted that the impact of stocking density on the use of the perches is not clear (Riber et al., 2018). One possible advantage of providing the perches is that it may help the birds thermoregulate their internal body temperature, since the birds can get off the litter and space themselves out from the other birds in the flock (Riber et al., 2018). There has been research showing that fast growing broilers will increase their use of perches, which are both cooled and metal, with age showing the birds have a preference for the cooled perches at 30-34°C (Zhao et al., 2013). In this same study, there was a decrease in the prevalence of hock burn,

foot pad dermatitis, and dirty plumage when cool perches were available (Zhao et al., 2013). There is an increase in corticosterone levels of female broiler breeders provided with perch environmental enrichments in an experimental setting showing that perches may influence stress measures (Adeniji, 2012). Despite the possible benefits perches may provide the birds, the availability of perches may not improve the leg health of broiler chickens. For example, one study found that the lameness scores and prevalence of tibial dyschondroplasia or tibia curvature did not change for fast growing broilers when there was access to rectangular or rounded wooden perches (Su et al., 2000).

There is not much evidence to support the improvement of foot pad condition of broilers provided with platforms compared to broilers provided with perches (Riber et al., 2018). There is a concern that perches may increase the cases of breast blisters (Nielson, 2004). Breast blisters occur when there is swelling of the sternal bursa (Riber et al., 2018). The thought is that a repeated pressure against the breast and keel bone will cause breast blisters, but there is evidence that the actual incidence of breast blisters is more heavily influenced by genotype and sex than the perches themselves (Nielson, 2004). The angularity of the keel bone is what is believed to increase the incidence of breast blisters in some genotypes (Riber et al., 2018). If a bird does have breast blisters, this may make perching painful because of the pressure on the keel bone (Nielson, 2004).

An alternative to a perch is a raised horizontal platform with slats. These platforms also serve as an elevated resting place, and often have a ramp to allow the birds to access the platform without having to jump up onto the platform (Riber et al., 2018). Overall, it has been found that the usage of the platforms is better than the perches for broilers (Baillie and O'Connell, 2015). In one study, researchers found that the presence of the platforms

improved both gait scores and incidences of tibial dyschondroplasia in broilers raised in a commercial setting (Kaukonen et al., 2017). It is possible that the birds prefer the platforms because the broilers have an easier time balancing on the platforms compared to the perches, and it is possible that the platforms may be offering the birds a raised resting place rather than increasing activity levels (Riber et al., 2018). It is possible that the provision of the resting places may decrease fearfulness in broilers (Pedersen et al., 2017), but more research needs to be conducted on this topic to be certain.

There are several other forms of enrichments which can be given to broilers including substrate (i.e. hay) bales, panels, barriers, and or even range access (Riber et al., 2018). Substrate bales can function as perches as well and increase exploration-based behavior. Additionally, the inclusion of substrate bales has been shown to decrease gait score (Bailie et al., 2013). It has been shown that the inclusion of panels, with or without solid fills can increase the dispersion of broilers (Cornetto and Estevez, 2001). In experimental setting, broilers were more evenly dispersed with panels and the birds rested more than their counterparts not provided the panels (Cornetto et al., 2002). It has been shown the addition of panels into a broiler environment do not affect daily growth or body weight at day 44 (Cornetto and Estevez, 2001), yet limited knowledge exists on the effect of panels on leg health.

Barriers are lower and wider than panels, allowing them to be used for perching as well as dispersing the flock (Riber et al., 2018). It is possible that the barriers can function as a raised resting place and increase the activity level of the birds (Riber et al., 2018). The barriers increase the activity level because the birds must walk around the barriers (Riber et al., 2018) and have not been shown to negatively affects broiler performance (Ventura

et al., 2010). It is also possible that the inclusion of the barriers into the broiler housing system could decrease the cases of foot pad dermatitis, and complex barriers could increase symmetry of the tibia bones in broilers (Ventura et al., 2010).

Chickens may also be raised with free access to outdoor range and the ability to go outdoors provides the birds with a more complex, and therefore enriched, environment. In a commercial system, birds typically are not raised free access to range unless the birds are raised in organic production (Riber et al., 2018). Fast-growing genotypes are not ideal for organic production because the broilers use outdoor access less than other genotypes, and are not very active (Nielsen, 2004). For the broilers to reach the target market weight for organic production the fast-growing birds must be feed restricted, and the view of feed restriction is not positive (Riber et al., 2018). The use of slow-growing genotypes is recommended for organic or free-range production because they do not need feed restriction and have lower incidences of foot pad dermatitis than fast-growing broilers raised in free-range production (van de Weerd et al., 2009).

Research into enrichments focused on sensory input such as visual or auditory stimuli provided to broilers is limited. It has been shown that chickens respond to visual stimuli provided in the form of videos, and to auditory stimuli such as music, or changes in noise levels (Campbell et al., 2019b). More research into the influence of sensory based enrichments on chickens is needed.

## **1.7 OBJECTIVES OF THIS STUDY**

Leg health is a concern in fast-growing broilers and, environmental factors may help lessen the incidence of leg health related illness and may influence tibial morphology.

It has been observed that different enrichments may have different influences on broilers, but little is known about the influence of enrichments on tibial morphology.

It is important to research and understand the influence potential enrichments have on broiler stress, welfare, and behavior, because in order to actually enrich the lives of the broilers, the potential enrichments have to improve welfare. There is little research on the effects of environmental enrichments on levels of stress in broilers. Welfare measures including contact dermatitis and plumage cleanliness have been evaluated with perches and platforms, but evaluation with visual enrichments is needed. The impact of environmental enrichments on fearfulness, particularly visual enrichments, should be further evaluated.

The goal of this study was to evaluate the effects of visual enrichment (spotlight), and structural enrichments (platforms) on production measures, welfare, morphology of the tibia bones, mineralization of the tibia, stress levels, and fear in broilers.



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**Table 1.1.** Some resource-based measures of on-farm broiler welfare in the National Chicken Council (NCC) guidelines and Welfare Quality® Assessment for Poultry protocol.

Resource	Organization	Requirements
Lighting	NCC	Darkness must be 10% or less of the light provided during the light hours.
		Minimum of 4 hours of darkness must be provided (except the first and last week of life). The 4-hour period does not have to be continuous
		There are no requirements for intensity, but lighting plans must be available to auditors.
	Welfare Quality®	No mention.
Litter Quality	NCC	Litter should be loosely compacted when squeezed in the hand. Litter should be dry throughout the house. Litter moisture is measured in the center of the house, not near the waterline.
	Welfare Quality®	Select 4-6 locations throughout the house to visually inspect to determine the variability in the litter moisture throughout the house.
		If it appears that there is a lot of variable litter moisture, sample multiple locations.
Enrichments	NCC	No mention.
	Welfare Quality®	Not discussed for broilers, but enrichment use is scored in laying hens.

**Table 1.2.** Animal-based measures of broiler welfare in the National Chicken Council (NCC) guidelines and Welfare Quality® Assessment for Poultry protocol.

Measure	Organization	Requirements
Gait score	NCC	To be performed within the week of slaughter using the US gait scoring system (0-2). Observe 100 birds and record the number of birds scoring 2 (unable to walk with gentle encouragement).
	Welfare Quality®	To be performed on farm close to slaughter. Catch 150 birds in 4 random locations throughout the house. Record each bird's individual score (0-5), then calculate the average gait score for the birds scored.
Foot Pad Dermatitis	NCC	Foot pad health is scored for 100 birds at catching, and another 100 birds are scored on a pass-fail system at the processing plant.
	Welfare Quality®	Score a total of 100 birds from 10 locations for skin lesions on the foot indicative of contact dermatitis (0-4).
Hock Burn	NCC	No mention.
	Welfare Quality®	Score a total of 100 birds from 10 locations for the presence of contact dermatitis (0-4).
Breast Blisters	NCC	No mention.
	Welfare Quality®	To be performed at the processing plant. Observe the line for 5-10 minutes and record if each bird has evidence of breast blisters, then calculate the percentage of birds with breast blisters.
Plumage Condition	NCC	No mention.
	Welfare Quality®	Plumage condition is scored (0-3) to determine the cleanliness of the feathers. Score a total of 100 birds from 10 locations.
Fear Tests	NCC	No mention.
	Welfare Quality®	The Avoidance Distance Test (ADT) is used to measure the human-animal relationship. The observer will approach a group of birds, squat for 10 seconds then extend the arm, counting birds within arm's length distance. This is repeated 21 times throughout the house.

**Table 1.3.** Animal-based measures of broiler positive affective state in the National Chicken Council (NCC) welfare guidelines and Welfare Quality® Assessment for Poultry protocol.

Measure	Organization	Requirements
Positive Affective State	NCC	No mention.
	Welfare Quality®	<p>The qualitative behavior assessment (QBA) is used to measure a positive emotional state in the birds. After entering the house, wait a few minutes for the birds to return to their normal behavior. Total observation length should not exceed 20 minutes. 20 total expressive terms are scored using a visual analogue scale. These terms are active, calm, friendly, relaxed, content, positively occupied, helpless, tense, scared, comfortable, inquisitive, drowsy, fearful, unsure, playful, agitated, energetic, nervous, confident, frustrated, distressed, depressed, and bored.</p> <p>The minimum on the visual analogue scale means the expressive term is not present in animals observed, and the maximum means the expressive term is present in all animals observed.</p>

**Chapter 2: THE IMPACT OF PLATFORM AND SPOTLIGHT ENRICHMENTS ON CONVENTIONAL BROILER PERFORMANCE, TIBIA MORPHOLOGY, AND BEHAVIOR**

## **2.1 ABSTRACT**

The ability to ensure welfare is an important consideration in proper management of broilers, and can be influenced by many different environmental factors. Management decisions can improve welfare on many levels, such as providing proper feed, access to water, lighting, stocking density, and environmental enrichments. Evaluation of potential enrichments is important to ensure these enrichments improve broiler welfare. The objective of this study was to evaluate the effect of no enrichments (C), or platform (P), spotlight (S), and a combination of spotlight and platform (S&P) enrichments on broiler production, welfare, stress, tibia morphology, tibia ash, and fear response. Body weight, feed conversion ratio, mortality, corticosterone, contact dermatitis, plumage condition, tibia length and tibia ash measures were unaffected by enrichment treatments. Compared to other treatments, P birds had wider ( $P \leq 0.02$ ) tibias at 90%, 75%, and 25% length locations and S birds had narrower ( $P \leq 0.04$ ) tibias at 90% and 75% length locations. Cortical bone thickness was greater on the anterior side of the right tibia at the 75% length location for broilers in the P treatment group ( $P = 0.002$ ). Fear response decreased for all treatments from week 3 to week 5 ( $P \leq 0.05$ ) as measured by the distance of broilers from the testing stimuli in the Avoidance Distance and Novel Object Tests. The results indicate that, although body weight was lower than expected, both structural (P) and visual (S) enrichments can influence tibial morphology without decreasing production values across treatment groups or animal-based measures of welfare. Platforms may be able to increase skeletal health as measured by increased widths of the tibia, and platform enrichments should be evaluated in a commercial setting. Further research into the potential influence of spotlight enrichments on broiler physiology is

needed to understand how the widths of the bone were decreased with S birds, but not S&P birds.



## **2.2 INTRODUCTION**

Animal welfare is a concern to both consumers of poultry products and the poultry industry. Animal welfare is influenced by many different factors within the environment, and the individual animal, meaning that decisions influencing the environment in which the birds live may affect the birds in many different ways. These decisions may influence stress, physiology, and welfare of the birds.

Stress is a response to an internal or external stimuli (Blas, 2015). In the short-term, stress responses may or may not be beneficial for the animal, but typically prolonged chronic stress harmful to the animal (Romero et al., 2015). Stress is a very complicated research topic, the stress response is essential to the survival of an animal, and maladaptive stress responses can decrease welfare (Blas, 2015). Currently, glucocorticoids are a common method to measure stress levels of an individual animal (Scanes, 2016). The primary glucocorticoid in avian species is corticosterone (CORT) (Scanes, 2016). Examples of stressors which may increase CORT levels include cold, heat, stocking density, immobilization, restraint, handling, fasting, and feed restriction (Scanes, 2016). Effects of stress on the body include changes in gastrointestinal functions, growth, metabolism, fear behaviors and tonic immobility, as well as the depression of immune system function (Blas, 2015; Scanes, 2016; Riber et al., 2018).

Fear can be defined as an “emotional state in response to real or perceived danger” (Forkman et al., 2007), and can be measured with behavioral tests. Overall tests of fearfulness are useful to evaluate the welfare and specifically emotional state of animals. The Novel Object Test is a fear test which can minimize the effects of handling on the fear response since the birds are not handled during the test and observers create distance

between themselves and broilers (Forkman et al., 2007). In a Novel Object Test, an unknown object is placed in the testing environment (Forkman et al., 2007). Typically, it is seen that broilers with enriched environments approach the novel object quicker than broilers with a barren environment (Jones and Waddington, 1992). The Avoidance Distance Test is another fear test performed to measure the human-animal relationship of broilers (Welfare Quality<sup>®</sup>, 2009). The Welfare Quality<sup>®</sup> Assessment for Poultry protocol (2009) provides detailed procedures for the Avoidance Distance Test in broilers, the observer enters the housing environment and assumes a crouching position. The number of birds within an arm's distance can then be measured (Welfare Quality<sup>®</sup>, 2009).

Leg health is important to the overall health of broiler chickens. Leg health in commercial broiler chickens has been a concern because of the rapid increase in body weight gain since the start of specific broiler chicken lines by breeder companies roughly 60 years ago (Havenstein, et al., 2003; Shim et al., 2012). In the 2019 management guide for the broiler breed used in this experiment, the day of hatch weight as 43 g which can be expected to increase to nearly 4,300 g in 59 days (Aviagen, 2019). There is concern that skeletal growth may not occur at the same speed as the overall growth rate of the broiler, and it has been observed that the fast-growing broilers have a poorer quality bone than the slow-growing broilers (Shim et al., 2012). To measure bone health and integrity, measures of bone morphology and mineral content can be used (Dilworth and Day, 1965; Bonser and Casinos, 2003; Guo et al., 2019).

Environmental enrichments are added to an animal's environment with the goal of improving animal welfare (de Azevedo et al., 2007). The housing systems often used for broiler chickens are considered barren (Bessei et al., 2006). Common enrichments which

may be provided in an enriched broiler house include perches, platforms, panels, barriers, bale of substrate, and outdoor access (Riber et al, 2018). It is thought that the addition of enrichments may increase activity levels in broilers and may help to reduce leg health issues, as activity levels seen in broilers typically decrease as birds age (Kestin et al., 1992; Weeks et al., 2000).

Measures of bone integrity and health, levels of stress, and fear, as influenced by the environment, especially enrichments, are an important topic of research to ensure broiler producers can provide broilers with the best possible environment based on scientific data. We compared the effect of no enrichments (C), or platform (P), spotlight (S), and a combination of spotlight and platform (S&P) enrichments on broiler production, welfare, stress, tibia morphology, tibia ash, and fear behavior.

## **2.3 MATERIALS AND METHODS**

### **Animals and Housing**

A total of 400 day-of-hatch Ross 708 broiler chicks were obtained from a commercial hatchery located on the eastern shore of Maryland. Chicks were sexed at the hatchery and only chicks sexed as male were used, but some female chicks were present. Chicks were placed into 2 rooms within the Animal Wing of the Animal and Avian Sciences building. All animal procedures were approved by the University of Maryland Institutional Animal Care and Use Committee (protocol R-JUL-20-35).

Each room housed eight 1.52 m x 3.05 m pens. Twenty-five chicks were randomly assigned to 1 of 16 pens (N = 25 chicks per pen). Chicks were fed *ad libitum* from a hanging tube feeder and water was provided from 6 nipple drinkers per pen. Commercial-type feed

was provided. Starter (22.2% crude protein, 3000 kcal/kg metabolizable energy), grower (20.1% crude protein, 3100 kcal/kg metabolizable energy), and finisher (18.3% crude protein, 3200 kcal/kg metabolizable energy) feeds were available to the broilers. The starter diet was fed from day 0-10, the grower diet from day 10-24, and the finisher diet from day 24-54. Room lighting consisted of incandescent light bulbs (60-watt equivalent incandescent light bulb, EcoSmart, Lighting Science Group Corporation, West Warwick, RI) hung 12.70 cm from the ceiling to the base of the bulb. One light bulb was hung above each pen near the front third of the pen to provide a light intensity of 45 lux at broiler height directly under the bulb.

The photoperiod was adjusted over the lifetime of the birds using automated timers (24-Hour Outdoor Mechanical 2 Outlet Light Timer, Fosmon, Woodbury, Minnesota). At placement, birds received 23 hours of light through day 3. Photoperiod light and dark hours (L:D) gradually changed, to decrease light hours, with age as follows; day 3-4 was 22L:2D, day 5-6 was 21L:3D, day 7-9 was 20L:4D, day 10-16 was 19L:5D, day 17-23 was 18L:6D, day 24-30 was 17L:7D, and was 16L:8D from day 31 to the end of study. The dark period always began at 23:00. At placement, the temperature was 32.00°C. Room set temperatures were also gradually decreased as follows; day 2 was 31.70°C, day 3 was 31.10°C, day 4 was 30.60°C, day 5 was 30.00°C, day 6 was 29.44°C, day 7 was 28.90°C, day 8 was 28.30°C, day 9 was 27.80°C, day 10 was 27.20°C, day 11 was 26.70°C, day 12 was 26.10°C, day 13 was 25.60°C, day 14 was 25.00°C, day 15 was 24.40°C, day 16 was 23.90°C, day 17 was 23.30°C, day 18 was 22.80°C, and day 19 until the end of the study was 22.20°C. To measure the actual temperature and humidity in the rooms 4 data loggers

(HOBO UX100-003, Onset Inc., Cape Cod, MA) in 2 locations were placed within each room.

### **Experimental Design and Enrichment Treatments**

A 2 x 2 randomized block trial design was utilized for this experiment. Each room had two blocks in the room, and treatment locations were randomized within the block using a random number generator. Each pen (N = 16 pens) was subjected to 1 of 4 treatments: 1) no enrichments (control, **C**), 2) a spotlight (**S**), 3) a platform (**P**), and 4) a spotlight and a platform (**S&P**) for a total of 4 replicates per treatment combination.

### ***Focal birds***

On day 4, birds were randomly selected for focal sampling. Five birds were selected in each pen (N = 5 focal birds per pen, 80 focal total birds) and labeled using black or blue marker on the top of the head or base of the tail. After the birds had lost their downy feathers, the same birds were relabeled with livestock markers. Due to unexpected mortality and incidence of illness in our experiment (confirmed as Infectious Bronchitis Virus, *Enterococcus durans*, *Enterococcus faecium*, and *Escherichia coli* by the Frederick Animal Health Laboratory on day 29), on day 37 of age 15 new focal birds were selected to replace the focal birds lost to illness. These birds were used for final focal bird collection data. An additional focal bird was selected from pens 9-16 (N = 8 additional focal birds) for a separate project on day 31, and were included in our focal measures.

### **Environmental Enrichments**

The enrichments were placed in the center of the pen (the center of the pen was located visually) located roughly 120 cm from the waterline, 40 cm from the feeder, and 60 cm from the sides of the pen (**Figure 2.1**).

### *Spotlight (S)*

Pin beam spotlights (B07V3BS773, GBGS, China) with a beam angle of 15° and 355 lux light intensity were used as spotlight treatments. A lux meter (Model LT300, Extech, Nashua, NH) was used to measure the light intensity (lux). Spotlights were on for 5 hours daily from 12:00 to 17:00. Spotlights were a blue toned light with a color temperature of 6000-6500 k. The area illuminated by the spotlights measured 55.88 cm in diameter (5.28% of the pen) and a lux meter was used to confirm that the spotlight did not illuminate neighboring pens.

### *Platform (P)*

Structural enrichments were 35.56 cm long x 25.40 cm wide x 12.70 cm tall platforms with a 35.56 cm long x 35.56 cm wide access ramp (**Figure 2.2**). The platform enrichments were crafted using 1.27 cm thick PVC pipe, and cross boards that were 1.27 cm thick PVC board screwed into to the frame. In total, 4.67% of the pen floor space was occupied by the platforms. Initially, we planned to include a 25.40 cm x 25.40 cm dust box attached to the platform enrichments, but it was determined while building the enrichments that this additional dust box took up too much space in the pen. If the dust box had been included, birds may not have been able to walk past the enrichment without touching the enrichment. We believed it was important that the birds had the option to interact with the enrichments in the narrow experimental pen environment when they would like to, and that they were able to avoid the enrichments if they chose to.

### *Platform+Spotlight (S&P)*

In the pens with the spotlight and the platform, the platform was positioned directly beneath the spotlight so that the spotlight illuminated the greatest surface area of the

platform (**Figure 2.3**). About 3.33% of the litter area surrounding the platform was illuminated by the spotlight in the S&P pens.

### **Video Recording**

Video was recorded for 24 hours on days 4, 5, 11, 12, 25, 26, 31, 32, 39, 40, 51, and 52 using a NVR and CCTV camera system (4KDIP164i, Lorex<sup>®</sup>, Ontario, Canada). Video was also recorded for 1.5-hour duration on the following days: day 23, 24, 37, 38, and were used for fear test analysis. The CCTV cameras (Model #LNB9272S) were mounted to the ceiling 2.64 m above each pen. These bullet-style CCTV cameras were connected via Ethernet cords to a network video recorder (Model# N881A63B). The video was recorded at 4K resolution at 30 frames per second. We realized that the intensity of the spotlight was too bright to view birds in the spotlight in the video, so the wide dynamic range (WDR) setting was applied, saturation was set to 70%, and brightness, sharpness, and gamma were set to 50% to ensure all birds were visible both in and out of the spotlight. Day and night settings were set to color to prevent the camera infrared light from turning on during the dark hours.

### **Data Collection**

#### ***Production***

Feed consumption, mortality counts, and mortality body weights were recorded daily per pen and used to calculate feed conversion ratio that was corrected for the body weight of the mortalities. Cause of mortality was recorded for each bird found dead or culled for health reasons.

Cumulative body weight (g) for all of the birds in each pen was recorded at placement, at feed changes (from starter to grower and from grower to finisher), and at

final collection (day 54). Individual body weights (g) of focal birds were also recorded at feed changes and end of study (day 53). Final collection weights occurred over two days, on day 53 body weight (g) for the individual focal birds were recorded and on day 54 we recorded cumulative body weight (g) of the remaining non-focal birds in the pen.

Feed conversion ratio (FCR) was calculated using cumulative body weight including focal birds (corrected to include mortality body weight at each phase) and feed consumption per pen. FCR was calculated for 3 phases (Phase 2 = placement to day 10 feed change from starter to grower, Phase 3 = day 11 to 24 feed change from grower to finisher feed, Phase 4 = day 25 to 54 at the end of the study) as well as over the entire lifespan (all 4 phases) of the birds. For the end of the study, and the cumulative lifespan FCR, the weight of individual focal broilers were recorded on day 53 of age, while feed and group non-focal broilers were recorded at day 54 of age. Thus, the calculation of FCR was not adjusted for the feed that would have been consumed by the focal birds between day 53 and 54.

### ***Sampling, Physical Welfare, Necropsy***

Final samples were collected on focal birds (N = 85 birds) on day 53. Blood was collected within 120 seconds from removal of the focal bird from the home pen using a 3 mL syringe attached to a 21-gauge 3.8 cm long needle inserted into the brachial vein.

Following blood collection, birds were euthanized by cervical dislocation. Following euthanasia, birds were scored for plumage dirtiness, foot pad dermatitis, and hock burn adapted from the Welfare Quality<sup>®</sup> guidelines (Welfare Quality<sup>®</sup> Protocol for Poultry, 2009) to a three-point scale where 0= no evidence, 1= mild evidence, 2= moderate to severe evidence (**Figure 2.4**).



The left and right tibiotarsus (referred to as tibia) bones were collected from focal birds by manually defleshing the bone and removing articular cartilage (Li et al., 2015). Each tibia was assigned a tag number and stored at -20°C.

Gross necropsy was recorded for focal birds by a researcher to confirm sex (by checking for testes).

## **Corticosterone and Tibia Bones**

### ***Corticosterone***

Blood samples were transferred into plasma separation tubes, plasma was separated from whole blood using a centrifuge for 15 minutes at 2000 x g at 15°C, and stored at -20°C. An ELISA kit (Arbor Assays, Ann Arbor, MI) was used to measure concentrations of plasma corticosterone (ng/mL) at a 1:20 dilution following manufacturer instructions.

### ***Tibia Volume***

Left and right tibia bones were removed from the freezer, thawed at 4°C overnight, and the weight (g) and volume were recorded. Bone volume (mL) was measured by submerging each bone into a graduated cylinder and the displacement of water was recorded.

### ***Tibia Ash***

Bones were dried in an oven at 70°C for 12 to 24 hours. Following drying, defatting was completed overnight using petroleum ether (Petroleum Ether, A.C.S. reagent, Merck KGaA, Darmstadt, Germany) boiled in a Soxhlet system. After defatting, the bones were dried once more in an oven at 70°C for 12 to 24 hours. Bones were then placed in crucibles and weighed (g). Crucibles with bones were placed in a muffle oven overnight at 600°C.

The following morning the ash and crucibles were weighed (g). Dry defatted percent ash of each tibia was calculated by subtracting the crucible weight from both the dry defatted bone ash weight, and dried defatted bone weight, and dividing the dry defatted bone ash weight (g) by dry defatted bone weight (g), then multiplying by 100.

### ***Tibia Superficial Morphology – Wet lab***

Wet lab morphology measures were performed on thawed tibia bones using a digital caliper to collect the length (mm) of the tibia, and widths (mm) at 90%, 75%, 50%, 25%, and 10% of the length (from the proximal to the distal head), and the depth (mm) of the medial and lateral intercondylar areas on the proximal, and the depth (mm) of the intercondylar area on the distal heads (N = 170 tibias, 20–22 broilers/treatment). The tibia length was used to calculate 90%, 75%, 50%, 25%, and 10% of the total length. The angle of the proximal head through the medial to lateral condyle was recorded using a protractor (**Figure 2.5**).

### ***Tibia Superficial Morphology – Digital***

Digital image morphological measures were also taken and recorded for the right and left tibia (N = 170 tibias, 20–22 birds/treatment). Bones were placed in a light box with an orange background during imaging. Images were taken using a digital SLR camera (a6400, Sony, Minato City, Tokyo, Japan) affixed with a macro lens (E 30mm f/3.5 macro lens, Sony, Minato City, Tokyo, Japan) and mounted to a tripod (**Figure 2.6**). The camera was set to manual mode to ensure the same exposure time and aperture were used for all images. ISO was set to 100 to optimize the resolution of the images. Exposure time was 1/8 seconds and the F-stop was set to 22 to maximize depth of field. Images were taken at a focal length of 18 cm from the bone for the anterior and posterior surfaces, and 12 cm

from the bone for the proximal and distal images. Direct manual focus (DMF) focus setting was used. A 1 cm grid paper printed on yellow paper was used as a background for anterior and posterior surface images. Images were taken of the tibia in four anatomical orientations: anterior, posterior, proximal, and distal.

Digital morphology measurements were collected using Fiji ImageJ software (National Institute of Health, Bethesda, Maryland). The 1 cm grid paper was used to set the scale as a ratio of pixels to millimeters (pixel:mm). Length (mm) of the tibia and width (mm) at 90%, 75%, 50%, 25%, and 10% of the length were collected in the anterior image. The line tool was used to record the total length of the tibia. The tibia length was used to calculate 90%, 75%, 50%, 25%, and 10% of the total length. The fixed length line tool macro was used to mark the width locations for each length percentage. Using the line tool, the width of the bone was measured at each width location (**Figure 2.7a**). The depth of the intercondylar areas were measured on the proximal (**Figure 2.7b**) and distal (**Figure 2.7c**) surface images by drawing a line across the most anterior portion of the condyles of the proximal head, and most distal portion of the condyle of the distal head to represent the highest point, then drawing a line perpendicular to the first line at the deepest point of the intercondylar area. The widths of the proximal and distal heads were also collected using the proximal and distal surface images by using the line tool. The proximal image was used to measure the angle of the proximal head through the medial to lateral condyle and was accomplished by drawing a line from the top of the anterior surface of the lateral condyle to the posterior surface of the bone. The midpoint of the first line was used as a reference through which the angle was drawn. The angle tool was used to measure the angle of the proximal head through the medial to lateral condyle (**Figure 2.6b**).

### ***Tibia Morphology - Cortical Bone Thickness and Surface Area***

Cortical bone thickness and surface areas of the cortical bone were measured in digital images of bones taken after defatting with the same SLR camera used to take superficial morphology images. Left and right tibias were cut at the 50% and 75% locations (described previously) using a fine-toothed hacksaw. Bones were placed on grid paper with the anterior surface on the paper. Images were taken of the cut bones at a focal distance of 12 cm from the cut surface. These cortical bone thickness images were measured at the 50% and 75% locations using Fiji ImageJ software. Cortical bone thickness was measured at four locations called the north, south, east, and west locations of each transverse section, and the total surface area of the cortical bone of the transverse surface was also collected. The north location corresponds with the posterior side of the bone, while the south location corresponds with the anterior side of the bone. The east and west locations represent the medial and lateral sides of the bone. For the left tibias the medial side corresponds to the west location, while for the right tibias the medial side corresponds to the east location. (Figure 2.8).

### **Behavior**

#### ***Fear Tests***

To measure fearfulness of the birds, the Avoidance Distance (AD) Test and Novel Object (NO) Test were performed at 2 time points on days 23-24 and 37-38. One observer conducted both tests in the home pen. At both time points, the tests were conducted over two days in the two rooms. On day 23, the NO test was applied in room 1 and the AD test was applied in room 2. On day 24, the NO test was applied in room 2 and the AD test was

applied in room 1. For days 37 and 38, the tests were applied in reciprocal order. Video recordings were used to analyze the AD and NO Tests. Platform enrichments remained in the pen during the fear response tests, but the spotlight enrichments were not on at the time of testing.

#### *Avoidance Distance (AD) Test*

During video recording, the non-novel observer (the observer conducting the tests also performed daily care) calmly entered the home pen and slowly walked to the left side of the pen next to the feeders. The observer slowly crouched into a squatted position where they remained for 10 seconds, head down, while looking at a stopwatch in their hand (**Figure 2.9a**). At 10 seconds, the observer slowly extended their right arm while staring straight ahead at the wall for an additional 10 seconds (**Figure 2.9b**). After 10 seconds with the arm extended the observer calmly left the pen and the test ended (Welfare Quality<sup>®</sup> Protocol for Poultry, 2009).

The avoidance distance (m) was categorized into 5 circular zones around the observer overlaid on screenshot images captured from the video at 10 and 20 seconds using the ellipse tool in ImageJ. Zones 1, 2, 3, and 4 were 0.5 m, 1 m, 1.5 m, and 2 m circles drawn from just in front of the midline of the observer, respectively, and Zone 5 was the remaining area in the pen (**Figure 2.10**). The number of birds in each zone was recorded at 2 time points - after the observer had squatted in the pen for 10 seconds and again after the observer had extended the arm for 10 seconds. If a bird was located on the edge of two zones, the zone containing over 50% of the bird was recorded.

#### *Novel Object (NO) Test*

During video recording, the observer calmly entered the pen and placed a 48 cm PVC stick wrapped with duct tape in the same location as the observer in the AD Test in each pen (**Figure 2.11**). The colors of duct tape were black, blue, green, yellow, and red. After the NO was placed in the pen, the observer calmly exited the room. The test time began once the room door was shut and lasted for 4 minutes (de Jong and Gunnink, 2019). After the 4 minutes, the observer entered the room and removed the object from the pen.

To determine locations and durations, the area around the NO was measured in ImageJ. Using the fixed length line macro tool 25 cm and 50 cm distances were measured from the center and ends of the NO. The ellipse tool was then used to draw the 25 cm and 50 cm areas around the NO. Both the 25 cm and 50 cm area around the NO was traced transparency overlay taped to a computer monitor and used for latencies and counts. The 25 cm and 50 cm distances were selected based on previous literature (de Jong and Gunnink, 2019; Giersburg et al., 2020; Jessen et al., 2021). Additional distances found in the literature include 1 bird distance from the NO (Adler et al., 2020), but measures in ImageJ found that 25 cm was roughly 1 bird's body length. The latency (seconds) of the first bird to approach the novel object was measured within 25 cm and 50 cm from the object. The latency of the first bird in the pen to peck the NO and the total duration of the pecking behavior for each bird was recorded. Additionally, a scan sample was performed to record the total number of birds within 25 cm and 50 cm of the novel object every 10 seconds.

### **Statistical Analysis**

This trial design was a 2 x 2 complete block factorial design. For production and behavior data, pen was the experimental unit and the individual bird was the experimental

unit for all other data. Main effects were spotlights and enrichments. Statistical analysis was completed using JMP Pro 14 software (SAS Institute Inc.). Production data (FCR, BW, and mortality), focal data (corticosterone, necropsy, and tibia morphology, tibia ash, and cortical bone, physical indicators of welfare), fear test data were analyzed separately. Production data were analyzed using an ANOVA test without random effects, because there were not enough degrees of freedom to include the random effect of pen nested within room. Focal data, except for physical indicators of welfare, and fear test data were analyzed using a two-way ANOVA test with random effect of pen nested within room. Focal data was analyzed at the level of the individual broiler as the experimental unit. For the Avoidance Distance (AD) Test, behavioral analysis, orthogonal contrasts were performed to compare the location of the birds at 3 weeks and to 5 weeks of age within each treatment. All other results from the fear test data was not analyzed in statistical software and are reported as descriptive statistics (mean and median percentages). A chi square test was performed to analyze the physical welfare data. Results are reported as LSMeans. For all statistical models, means with  $P \leq 0.05$  were considered significant.

## **2.4 RESULTS**

### **Experimental Measures**

**Figure 2.12** shows room average temperature and humidity recorded from 4 data loggers in 2 locations within each room (Room 1 and Room 2) throughout the duration of this study. We did not run statistical analysis on the temperature and humidity data between the two rooms, but both humidity and temperature are numerically similar throughout the study.

## **Production**

### ***Mortality***

**Table 2.1** shows the data for mortality as a percentage (of DOA and culled birds) within each pen at Phases 2 through 4 and cumulative (total) mortality throughout the study. **Figure 2.13** shows average mortality in each treatment in each phase and cumulatively throughout the study (Total). There was no effect of room, nor main or interaction effects of treatment on mortality at each phase or cumulatively ( $P > 0.05$ ). Cumulative mortality within each pen ranged from 8-36% and the average cumulative mortality across all pens was  $18\% \pm 4\%$  SEM (standard error of the mean). In Phase 2 (placement – day 10) average mortality was  $4\% \pm 1\%$  SEM, in Phase 3 (day 11 – 25) average mortality was  $2\% \pm 1\%$  SEM, in Phase 4 (day 26 -54) average mortality was  $13\% \pm 1\%$  SEM. Of the mortalities recorded in Phase 2, birds that were found dead on arrival (DOAs) accounted for 71.43%, in Phase 3 50% were DOA, and in Phase 4 2.04% were DOA. Conversely, of the mortalities recorded in Phase 2 28.57% were culled due to health issues, 50% were culled due to health issues in Phase 3, and 97.96% were culled due to health issues in Phase 4. On day 29, 7 euthanized birds from 6 different pens in both room 1 and room 2 were taken to the Frederick Animal Health Laboratory in Frederick, Maryland for diagnosis. The birds were diagnosed with 3 bacterial infections: *Escheria coli*, *Enterococcus durans*, and *Enterococcus faecium*, and 1 viral infection: Infectious Bronchitis Virus (MASS and GA08).

### ***Body weight***

**Table 2.2** shows the average body weight (BW) in each pen at each phase. At the end of Phase 4 (day 53 for focal broilers, and day 54 for non-focal broilers), average



individual broiler BW per pen ranged from 2,630.23 to 4,411.36 g (**Table 2.2**) with an overall average individual BW of 3,409.78 g across treatments. There were no main or interaction treatment effects on BW ( $P > 0.05$ ; **Table 2.3**). Cumulative pen FCR ranged from 1.35 to 2.44 with an overall average FCR of 1.83 (**Table 2.4**). There were no main or interaction effects of enrichment treatments on total FCR or FCR for each feed phase, but there was a trend ( $P = 0.08$ ) for S and S&P pens to be lower in FCR than P pens. (**Table 2.5**).

### ***Physical Welfare***

There were no main or interaction effects ( $P > 0.05$ ) of enrichment treatments on plumage condition, foot pad dermatitis, or hock burn scores (**Table 2.6**). All (100%) of the birds had a plumage condition score of 1 (slightly dirty). Across treatments, the percentage of birds with a foot pad dermatitis score of 0 ranged between 80-100%, 0-20% had a score of 1, and no (0%) of birds had a score of 2. Across treatments, the percentage of birds with a hock burn score of 0 ranged from 60-76.2%, 23.8-36.4% had a score of 1, and 0-4.6% had a score of 2.

### **Corticosterone and Tibia Bones**

#### ***Corticosterone***

There were no main or interaction effects ( $P > 0.05$ ) of enrichment treatments on corticosterone levels in blood plasma ( $N = 82$  broilers). There was a time of day at blood draw effect, where the concentration of plasma corticosterone decreased with time of day ( $P = 0.04$ ,  $R^2 = 0.06$ , data not shown). Three focal birds were excluded from analysis because we were unable to collect blood within 120 seconds of first handling. Across

treatments, average corticosterone concentrations ranged from 4.25ng/mL to 5.79 ng/mL (Figure 2.14).

### ***Tibia Ash***

There were no main or interaction effects ( $P > 0.05$ ) of enrichment treatments on percent tibia ash of the left, right, or average (of the left and right) and average percent tibia ash across treatments ranged from 53.25% to 54.49% (Table 2.7). There was a tendency for focal broilers in the pens with platforms to have slightly ash for the average of the left and right tibias (Table 2.7).

### ***Tibia Bone Morphology***

#### ***Wet lab Superficial***

For wet lab tibia length, width, and angle measures, there was no significant difference in the comparison of the left and right tibia (data not shown), so the data for wet lab tibia measures is reported as the average of the left and right tibia for each bird.

Table 2.8 shows the average tibia length, proximal and distal head width, proximal head angle, widths at 90%, 75%, 50%, 25%, and 10% of tibia length, and medial, lateral, and distal intercondylar depths of the right and left tibia for each treatment. There were no main or interaction effects ( $P > 0.05$ ) of enrichment treatments on the tibia length, width of the distal head, width at 50% or 10% of the length, angle of the proximal head, or depths of the medial and distal intercondylar areas (Table 2.8). Birds in pens with P and S&P treatments had a greater tibia width at the proximal head ( $P = 0.02$ ) compared with S and C treatments. Birds in pens with the S treatment tended to have more narrow proximal head ( $P = 0.06$ ) compared with other treatments. At 90% of the tibia length, birds in pens with the S treatment had the most narrow ( $P = 0.006$ ) tibia, birds in pens with the P treatment

had the widest ( $P = 0.003$ ) tibia, and tibias from birds in pens with the C and S&P treatments were intermediate. At 75% of the tibia length, birds in pens with the P treatment had a wider ( $P = 0.01$ ) tibia than the S ( $P = 0.009$ ) treatment. At 25% of the tibia length, birds in pens with the P treatment had a wider ( $P = 0.04$ ) tibia compared with the S treatment (**Figure 2.15**). The birds in the pens with the S treatment had a more shallow ( $P = 0.03$ ) depth of the proximal head lateral intercondylar area compared with P and C treatments (**Figure 2.16**). Tibia weight and volume were both greater ( $P \leq 0.05$ ) for birds in pens with the P and S&P treatments compared with C and S treatments and there was a tendency ( $P = 0.09$ ) for birds in pens with the S treatment to have lighter tibias than birds in the other treatments ( $P = 0.09$ ) (**Table 2.9**).

#### *Digital Superficial*

There were significant differences in the length, width, and angle measures between the left and right tibia in the digital image morphology measures. No main or interaction effects ( $P > 0.05$ ) were seen in the tibia length, proximal and distal head width, proximal head angle, widths at 50% of tibia length, and lateral and distal intercondylar depths of the right (**Table 2.10**), left (**Table 2.11**), and average of the right and the left tibia (**Table 2.12**) across treatments.

While there were no treatment effects on either the right or left tibia width at 90% length location, the birds in pens with the P and S&P treatments had a wider ( $P = 0.04$ ) average tibia width at 90% compared with C and S treatments (**Table 2.12**). The birds in pens with the S treatment had a narrower ( $P = 0.03$ ) left tibia at the 75% length location and birds in pens with the P treatment had wider ( $P = 0.02$ ) left tibias at the 75% length location, while the C and S&P treatments were intermediate (**Table 2.10**). A similar pattern

was seen with the average widths at 75% length, where the birds in pens with the S treatment tended to have narrower ( $P = 0.06$ ) tibias and birds in pens with the P treatment had wider ( $P = 0.008$ ) tibias, while the C and S&P treatments were intermediate (**Table 2.12**). For the right width at 75% length, the birds in pens provided the P and S&P treatments had wider ( $P = 0.006$ ) tibias compared with C and S treatments (**Table 2.11**). There was a tendency for the average tibia widths to be greater ( $P = 0.09$ ) for birds in pens with P treatment at 50% and 25% length locations compared with other treatments (**Table 2.12**).

The width of the right tibia was greater ( $P = 0.03$ ) for birds in pens with the P and S&P treatments at the 25% and 10% locations compared with C and S treatments, and there was a tendency for birds in pens with the S treatment to have a more narrow ( $P = 0.07$ ) tibia at 25% length compared to other treatments (**Table 2.11**). The depth of the medial intercondylar area of the right tibia proximal head was shallower ( $P = 0.02$ ) for birds in pens provided the P and S&P treatments compared with C and S treatments (**Table 2.11**). The depths of the lateral proximal and distal intercondylar areas of the right tibia for birds in pens with the S treatment tended ( $P = 0.08$ ) to be more shallow than other treatments (**Table 2.11**).

#### *Wet lab vs. Digital Superficial Tibia Morphology*

Apart from the proximal head width and medial intercondylar depth, there were significant differences in the superficial tibia morphology depending on whether the Wet lab or the Digital method was used to collect measures (**Table 2.13**). Measures that were greater for the Wet lab method included the distal tibial head width by 0.26 mm ( $P = 0.009$ ), width at 90% of the length by 0.98 mm ( $P = 0.003$ ), and the depth of the lateral intercondylar area

by 0.29 mm ( $P < 0.0001$ ) compared with these measures collected with the Digital method. Measures that were greater for the Digital method included the tibia length by 9.94 mm ( $P < 0.0001$ ), proximal head angle by 1.29° ( $P = 0.007$ ), width at 75% length location by 1.08 mm ( $P < 0.0001$ ), width at 50% length location by 0.76 mm ( $P < 0.0001$ ), width at 25% length location by 1.02 mm ( $P < 0.0001$ ), width at 10% length location by 2.54 mm ( $P < 0.0001$ ), and the depth of the intercondylar area of the distal head by 0.26 ( $P = 0.007$ ) (**Table 2.13**).

#### *Digital Tibia Morphology -Cortical Bone*

At the 50% length location, there were no main or interaction effects ( $P > 0.05$ ) of enrichment treatments on the North, East, South, West thickness, or surface area of the left or right (**Table 2.14**) or the average (**Table 2.15**; **Figure 2.17a**) cortical bone of the tibia. At the 75% length location, there were no main or interaction effects ( $P > 0.05$ ) of enrichment treatments on the surface area of the cortical bone of the left and right tibia (**Table 2.16**; **Figure 2.17b**), or average of the left and right tibias (**Table 2.17**). However, for birds in pens with the P treatment, the surface area of the right tibia at 75% length location tended to be greater ( $P = 0.08$ ) compared with the other treatments (**Table 2.16**). For birds in pens with the P and S&P treatments, the cortical bone thickness at the South (anterior) location of the right tibia at 75% length was thicker ( $P = 0.002$ ) compared with C and S treatments (**Table 2.16**; **Figure 2.18**).

## **Behavior**

### *Fear Response Tests*

#### *Avoidance Distance (AD)*

At week 3, the average percentage of broilers in the pen was 1.99% in Zone 1, 14.78% in Zone 2, 34.85% in Zone 3, 26.42% in Zone 4, and 21.97% in Zone 5, and the median for each zone was 0% in Zone 1, 15.14% in Zone 2, 33.33% in Zone 3, 25% in Zone 4, and 18.61% in Zone 5. At week 5, the average percentage of broilers in the pen was 6.53% in Zone 1, 23.14% in Zone 2, 33.97% in Zone 3, 24.34% in Zone 4, and 12.02% in Zone 5, and the median for each zone was 4.26% in Zone 1, 23.27% in Zone 2, 32.76% in Zone 3, 25.54% in Zone 4, and 10.26% in Zone 5. There was no difference ( $P > 0.05$ ) in the percentages of birds in Zones 1-5 at 10 s compared with 20 s after the observer entered the pen and squatted during the avoidance distance (AD) test at 3 weeks and 5 weeks of age (data not shown) so the average of 10 and 20 s data was used for analysis. The average percentage of birds in each location at week 3 are reported in **Table 2.18** and in **Table 2.19** at week 5. The average percentages of birds in each location at both week 3 and 5 is visualized in **Figure 2.19**. There were no treatment main or interaction effects on the percentages of birds in Zone 2, 4, or 5 at week 5 or week 3 ( $P > 0.05$ ). However, in pens with P and S&P treatments there was a lower percentage of birds in Zone 1 at week 3 ( $P = 0.02$ ) compared with S treatment (**Table 2.18**). In pens with P treatment there was a greater percentage of birds in Zone 3 at week 5 ( $P = 0.04$ ) compared with S treatment (**Table 2.19**).

Within Zones 1 and 2, there was a numerically greater percentage of birds by 0.59-7.44% in Zone 1 and 2.29-10.31% in Zone 2 at week 5 compared to week 3 for pen with C, P, and S treatments (**Table 2.20**). Within Zone 1, the pens with S&P treatment had a 3.3% numerical increase in percentage of the birds in at week 5, but in Zone 2 there was a statistically greater increase of 13.64% ( $P = 0.0009$ ) more birds at week 5 (**Table 2.20**).

Zone 5 had a numerically greater percentage of C birds at week 3 than week 5 by 2.47%. There was a statistically greater increase of 11-14.37% increase of birds in Zone 5 in pens with P ( $P = 0.01$ ), S ( $P = 0.03$ ), and S&P ( $P = 0.0005$ ) treatments compared with C at week 3 (**Table 2.20**).

#### *Novel Object (NO)*

The results from the novel object (NO) test are mean and median counts and percentages. The mean percentage of birds to approach within 25 cm of the NO during week 3 was 3.64% and the median was 0%, the mean to approach within 26-50 cm was 14.71% and the median was 15.79%. The mean percentage of birds to approach within 25 cm of the NO during week 5 was 9.50% and the median was 8.70%, the mean to approach within 26-50 cm was 15.43% and the median was 14.29%.

During the NO test, birds in only one pen, pen 16, pecked the NO at week 3 (**Table 2.21**). In pen 16, 3 birds pecked at the NO 21 times in total and had a latency of 2 seconds to the first peck at week 3, which may represent an outlier since only this pen had any birds peck at the NO (**Table 2.21**). At week 3 the mean number of broilers to peck at the NO was 0.19 and median was 0 broilers. Mean latency to first peck was 225 sec and median was 240 sec.

At week 5, birds in nearly all of the pens (14 of 16 pens) pecked at least once at the NO and the latency to first peck ranged from 7 sec to 169 sec for birds within pens that did peck the NO (**Table 2.22**). The mean latency to first peck was 73 sec and a median of 25 sec. Number of birds to peck at the NO ranged from 1 to 8 birds and the number of total times the birds in the pen pecked the NO ranged from 1 to 84 pecks (**Table 2.22**). The mean number of birds to peck was 2.69 and the median was 2 broilers.

Latency to approach within 50 cm (Zone 2) was mostly 0 sec for both week 3 (range: 0–57 sec, mean: 3.56 sec, median 0 sec) and week 5 (range: 0–1 sec, mean: 0.06 sec, median: 0 sec; **Table 2.21; Table 2.22**), meaning that the birds had entered Zone 2 within the time it took for the observer to exit the room and begin the test. At week 3 of age, birds within 7 of the 16 pens had a latency of greater than 0 sec to approach within 25 cm (Zone 1) of the NO and latency to approach within 25 cm (Zone 1) of the NO at week 3 ranged from 0 sec to 240 sec (**Table 2.21**). Of the 16 pens, 3 did not have a latency of 0 sec at week 5 to approach within 25 cm of the NO. Latency to approach within 25 cm of the NO at week 5 ranged from 0 sec to 16 sec (**Table 2.22**).

Scan sampling of the NO test showed there was more variation between treatments and over time at week 3 (**Figure 2.20**) than week 5 (**Figure 2.21**). All treatments, except birds in the C pens, had no birds within 25 cm (Zone 1) of the NO by the end of the test at week 3 (**Figure 2.20a**). The C treatment pens also had the most birds within 26–50 cm (Zone 2) of the NO at the start of the test, and the number of birds decreased overtime (**Figure 2.20b**). At week 5, all treatments had birds within 25 cm (Zone 1) (**Figure 2.21a**) and 26–50 cm (Zone 2) (**Figure 2.21b**) of the NO throughout the duration of the test, and the greatest variation between treatment groups was seen at the beginning of the test, particularly within the first 60 sec (**Figure 2.21**). Birds were more evenly distributed in Zones 1 and 2 across treatments throughout the duration of the test at week 5 (**Figure 2.21**) compared with week 3 (**Figure 2.20**). There were numerically more birds within 26–50 cm (Zone 2) of the NO than birds within 25 cm (Zone 1) of the NO at the end of the test, but for all treatments there were birds in both locations at all time points at week 5 (**Figure 2.21**).



Overall, there was a greater percentage of birds in each treatment group within 25 cm (Zone 1) of the NO at week 5 (range: 8.01%-11.90%) compared with week 3 (range: 1.08%-6.77%) (**Table 2.23**). Within each treatment group, there was a greater ( $P < 0.0001$ ) percentage of birds in Zone 1 at week 5 than week 3 (**Table 2.23**). While there was no difference in the percentage of birds in Zone 2 within the S&P pens ( $P = 0.17$ ), there was a greater ( $P \leq 0.002$ ) percentage of birds within Zone 2 in S and P treatment pens and a lower percentage ( $P = 0.001$ ) of birds in C treatment pens at week 5 compared with week 3 (**Table 2.23**). For all treatment group pens, there was a lower percentage ( $P \leq 0.05$ ) of birds in Zone 3 (greater than 50 cm from NO) at week 5 than week 3 (**Table 2.23**).

## **2.5 DISCUSSION**

### **Production**

#### ***Feed Conversion and Body Weight***

In this study, there was no effect of environmental enrichment treatments on feed conversion ratio (FCR) and this finding is in agreement with broiler research performed on commercial farms with environmental enrichments (de Jong and Gunnink, 2019). The average cumulative FCR was calculated based on feed consumption from day 54 and final body weights (day 53 for focal birds, and 54 for the remaining birds in the pen) of 1.83 was slightly higher FCR than the expected 1.804 for the male 708 broiler (Aviagen, 2019). This difference might be explained by the disease experienced by birds in our study, as the presence of a diseased state may increase FCR (Aviagen, 2011). However, the 1.83 FCR was similar to the Ross 708 Management guide value of 1.83 for as-hatch (mixed sex) broilers (Aviagen, 2019). The birds used in this study were sourced from a hatchery with

chick sexers and the chicks were predominantly males, but there was a total of 7 female focal birds, and several non-focal females (29 females at end of study) present in this experiment and this could have affected FCR and other measures.

The FCR is a very important value to the broiler industry because it shows how efficient the broiler chickens are at converting feed to body weight gain. High feed efficiency (low FCR) means that there will be heavier birds, and ultimately more meat available to sell, per unit of feed consumed by the bird than a bird with a lower efficiency (higher FCR). In addition to FCR, body weight is a very important value for similar reasons. If a bird is highly efficient at converting feed to weight gain, but is lighter than other birds, it will yield less meat. This could happen if the birds consumed less feed than a larger bird with the same or higher FCR and is likely what occurred in this experiment. The final body weight was estimated to be 3,410 g in our study, which is considerably lighter than the expected weight of 4,226 g for male broilers or 3,877 g in an as-hatch at day 54 (Aviagen, 2019). There was a high incidence of illness and ill birds were culled. As discussed in more detail in the mortality section, the birds were found to have multiple bacterial and a viral infections. It is possible that the illness experienced by these birds led to a greater time resting and less time spent eating that was affected by infection or the metabolic cost of the recovery from infection (Snyder et al., 2021) prevented birds from gaining more weight in the current study.

### ***Mortality***

In this study, there was no effect of environmental enrichment treatments on mortality. It is important to note that the average cumulative mortality in our study was 18%, which is considerably higher than other studies performed in a commercial setting

concerning environmental enrichments in which mortality normally ranges between less than 1%- 4% (Bailie and O'Connell, 2015; de Jong and Gunnink, 2019). At day 27 of age researchers noticed there were a considerable number of sick broilers. This high rate of morbidity led us to euthanize 29 broilers on day 27. Due to continued concerns with morbidity after day 27, researchers took 7 euthanized birds from 6 different pens in both room 1 and room 2 to the Frederick Animal Health Laboratory in Frederick, Maryland for diagnosis on day 29. The birds were diagnosed with 3 bacterial infections: *Escheria coli*, *Enterococcus durans*, and *Enterococcus faecium*, and 1 viral infection: Infectious Bronchitis Virus (MASS and GA08). Infectious Bronchitis Virus is a respiratory disease that is clinically characterized by coughing, sneezing, tracheal rales, conjunctivitis, and dyspnea (Jackwood, 2019). Interestingly, Infectious Bronchitis Virus has been identified to induce secondary colibacillosis (*E. coli* infection) in broilers (Gross, 1990; Dwars et al., 2008). Colibacillosis can be local or systemic in broilers and is often seen as a respiratory infection and pericarditis. If the infection travels from the initial entry point into the body, then the infection becomes systemic. The bacteria are able to travel through the bloodstream and proliferate to cause lesions elsewhere in the body and ultimately septicemia (Dwars et al., 2008). Notably, lesions caused by bacterial infections have been seen in leg bones of broilers and these infections are called; femoral head necrosis (FHN), tibial head necrosis (THN), bacterial chondronecrosis with osteomyelitis (BCO) (Thorp, 1994; Wideman, 2016). In this study, tibia bones were not scored for THN or BCO because they would not have been viable for morphological measurement, but no obvious lesions indicative of either disease were observed during morphology measures, and femurs were

not observed during this study. Still it is possible that the diseased state could have influenced both physiology and behavior of the broilers during this study.

### **Physical Indicators of Welfare**

We observed no effect of environmental enrichments on physical indicators of welfare (foot pad dermatitis, hock burn, and plumage cleanliness) measures. This is in agreement with commercial studies on environmental enrichments (Bailie et al., 2013; 2018a; 2018b; de Jong and Gunnink, 2019). We saw that incidence of hock burn (score > 0) was 24-40%, incidence of foot pad dermatitis was 0- 20%, and 100% of birds were slightly dirty with a plumage condition score of 1. Our hock burn scores were higher than some research performed on commercial broiler farms in which the prevalence of hock burn ranged from 9-15% (Bailie et al., 2013; 2018a; 2018b). Although there is no literature on the impact of spotlights on physical welfare measures, there was no impact of natural lighting through windows compared to no windows (fluorescent lighting) in a study that also evaluated physical indicators of welfare including foot pad dermatitis, hock burn, plumage condition, and injuries (de Jong and Gunnink, 2019).

A few factors may have contributed to the lack of significant physical welfare findings. First, throughout the experiment additional litter was added to areas where moisture had begun to build up, likely preventing any buildup of moisture and ammonia. This would decrease the likelihood of ammonia burns and contact dermatitis. Additionally, birds showing signs of illness were euthanized throughout the study. Since the euthanized birds were not scored for physical welfare measures, we cannot be certain that these birds were unaffected. Finally, we utilized a low stocking density in this experiment, and it is possible that the low stocking density is related to our low incidence of dermatitis (Bailie

et al., 2018a). The higher than expected incidence of hock burn may be due the diseased state of the broilers used in this experiment, as greater time spent sitting can increase the risk of hock burn due to greater contact with litter (de Jong et al., 2016) but activity levels may decrease in response to infection to facilitate healing (Snyder et al., 2021).

## **Corticosterone and Tibia Bones**

### ***Corticosterone***

We observed no change in basal plasma corticosterone levels of broilers in any treatment group at final collection on day 53. While these findings may suggest there is not a change in the basal levels of stress in birds provided enrichments, it is important to consider that plasma corticosterone levels were only measured at one time point during the study, and we do not know how the corticosterone levels may or may not have shown differences in response to a stressor or other stimuli. Additionally, levels of corticosterone can be influenced by the circadian rhythm and will vary throughout the day (de Jong et al., 2001; Scanes, 2016). We could have collected blood to measure plasma corticosterone at multiple time points. With multiple corticosterone measures we could have compared basal levels over time to better detect how the environmental enrichments were, or were not, influencing basal levels of stress. However, one major limitation to collection of blood is the time limit of handling before stress levels related to handling are reflected in the plasma corticosterone levels. In this study, we collected all blood samples within 120 seconds of initial handling of the bird at collection and do not believe handling has impacted our plasma measure of corticosterone since levels of corticosterone from handling stress increase after 2 minutes (Chloupek et al., 2011). If we had collected blood for plasma corticosterone at multiple time points, it is possible that the focal birds would have

developed a greater fear of humans because of negative experiences, and this might have influenced the corticosterone measures for focal birds since corticosterone may have increased even before handling for blood draw.

In our study we utilized an ELISA to measure the concentration of corticosterone, but this is not the only method used to measure corticosterone. Other studies have used Radioimmunological assays (RIA) to measure corticosterone in blood samples (Kang and Kuenzel, 2014). Still, other less invasive measures of corticosterone levels may have provided a greater understanding of corticosterone levels over time. Feather corticosterone can be used to understand stress levels in a life stage of the birds (Bortolotti et al., 2008). Although we were not able to find literature on environmental enrichments and feather corticosterone (or other measures of corticosterone) response in broilers, feather corticosterone has been used to measure the short term (10 days) and long term (3 months) stress response of a wild caught bird species (*Nucifraga columbiana*) to enrichments in their housing environment (Fairhurst et al., 2011). The results from this study showed that the enrichments may have served as a stressor in the short term, but after acclimation, the enrichments may have decreased stress levels in the long term (Fairhurst et al., 2011). Measuring corticosterone levels in broilers over time would be useful to better understand the impacts environmental enrichments have on broiler physiology, and could help to find what, if any, enrichments lead to the greatest decrease in stress levels in broilers.

Additionally, the birds in this study experienced both bacterial and viral infections. Viral infections are known to influence corticosterone levels in broilers (Davidson et al., 2020). The corticosterone levels measured in this study may have been influenced by illness. Although birds with evident signs of the illness were euthanized, it is possible that

some focal birds were still infected, but not presenting with evident signs of illness at the time of focal sampling. Despite the potential influence of infection on corticosterone levels the broilers in this study had an average corticosterone levels ranging from 4.25ng/mL to 5.79 ng/mL, which is comparatively low levels of plasma corticosterone in comparison to the average corticosterone level of 11 ng/mL (Blas, 2015).

### ***Tibia Superficial Morphology***

For the digital method, we found treatment differences on the right tibiotarsus (tibia) width at 75%, 25%, and 10% of the total bone length and also on the left tibia at 75% length. Treatment differences were also found on the average of left and right (tibia) width at 90%, and 75% length for digital methods. We also found differences in the average tibia width of the proximal head and at 90%, 75%, and 25% locations using the wet lab methods. Birds in pens with the platform had a greater tibia width at the proximal head, while birds in pens with the spotlight tended to have a more narrow proximal head compared with other treatments for data collected using the average (of left and right tibia) wet lab method. Bone tissue is undergoing constant change through cellular remodeling during growth, and can be influenced by internal factors such as endocrine signals, transcription factors (Mackie et al., 2008), external stimuli such as body weight, physical activity, and calcium requirements of the body (Glimcher, 1998). The proximal head of the tibia is the location of the fastest growing growth plate (Angel, 2007), so it may be likely that the environment the birds were experiencing did influence the shape and perhaps other characteristics of the bone.

The birds in pens with the platform enrichments had greater average (both wet lab and digital) tibia widths at the 90% and 75% length locations compared to other birds.

Birds in pens with the platform were provided opportunities to perform additional activities, such as hiding under the enrichment (at a young age), hopping on or off the platform, and walking or running up the platform, that birds without the platform treatment did not have. The ability to perform these activities may have put different physical pressures on the legs, and ultimately the tibia, which could have led to changes in the width of the bone. It has been reported that broilers subjected to an exercise treatment (4 periods of 15 minutes per day for 5 days in a treadmill-like carousel cage) had less abnormalities in the vascularization of the proximal tibia head compared with birds that were not exercised (Thorp and Duff, 1988). Vascularization of the bone is very important for proper bone ossification of the cartilage tissue to form bone at the growth plate and the prevention of disease, such as tibial dyschondroplasia (TD). Although other bone health studies have scored for TD (Bizeray et al., 2002; Kaukonen et al., 2017), we chose not to because we had we could not have been able to measure tibia morphology (damaged tibial heads), yet it is not likely that we would have had a high enough incidence in TD to measure differences (Bizeray et al., 2002). It is worth noting that we found one bird with a very evident case of TD after collecting morphology measures. This bird showed TD on both the left and right tibia. If the same environmental enrichments utilized in our study were used in a conventional housing environment, it may be useful to score the birds for leg diseases, including but not limited to Tibial Dyschondroplasia (TD), Bacterial Chondronecrosis with Osteomyelitis (BCO), Tibial Head Necrosis (THN), Femoral Head Necrosis (FHN), to further investigate any changes in bone quality and health. If these findings are in fact repeatable this could lead to another environmental factor which may



be utilized to improve broiler health as leg health in conventional growing broilers is a current welfare concern.

Birds in pens with the spotlight had a narrower tibia at 90% and 75% of the length compared to other birds for the average wet lab method. Birds in pens with the spotlight had a narrower tibia at 75% of the length compared to other birds for the left digital method, while this effect was a tendency for the average of both tibias. The decrease in the width of the tibias for birds provided the spotlights is likely not explained by a difference in activities available to the birds, because the spotlight was an addition to the environment which stimulated the birds visually. Although the spotlight may or may not have stimulated certain activities, it was not a physical object, which the birds could physically use for hiding, climbing, or roosting, compared to the platform which was structure in which the birds could to interact with in a 3-dimensional way. It has been shown that the wavelength and/or intensity of lighting influence the development of the skeletal system through the endocrine system (Manser, 1996; Mackie et al., 2008). Light is known to influence circadian rhythm and metabolism (Manser, 1996; Mackie et al., 2008). Additionally, factors such as growth hormone and thyroid hormones have an influence on the formation of bone tissue (Mackie et al., 2008). Broilers incubated under green lights were shown to have higher levels of growth hormone and insulin-like growth factor 1 compared to broilers incubated in the dark (Zhang et al., 2014). This finding suggests that wavelengths of light can influence the endocrine system of broilers, which may help to explain the decrease in tibia width of broilers raised in spotlight pens compared with the other treatments in the current study.

The spotlight utilized in this study was a cool toned (6000-6500 Kelvin) light at 355 lux intensity and appeared slightly blue in color. The exact wavelength of light produced is not known or was measured, but it was evidently different compared to the soft white (2,920 Kelvin) incandescent room lights at 45 lux light intensity which were utilized in this study. This blue colored light could have influenced levels of hormones in the broilers, specifically through an increase in testosterone levels. In an experimental setting, Cao et al. (2008) found that male broilers raised under blue light had greater testosterone levels compared with those raised under white or red LED lights. Although the birds in our study were not incubated with the spotlight, the light was introduced early in life (day-of-hatch) and may have influenced production of growth hormone or other hormones post hatch, possibly leading to a slight decrease in rate of endochondral ossification (mineralization of the bone at the growth plate) (Mackie et al., 2008).

The spotlight could have had an influence on skeletal development or the breakdown and regeneration of bone tissue, potentially through osteoblast and osteoclast activity. The wavelength of the spotlight may have led to the differences in bone morphology in birds with the spotlight compared with birds not provided this spotlight. In a 2018 study, it was shown that a mixture of yellow and white LED lighting increased the shank length of chickens, suggesting that the effects of lighting wavelength are able to influence skeletal development (Yang et al., 2018). It was observed that the birds in pens with the spotlight tended to congregate around the edge of the illuminated area. It has been shown that broilers raised under blue light spend more time sitting and dozing than birds raised under red or white lights (Prayitno et al., 1997). The blue-colored spotlight provided

may have influenced the birds' behavior, such as time spent sitting in the area of the spotlight, which may have also contributed to the decrease in tibia width.

Bone breaking strength is influenced by both organic matrix and inorganic (ash) present in the bone (Rath et al., 1999) and is a common measure of skeletal integrity in research. Although, to our knowledge, no research on the effect of platforms or spotlights on the tibia breaking strength of broilers have been published, other research evaluating the effects of laser light enrichments on tibia breaking strength have not seen an effect (Meyer et al., 2019). Notably bone breaking strength is performed at the 50% location on the bone (Pedersen et al., 2020), which limits the information or understanding of bone strength to only one location on the bone, but including multiple morphology measures at various locations along the bone, can provide more detail about multiple points on the tibia. We cannot say for certain that the tibia morphology differences in our study were the result of an increase or decrease in the quality of the tibia because there was not a difference in whole bone mineralization, measured with percent tibia ash.

We found no effect of environmental enrichment treatments on the tibia width at 50% of the length. These results are supported by research conducted by others, which found no difference in the effect of environmental enrichments on the width at the mid-diaphysis (50%) and or total length of the tibia (Bizeray et al., 2002; Pederson et al., 2020). We did not find significant differences in the width of the distal tibial head with the platform and spotlight environmental enrichments in the current study. Pederson et al. (2020) found that broilers with a greater distance between feed and water had greater width at the distal end of the tibia than birds provided straw bales. The researchers reported that the difference in tibia width at the distal end was likely due to differences in activity levels,

specifically the time spent locomoting (Pederson et al., 2020). This shows that different forms of enrichments can influence tibial morphology in different ways.

We did not observe any differences in the depth of the intercondylar area of the distal head with enrichments, but we did observe differences on the proximal head. The intercondylar area of the lateral side of tibia was shallower in the data collected in the wet lab and more shallow intercondylar areas on the medial of the right tibias in the data collected in ImageJ software on 2D images (digital measures). Although the differences in intercondylar depths were not consistent across measurement methods, a similar point can be made about the intercondylar depths of the proximal tibial head, irrespective of the measurement method. Cruickshank and Sim (1986) observed that birds with twisted legs (varus valgus deformity) had more shallow intercondylar depths at the distal end of the tibia, wider tibias at the mid diaphysis (50% length), and shorter tibial length. The shallow intercondylar depth may allow for minor displacement of tendons, which leads to strain, ultimately causing abnormal bone curvature along the shaft of the tibia. These morphological changes can then be seen at a clinical level as a varus valgus deformity (Cruickshank and Sim, 1986). We observed no effect of enrichments on the length of the tibias, distal intercondylar depth, or width of the bone at the 50% length location for the left, right, or average of both the wet lab and digital measures. When compared to Cruickshank and Sim (1986), our results may not have been affected by clinical varus valgus deformities, but twisting of the tibia was not measured. However, we did not score for varus valgus deformities so we cannot say for certain that the population of birds tested did or did not have twisted legs. In future studies, it would be beneficial to measure the degree of twisting of the tibia or to score for varus and valgus deformities of the leg.

Although it was not recorded, some birds in our study were culled for apparent splay leg, which is indicative of slipped tendons or infection causing vertebral osteomyelitis (Braga et al., 2018). Vertebral osteomyelitis, also called spondylitis or “kinky back”, is an inflammation of the thoracic vertebra T4 by bacteria (Braga et al., 2018). Interestingly, we did observe changes in the intercondylar depths of the proximal head with both the spotlight (wet lab average) and platform (digital average) enrichments, which may show evidence that changes to the proximal end of the bone should be further analyzed in order to understand if these enrichments induce conformational abnormalities other than varus or valgus deformities. It is also possible the bacterial infections diagnosed in these birds could have led to vertebral osteomyelitis as 2 of the bacteria (*Escherichia coli* and *Enterococcus durans*) possibly responsible for infection in these broilers are known to cause vertebral osteomyelitis (Braga et al., 2018).

***Wet lab vs. Digital Superficial Tibia Morphology:***

The comparison of the wet lab and ImageJ (digital) methodologies used to measure tibia morphology showed multiple differences in the data. The measures collected in ImageJ were greater for the total length, proximal head angle, 75% length, 50% length, 25% length, and 10% length, and distal intercondylar area. The distal head width, 90% length, and lateral intercondylar depth of the proximal head were greater for the wet lab method than the digital method.

The nature of these differences may be a result of increased precision with the ImageJ methodology. The ImageJ method was more precise because we were able to measure the exact location of the width at percentages of total length measures but with the wet lab method the bone was marked using a permanent marker. When measuring the

bone at the mark, the calipers may have been placed at any point along the width of the mark, but we were able to eliminate this issue in the ImageJ method through the use of the fixed length line macro tool. This macro tool was used to mark the exact location at the 90%, 75%, 50%, 25%, and 10% total length, first the line tool was used for measuring the total length of the bone and then observers calculated the length of the percent length locations from the distal end (0% length) then creating a line of that exact length using the macro tool. Additionally, we were only able to measure values out to 2 decimal places with the digital calipers used in the wet lab collected data, but we were able to measure out to 4 decimal places with the ImageJ methods.

Despite the benefit of increased precision with the ImageJ method, there are still limitations. The superficial images used for this analysis are 2D, but the bone measured is 3D in nature. The inability to look at the tibia in 3D space may have led to the loss of detail as only one surface can be used in the images. Any minor changes in bone morphology may have impacted how the bone rested on the surface on which it was photographed, which would in turn have an impact on the data collected from the image. Additionally, the time spent on measurements of bone morphology in ImageJ was greater than the time spent measuring with the digital calipers for the wet lab measures. Due to the limitations associated with the ImageJ methods, we would recommend using the wet lab methods, but if 3D images were available to be used in ImageJ for bone morphology (e.g. torsion), then the digital methods would be a better methodology than wet lab methods.

### ***Digital Tibia Morphology -Cortical Bone***

From the ImageJ digital measures in this study, there was no effect of environmental enrichments on the surface area of the cortical bone, or thickness of the

cortical bone on the posterior, medial, or lateral side at 50% or 75% of the length of the tibia. We chose to measure the cortical bone at the 75% of the length location because we saw differences in the width of the bone observed at the 90% and 75% length locations. We chose not to include the 90% length location in our cortical bone analysis because the development of the cortical bone at this location may not be far along enough to properly compare the cortical bone between birds (C. R. Angel, personal communication).

The increase in cortical bone thickness has been reported to be due to the decrease in bone resorption and the increase in periosteal bone formation at areas where morphological deformities can increase strain on the leg bone (Cruickshank and Sim, 1986), but this is not the only reason for the thickening of the cortical bone. Cortical bone is important for mechanical strength of the bone and loss of cortical bone can be associated with risk of fractures (Rath et al., 2000). Interestingly, the physical loading is needed to maintain the cortical bone mass, and physical activity has shown to improve bone strength in laying hens (Rath et al., 2000). Since cortical bone thickening can be associated with potential increases in mechanical strength, a thicker cortical bone may be beneficial to broiler leg health.

We expected that the cortical bone at the 50% location would not be significantly different across treatment groups and this hypothesis was supported by the data. We also expected that differences seen in bone morphology at the 75% location would be reflected in the cortical bone thickness and surface area at this location. Our hypothesis was only partially supported because the anterior (south) location of the right tibia, but not the left, was thicker when birds were provided the platform enrichments, but no spotlight effects. Sanchez-Rodriguez and team (2019) have shown that the cortical bone at the mid-diaphysis

(50% length) is fully mineralized and porosity is low at day 37 of age. In addition to the mineralization, porosity is another measure of skeletal integrity of bone (Black and Mattson, 1982). If we had measured the porosity of the bone, we likely would not have seen differences at the 50% length location due to the age of the birds at sampling (53 days), but it is possible that differences at 75% length location could correspond to differences in rate at which the bones are becoming fully mineralized. In future studies, microscopy could be used to evaluate the porosity of the bone near the proximal head.

Differences in tibial morphology can be the result of a morphological deformity, as other researchers have found that birds with varus valgus deformities had greater cortical thickness as a percentage of the tibial diameter (measures on the medial and lateral sides) (Cruickshank and Sim, 1986). In our study, we saw no changes in the width at the 50% length location (mid diaphysis), but there was an increase in cortical bone thickness on the anterior (South) side at the 75% length location of the right tibia for birds in pens with platforms. Compared to the 50% location, the 75% location is closer to the proximal growth plate, and this may be why we saw a difference in the 75% length cortical bone thickness, but not the 50% location. Additionally, areas closer to the proximal growth plate are more sensitive to mineralization during development than locations further from the growth plate. Notably, these differences were only seen in the right tibia, and not the left. This asymmetry in the tibias may be the result of increased stress levels, as stress has been suggested to cause deviation from bilateral symmetry (Knierim et al., 2007). Despite having no difference in basal corticosterone levels at day 53, our results suggest that broilers provided platform enrichments may have experienced greater physical loading



stress on the bone leading to increased cortical bone mass and greater mechanical strength at 75% for the right bone (Rath et al., 2000).

### ***Ash***

We observed no effect of environmental enrichment treatments on percent tibia ash. This finding is in agreement with other research on the impact of environmental enrichments on broiler tibia bone percent ash (Kaukonen et al., 2017). The process of mineralization is required for the development of bone at the growth plate and provides strength to the bone and percent tibia ash is an indirect measure of total mineralization in the bone (Thorpe, 1994). The percent tibial ash in this study ranged between 53-55%, which was slightly higher than percent ash reported in other research by 4-15% (Shim et al., 2012; Pritchard et al., 2020). Since there were no differences in tibia ash, there seemed to be no difference in overall bone mineralization showing there were not clear differences in skeletal development of the tibia in this experiment.

## **Behavioral Indicators of Welfare**

### ***Fear Tests***

We performed the Avoidance Distance and Novel Object Tests to determine if the platform and/or spotlight enrichments affected fearfulness of the broilers in this study. At week 3 of age, we have seen that the S&P pens had a numerically greater proportion of birds within 25 cm of the NO for about the first 60 sec and last 50 sec of the NO fear test. This shows that the birds in S&P groups may be more interested or less fearful of the NO at this age. At the end of the test, all other treatment groups did not have any broilers within 25 cm of the NO, suggesting that a full 4 minute duration for this test may not have been

necessary. Other research teams have performed the NO test with a shorter duration (Bassler et al., 2013).

The potential decrease in fear levels in S&P pens may have been due to the increase in environmental complexity for these broilers, as they had the most complex housing environment provided in this study. The broilers in our study were used to having a structural enrichment, in the form of the platform, and a solely visual enrichment (spotlight), but the spotlight was off during the test. This may be why they approached the NO more often than other treatments, because the combination of enrichments may have predisposed the broiler to be more familiar with increased variable levels of environmental complexity through both a constantly present (platform), and a transient enrichment (spotlight). This combination of experience may have allowed the broilers to better cope with changes in their environment. Other research may support this hypothesis because it has been reported that broilers tended to approach within 50 cm of a NO at 39 days of age more when they were housed in an environment enriched with wood shavings, round metal perches, and metal chains with supplementary window lighting, compared with enriched or control (no window or enrichment) broilers (de Jong and Gunnink, 2019)

The same pattern did not apply at 26-50 cm from the NO, because there were broilers in this area throughout the duration of this test, at both 3 and 5 weeks of age, but at 3 weeks there were more broilers from the C group in the area for roughly the first 60 sec of the test. Previous research on broilers in enriched housing showed that perches with dust boxes decreased the fear of humans compared to the control group, but our AD Test findings did not show a decrease in fear of humans with either the spotlight or platform enrichments (Baxter et al., 2019). This also suggests that the entire 4 minute duration may

not be needed to perform this test. At week 5 of age the percent of broilers in Zones 1 and 2 appeared to be similar between treatments across the duration of the NO test.

During the AD test at week 3 of age there were marginally less broilers (3.65% less) in Zone 1 for the P and S&P group. Despite the potential decrease in fear response during the NO Test in the S&P group, there seemed to be a slight increase in distance of the broilers from the observer during the AD Test, showing that the broilers may have had different responses to different stimuli, and the presence of the observer may have elicited more fear, than the novel object. This same pattern was not significant at week 5, but it was trending towards significance.

We observed that birds at week 5 of age were less fearful of the observer in the pen during the Avoidance Distance Test because there was a greater percentage of birds in the pen closer to the observer (within 1 m of the observer) at week 5 for all treatment groups than birds at week 3 of age. It was also observed that birds at week 5 of age were less fearful of the novel object in the pen than birds at week 3 of age, because there was a greater percentage of birds in the pen closer to the novel object (within 50 cm of the novel object) at week 5 for all treatment groups than birds at week 3 of age. The increase in birds in closer proximity to the observer and the novel object during the Avoidance Distance and Novel Object Tests suggests that the birds were less fearful of the observer at week 5 than week 3.

Other researchers have also seen an effect of age on fearfulness. Notably, some research findings are in agreement with ours and have found that birds are less fearful at an older age, as measured by Avoidance Distance and Novel Object Tests (Giersberg et al., 2021). Contrarily, others have found that broilers' fearfulness increased in the Novel

Object test as age increased (Bailie et al., 2018b). Others broiler studies have reported that fearfulness of humans decreased with increasing age, but that fear of a novel object increased with age (Bailie and O'Connell, 2014). In 2019, de Jong and Gunnink found that birds pecked at a novel object less at 5 weeks of age than at 3 weeks of age, but in the current study, we found the opposite (de Jong and Gunnink, 2019).

Notable limitations of fear-based behavior tests is that proximity measures rely on the movement, or lack thereof, of the birds subjected to the test. Potentially lame, uninterested, or ill broilers may not move away from, or towards, the test stimuli (observer or novel object), affecting test results. These limitations can impact the interpretation and application of the fear response tests.

It is important to understand that lameness increases incidence with age in broilers (Weeks et al., 2000). Lameness can impact a broiler's motivation and physical capacity to walk (Weeks et al., 2000), meaning the bird may be less likely to move away from the fear stimuli. To measure the relationship between fear and lameness in broilers, Bassler et al. (2013) did not find a significant correlation between lameness (gait score) and the Touch Test and Avoidance Distance fear tests and suggested that the length of the dark period on farm had a greater impact on fearfulness than lameness in the Touch Test. The dark periods in this commercial survey of potential risk factors associated broiler welfare, the farm photoperiods recorded ranged from 0-6.5 hours daily at week 3 of age, and the authors found that longer dark periods increased measure of fear (Novel Object and Touch Test) (Bassler et al., 2013). The dark period during week 3 of our study was 7 hours, and the dark period at week 5 was 8 hours. This indicated that the dark period used in this study likely did not negatively influence the welfare measures in our study, but the longer dark

period at week 5 may be related to the decrease in measurable fear. These findings show that changes in fearfulness in broilers is likely multifactorial and cannot be attributed to walking ability only. The fearfulness of the birds in our study were likely not affect by lameness.

On the other hand, in another commercial broiler study, Vasdal et al. (2018) found that the Touch Test results were confounded by the lameness measured by walking ability of the broilers. In both Bassler et al. (2013) and Vasdal et al. (2018) studies, the Touch Test methodology was adopted from the Avoidance Distance Test in the Welfare Quality<sup>®</sup> assessment protocol for broilers (Welfare Quality<sup>®</sup>, 2009), and as such, can be compared to the Avoidance Distance Test performed in our study. Since these studies have conflicting results (Bassler et al., 2013; Vasdal et al., 2018), we cannot conclude that there was not an effect of walking ability on the fearfulness of the birds during the fear tests in our study, but it is unlikely that our birds were experiencing lameness because we checked all broilers daily, and birds with an inability to walk (lameness) or very abnormal gait were euthanized.

Additionally, it was very likely that birds at week 3 of age were experiencing viral or bacterial infection (see mortality section of this discussion) in our study, which may have changed the magnitude or nature of their fear response. The veterinary diagnosis of infection in our broiler population was performed 5 days after the week 3 behavioral tests had been performed, but clear clinical signs of illness were not present at the time the tests were conducted. It is possible that some birds may have been less likely show measurable fearfulness due to lethargy or dullness caused by illness (Snyder et al., 2021). This may help to explain the greater latency to approach to the NO test, lack of pecking at the NO, and overall more birds at Zone 5 of the AD test during week 3.

Within the methodologies and interpretations of the Avoidance Distance and Novel Object Tests, the birds in closer proximity to the stimuli are assumed to be less fearful than the birds further away. However, a limitation of this interpretation is that the birds in closer proximity are not further classified as being present from the start or actively approached the stimuli. It could be that the birds in our study may have been indifferent or unbothered by the presence of the human or novel object because some birds did not have move at all during the Avoidance Distance and Novel Object Tests. Some of the birds that did not move were in close proximity to the human and the novel object at the start of the Avoidance Distance and Novel Object Tests. Although we cannot confirm, it is possible that the birds who did not approach the human or novel object were not fearful may have had no interest in the test stimuli. If there were birds uninterested in the testing stimuli, then our results reflect a combination of fearful and uninterested birds.

Birds that did not move while the observer was placing the novel object in the pen (prior to the start of the Novel Object Test) were excluded from latency of approach, in an attempt to eliminate the effects of lameness, and ill birds, on our fear-based measures. We could have excluded the birds in the scan samples for the NO and AD tests that did not move prior to the start of the tests, but we chose to include these birds as we could not confirm the birds were unable to walk. Limiting any potential effect of lameness was important to our study to minimizing this potential confounding factors in our interpretation of the fear based behavioral tests performed. Since these effects were limited, we are more confidently able to say any differences, or similarity, in the response of broilers to the fear testing stimuli are likely a result of our enrichment treatments.

During this experiment, we used the same novel object at both ages. This was done to eliminate any effects that different objects may have had on broiler behavioral response because different objects may elicit more or less fear or preference for one of the objects over the other. However, it is worth noting that by eliminating those potential confounding factors, we did introduce another factor. Since the same novel object was provided at both 3 and 5 weeks of age, the object was no longer 100% novel to broilers during the test at 5 weeks of age. Although we believe that it is unlikely that the previous interaction with the object influenced the response of the broilers, since only three birds in one pen pecked at the object at week 3, and an average of at least 75% of the birds were greater than 50 cm from the object for the duration of the test across all pens at week 3. It is still possible that the decrease in fear response for all treatment groups from week 3 to week 5 is related to the slight familiarity of the broilers with the object used in the Novel Object Test.

On a similar note, the Avoidance Distance Test was performed by the same human, who was also a caretaker for these broilers, at both 3 and 5 weeks that also regularly entered each pen during caretaking, so the results in this study could have been affected by the bird's familiarity with a known human. Additionally, all data collection throughout video analysis was performed by one observer, eliminating the interobserver variation, but not intraobserver variation. Other researchers have used more than one observer for behavioral tests (Giersberg et al., 2021), while other do not report if one or more observers were used (Bassler et al., 2013). In future studies, the Avoidance Distance Test could be performed by both a familiar and unfamiliar (novel) caretaker and broiler fear responses could be compared. This test is can be performed with a novel observer, but this was not possible during the time of our study to limited personnel as this experiment was performed during

the COVID-19 pandemic. The effect of novelty could be a factor in the fear response and therefore the measure of the human-animal relationship. This could be important because the individuals utilizing the methods described in the Welfare Quality<sup>®</sup> assessment protocol for broilers (Welfare Quality<sup>®</sup>, 2009) will not be the caretakers with whom the broilers are interacting with regularly. Although we expect that the nature of the interactions occurring within the caretaker-animal relationship to influence all human-animal relationships, it would still be beneficial to know if the degree of fear response in broilers is stronger in response to an unknown individual.

The spotlight treatment was on for 5 hours during the afternoon every day of this study and the Avoidance Distance and Novel Object Tests were conducted in the morning hours. Thus, the response of the broilers provided the spotlight treatment only represents the behavioral response to fear stimuli of the broilers while spotlight enrichment were off. It is possible that the broilers may have responded differently if the test had been performed in the afternoon hours when the spotlight treatment was on.

The platform enrichments remained in the pens during fear testing and were located predominately in Zones 2 and 3 (0.5 – 1.5 m from the observer). Since the birds provided the spotlight and control birds did not have an additional physical structure (platform) in their environment at the time of the fear testing, this may explain why a greater percentage of the birds without enrichments and with spotlight only enrichments approached within 0.5 m of the observer during the Avoidance Distance test, than the platform or combination of spotlight and platform groups. The presence of the platform in the pen during the fear tests may also explain the greater percentage of birds in Zone 3 (1-1.5 m) for pens provided the platform at week 5 than other treatments. The birds were able to use the platform, either



as an elevated resting place from which to observe the human, or to attempt to hide from the human during this test. Both behaviors were observed during fear test analysis but were included in the data. Additionally, we observed resource guarding behaviors and aggression in pens with platforms throughout the study. This may be that suggestion that although there was no difference in basal corticosterone levels there may be an increase in stress from interactions with conspecifics, therefor supporting the idea that differences in morphology measures of the left compared to right tibia may represent a lack of bilateral symmetry as a result of increased stress levels. We did observe any resource guarding behaviors in the spotlight pens, and one bird in a spotlight pen not only approached the observer, but also pecked at the observer during the testing time, and as the observer stood to leave the pen. This suggests that the spotlight treatment may have influence the birds' reactivity to the presence of the human in the AD test. The influence of light on the human-animal relationship warrants further investigation.

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## Tables

**Table 2.1.** Mortality (%) of pens (N = 16 pens) of broilers subjected to environmental enrichment treatments (N = 4 pens/treatment) of broilers at Phase 2-4 and cumulative (Total) mortality throughout the study.

Pen	Room	Treatment <sup>2</sup>	<i>Mortality (%)</i> <sup>1</sup>			Total
			Phase 2 <sup>3</sup>	Phase 3	Phase 4	
1	1	C	4	0	13	16
2	1	S&P	8	4	18	28
3	1	P	8	0	14	21
4	1	S	12	14	16	36
5	1	S&P	8	0	13	20
6	1	S	0	4	4	8
7	1	C	8	0	13	20
8	1	S	4	4	13	20
9	2	P	0	0	12	12
10	2	C	0	4	8	12
11	2	S&P	0	0	20	20
12	2	S	0	0	16	16
13	2	C	0	0	16	16
14	2	S	0	0	8	8
15	2	P	4	0	17	20
16	2	S&P	0	4	8	12

<sup>1</sup> Mortality includes both dead on arrival (DOA) culled broilers.

<sup>2</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

<sup>3</sup> Phase 2 included days 0 - 10, Phase 3 included days 11 - 24, Phase 4 included days 25 – 54, and Total included days 0-54.

**Table 2.2.** Average body weight (BW, g) of each pen (N = 16 pens) of broilers subjected to environmental enrichment treatments (N = 4 pens/treatment) in each pen at Phases 1-4.

Pen	Room	Treatment <sup>1</sup>	<i>BW (g)</i>			
			Phase 1 <sup>2</sup>	Phase 2	Phase 3	Phase 4
1	1	C	38.40	308.40	943.58	3,428.33
2	1	S&P	38.83	284.70	942.59	4,411.36
3	1	P	38.72	260.35	760.87	2,630.23
4	1	S	36.88	375.28	965.05	3,176.32
5	1	S&P	37.60	312.73	1,097.73	3,536.09
6	1	P	38.08	322.72	829.95	3,785.21
7	1	C	38.32	313.17	967.22	3,322.39
8	1	S	37.92	318.74	1,135.18	3,386.09
9	2	P	37.36	330.19	1,009.80	3,583.00
10	2	C	38.16	305.11	1,009.80	3,557.71
11	2	S&P	37.92	331.81	1,004.51	3,173.40
12	2	S	39.28	311.45	920.92	3,178.40
13	2	C	38.24	325.28	1,000.94	3,265.60
14	2	S	37.44	327.18	904.56	3,409.20
15	2	P	38.00	257.56	888.44	3,030.00
16	2	S&P	37.12	315.51	998.52	3,683.13

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

<sup>2</sup> Phase 1 was day 0, Phase 2 was day 10, Phase 3 was day 24, Phase 4 was day 53 and 54.

**Table 2.3.** Average body weight (BW, g) of pens (N = 16 pens) of broilers subjected to environmental enrichment treatments (N = 4 pens/treatment) at Phases 1-4.

	<i>BW (g)</i>							
	<b>Treatment<sup>1</sup></b>				<b>SEM</b>	<b>P value</b>		
	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>		<b>P</b>	<b>S</b>	<b>S&amp;P</b>
<b>Phase 1<sup>2</sup></b>	38.28	38.44	37.88	37.48	0.42	0.79	0.13	0.52
<b>Phase 2</b>	312.99	292.71	333.16	311.19	13.28	0.14	0.17	0.95
<b>Phase 3</b>	980.38	872.27	981.43	1,010.84	41.35	0.36	0.12	0.12
<b>Phase 4</b>	3,393.51	3,257.11	3,287.50	3,700.99	190.31	0.48	0.39	0.17

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

<sup>2</sup> Phase 1 was day 0, Phase 2 was day 10, Phase 3 was day 24, Phase 4 was day 53 and 54.

**Table 2.4.** Feed conversion ratio (FCR) of each pen (N = 16 pens) of broilers subjected to environmental enrichment treatments (N = 4 pens/treatment) each pen (N = 16) of broilers at Phase 2-4 and cumulative (Total) FCR.

Pen	Room	Treatment <sup>2</sup>	FCR <sup>1</sup>			Total
			Phase 2 <sup>3</sup>	Phase 3	Phase 4	
1	1	C	1.19	1.87	1.84	1.81
2	1	S&P	1.21	1.76	1.28	1.35
3	1	P	1.12	1.80	2.83	2.44
4	1	S	2.07	1.81	1.95	1.93
5	1	S&P	1.13	2.15	1.81	1.83
6	1	P	1.38	1.61	1.96	1.85
7	1	C	1.35	2.10	1.78	1.81
8	1	S	1.25	1.99	1.80	1.80
9	2	P	1.18	1.63	1.99	1.86
10	2	C	1.05	1.69	2.03	1.89
11	2	S&P	1.22	1.67	1.92	1.83
12	2	S	1.17	1.70	1.95	1.85
13	2	C	1.18	1.58	1.96	1.82
14	2	S	1.51	1.54	1.92	1.83
15	2	P	1.34	1.51	1.97	1.84
16	2	S&P	1.29	1.58	1.72	1.67

<sup>1</sup> FCR was calculated as feed consumed divided by corrected weight gained (weight gained included gains from DOA and culled broilers).

<sup>2</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>3</sup> Phase 2 day 0-10, Phase 3 was day 11-24, Phase 4 was day 25, Phase 4 was day 25-54.

**Table 2.5.** Feed conversion ratio (FCR) of pens (N = 16 pens, 4 pens/treatment) of broilers subjected to environmental enrichment treatments at Phase 2-4 and cumulative (Total) throughout the study corrected for mortality.

	<i>FCR</i> <sup>1</sup>							
	<b>Treatment</b> <sup>2</sup>				<b>SEM</b>	<b>P-value</b>		
	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>		<b>P</b>	<b>S</b>	<b>S&amp;P</b>
Phase 2 <sup>3</sup>	1.19	1.25	1.50	1.21	0.11	0.33	0.25	0.14
Phase 3	1.81	1.64	1.76	1.79	0.10	0.50	0.61	0.34
Phase 4	1.90	2.19	1.91	1.68	0.13	0.81	0.08	0.08
Total	1.83	2.00	1.85	1.67	0.09	0.92	0.12	0.09

<sup>1</sup> FCR was calculated as feed consumed divided by corrected weight gained (weight gained included gains from DOA and culled broilers).

<sup>2</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

<sup>3</sup> Phase 2 day 0-10, Phase 3 was day 11-24, Phase 4 was day 25-54, and Total was day 0-54.

**Table 2.6.** Percentages (%) of focal broilers (N = 85 broilers, 20–22 broilers/treatment) subjected to environmental enrichment treatments on day 53 of age.

<b>Treatment<sup>2</sup></b>	<b>Plumage Condition<sup>1</sup></b>			<b>Foot Pad Dermatitis</b>			<b>Hock Burn</b>		
	<b>0</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>2</b>
C	0.0	100.0	0.0	90.9	9.1	0.0	68.2	31.8	0.0
P	0.0	100.0	0.0	90.9	9.1	0.0	59.1	36.4	4.6
S	0.0	100.0	0.0	80.0	20.0	0.0	60.0	40.0	0.0
S&P	0.0	100.0	0.0	100.0	0.0	0.0	76.2	23.8	0.0
P-value	no estimate			0.11			0.64		

<sup>1</sup> Plumage condition, foot pad dermatitis, and hock burn were all scored on a 0-2 scale, where a score of 0 represented no signs, a score of 1 represented a mild to moderate case, and a score of 2 represented severe cases.

<sup>2</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

**Table 2.7.** Average percent ash (%) of left (N= 85 tibias), right (N = 85 tibias), and average (of the left and right tibia; N = 170 tibias, 20–22 broilers/treatment) tibias ( $\pm$  SEM) of broilers subjected to environmental enrichment treatments on day 53 of age.

<b>Tibia</b>	<b>Tibia Percent Ash<sup>1</sup></b>				<b>P value</b>			
	<b>Treatment<sup>2</sup></b>	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>
Left		54.87 $\pm$ 0.68	53.53 $\pm$ 0.69	54.48 $\pm$ 0.72	53.49 $\pm$ 0.70	0.12	0.76	0.81
Right		54.13 $\pm$ 0.33	54.03 $\pm$ 0.33	53.97 $\pm$ 0.36	53.25 $\pm$ 0.34	0.25	0.19	0.38
Average		54.48 $\pm$ 0.40	53.79 $\pm$ 0.40	54.25 $\pm$ 0.42	53.35 $\pm$ 0.40	0.07	0.42	0.80

<sup>1</sup>Ash percentage was calculated as ((tibia ash weight divided by dried defatted tibia weight) \*100).

<sup>2</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).



**Table 2.8.** Average wet lab length (mm), width (mm) at 10%, 25%, 50%, 75%, and 90% length, and proximal head angle (°) morphology measures ( $\pm$  SEM) of tibias from broilers (N = 170 left and right tibias, 20–22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.

Measure (mm)	<i>Wet Lab Morphology</i>				P value		
	Treatment <sup>1</sup>				P	S	S&P
	C	P	S	S&P			
Length	114.21 $\pm$ 0.92	113.91 $\pm$ 0.93	112.78 $\pm$ 0.97	114.55 $\pm$ 0.93	0.44	0.68	0.29
Proximal Head Width	30.66 $\pm$ 0.30 <sup>b</sup>	31.16 $\pm$ 0.31 <sup>a</sup>	29.74 $\pm$ 0.32 <sup>b</sup>	30.84 $\pm$ 0.30 <sup>a</sup>	0.02	0.06	0.35
Proximal Head Angle	38.22 $\pm$ 0.55	37.43 $\pm$ 0.57	37.26 $\pm$ 0.58	37.02 $\pm$ 0.55	0.37	0.24	0.64
Distal Head Width	23.00 $\pm$ 0.35	23.08 $\pm$ 0.35	22.11 $\pm$ 0.37	22.90 $\pm$ 0.36	0.24	0.15	0.34
Widths at Percentages of Length							
90%	25.61 $\pm$ 0.41 <sup>b</sup>	27.03 $\pm$ 0.41 <sup>a</sup>	24.31 $\pm$ 0.43 <sup>c</sup>	25.74 $\pm$ 0.41 <sup>b</sup>	0.003	0.006	0.99
75%	14.49 $\pm$ 0.19 <sup>ab</sup>	14.80 $\pm$ 0.19 <sup>a</sup>	13.64 $\pm$ 0.21 <sup>b</sup>	14.46 $\pm$ 0.19 <sup>ab</sup>	0.01	0.009	0.22
50%	11.11 $\pm$ 0.18	11.18 $\pm$ 0.18	10.62 $\pm$ 0.19	11.30 $\pm$ 0.18	0.06	0.33	0.12
25%	12.40 $\pm$ 0.19 <sup>a</sup>	12.54 $\pm$ 0.19 <sup>a</sup>	11.80 $\pm$ 0.20 <sup>b</sup>	12.54 $\pm$ 0.19 <sup>a</sup>	0.04	0.14	0.14
10%	18.20 $\pm$ 0.36	18.22 $\pm$ 0.35	17.31 $\pm$ 0.37	18.05 $\pm$ 0.37	0.30	0.15	0.31
Intercondylar depth							
Medial	1.87 $\pm$ 0.08	1.82 $\pm$ 0.08	1.87 $\pm$ 0.08	1.76 $\pm$ 0.08	0.32	0.71	0.76
Lateral	2.19 $\pm$ 0.07 <sup>a</sup>	2.34 $\pm$ 0.08 <sup>a</sup>	2.12 $\pm$ 0.08 <sup>b</sup>	2.05 $\pm$ 0.07 <sup>b</sup>	0.60	0.03	0.18
Distal	6.42 $\pm$ 0.12	6.50 $\pm$ 0.12	6.11 $\pm$ 0.12	6.36 $\pm$ 0.12	0.20	0.08	0.49

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

**Table 2.9.** Average weight before drying (g) and volume (mL) ( $\pm$  SEM) of tibias from broilers (N = 170 tibias, 20–22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.

<i>Wet Lab Morphology</i>							
<b>Measure</b>	<b>Treatment<sup>1</sup></b>				<b>P value</b>		
	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>
Weight (g)	19.35 $\pm$ 0.65 <sup>b</sup>	19.93 $\pm$ 0.65 <sup>a</sup>	17.31 $\pm$ 0.68 <sup>b</sup>	19.62 $\pm$ 0.67 <sup>a</sup>	0.04	0.09	0.21
Volume (mL)	16.42 $\pm$ 0.54 <sup>b</sup>	16.59 $\pm$ 0.54 <sup>a</sup>	14.50 $\pm$ 0.56 <sup>b</sup>	16.96 $\pm$ 0.54 <sup>a</sup>	0.03	0.18	0.05

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

**Table 2.10.** Digital length (mm), width (mm) at 10%, 25%, 50%, 75%, and 90% length, and proximal head angle (°) morphology measures ( $\pm$  SEM) of the left tibias from broilers (N = 170 left and right tibias, 20–22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.

<b>Measure (mm)</b>	<b><i>Left Digital Morphology</i></b>				<b>P value</b>			
	<b>Treatment<sup>1</sup></b>	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>
Tibia Length		122.88 $\pm$ 1.57	124.16 $\pm$ 1.57	122.55 $\pm$ 1.62	125.02 $\pm$ 1.59	0.26	0.87	0.72
Proximal Head Width		30.78 $\pm$ 0.65	31.00 $\pm$ 0.65	29.89 $\pm$ 0.67	30.98 $\pm$ 0.66	0.34	0.50	0.52
Proximal Head Angle		39.69 $\pm$ 0.83	38.59 $\pm$ 0.83	39.76 $\pm$ 0.86	38.66 $\pm$ 0.84	0.21	0.94	1.00
Distal Head Width		22.18 $\pm$ 0.52	22.44 $\pm$ 1.52	21.41 $\pm$ 0.54	22.35 $\pm$ 0.53	0.28	0.42	0.53
<b>Widths at Percentages of total length</b>								
90%		24.68 $\pm$ 0.64	25.95 $\pm$ 0.64	25.95 $\pm$ 0.66	25.20 $\pm$ 0.65	0.12	0.39	0.79
75%		15.35 $\pm$ 0.20 <sup>ab</sup>	15.98 $\pm$ 0.20 <sup>a</sup>	14.78 $\pm$ 0.22 <sup>b</sup>	15.42 $\pm$ 0.21 <sup>ab</sup>	0.02	0.03	0.80
50%		12.03 $\pm$ 0.25	11.98 $\pm$ 0.25	11.39 $\pm$ 0.27	12.00 $\pm$ 0.26	0.30	0.26	0.22
25%		14.67 $\pm$ 0.74	13.70 $\pm$ 0.74	12.77 $\pm$ 0.75	13.54 $\pm$ 0.74	0.89	0.20	0.26
10%		19.44 $\pm$ 0.61	21.18 $\pm$ 0.61	19.71 $\pm$ 0.63	20.60 $\pm$ 0.62	0.06	0.81	0.50
<b>Intercondylar depth</b>								
Medial		1.94 $\pm$ 0.08	1.87 $\pm$ 0.07	1.91 $\pm$ 0.08	1.83 $\pm$ 0.08	0.35	0.69	0.90
Lateral		1.79 $\pm$ 0.08	2.02 $\pm$ 0.08	1.87 $\pm$ 0.08	1.86 $\pm$ 0.08	0.20	0.67	0.16
Distal		6.68 $\pm$ 0.19	6.82 $\pm$ 0.19	6.36 $\pm$ 0.20	6.57 $\pm$ 0.20	0.39	0.17	0.87

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

**Table 2.11.** Digital length (mm), width (mm) at 10%, 25%, 50%, 75%, and 90% length, and proximal head angle (°) morphology measures ( $\pm$  SEM) of the right tibias from broilers (N = 170 left and right tibias, 20-22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.

<b>Measure (mm)</b>	<b>Right Digital Morphology Treatment<sup>1</sup></b>				<b>P value</b>		
	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>
Tibia Length	124.32 $\pm$ 1.17	124.17 $\pm$ 1.17	122.34 $\pm$ 1.23	124.89 $\pm$ 1.19	0.33	0.61	0.28
Proximal Head Width	31.20 $\pm$ 0.49	31.29 $\pm$ 0.49	30.12 $\pm$ 0.51	30.69 $\pm$ 0.50	0.52	0.12	0.64
Proximal Head Angle	38.91 $\pm$ 1.06	38.90 $\pm$ 1.06	38.62 $\pm$ 1.09	37.37 $\pm$ 1.07	0.57	0.41	0.57
Distal Head Width	22.23 $\pm$ 0.43	22.62 $\pm$ 0.43	21.35 $\pm$ 0.44	22.40 $\pm$ 0.43	0.12	0.28	0.46
<b>Widths at Percentages of total length</b>							
90%	23.56 $\pm$ 0.61	24.98 $\pm$ 0.61	24.33 $\pm$ 0.63	24.70 $\pm$ 0.62	0.17	0.70	0.41
75%	15.34 $\pm$ 0.22 <sup>b</sup>	16.05 $\pm$ 0.22 <sup>a</sup>	14.93 $\pm$ 0.23 <sup>b</sup>	15.69 $\pm$ 0.23 <sup>a</sup>	0.006	0.11	0.91
50%	11.79 $\pm$ 0.24	11.98 $\pm$ 0.24	11.35 $\pm$ 0.25	12.01 $\pm$ 0.24	0.10	0.41	0.35
25%	13.46 $\pm$ 0.23 <sup>b</sup>	13.75 $\pm$ 0.23 <sup>a</sup>	12.68 $\pm$ 0.25 <sup>b</sup>	13.57 $\pm$ 0.24 <sup>a</sup>	0.03	0.07	0.24
10%	20.58 $\pm$ 0.45 <sup>b</sup>	21.72 $\pm$ 0.45 <sup>a</sup>	19.81 $\pm$ 0.46 <sup>b</sup>	20.87 $\pm$ 0.45 <sup>a</sup>	0.03	0.10	0.92
<b>Intercondylar depth</b>							
Medial	1.95 $\pm$ 0.07 <sup>b</sup>	1.76 $\pm$ 0.07 <sup>a</sup>	2.02 $\pm$ 0.07 <sup>b</sup>	1.83 $\pm$ 0.07 <sup>a</sup>	0.02	0.36	0.97
Lateral	1.82 $\pm$ 0.06	2.03 $\pm$ 0.06	1.85 $\pm$ 0.06	1.79 $\pm$ 0.06	0.21	0.10	0.04
Distal	6.54 $\pm$ 0.17	6.95 $\pm$ 0.17	6.34 $\pm$ 0.17	6.57 $\pm$ 0.17	0.12	0.08	0.61

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

**Table 2.12.** Average digital length (mm), width (mm) at 10%, 25%, 50%, 75%, and 90% length, and proximal head angle (°) morphology measures ( $\pm$  SEM) of tibias from broilers (N = 170 left and right tibias, 20–22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.

Measure (mm)	<i>Digital Morphology Averages</i>				P value			
	Treatment <sup>1</sup>	C	P	S	S&P	P	S	S&P
Tibia Length		124.13 $\pm$ 1.21	123.68 $\pm$ 1.22	122.32 $\pm$ 1.24	125.21 $\pm$ 1.27	0.34	0.91	0.20
Proximal Head Width		31.04 $\pm$ 0.55	31.10 $\pm$ 0.54	30.00 $\pm$ 0.55	30.87 $\pm$ 0.57	0.28	0.40	0.48
Proximal Head Angle		39.42 $\pm$ 0.89	38.64 $\pm$ 0.88	39.47 $\pm$ 0.89	37.65 $\pm$ 0.93	0.16	0.62	0.56
Distal Head Width		22.34 $\pm$ 0.45	22.40 $\pm$ 0.45	21.40 $\pm$ 0.46	22.40 $\pm$ 0.47	0.25	0.33	0.31
Widths at Percentages of total length								
90%		23.78 $\pm$ 0.58 <sup>b</sup>	25.67 $\pm$ 0.58 <sup>a</sup>	24.22 $\pm$ 0.58 <sup>b</sup>	25.08 $\pm$ 0.60 <sup>a</sup>	0.04	0.81	0.44
75%		15.36 $\pm$ 0.21 <sup>ab</sup>	15.96 $\pm$ 0.21 <sup>a</sup>	14.84 $\pm$ 0.22 <sup>b</sup>	15.60 $\pm$ 0.22 <sup>ab</sup>	0.008	0.06	0.73
50%		11.86 $\pm$ 0.23	12.03 $\pm$ 0.24	11.35 $\pm$ 0.24	12.06 $\pm$ 0.25	0.09	0.33	0.28
25%		13.52 $\pm$ 0.46	14.26 $\pm$ 0.46	12.70 $\pm$ 0.47	13.63 $\pm$ 0.48	0.09	0.16	0.85
10%		20.46 $\pm$ 0.48	21.01 $\pm$ 0.48	19.80 $\pm$ 0.48	20.75 $\pm$ 0.50	0.14	0.37	0.69
Intercondylar depth								
Medial		1.95 $\pm$ 0.07	1.82 $\pm$ 0.06	1.94 $\pm$ 0.07	1.85 $\pm$ 0.07	0.10	0.87	0.80
Lateral		1.83 $\pm$ 0.07	1.99 $\pm$ 0.07	1.87 $\pm$ 0.07	1.82 $\pm$ 0.07	0.41	0.34	0.16
Distal		6.62 $\pm$ 0.17	6.87 $\pm$ 0.17	6.37 $\pm$ 0.18	6.57 $\pm$ 0.18	0.22	0.14	0.88

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

**Table 2.13.** Comparison of average tibia morphology (mm) and proximal head angle (°) ( $\pm$  SEM) of wet lab and digital measures of left and right tibias from broilers (N = 170 tibias, 20–22 broilers/treatment) subjected to environmental enrichment treatments on day 53 of age.

<i>Wet Lab vs Digital Methodology</i>			
<b>Measure</b>	<b>Method</b>		<b>P value</b>
	<b>Wet Lab<sup>1</sup></b>	<b>Digital<sup>2</sup></b>	
Tibia Length	113.88 $\pm$ 0.60 <sup>b</sup>	123.82 $\pm$ 0.46 <sup>a</sup>	<0.0001
Proximal Head Width	30.63 $\pm$ 0.18	30.76 $\pm$ 0.22	0.64
Proximal Head Angle	37.51 $\pm$ 0.31 <sup>b</sup>	38.80 $\pm$ 0.35 <sup>a</sup>	0.007
Distal Head Width	22.79 $\pm$ 0.16 <sup>a</sup>	22.15 $\pm$ 0.18 <sup>b</sup>	0.009
<b>Widths at Percentages of length (mm)</b>			
90%	25.69 $\pm$ 0.21 <sup>a</sup>	24.71 $\pm$ 0.24 <sup>b</sup>	0.003
75%	14.37 $\pm$ 0.13 <sup>b</sup>	15.45 $\pm$ 0.14 <sup>a</sup>	<0.0001
50%	11.06 $\pm$ 0.10 <sup>b</sup>	11.82 $\pm$ 0.12 <sup>a</sup>	<0.0001
25%	12.33 $\pm$ 0.11 <sup>b</sup>	13.35 $\pm$ 0.12 <sup>a</sup>	<0.0001
10%	17.97 $\pm$ 0.14 <sup>b</sup>	20.51 $\pm$ 0.20 <sup>a</sup>	<0.0001
<b>Intercondylar depth (mm)</b>			
Medial	1.83 $\pm$ 0.03	1.89 $\pm$ 0.03	0.23
Lateral	2.18 $\pm$ 0.04 <sup>a</sup>	1.89 $\pm$ 0.04 <sup>b</sup>	<0.0001
Distal	6.35 $\pm$ 0.06 <sup>b</sup>	6.61 $\pm$ 0.08 <sup>a</sup>	0.007

<sup>1</sup> Wet lab method was performed using digital calipers on postmortem tibias of focal broilers.

<sup>2</sup> Digital method was performed using ImageJ software on 2D images of the postmortem tibias of focal broilers.

**Table 2.14.** Cortical bone thickness (mm) and surface area (mm<sup>2</sup>) ( $\pm$  SEM) at 50% length (mid diaphysis) from digital images of the left (N = 82, 20–22 broilers/treatment) and right (N = 83, 19–22 broilers/treatment) tibias from broilers on day 53 of age that were subjected to environmental enrichment treatments.

		<i>50% Location Cortical Bone thickness and surface area</i>				<i>Treatment<sup>1</sup></i>		
<b>Side</b>	<b>Measure</b>	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>	<b>P value</b>		
						<b>P</b>	<b>S</b>	<b>S&amp;P</b>
Left	North (posterior) <sup>2</sup>	0.98 $\pm$ 0.06	0.97 $\pm$ 0.06	1.02 $\pm$ 0.06	0.98 $\pm$ 0.07	0.70	0.77	0.86
	East (lateral)	0.97 $\pm$ 0.12	1.08 $\pm$ 0.12	1.04 $\pm$ 0.12	1.07 $\pm$ 0.12	0.55	0.81	0.77
	South (anterior)	1.43 $\pm$ 0.10	1.43 $\pm$ 0.10	1.45 $\pm$ 0.10	1.35 $\pm$ 0.10	0.64	0.77	0.62
	West (medial)	1.63 $\pm$ 0.10	1.51 $\pm$ 0.10	1.57 $\pm$ 0.10	1.54 $\pm$ 0.10	0.45	0.87	0.66
	Surface Area	35.07 $\pm$ 1.19	36.20 $\pm$ 1.11	34.46 $\pm$ 1.19	35.57 $\pm$ 1.18	0.60	0.35	1.00
Right	North (posterior)	0.99 $\pm$ 0.06	1.10 $\pm$ 0.06	0.97 $\pm$ 0.07	0.90 $\pm$ 0.06	0.78	0.12	0.17
	East (medial)	1.67 $\pm$ 0.12	1.60 $\pm$ 0.12	1.59 $\pm$ 0.12	1.49 $\pm$ 0.12	0.49	0.46	0.88
	South (anterior)	1.22 $\pm$ 0.09	1.36 $\pm$ 0.09	1.34 $\pm$ 0.09	1.19 $\pm$ 0.10	0.92	0.80	0.15
	West (lateral)	0.90 $\pm$ 0.10	0.99 $\pm$ 0.10	1.02 $\pm$ 0.10	0.94 $\pm$ 0.10	0.95	0.68	0.42
	Surface Area	33.18 $\pm$ 1.15	36.25 $\pm$ 1.19	32.58 $\pm$ 1.29	33.47 $\pm$ 1.19	0.13	0.19	0.39

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>2</sup> North location represents the posterior side, South represents the anterior side, and the East and West locations represent the medial and lateral sides of the tibia. The left tibia medial side was the West location, while the right tibia medial side was the East location.

**Table 2.15.** Average cortical bone thickness (mm) and surface area (mm<sup>2</sup>) ( $\pm$  SEM) at 50% length (mid diaphysis) from digital images of left and right tibias that were averaged (N = 165 tibias, 19–22 broilers/treatment) from broilers on day 53 of age that were subjected to environmental enrichment treatments.

<b>Measure</b>	<b>50% Location Average Cortical Bone Morphology</b>				<b>P value</b>		
	<b>Treatment<sup>1</sup></b>				<b>P</b>	<b>S</b>	<b>S&amp;P</b>
	<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>			
North (posterior)	0.99 $\pm$ 0.06	1.03 $\pm$ 0.06	0.98 $\pm$ 0.06	0.94 $\pm$ 0.06	0.97	0.36	0.53
East <sup>2</sup>	0.90 $\pm$ 0.10	1.07 $\pm$ 0.10	1.03 $\pm$ 0.10	1.03 $\pm$ 0.10	0.42	0.66	0.41
South (anterior) <sup>2</sup>	1.35 $\pm$ 0.10	1.39 $\pm$ 0.10	1.43 $\pm$ 0.10	1.28 $\pm$ 0.10	0.62	0.85	0.34
West	1.65 $\pm$ 0.10	1.54 $\pm$ 0.09	1.55 $\pm$ 0.10	1.57 $\pm$ 0.10	0.42	0.66	0.41
Surface Area	34.47 $\pm$ 1.09	36.46 $\pm$ 1.05	33.69 $\pm$ 1.09	35.79 $\pm$ 1.10	0.18	0.28	0.70

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

<sup>2</sup> The left tibia medial side was the West location, while the right tibia medial side was the East location and was taken into consideration for the average calculation.



**Table 2.16.** Cortical bone thickness (mm) and surface area (mm<sup>2</sup>) ( $\pm$  SEM) at 75% length from digital images of the left (N = 83, 20-22 broilers/treatment) and right (N = 84, 20–22 broilers/treatment) tibias from broilers on day 53 of age that were subjected to environmental enrichment treatments.

		<i>75% Location Cortical Bone thickness and surface area</i>				<b>P value</b>		
<b>Side</b>	<b>Measure</b>	<b>Treatment<sup>1</sup></b>				<b>P</b>	<b>S</b>	<b>S&amp;P</b>
		<b>C</b>	<b>P</b>	<b>S</b>	<b>S&amp;P</b>			
Left	North (posterior) <sup>2</sup>	0.68 $\pm$ 0.07	0.70 $\pm$ 0.06	0.74 $\pm$ 0.06	0.63 $\pm$ 0.07	0.56	0.97	0.39
	East (lateral)	0.62 $\pm$ 0.09	0.91 $\pm$ 0.09	0.73 $\pm$ 0.09	0.65 $\pm$ 0.09	0.28	0.41	0.06
	South (anterior)	1.25 $\pm$ 0.13	1.37 $\pm$ 0.13	1.60 $\pm$ 0.13	1.25 $\pm$ 0.13	0.39	0.39	0.08
	West (medial)	0.78 $\pm$ 0.09	0.79 $\pm$ 0.09	0.74 $\pm$ 0.10	0.68 $\pm$ 0.10	0.42	0.80	0.72
	Surface Area	31.87 $\pm$ 1.67	36.60 $\pm$ 1.62	34.51 $\pm$ 1.72	33.05 $\pm$ 1.72	0.35	0.79	0.09
Right	North (posterior)	0.61 $\pm$ 0.09	0.72 $\pm$ 0.09	0.72 $\pm$ 0.09	0.65 $\pm$ 0.09	0.83	0.82	0.36
	East (medial)	0.73 $\pm$ 0.07	0.62 $\pm$ 0.07	0.73 $\pm$ 0.07	0.65 $\pm$ 0.07	0.22	0.87	0.84
	South (anterior)	1.14 $\pm$ 0.05 <sup>b</sup>	1.49 $\pm$ 0.06 <sup>a</sup>	1.16 $\pm$ 0.06 <sup>b</sup>	1.33 $\pm$ 0.06 <sup>a</sup>	0.002	0.24	0.16
	West (lateral)	0.66 $\pm$ 0.11	0.75 $\pm$ 0.11	0.68 $\pm$ 0.11	0.70 $\pm$ 0.11	0.63	0.90	0.77
	Surface Area	30.39 $\pm$ 1.62	35.30 $\pm$ 1.66	30.55 $\pm$ 1.70	31.84 $\pm$ 1.66	0.08	0.34	0.29

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (**C**, no enrichments), a platform (**P**), a spotlight (**S**), and a spotlight and a platform (**S&P**).

<sup>2</sup> North location represents the posterior side, South represents the anterior side, and the East and West locations represent the medial and lateral sides of the tibia. The left tibia medial side was the West location, while the right tibia medial side was the East location.

**Table 2.17.** Average cortical bone thickness (mm) and surface area (mm<sup>2</sup>) ( $\pm$  SEM) at 75% length from digital images of the left and right tibias that were averaged (N = 166 tibias, 19-22 broilers/treatment) from broilers on day 53 of age subjected to environmental enrichment treatments.

Measure	Treatment <sup>1</sup>				P-value		
	C	P	S	S&P	P	S	S&P
North (posterior)	0.65 $\pm$ 0.06	0.71 $\pm$ 0.06	0.73 $\pm$ 0.06	0.65 $\pm$ 0.06	0.89	0.91	0.27
East <sup>2</sup>	0.68 $\pm$ 0.08	0.70 $\pm$ 0.08	0.70 $\pm$ 0.08	0.77 $\pm$ 0.08	0.61	0.64	0.75
South (anterior)	1.21 $\pm$ 0.08	1.46 $\pm$ 0.08	1.37 $\pm$ 0.08	1.30 $\pm$ 0.08	0.27	0.95	0.07
West	0.78 $\pm$ 0.06	0.67 $\pm$ 0.06	0.76 $\pm$ 0.06	0.67 $\pm$ 0.06	0.12	0.94	0.81
Surface Area	31.39 $\pm$ 1.46	36.23 $\pm$ 1.46	32.56 $\pm$ 1.50	33.67 $\pm$ 1.49	0.12	0.43	0.13

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>2</sup> The left tibia medial side was the West location, while the right tibia medial side was the East location and was taken into consideration for the average calculation.

**Table 2.18.** Average percentage (%) ( $\pm$  SEM) of broilers in Zones 1-5 throughout the pen (N = 16 pens, 4 pens/treatment) during the avoidance distance (AD) test at 3 weeks of age.

Zone <sup>2</sup>	Average percentage of broilers in each Zone location at week 3				P-value		
	Treatment <sup>1</sup> C	P	S	S&P	P	S	S&P
1	2.65 $\pm$ 0.78 <sup>ab</sup>	0.50 $\pm$ 0.50 <sup>b</sup>	4.19 $\pm$ 1.16 <sup>a</sup>	0.60 $\pm$ 0.60 <sup>b</sup>	0.02	0.47	0.52
2	24.83 $\pm$ 3.34	12.68 $\pm$ 1.37	10.52 $\pm$ 4.27	11.07 $\pm$ 2.54	0.23	0.10	0.19
3	31.9 $\pm$ 2.91	36.84 $\pm$ 3.37	38.5 $\pm$ 2.96	32.15 $\pm$ 3.75	0.88	0.83	0.23
4	26.37 $\pm$ 1.91	25.82 $\pm$ 3.73	22.65 $\pm$ 3.64	30.83 $\pm$ 4.19	0.41	0.89	0.35
5	14.25 $\pm$ 4.00	24.16 $\pm$ 3.18	24.14 $\pm$ 4.56	25.35 $\pm$ 2.97	0.33	0.34	0.45

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>2</sup> Zones 1, 2, 3, and 4 were 0.5 m, 1 m, 1.5 m, and 2 m circles drawn from just in front of the midline of the observer, respectively, and zone 5 was the remaining area in the pen.

**Table 2.19.** Average percentage (%) ( $\pm$  SEM) of broilers in Zones 1-5 throughout the pen (N = 16 pens, 4 pens/treatment) during the avoidance distance (AD) test at 5 weeks of age.

Zone <sup>2</sup>	Average percentage of broilers in each zone location at week 5				P-value		
	Treatment <sup>1</sup> C	P	S	S&P	P	S	S&P
1	9.52 $\pm$ 3.23	1.09 $\pm$ 0.71	11.64 $\pm$ 4.18	3.90 $\pm$ 1.27	0.07	0.56	0.93
2	27.12 $\pm$ 2.00	19.86 $\pm$ 1.94	20.84 $\pm$ 4.21	24.72 $\pm$ 2.58	0.68	0.86	0.19
3	30.44 $\pm$ 2.23 <sup>ab</sup>	42.12 $\pm$ 3.44 <sup>a</sup>	28.23 $\pm$ 1.91 <sup>b</sup>	35.09 $\pm$ 3.37 <sup>ab</sup>	0.04	0.26	0.55
4	21.16 $\pm$ 1.46	23.78 $\pm$ 3.99	27.13 $\pm$ 3.45	25.31 $\pm$ 1.86	0.93	0.39	0.60
5	11.78 $\pm$ 3.29	13.15 $\pm$ 2.29	12.17 $\pm$ 5.36	10.98 $\pm$ 2.54	0.99	0.87	0.82

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>2</sup> Zones 1, 2, 3, and 4 were 0.5 m, 1.0 m, 1.5 m, and 2.0 m circles drawn from the front of the midline of the observer, respectively, and Zone 5 was the remaining area in the pen.

**Table 2.20.** Contrast comparisons of the difference in the percentage (%) ( $\pm$  SEM) of broilers in Zones 1-5 throughout the pen (N = 16, 4 pens/treatment) during the avoidance distance (AD) test at 3 and 5 weeks of age.

<i>Contrast comparing weeks 3 and 5 of age</i>										
	<b>Zone<sup>1</sup></b>					<b>P-value</b>				
	<b>1<sup>3</sup></b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
C <sup>2</sup>	-6.86 $\pm$ 3.80	-2.29 $\pm$ 3.80	1.47 $\pm$ 3.80	5.21 $\pm$ 3.80	2.47 $\pm$ 3.80	0.08	0.55	0.70	0.17	0.52
P	-0.59 $\pm$ 3.87	-7.19 $\pm$ 3.87	-5.28 $\pm$ 3.87	2.05 $\pm$ 3.87	11.00 $\pm$ 3.87*	0.88	0.07	0.18	0.60	0.01
S	-7.44 $\pm$ 5.32	-10.31 $\pm$ 5.32	10.27 $\pm$ 5.32	-4.49 $\pm$ 5.32	11.97 $\pm$ 5.32*	0.17	0.06	0.06	0.40	0.03
S&P	-3.30 $\pm$ 3.92	-13.64 $\pm$ 3.92	-2.94 $\pm$ 3.92	5.52 $\pm$ 3.92	14.37 $\pm$ 3.92*					

<sup>1</sup>Zones 1, 2, 3, and 4 were 0.5 m, 1.0 m, 1.5 m, and 2.0 m circles drawn from in front of the midline of the observer, respectively, and Zone 5 was the remaining area in the pen.

<sup>2</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>3</sup> Negative contrasts indicate that there were a greater percentage (%) of the broilers in each zone at the older age (5 weeks).

**Table 2.21.** Latencies (s) to approach within 25 cm and 50 cm and of the first peck, numbers of broilers, pecks, and broilers that pecked, and duration (s) of the time spent pecking the novel object of broilers in pens (N = 16 pens, 4 pens/treatment) during the novel object (NO) test at 3 weeks of age.

<i>Latency and numbers of pecks in pens of broilers at week 3</i>									
Pen	Room	Week	Treatment <sup>1</sup>	Latency to approach 25 cm (sec) <sup>2</sup>	Latency to approach 50 cm (sec)	Number of broilers to peck NO	Number of pecks	Duration of time pecking (sec)	Latency of first peck (sec)
1	1	3	C	88	0	0	0	0	240
2	1	3	S&P	0	0	0	0	0	240
3	1	3	P	240	57	0	0	0	240
4	1	3	S	7	0	0	0	0	240
5	1	3	S&P	0	0	0	0	0	240
6	1	3	P	2	0	0	0	0	240
7	1	3	C	0	0	0	0	0	240
8	1	3	S	0	0	0	0	0	240
9	2	3	P	0	0	0	0	0	240
10	2	3	C	0	0	0	0	0	240
11	2	3	S&P	2	0	0	0	0	240
12	2	3	S	0	0	0	0	0	240
13	2	3	C	0	0	0	0	0	240
14	2	3	S	6	0	0	0	0	240
15	2	3	P	12	0	0	0	0	240
16	2	3	S&P	0	0	3	21	123	2

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>2</sup> Pens where broilers were already within 25 cm (Zone 1) or 50 cm (Zone 2) of the NO at the start of the test the latencies were recorded as 0 seconds. Pens where no broilers approached the NO the latencies were recorded at 240 seconds.

**Table 2.22.** Latencies (s) to approach within 25 cm and 50 cm and of the first peck, numbers of broilers, pecks, and broilers that pecked, and duration (s) of the time spent pecking the novel object of broilers in pens (N = 16 pens, 4 pens/treatment) during the novel object (NO) test at 5 weeks of age.

<i>Latency and numbers of pecks in pens of broilers at week 5</i>										
Pen	Room	Week	Treatment <sup>1</sup>	Latency to approach 25 cm (sec) <sup>2</sup>	Latency to approach 50 cm (sec)	Number of broilers to peck NO	Number of pecks	Duration of time pecking (sec)	Latency of first peck (sec) <sup>3</sup>	
1	1	5	C	0	0	1	2	21	13	
2	1	5	S&P	0	0	1	12	33	7	
3	1	5	P	0	0	4	31	270	21	
4	1	5	S	16	0	1	2	6	166	
5	1	5	S&P	0	0	5	30	131	20	
6	1	5	P	6	0	1	1	2	87	
7	1	5	C	0	0	4	21	130	8	
8	1	5	S	0	0	6	65	407	15	
9	2	5	P	1	0	0	0	0	240	
10	2	5	C	0	0	2	2	3	28	
11	2	5	S&P	0	0	1	1	6	169	
12	2	5	S	0	1	4	6	38	8	
13	2	5	C	0	0	2	6	69	29	
14	2	5	S	0	0	0	0	0	240	
15	2	5	P	0	0	3	7	32	102	
16	2	5	S&P	0	0	8	84	552	13	

<sup>1</sup> Each pen of 25 broilers was randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).

<sup>2</sup> Pens where broilers were already within 25 cm (Zone 1) or 50 cm (Zone 2) of the NO at the start of the test the latency to approach was recorded as 0 seconds.

<sup>3</sup> Pens where no broilers approached the NO the latency was recorded at 240 seconds.

**Table 2.23.** Average (%) ( $\pm$  SEM) of broilers in the pen (N = 16, 4 pens/treatment) subjected to environmental enrichment treatments in zones 1-3 throughout the duration of the novel object (NO) test at weeks 3 and 5 of age.

<i>Comparison of mean percentage of broilers in all zones</i>			
Zone <sup>1</sup>	Week		P value
	3	5	
<b>C</b>			
1	3.56 $\pm$ 1.03 <sup>b</sup>	9.10 $\pm$ 1.03 <sup>a</sup>	<0.0001
2	20.58 $\pm$ 1.03 <sup>a</sup>	17.57 $\pm$ 1.03 <sup>b</sup>	0.001
3	75.86 $\pm$ 2.30 <sup>a</sup>	73.33 $\pm$ 2.30 <sup>b</sup>	0.05
<b>P</b>			
1	3.18 $\pm$ 2.24 <sup>b</sup>	8.01 $\pm$ 2.24 <sup>a</sup>	<0.0001
2	11.15 $\pm$ 2.65 <sup>b</sup>	13.43 $\pm$ 2.65 <sup>a</sup>	0.002
3	85.68 $\pm$ 3.87 <sup>a</sup>	78.57 $\pm$ 3.87 <sup>b</sup>	<0.0001
<b>S</b>			
1	1.08 $\pm$ 2.07 <sup>b</sup>	9.03 $\pm$ 2.07 <sup>a</sup>	<0.0001
2	11.70 $\pm$ 1.52 <sup>b</sup>	16.90 $\pm$ 1.52 <sup>a</sup>	<0.0001
3	87.22 $\pm$ 2.32 <sup>a</sup>	74.07 $\pm$ 2.32 <sup>b</sup>	<0.0001
<b>S&amp;P</b>			
1	6.77 $\pm$ 3.34 <sup>b</sup>	11.90 $\pm$ 3.34 <sup>a</sup>	<0.0001
2	15.41 $\pm$ 1.01	13.80 $\pm$ 1.01	0.17
3	77.82 $\pm$ 3.81 <sup>a</sup>	74.29 $\pm$ 3.81 <sup>b</sup>	0.002

<sup>1</sup>Zones 1 and 2 were 25 cm and 50 cm oval areas drawn from the edges of the NO, respectively, and Zone 3 was the remaining area in the pen.

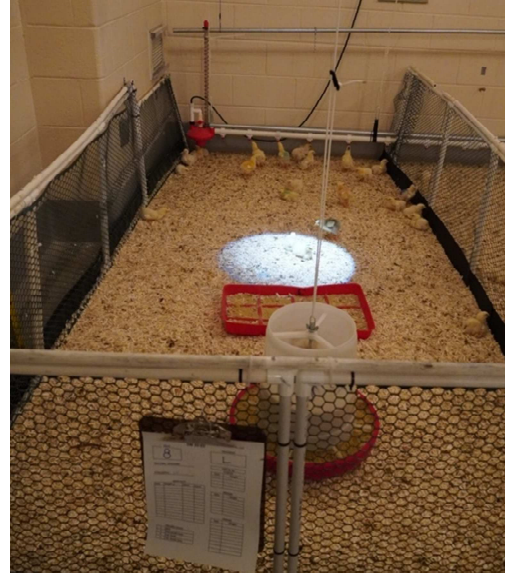


## Figures

a)



b)



c)

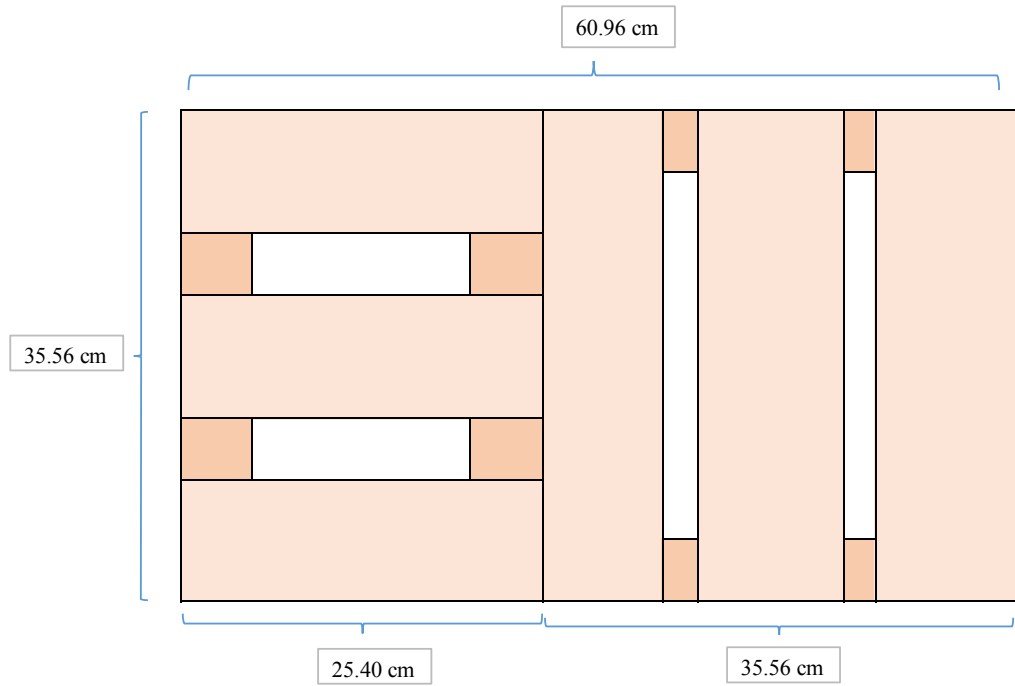


d)

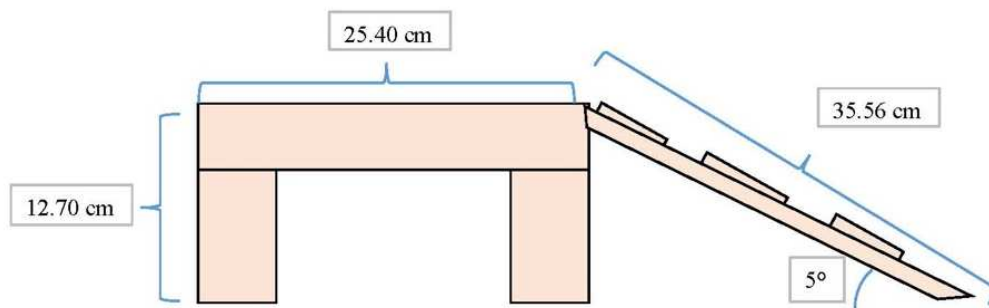


**Figure 2.1.** Photograph of the pens with four enrichment treatment groups: a) control (C), b) spotlight (S), c) platform (P) and d) spotlight and platform (S&P) that were in each pen throughout the experiment.

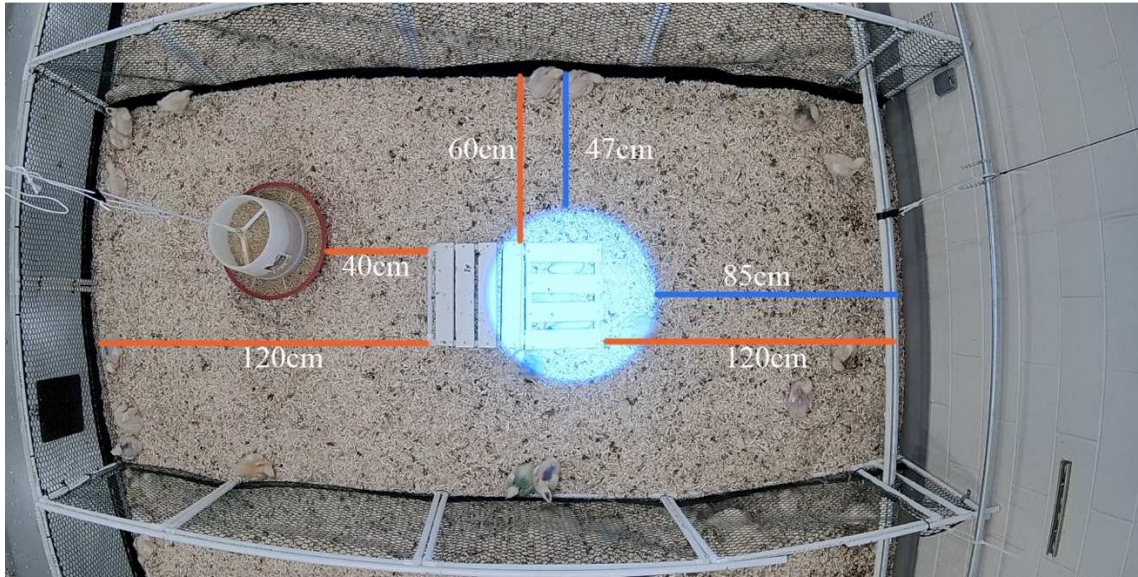
a)



b)

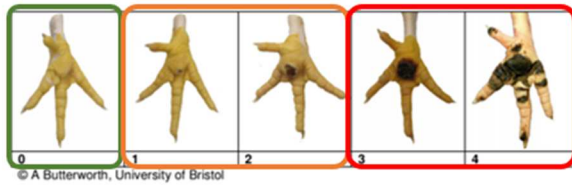


**Figure 2.2.** Platform enrichment a) top view and b) side view.



**Figure 2.3.** Schematic of the distance of the spotlight and platform (S&P) enrichments from the feeder, waterline, front, back, and sides of the pen.

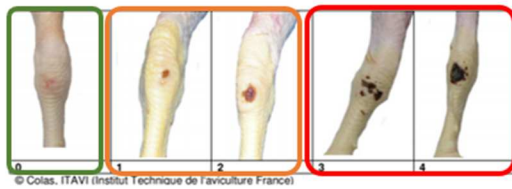
a)



**SW 20-03 Foot Pad Dermatitis Scoring**

- 0- No evidence of FPD (score 0 on the scale to the left)
- 1- Mild-moderate evidence of FPD (score 1-2 on scale to the left)
- 2- Clear case of FPD (Score 3-4 on scale)

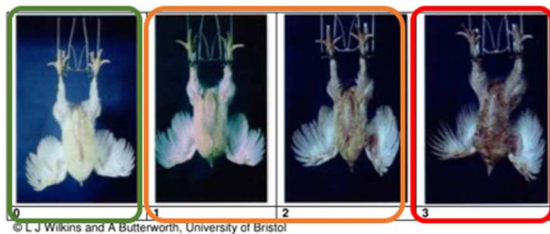
b)



**SW20-03 Hock Burn Scoring**

- 0- No hock burn (score 0 on the scale to the left)
- 1- Mild evidence of hock burn (score 1-2 listed to the left)
- 2- Clear case of hock burn (score 3-4)

c)



**SW 20-03 Plumage Condition Scoring**

- 0- Feathers are clean
- 1- Mild staining, may have some feces
- 2- Serious staining and/or feather loss, and/or very dirty plumage

**Figure 2.4.** 0-2 scales for welfare scores modified from Welfare Quality<sup>®</sup> Protocol for Poultry. a) foot pad dermatitis, b) hock burn, and c) plumage dirtiness. Green boxes indicate score 0, orange boxes score 1, and red boxes score 2.



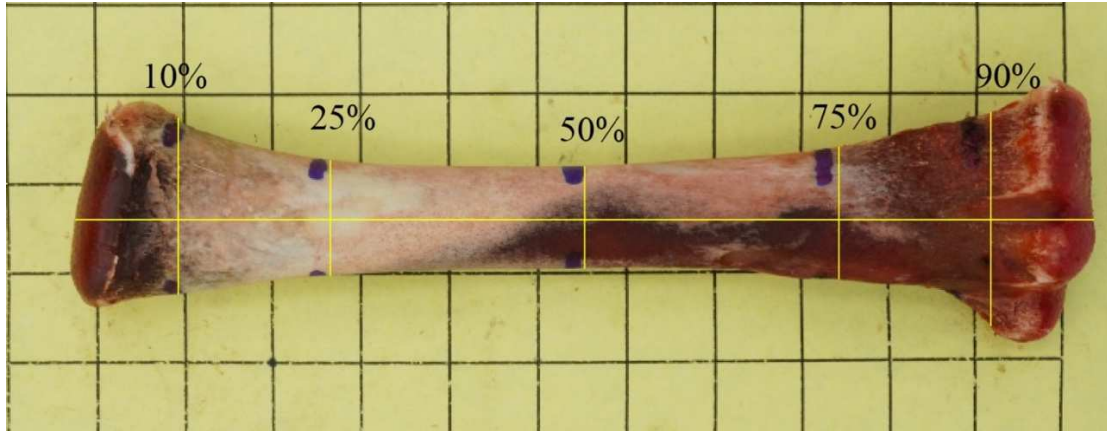


**Figure 2.5.** Plexiglas instrument used to measure the angel of the proximal tibial head.

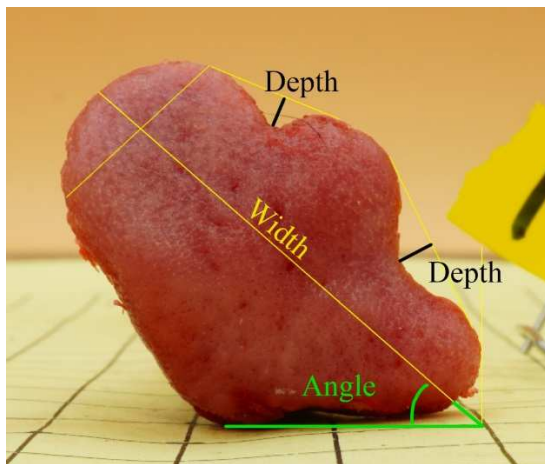


**Figure 2.6:** Digital camera set up for imaging tibias for digital morphology and cortical bone measures in the lab space.

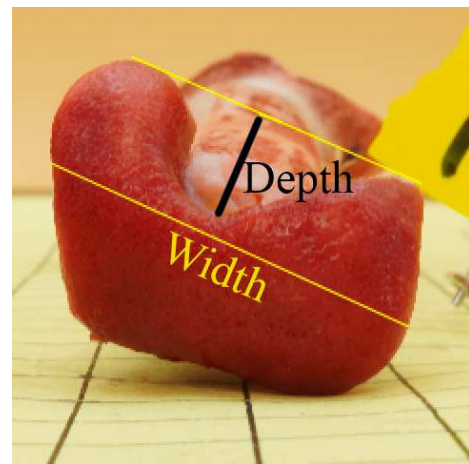
a)



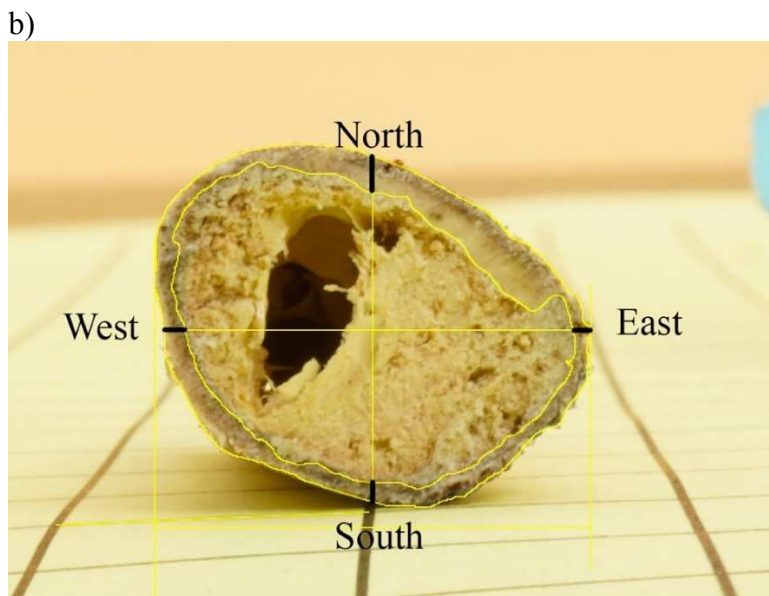
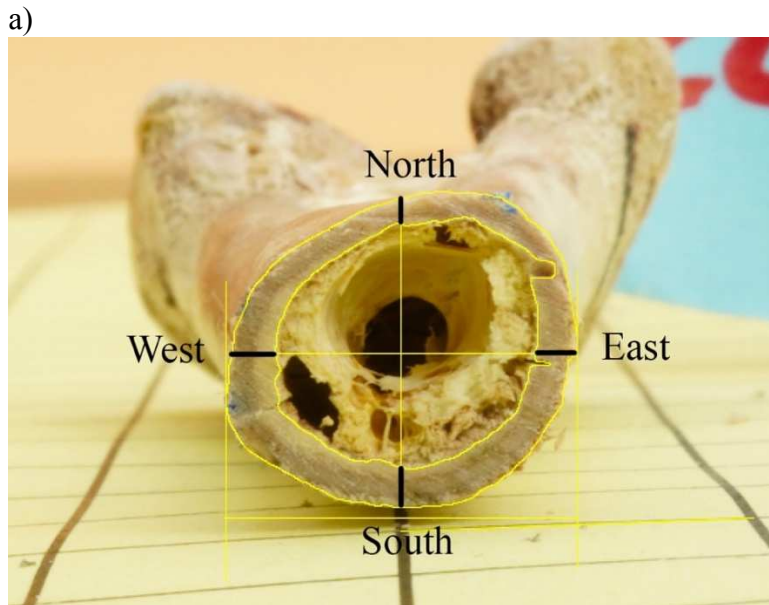
b)



c)



**Figure 2.7.** Morphology measures collected in ImageJ on tibias a) length and widths at various 10%, 25%, 50%, 75%, 90% percent of the length, b) proximal head morphology including angle, width, and intercondylar depth, c) distal head morphology including width and intercondylar depth.



**Figure 2.8.** Cortical bone surfaces at the a) 50% and b) 75% location. The north thickness represents the posterior side of the bone, while the south thickness represents the anterior side of the bone. The west location represents the medial side of left tibias and the lateral side of right tibias. The east location represents medial side of right tibias and the lateral side of left tibias.



a)

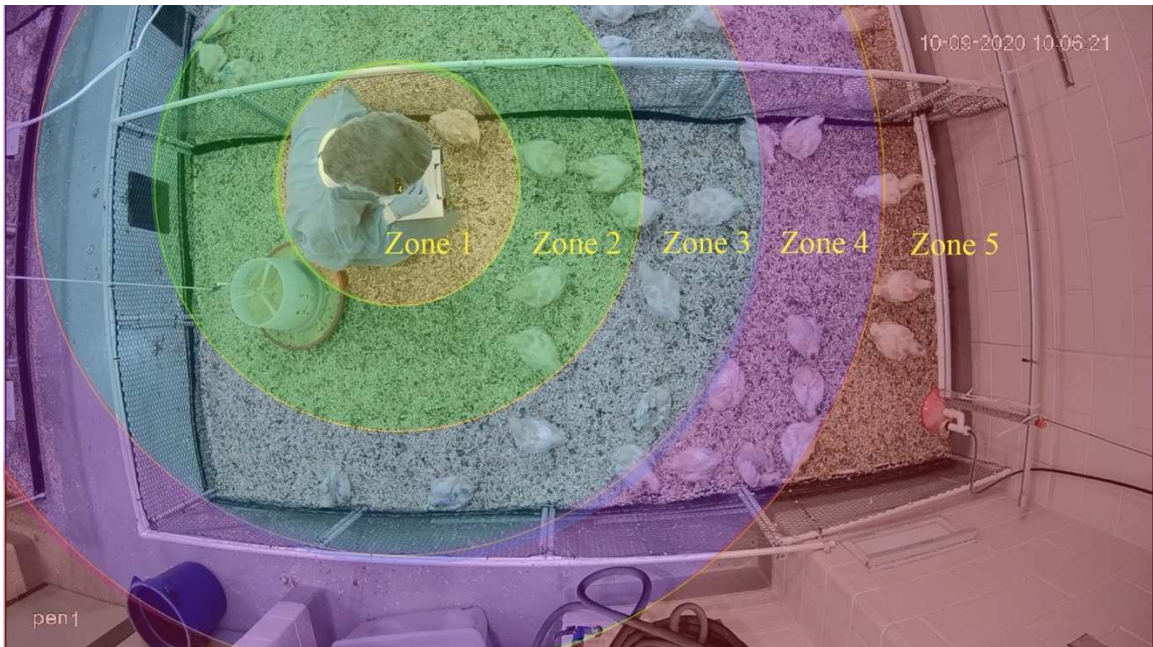


b)

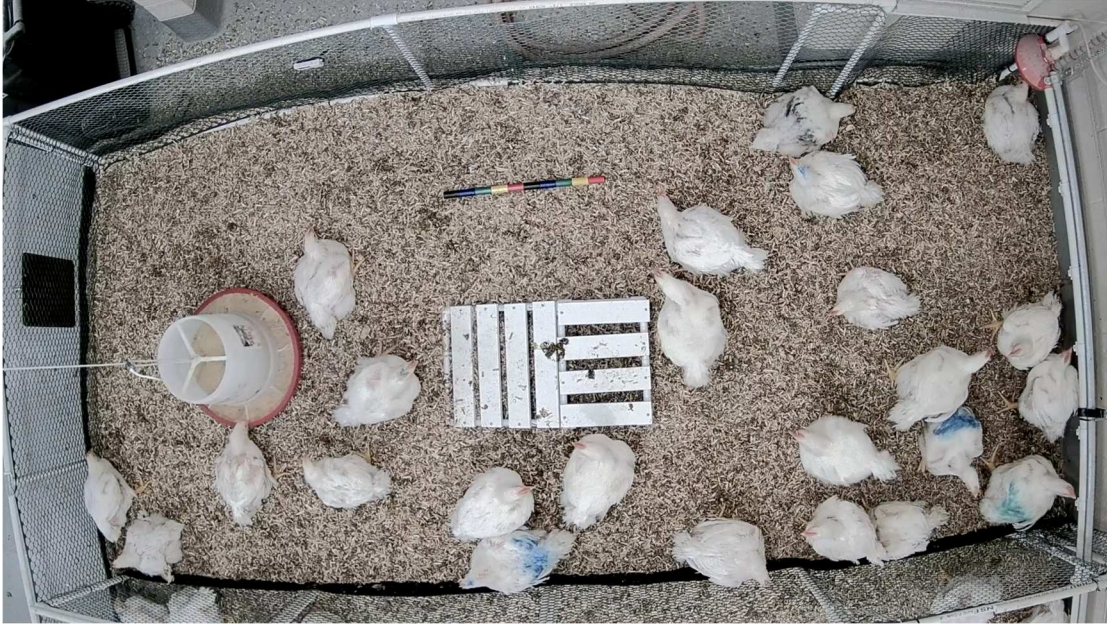


**Figure 2.9:** Observer in the pen during the avoidance distance (AD) test at 2 time points: a) prior to extension of the arm and b. after extension of the arm.

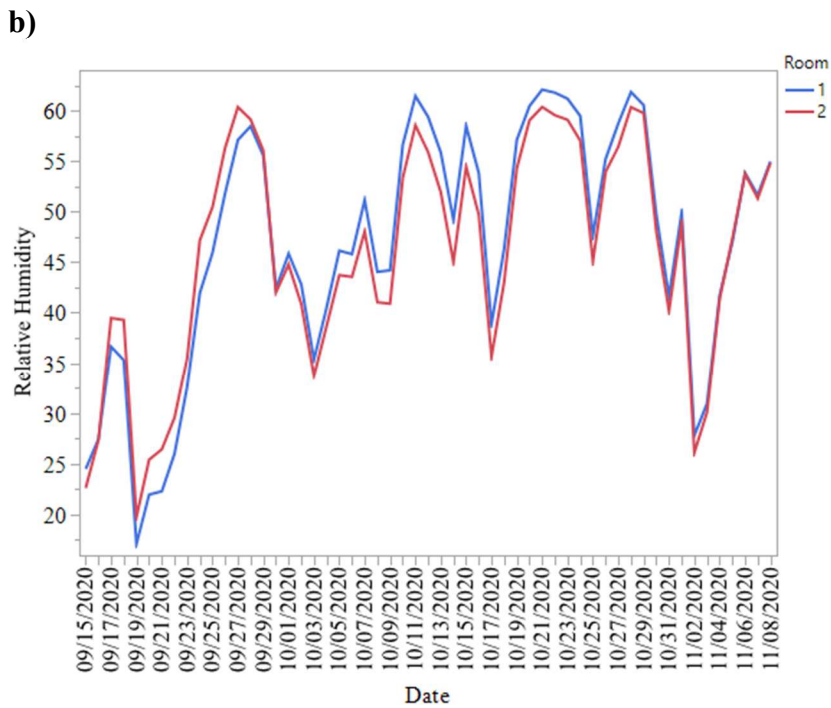
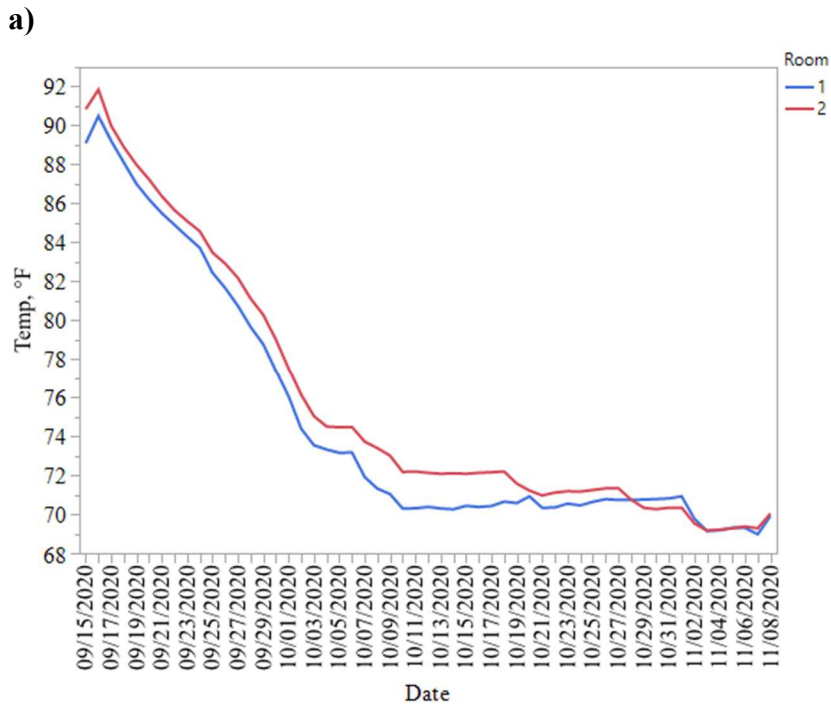




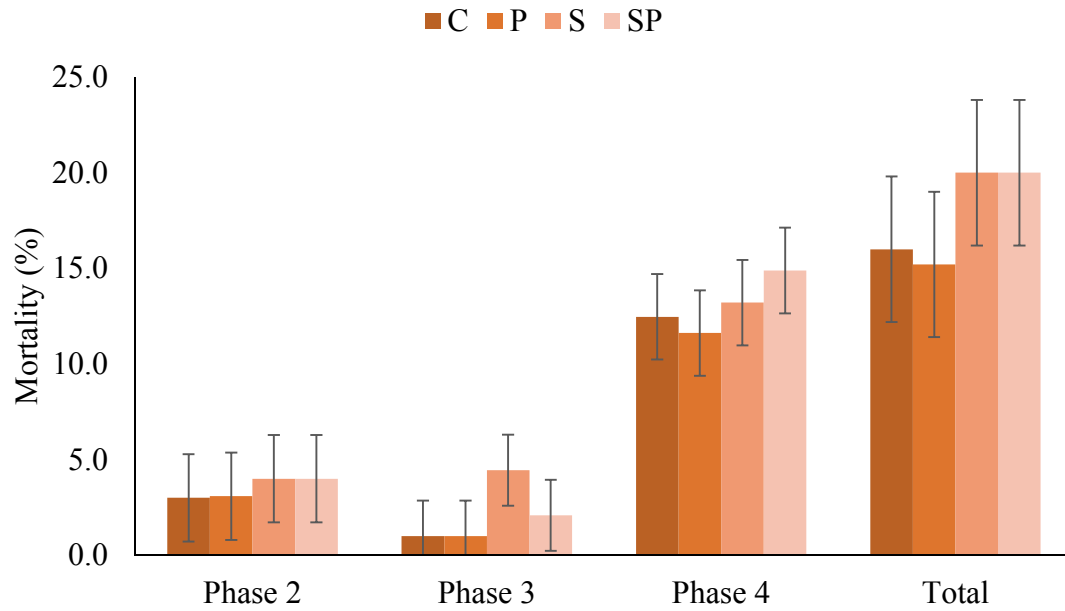
**Figure 2.10.** Zones in ImageJ during the avoidance distance (AD) test. Zones 1, 2, 3, and 4 were 0.5 m, 1 m, 1.5 m, and 2 m radius circles drawn from just in front of the midline of the observer, respectively, and zone 5 was the remaining area in the pen.



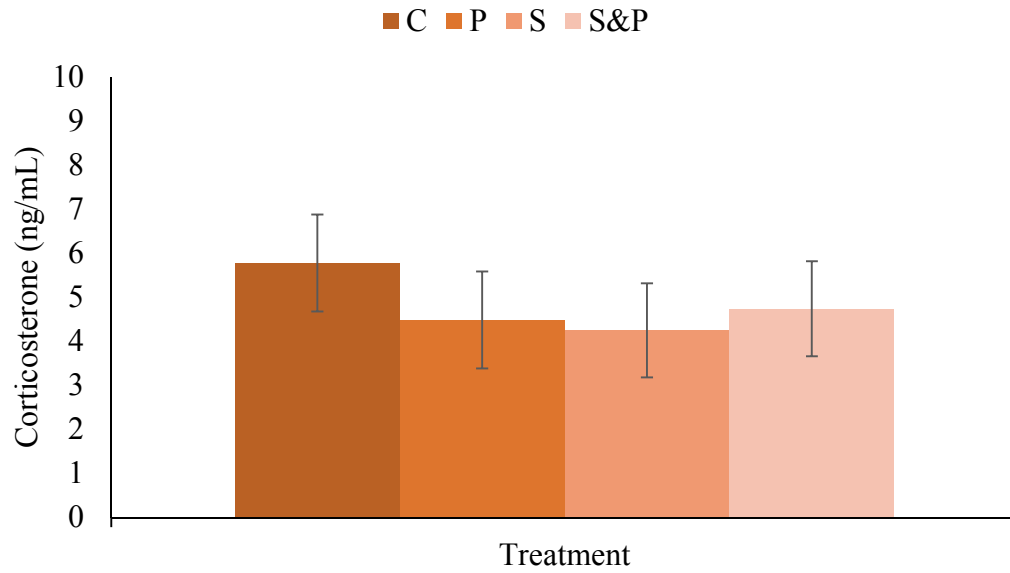
**Figure 2.11.** The novel object (NO) in the pen during the NO test.



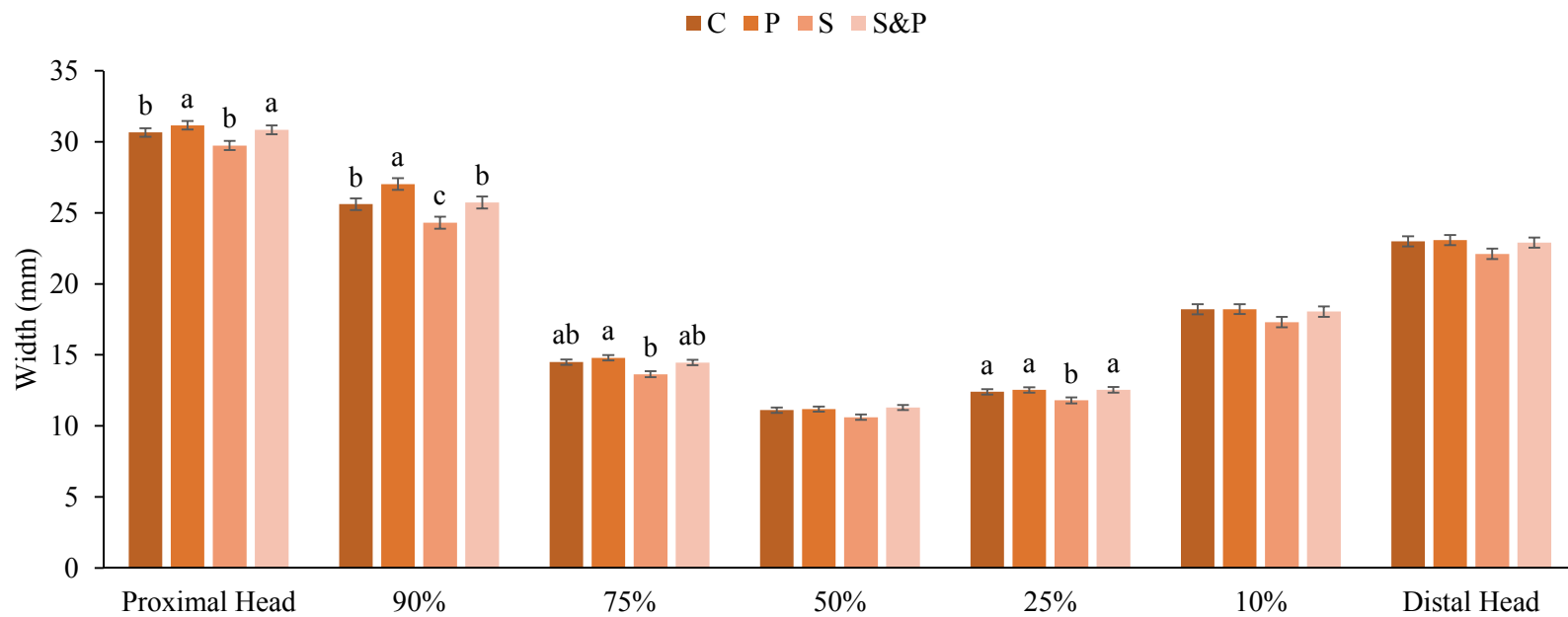
**Figure 2.12.** Ambient a) temperature (°F) and b) relative humidity (%) in Room 1 (pens 1-8) and Room 2 (pens 9-16) collected throughout the duration of the study.



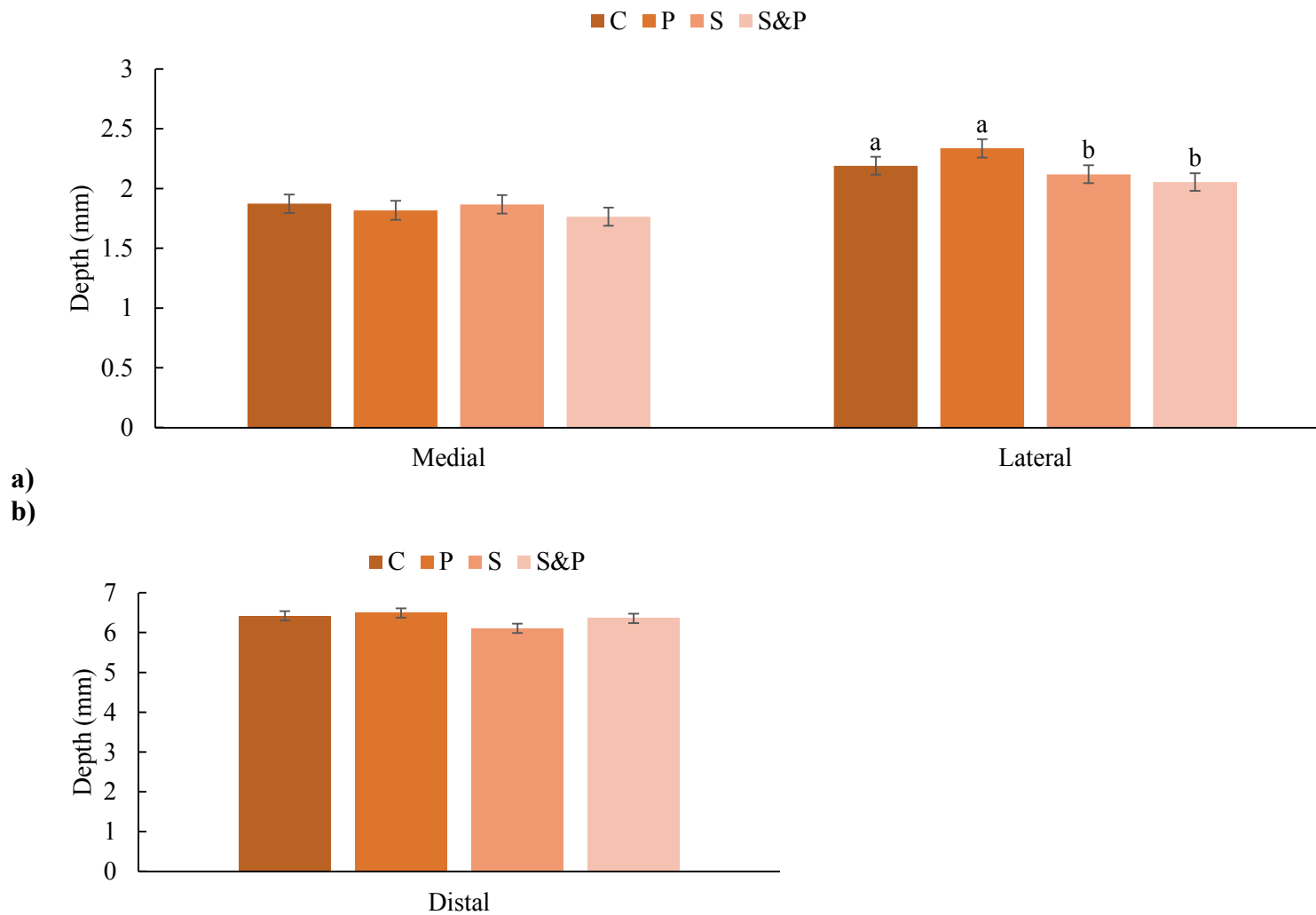
**Figure 2.13.** Mortality (%) within each phase (Phase 2-4) and cumulative (Total) mortality of pens (N = 16 pens, 4 pens/treatment) of broilers subjected to environmental enrichment treatments (N = 4 pens/treatment).



**Figure 2.14.** Average plasma corticosterone (ng/mL) concentrations focal broilers (N = 82 broilers, 19-22 broilers/treatment). Broilers were randomly assigned to 1 of 4 treatments: control (C, no enrichments), a platform (P), a spotlight (S), and a spotlight and a platform (S&P).



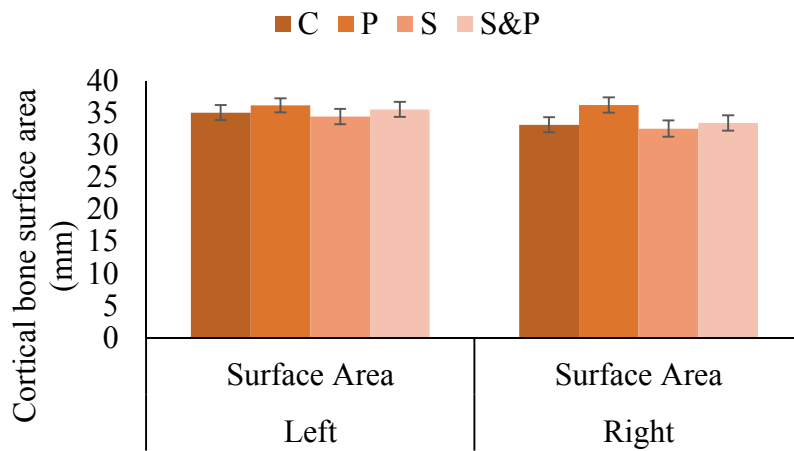
**Figure 2.15.** Average wet lab widths (mm) at 10%, 25%, 50%, 75%, and 90% of the length and the proximal and distal head of tibias from broilers (N = 170 tibias, 20-22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments (C = control, P = platform, S = spotlight, S&P = spotlight and platform).



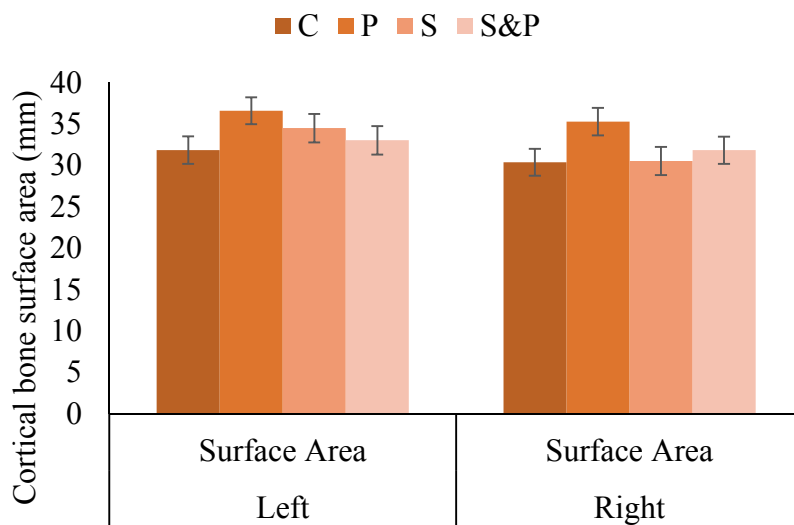
**Figure 2.16.** Average wet lab depths (mm) of the **a)** medial and distal intercondylar areas and **b)** distal intercondylar area of tibias from broilers (N = 170 tibias, 20-22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.



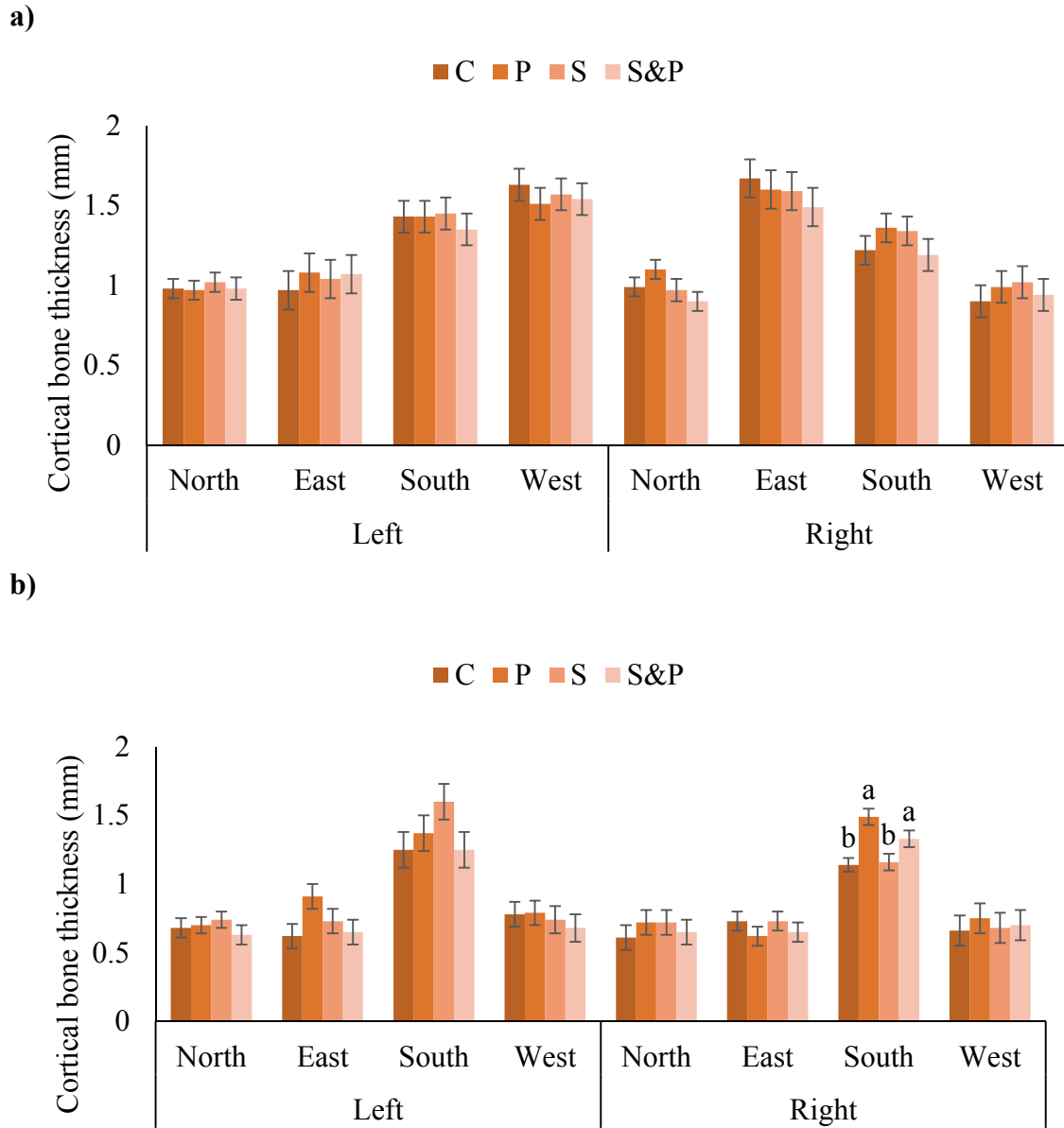
a)



b)

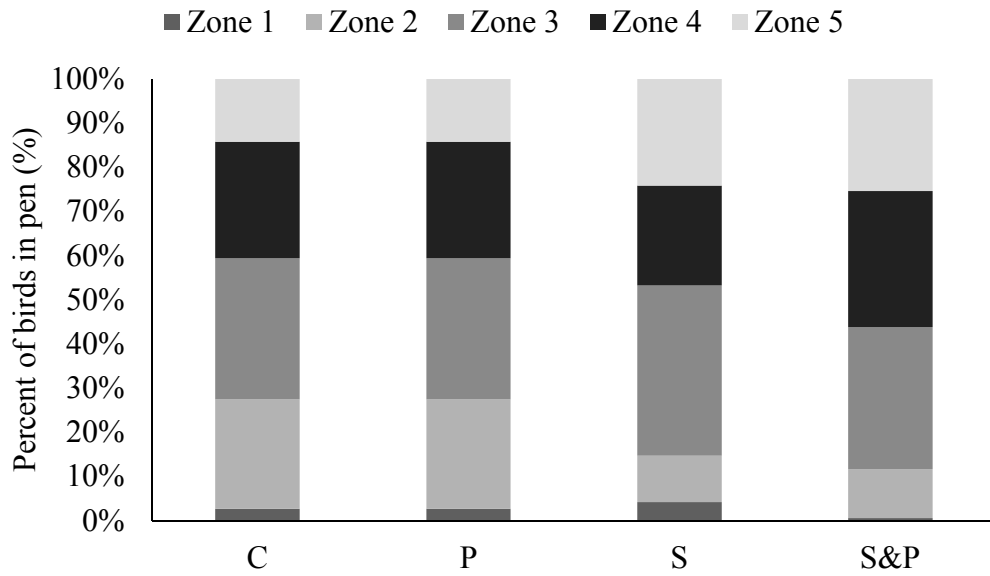


**Figure 2.17.** Cortical bone surface area ( $\text{mm}^2$ ) at the **a)** 50% length and **b)** 75% length locations from digital images of the left and right tibiae from broilers (N = 170 tibiae, 20-22 broilers/treatment) on day 53 of age that were subjected to environmental enrichment treatments.

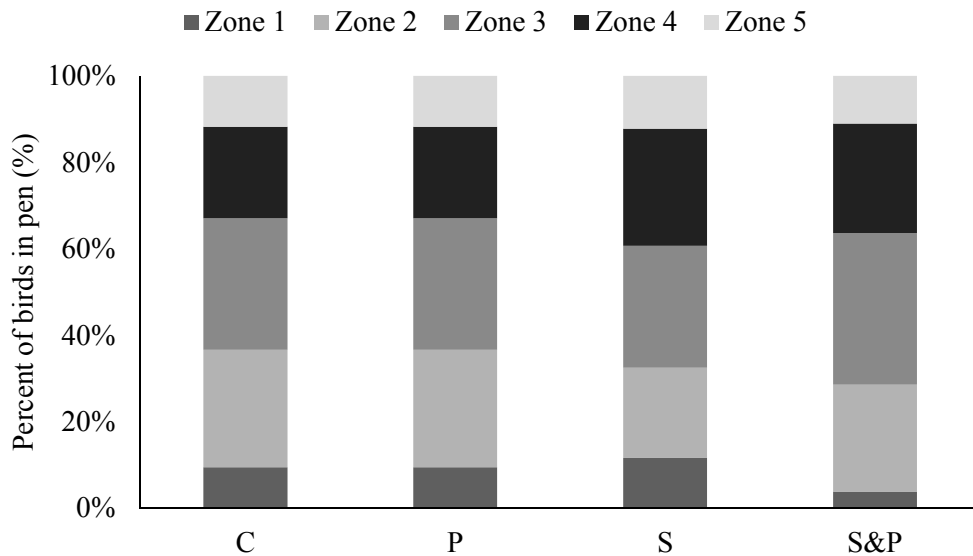


**Figure 2.18.** Cortical bone thickness (mm) at **a)** 50% length and **b)** 75% length locations from digital images of the left and right tibias from broilers (N = 170 tibias, 20-22 broilers/treatment) subjected to environmental enrichment treatments on day 53 of age.

a)

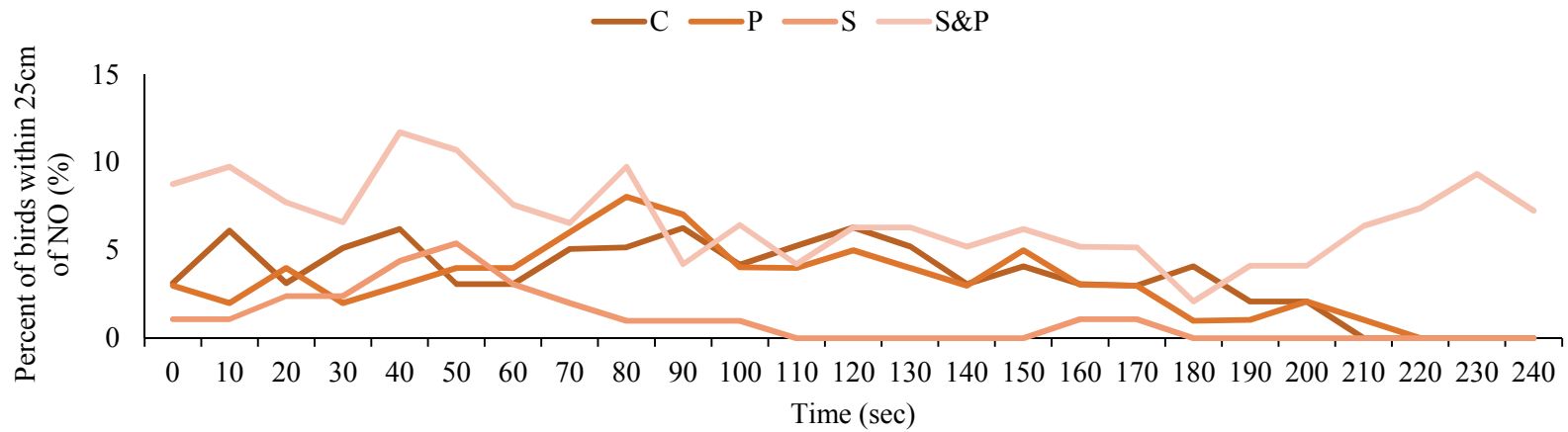


b)

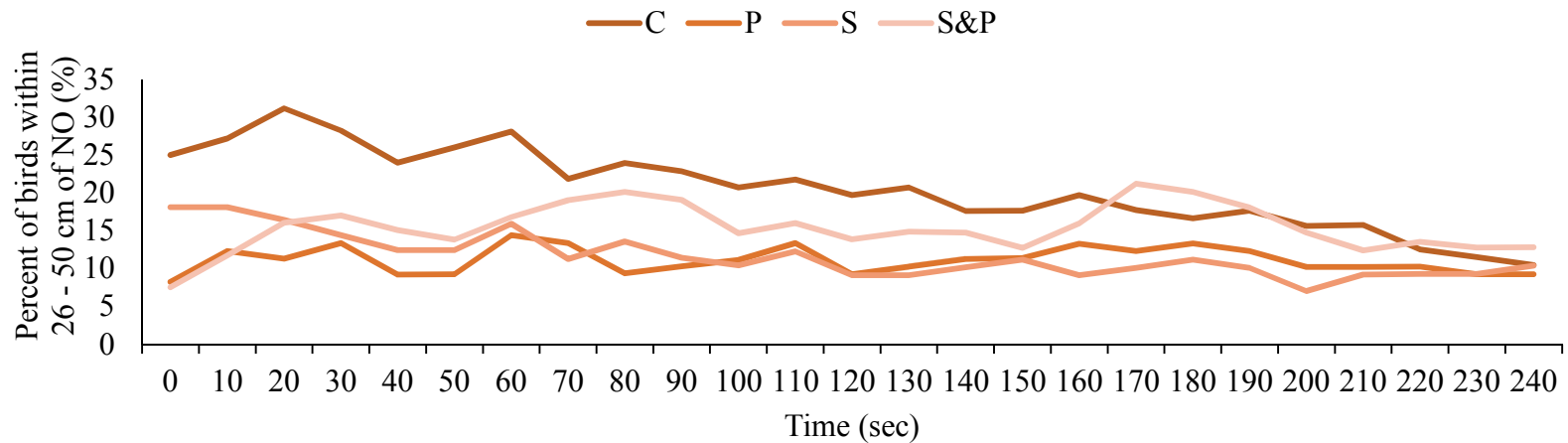


**Figure 2.19.** Average percentage (%) of broilers in Zones 1-5 throughout the pen (N = 16 pens, 4 pens/treatment) during the avoidance distance (AD) test at **a)** week 3 of age and **b)** week 5 of age.

a)

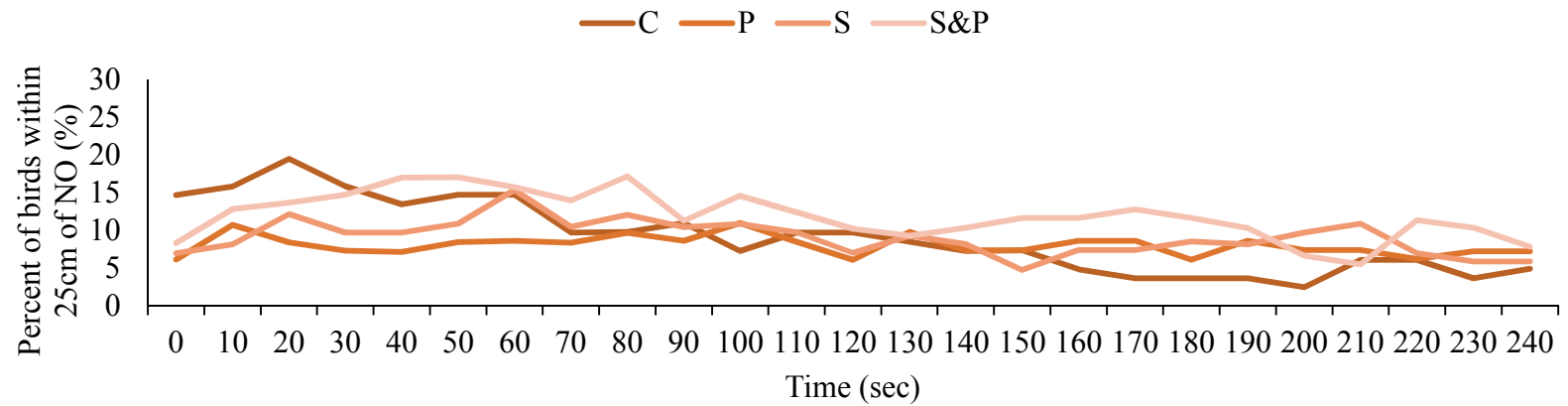


b)

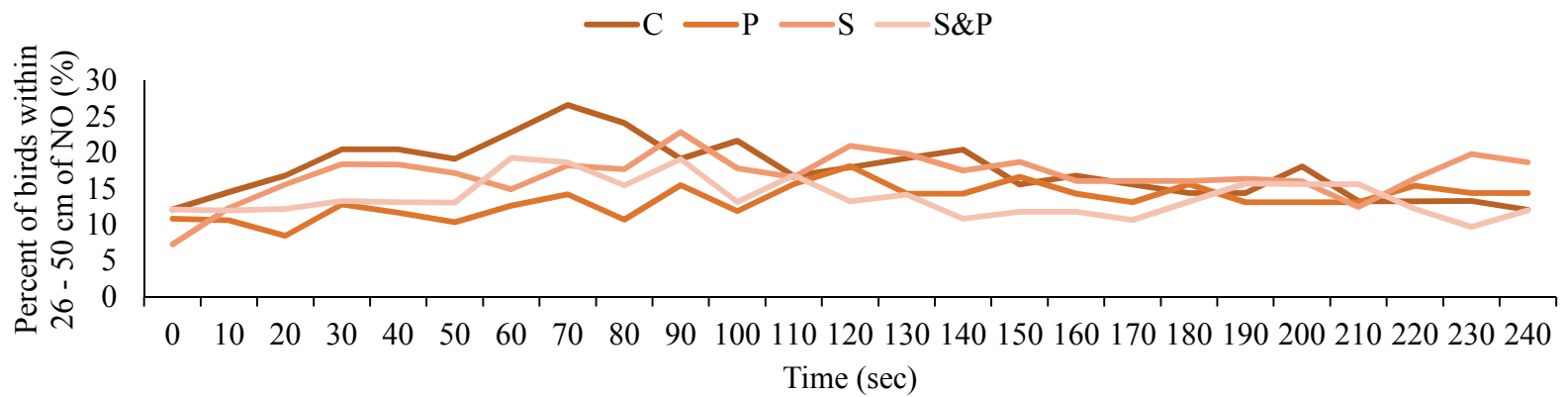


**Figure 2.20.** Average percentage (%) of broilers within **a)** 0 -25cm (Zone 1) and **b)** 26-50 cm (Zone 2) of the novel object throughout the pen (N = 16 pens, 4 pens/treatment) during the novel object (NO) test at 3 weeks of age.

a)



b)



**Figure 2.21.** Average percentage (%) of broilers within **a)** 0-25cm (Zone 1) and **b)** 26-50 cm (Zone 2) of the novel object throughout the pen (N = 16 pens, 4 pens/treatment) during the novel object (NO) test at 5 weeks of age.

## **Chapter 3: CONCLUSION**

### **3.1 SUMMARY AND IMPACT**

Overall, the platform enrichments may have improved leg health in our study, as shown by wider tibias at 90% length, 75% length, and 25% length locations along the tibia bone compared to the control, spotlight, and platform and spotlight combination treatments. We hypothesize that this is likely because the platforms provided opportunities for additional activities, and therefore different physical pressures on the legs that were not experienced by broilers in pens without the platform. The platform did not decrease fear of humans, as measured through the Avoidance Distance Test, or the oval object (48 cm PVC stick with multiple colors of tape) in the Novel Object Test at week 3 or week 5. Although this was not supported by the plasma corticosterone levels, the platform may have increased different stress levels as seen through differences in morphology measures of the left compared to right tibia may represent a lack of bilateral symmetry.

The spotlight enrichments have the potential to provide environmental complexity through stimulation of the visual system. In this study, broilers in pens with the spotlight treatments had narrower tibias at 90% length, and 75% length locations, showing that there was a change in skeletal morphology, but there was no difference in tibia ash as a percentage of bone weight. The similarity in bone ash across treatments show that skeletal development, tibia bone strength, or bone health were likely not influenced by the platform or spotlights. More broilers in the pens with spotlight enrichments were closer to the observer in the Avoidance Distance Test at week 3 (but not week 5), showing a decrease in fear of humans compared to the platform and the combination of spotlight and platform treatments. Further research into the effect of spotlights enrichments on skeletal

development and integrity should be done to better understand the influence of lighting on broiler behavior and physiology.

Finally, the interaction of the spotlight and the platform remained most similar to the control in most measures of bone morphology, indicating the combination of these treatments may not have a much of an influence on the broilers behaviorally or physiologically.

Overall, the enrichment treatments did not influence physical indicators of animal-based measures of welfare or production. The platform enrichments may have improved leg health in our study, as shown by wider tibias, and thicker cortical bone. This may be related to physical load stress on the tibia from the used of platforms. Spotlight treatment broilers had narrower tibias but there was no difference in mineral content (ash) as a percentage of bone weight showing that skeletal development likely was not influenced by the spotlights. The similarity in bone ash across treatments show that skeletal development, tibia bone strength, or bone health were likely not influenced by the platform or spotlights. Broilers within the control pens remained similar to pens with the combination of spotlight and platform, showing that the combination of this structural and visual enrichment may not have impact the broilers in measures analyzed. Basal corticosterone measures at 53 days of age were not influenced by treatments showing that spotlight and platform enrichments did not influence basal stress levels in broilers.

Given these results, we recommend that platform enrichments be evaluated in a commercial setting to test if our results are repeatable in commercial settings. Due to the more narrow tibias reported in this study we suggest further experimental research into



the potential influence of spotlight enrichments on the endocrine system and physiology. Since adding spotlights may not be practical to implement on-farm, more research is needed to understand the effects of spotlights on broiler health and welfare is necessary.