A novel environmental enrichment laser device stimulated broiler chicken active behavior and improved performance without sacrificing welfare outcomes

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A novel environmental enrichment laser device stimulated broiler chicken active behavior and improved performance without sacrificing welfare outcomes

Meaghan M. Meyer

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Science

Program of Study Committee:
Elizabeth Bobeck, Major Professor
Anna Johnson
Shannon Coleman

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2019

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DEDICATION

This thesis is dedicated my family. Without your confidence in me, unwavering support in what I do, and constant provision of a place to escape or to go home I would never have gotten through this degree. Thank you, I love you, and you are all dynamite. Of course, I would also like to dedicate this work to my beloved cats, Shorty and The Nert.
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The modern commercial broiler chicken is a genetic hybrid selected for fast growth, feed efficiency, and a large proportion of breast muscle tissue. As birds reach market weight within a typical 6-week commercial cycle, they are increasingly inactive and prone to leg disorders and contact dermatitis of the feet and breasts. Environmental enrichment can be used to increase activity and natural behavior in captive animals. The goal of this thesis was to incorporate environmental enrichment to stimulate broilers to voluntarily move and feed, whilst maintaining leg health and performance. The environmental enrichment was a novel device that projected red, randomly moving laser dots onto the pen floor to promote natural foraging and predatory behavior. A secondary goal was to validate broiler welfare measures recommended by the National Chicken Council (NCC) to understand and improve broiler welfare auditing. The three experimental objectives to meet these goals were to: 1) validate and modify measures of broiler welfare to determine the effects of enrichment and make recommendations to broiler producers and researchers; 2) measure the impact of enrichment on broiler production, leg health, and environmental parameters; and 3) study the effects of enrichment on broiler behavior and walking distance within the home pen.

Results showed that broilers in laser-enriched pens had greater feed intake and weight gain in each performance period and overall, increased average daily gain in the starter and finisher periods and overall, and improved feed conversion in the grower and finisher periods, as well as throughout the 6-week trial. Active behaviors (% of birds’ time) and distance walked (in centimeters) were increased, and inactive behaviors (% of birds’ time) were reduced in laser-enriched birds. Time spent at feeder was increased in laser-enriched broilers. Gait score was not
negatively affected by treatment. The condition of laser-enriched birds’ footpads, breasts, and tibia was not diminished, nor was the air or litter quality in the enriched room of the barn.

In conclusion, this novel environmental enrichment device was successful in stimulating broilers to voluntarily move and feed within the home pen, leading to improved performance outcomes without negatively impacting bird welfare, utilizing the measures collected in this study. This environmental enrichment option never comes into physical contact with the broilers, hence could be used over multiple flocks. This vision-oriented form of environmental enrichment is a promising option for commercial broiler producers that does not require alterations to management or increased human labor.
CHAPTER 1: GENERAL INTRODUCTION

Due to intense genetic selection, modern broilers weigh 4 to 5 times more than broiler lines from the 1950’s and are 1.5-3 times more feed efficient (Havenstein et al., 2003, Zuidhof et al., 2014). However, this selection for increased growth rate has contributed to up to 30% of modern commercial broilers being affected by leg lameness or poor locomotion (Knowles et al., 2008; Bassler et al., 2013). Lameness leads to increased inactivity, which in turn increases litter contact and could result in a higher occurrence of contact dermatitis in the footpad and breast (Weeks et al., 2000; Bassler et al., 2013; Nääs et al., 2018). Contact dermatitis-driven tissue damage may be a result of urea in the litter generating ammonia, creating a chemical burn effect that is likely painful. The severity of this issue may be reflective of air and litter quality in the house (Haslam et al., 2007). In consideration of these concerns, the National Chicken Council (NCC, 2017) Animal Welfare Guidelines and Audit Checklist for Broilers requires that ammonia in the air never exceed 25 ppm at bird height, litter must be evaluated throughout each house for friability, and 200 paws must be scored at slaughter for dermatitis.

Lameness that hinders birds from accessing feed and water negatively impacts the industry economically, necessitating up to 2% culls in a $30 billion industry (Dunkley, 2007; USDA, 2017). In addition, studies have reported that broilers with severe leg lameness eat more analgesic-containing feed than healthy birds, and birds fed an analgesic diet showed improved walking speed, indicating relief from pain and discomfort (McGeown et al., 1999, Danbury et al., 2000). The NCC broiler audit guidelines (2017) have recognized lameness as a detrimental welfare issue and recommend gait scoring 100 birds per flock to evaluate leg health within one week of slaughter.
Past research has shown that physical activity increased tibia strength and decreased broiler lameness (Reiter and Bessei, 2009), therefore, promotion of movement might positively affect bird welfare. Typical broiler behavior shows a preference for the bird to spend more time lying and less time/decreased distance walking as it reaches market weight (Newberry, 1990; Weeks et al., 1994). However, as market-weight broilers continue to access feeders and waterers, even when lame (Weeks et al., 2000), we can hypothesize that this is not because they physically cannot move, but rather they are not motivated to do so. It has been well established that birds are visual foragers/feeders (Fernandez-Juricic, 2004) that can see into the ultraviolet A spectrum and consistently prefer red when presented with novel colors (Prescott and Wathes, 1999; Ham and Osorio, 2007). Environmental enrichment is defined as a modification to the environment of captive animals that improves biological functioning (Newberry, 1995). Bizeray et al. (2002) studied the effects of red, blue, green, and yellow moving spotlights but did not see a change in broiler physical activity. The authors concluded that that the spotlights were moving too quickly for the broiler birds to follow; light efficacy designed to motivate broiler activity is still lacking in the literature. Bobeck and Johnson (2016) identified an instinct common to all poultry (visual-oriented foraging) and a potential method for motivating movement, which in turn may improve welfare outcomes.

The overall thesis goal was to implement an environmental enrichment device designed to stimulate foraging and predatory behavior to motivate broiler movement and improve performance. A secondary goal was to validate broiler welfare measures recommended by the NCC to understand and improve broiler welfare auditing. The three experimental objectives to meet these goals were to: 1) validate and modify measures of broiler welfare to determine the effects of enrichment and make recommendations to broiler producers and researchers; 2)
measure the impact of enrichment on broiler production, leg health, and environmental parameters; and 3) study the effects of enrichment on broiler behavior and walking distance within the home pen.

**Hypothesis**

The hypothesis was that the introduction of a laser enrichment device would increase broiler bird physical activity while leading to increased feed intake, increased weight gains, and improved feed conversion.

**Expected Outcomes**

Outcomes will include one manuscript submitted to Journal of Applied Animal Welfare Science titled “Development and validation of broiler welfare assessment methods for research and on-farm audits” (2019), two manuscripts submitted to Journal of Poultry Science: “A novel environmental enrichment device improved broiler performance without sacrificing bird physiological or environmental quality measures” (2019); and “A novel environmental enrichment device increased physical activity and walking distance in broilers” (2019). Two abstracts have been accepted for presentation at the Poultry Science Association annual conference (Montreal, Quebec, 2019), two Iowa State animal industry reports have submitted, and one Iowa State University MS thesis will be the final summary of this work.

**Practical Implications**

The results from this research will aid the broiler industry in advancing enrichment, a field which is currently lacking, while simultaneously improving production outcomes. Additionally, validation and recommendations on methods to measure broiler welfare and behavior can be practically applied to poultry producers and/or researchers.
CHAPTER 2: LITERATURE REVIEW

Introduction

Commercial broiler chickens are genetically-selected for feed efficiency and growth that culminates in a market-weight bird at six weeks. This fast rate of growth leads to birds that are 4-5 times heavier and 1.5-3 times more feed efficient than broilers 50 years ago (Havenstein, et al., 2003; Zuidhof, et al., 2014). Up to 30% of today’s heavier birds are prone to leg lameness, a result of skeletal disorders (tibial dyschondroplasia, osteodystrophy, femoral head separation) caused by an out-of-synch leg bone development to match rapid muscle mass accumulation (Bessei, 2006; Knowles, et al., 2008; Bassler, et al., 2013). Contact dermatitis (breast blisters, hock burns, and footpad dermatitis) develops due to extended periods of time spent in contact with the litter (Weeks, et al., 2000; Nääs, et al., 2009). Research suggests leg disorders are painful, evidenced by improved walking ability in broilers administered carprofen and self-selection of a carprofen-containing feed in lame birds, and ultimately lead to increased inactivity/lying on the litter (McGeown, et al., 1999; Danbury, et al., 2000). Consumer concerns have arisen in light of these negative welfare issues (Hall and Sandilands, 2007).

A viable method to improve broiler leg condition is to increase physical activity (Reiter and Bessei, 2009). The use of environmental enrichment, particularly utilizing the natural vision-oriented foraging behavior of birds, is an option that may not sacrifice production outcomes. However, in current U.S. broiler systems, environmental enrichment is underused (Bessei, 2006; Riber, et al., 2017). Furthermore, environmental enrichment is not scored by U.S. broiler welfare auditing tools, and there is a lack of validated methods to measure overall bird welfare and the environmental enrichment effects. Thus, this literature review will cover the current state of broiler
chicken welfare, previously studied broiler enrichment options, and general bird vision/vision-orientated behavior.

**Broiler Welfare**

**Primary Concerns Overview**

Livestock welfare continues to be important to consumers in the United States (Spain, et al., 2018). In poultry, conventional cage laying hen housing has garnered the greatest attention from stakeholders that has ultimately driven a movement towards alternative or cage-free systems allowing more natural hen behavior (Blatchford, 2017). However, broiler, or meat-type, chickens account for 71% of the industry, compared with 18% from egg production (USDA, 2017). Broiler welfare issues have been relatively less discussed by the general public and are thus less researched (Blatchford, 2017). Still, consumer concerns regarding housing and the use of fast-growing breeds are increasing (Hall and Sandilands, 2007; de Jonge and van Trijp, 2013). Critical animal-based concerns, as identified in Bessei’s (2006) review of broiler welfare, are comprised of three major categories within an overarching theme termed “genetic issues”: 1) fast early growth rates and resulting diseases, 2) skeletal disorders, and 3) poor locomotion. This section will focus on two primary welfare concerns; leg disorders and contact dermatitis, which derive from and contribute to the three afore mentioned “genetic issues”, rather than genetically-driven metabolic disorders (i.e.; ascites and sudden death syndrome).

**Leg disorders**

Leg lameness is a multi-factorial welfare issue leading to poor locomotion and pain when walking, and is affected by the animal’s genetic pre-disposition, environment, and behavior (Knowles, et al., 2008). Economically, leg disorders hurt the broiler industry by necessitating 2% of on-farm culls and downgraded products at slaughter. This is an estimated cost of $600 million
in the $30 billion industry (Dunkley, 2007; Nääs, et al., 2009; USDA, 2017). The number of affected birds is even higher, with 26-31% of broilers showing lameness (Kestin, et al., 1992; Knowles, et al., 2008).

Genetically, the introduction of quantitative rather than Mendelian genetics and the use of hybrids in research around the 1950’s, and their subsequent speedy adoption by commercial breeders, are credited to the massive improvements seen in broiler growth and feed conversion (Griffin and Goddard, 1994; Hunton, 2006). For comparison, broiler chickens grow at four times the rate of laying hens and put on breast muscle eight times faster (Bulfield, et al., 1988; Griffin and Goddard, 1994). Further, today’s broilers weigh 4-5 times more than their 1950’s counterparts (Havenstein, et al., 2003; Zuidhof, et al., 2014). While other factors including disease and nutrition certainly play into leg disorders (Butterworth, 1999), this extreme speed of growth “must be considered as the main factor” (Bessei, 2006).

From a bird-based perspective, there is behavioral evidence indicating that leg conditions are painful, particularly when weight bearing (Weeks, et al., 2000; Bassler, et al., 2013). Elegant work done by Danbury et al. (2000) and McGeown et al. (1999), respectively, showed that broilers with advanced lameness chose to eat more carprofen-containing feed (an analgesic/pain-reliever) than sound birds, and that birds administered the analgesic via subcutaneous injection had improved walking speeds; both clear indications that the drug provided relief from pain and discomfort caused by leg disorders. It is well-documented that typically, broilers spend 75-85% of their time inactive. Even sound birds spend a meager 3.3% of the day walking, and lame birds only 1.5% at harvest weight (Weeks, et al., 2000). One causative factor may be that the genetic selection for a large proportion of breast tissue has effectively shifted the birds’ center of gravity forward, and consequently thrown off the natural balance (Weeks, et al., 2000). Hence, the bird’s
gait is altered, forcing a modified or abnormal method of walking (Nääs, et al., 2009). An interesting outcome of work by Weeks et al. (2000) was that lame birds walked the bare minimum to access feed and water, and did not show decreased performance or feed efficiency, but rather made fewer trips/feeding bouts of longer duration than sound counterparts. Lame birds were also observed eating while lying down, which is a behavior not observed in healthy birds or the modern chicken’s junglefowl ancestor. Junglefowl continuously move while foraging, and are not observed to eat in one spot for extended periods of time (Arshad, et al., 2000). This work is a clear example of how lameness decreases locomotion and increases litter contact, even while the bird is feeding.

While genetics may be the main contributing factor to leg disorders, and infectious diseases can decrease leg health (Butterworth, 1999; McNamee and Smyth, 2000), there are other, perhaps more preventable, contributors. Research has shown that lameness prevalence is affected by the environment. Decreased stocking density, improved air ventilation and quality, improved litter quality (Dawkins, et al., 2004; Sorensen, et al., 2000; Tullo, et al., 2017) and increased hours of darkness (Bassler, et al., 2013) decrease lameness. Increased hours of darkness are effective because bone mineralization peaks during dark hours (Russell, et al., 1984), and birds then show greater activity during light hours (Schwean-Lardner, 2012).

It is thought that most skeletal support is established in broiler birds by day 18, following “intensive bone formation to provide rapid mineralization” (Williams, et al., 2000). However, bone porosity or density must change more slowly over time to support increasing bird weight (Williams, et al., 2000). Bone strength and mass increase with activity (Lanyon, 1992; Rath, et al., 2000), and load-bearing bones need to bear weight during development or will immediately fail to do so when given the opportunity (Lanyon, 1993). Previous work has shown that when
broiler birds were forced to exercise using treadmills, tibia strength was increased and lameness was decreased (Reiter and Bessei, 2009). Prayitno and others (1997) raised birds in different colored lighting systems and determined that red light increased activity and reduced leg disorders. Conversely, restricting exercise in laying hens resulted in severely decreased leg health (Shipov, et al., 2010). Thus, it has been established that an increase in exercise improves avian leg condition.

**Contact dermatitis**

Commercially, contact dermatitis including breast blisters and footpad dermatitis has necessitated downgrading of up to 15-30% of broiler carcasses/week (Greene, et al., 1985). Breast blisters, also referred to as bursitis, range from brownish-colored scabs to exudate and litter-filled ulcers, aggravated because broilers rest 60% of their body weight on the keel while lying. This causes the bursa to become swollen and filled with fluid when birds spend the majority of their time lying down (Nielsen, 2004). Footpad dermatitis is a similar condition, but on the plantar surface of feet and, when severe, toes, with symptoms of inflammation and necrotic lesions. Footpad dermatitis, in particular, represents a considerable monetary loss to the industry, where paws are “the third most important economic part of the chicken behind the breast and wings...accounting for approximately $280 million a year” (Shepherd and Fairchild, 2010). Closely related to footpad dermatitis are hock burns, a condition leading to darkened or scabbed skin on the birds’ hock (Kjaer, et al., 2006).

Breast blisters, footpad dermatitis, and hock burns are reflective of diminished air and litter quality in the barn (Allain, et al., 2009). These conditions are likely caused by ammonia originating from urea (converted from uric acid by organisms) in the litter, causing a chemical burn effect on the birds’ skin. Haslam and others (2006) showed that the percent of birds with
footpad concerns was correlated with ammonia concentrations in the house and litter moisture, although this association was not found with breast burns. Allain and others (2009) found that common fast-growing broiler genotypes were afflicted by a greater degree of lesions than slow-growing birds; the authors surmised that this was due to leg disorders in the fast-growing line, and thus increased time in contact with the litter. Sorensen and others (2000) likewise concluded that a greater prevalence of footpad dermatitis and hock burns were seen in worsening litter conditions, and likely contributed to poor walking ability. In summary, leg disorders and contact dermatitis are interrelated issues that are multi-factorial and predictive of one another. Genetics contributing to worse leg health and walking ability lead to increased time lying and greater litter contact, thus increased dermatitis. These animal welfare concerns can be mediated by improved air and litter quality, decreased stocking density, increased dark hours, and increased physical activity.

The Five Freedoms, Schools of Welfare, and a Life Worth Living

How far have we the right to take our domination of the animal world? Have we the right to rob them of all pleasure in life simply to make more money more quickly out of their carcasses? Have we the right to treat living creatures solely as food converting machines? (Harrison, 1964)

This powerful statement comes from the revolutionary book, Animal Machines, written by Ruth Harrison in 1964. Harrison’s book was the first of its kind to shed light on the food animal industry (in the United Kingdom [UK]) at the time and unearthed many human food safety as well as animal welfare concerns. As a direct result of public upset stemming from Harrison’s book, a provisional outline for the Five Freedoms were proposed by the Brambell Committee (UK), which were finally crafted in 1979 by the Farm Animal Welfare Council.
These freedoms have become the gold standard of animal welfare and are interwoven in educational assessment, auditing, and law (Webster, 2016). The five freedoms are as follows:

1) Freedom from Hunger and Thirst: by ready access to fresh water and diet to maintain full health and vigor; 2) Freedom from Discomfort: by providing an appropriate environment including shelter and a comfortable resting area; 3) Freedom from Pain, Injury, or Disease: by prevention or rapid diagnosis and treatment; 4) Freedom to Express Normal Behavior: by providing sufficient space, proper facilities and company of the animal’s own kind; and 5) Freedom from Fear and Distress: by ensuring conditions and treatment which avoid mental suffering.

(Farm Animal Welfare Council, 1979)

The World Organisation for Animal Health (OiE) has provided a globally recognized definition of animal welfare that recognizes welfare as a multifaceted and multidimensional issue (Johnson, 2019). This definition is laid out in the Terrestrial Animal Health Code:

Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and appropriate veterinary treatment, shelter, management and nutrition, humane handling and humane slaughter or killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.

(OiE, 2008)
Another method for classifying animal welfare was proposed by Fraser et al. (1997), using three schools: 1) natural-living, 2) feelings-based or affective states, and 3) functioning-based. The first school pertains to allowing animals to express a behavioral repertoire natural to their species, a concern also addressed in the Five Freedoms. For example, conventional laying hen cages do not provide substrate for natural dust-bathing behavior, which may be inherently frustrating for the hens, who will even “sham dust-bathe” despite the lack of resulting oil distribution and feather maintenance thought to drive the behavior (Olsson, et al., 2005).

The second, feelings-based conception of animal welfare refers to affective states of animals; i.e. freedom from suffering. Suffering can be explained as emotional states that “are unpleasant enough that, if we could, we would endeavor to get out of” (Dawkins, 2008). Positive affective states such as comfort and contentment are emphasized in this school of thought (Fraser, et al., 1997). Through motivation and preference tests, animal welfare researchers have identified a scientific method to determine what situations animals will choose or choose to avoid when given control over their environment, indicating affective states (Kirkden, et al., 2006). For example, a study testing motivation to forage in broilers showed that feed-restricted birds went to a wood shavings-filled foraging area faster and spent more time foraging than birds that were adequately fed, although food was never presented there, indicating a hungry, negative affective state (Dixon, et al., 2014).

The third school of welfare is functioning-based, where normal/healthy biological functioning of the animal is key. This conception of animal welfare emphasizes nutrition, physical health, and physiological outcomes. (Fraser, et al., 1997). The physiological measures (body temperature, blood chemistry, heterophil: lymphocyte ratio, and intracellular enzyme activity) collected by Mitchell and Kettlewell (1998) during a study of transport stress in broilers
are reflective of this school of thought. These measures tend to be particularly valued by veterinarians and livestock farmers (Fraser, et al., 1997). Ultimately, a clear picture of animal welfare must include all three schools.

A more contemporary and growing school of thought in animal welfare science is the idea of “quality of life” or as it is often described, “a life worth living”. In this concept, the quality of an animal’s life is defined in-regards-to the balance of positive and negative experiences or affective states of that animal. The aim is for good experiences to outweigh bad (Mellor, 2016). A more exact scale was proposed by Green and Mellor (2011) with the following five categories (from lowest to highest quality of life): 1) a life not worth living, 2) a life worth avoiding, 3) neutral point of balance, 4) a life worth living, and 5) a good life. A neutral point of balance (3) is likely achieved when the commercial system is in full compliance with welfare codes/audits that are focused solely on nutritional, health, and performance outcomes. A life worth living or a truly good life, according to this system, are unlikely to be achieved unless the operation abides to a welfare tool that incorporates assurance of natural animal behavior, a positive human-animal relationship, or environmental enrichment that allows the animal control over the environment or rewarding experiences (Mellor, 2016). Current animal welfare legislation and on-farm auditing tools for broiler birds are reviewed in the next section.

**Broiler Legislation and On-Farm Auditing**

The Animal Welfare Act (AWA) is the sole federal law in the U.S. regulating farm and research animal welfare. The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), and USDA Animal Care enforce the AWA, considered the “minimum acceptable standard” of animal care nationally (2019). Briefly, the AWA contains standards of housing, sanitation, food, water, medical care, and thermal regulation for specific
species being commercially sold and transported. Compliance with these standards is assured in inspections carried out by trained USDA veterinary medical officers (USDA, 2015). Within the European Union, the European Food Safety Authority Council Directive 2007/43/CE, similar to the AWA in the U.S., is a government-regulated standard of minimum care guidelines, in this case specifically for broiler chickens. This directive covers training, meat product-labelling, management, housing, stocking density, slaughter, etc. Similar to USDA inspections, the EU Council Directive requires non-optional federal inspections to assure compliance (EU, 2007).

Separate from the AWA and EU Council Directive, voluntary broiler chicken-specific assessment and auditing programs exist. The most followed in the U.S. are the National Chicken Council (NCC) Animal Welfare Guidelines and Audit Checklist for Broilers (2017). The NCC guidelines for producers are used nationally to audit personnel training, hatchery operations, grow-out operations (management, nutrition, comfort and shelter, health care monitoring, and flock husbandry), catching and transportation, and processing of commercial broilers. The NCC guidelines are largely functioning-based, with mostly physical or environmental-based measures, but do recommend gait-scoring. Missing from the NCC guidelines are critical broiler behavior and affective state-based measures, suitable environmental enrichment options, and methods to assess enrichment (NCC, 2017).

Internationally, the Welfare Quality® assessment tool for poultry, developed by the European Union (2009), is widely used in research and on-farm. This auditing-tool is in certain aspects stronger and more inclusive of Fraser’s three schools of welfare (natural-living, feelings-based/affective states, and functioning-based). Welfare Quality involves collection of behavioral data on-farm including: measurement of social and other normal bird behaviors, the Avoidance distance test for assessment of human-animal relationship and fearfulness, and the Qualitative
behavioral assessment to measure affective state. Environmental enrichment is not required by this audit. Scoring feather condition is a requirement of Welfare Quality that is lacking in NCC audit checklist. This tool was validated during on-farm audits across the EU and has more recently been validated in research (Blatchford, et al., 2016), and is certainly stronger in some welfare aspects than the N.C.C. audit guidelines (U.S.), which require further validation and inclusion of broiler behavior- and affective-state- based measures.

**Broiler Environmental Enrichment**

**Enrichment Definition and Forms**

Environmental enrichment is defined as “*an improvement in the biological functioning of captive animals resulting from modifications to their environment*” (Newberry, 1995). The overarching goal of environmental enrichment is to allow animals to perform more of their natural behavioral repertoire, to maintain or improve health, and to improve economics, all while maintaining practicality (van de Weerd and Day, 2009). Additionally, enrichment must not be thought of as the implementation of one single thing to meet one behavioral need, but rather an “*adequately complex program...within this program there should be opportunities to work and also for leisure activities such as play and curiosity.*” (Poole, 1992). It is crucial to evaluate and consider enrichment in terms of animal-based welfare outcomes rather than considering any simple modification to the environment, a change improving production, or a modification that is positive only from a producer’s perspective as true enrichment (Newberry, 1995). Environmental enrichment that is truly filling a biological need missing from the animal’s current captive environment can reduce negative stereotypical behavior and will measurably improve the animal’s welfare (Mason, et al., 2007).
Five categories of environmental enrichment were described by Bloomsmith et al. (1991) and are summarized in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010). These classifications are a commonly used, practical way to evaluate and describe enrichment and its relevance to the animal. The categories include: 1) social enrichment; contact with conspecifics or people which may be direct or indirect, 2) occupational enrichment; encourages exercise or provides psychological control or challenges, 3) physical enrichment; changes in size or complexity of the environment, addition of objects or substrates, 4) sensory enrichment; visual, auditory, olfactory, tactile, or taste stimuli, and 5) nutritional enrichment; novel food or food delivery methods.

Another method of categorizing enrichment involves splitting into two categories: 1) “point-source objects” (perches, straw bales, string, etc.) added to conventional environments, and 2) complex enriched environments (indoor or with outdoor access) that are drastically different from conventional housing systems (van de Weerd and Day, 2009). Environmental enrichment success will vary depending on which behavioral outcomes they aim to achieve, or which behavioral stereotypies they aim to eradicate. Current enrichment for broilers will be explored in the next section, in the context of biological relevance and which of the five forms or two categories of enrichment they provide.

**Broiler Enrichment**

In a review of environmental enrichment for broilers, Riber et al. (2018) concluded that current enrichment for broilers needs further development, and there is a dearth of commercially validated trials/applications. Further, the authors identified a lack of knowledge on current broiler bird environmental enrichment practicality and its effects on stress, fearfulness, and disease-state; these need to be resolved before practical application. In a 2017 review (Riber, et
al.), the authors identified very few studies on broiler breeder enrichment, and a strong need for enrichment stimulating foraging behavior. Foraging behavior may be particularly important because broiler breeders are feed restricted, a practice that leads to hunger and frustration (Dixon, et al., 2014). Additionally, enrichment that encourages more healthy breeding behavior is lacking. Thus far, enrichment is not required by the AWA or NCC welfare auditing guidelines for broilers (U.S.), for either commercial broilers or the breeding stock. This issue may be resolved if enrichment were validated and practically applied in commercial studies as proposed in Riber et al. (2018).

**Perches, platforms, panels, and barriers**

Perching during dark hours is a natural jungle fowl behavior (Collias and Collias, 1967), and is observed in modern commercial laying hens when housing allows it (Sandilands, et al., 2009). However, perch use by commercial broilers has shown varied success. Some research has indicated a positive effect with perch usage of improved footpad condition, likely due to decreased litter contact, a positive outcome (Ventura, et al., 2012; Kiyma, et al., 2016; Gebhardt-Henrich, et al., 2017). Perch use greatly depends on bird age, as there is a decline in use over time, particularly after week 4 (LeVan, et al., 2000; Sandilands, et al., 2016). Perch ergonomics can also affect usage, with broilers using 10 cm tall perches more than 15 cm perches (Norring, et al, 2016), and work in laying hens showing that birds preferred wooden or rubber-coated perches to steel (Bessei, 2006; European Food Safety Authority, 2015). Su and others (2000) found no perch effect on body weight or leg health.

LeVan, et al., (2000) and Pettit-Riley and Estevez, (2001) reported that perching behavior can be as low as 2-2.6% as birds reach market weight and concluded this could be due to heavy body weight overriding motivation and/or physical ability to perch. In contrast,
Ventura, et al. (2012) observed increased perching behavior, with up to 25% perching at a low stocking density and up to 40% perching in a slow growing broiler line, comparable to laying hen perch use. However, heavier, fast-growing broilers perched significantly less, particularly after week 5 (Bokkers and Koene, 2003). Elevated platforms, which are normally plastic, slatted, and ramp-accessible, are currently in use internationally in broiler breeder barns (Riber, et al., 2018). While these platforms have shown greater use than traditional perches and are considered a viable alternative, they do not serve to stimulate activity (Norring, et al., 2016), nor are platforms the natural preference for birds who would prefer a perch they are able to physically grasp (European Food Safety Authority, 2015).

Vertical panels and barriers have been implemented into broiler environments to add a degree of complexity (Riber, et al., 2018), and to more evenly distribute birds and stocking density, as broilers are well known to prefer resting along barn sides. This pattern leads to heavily used areas with diminished litter quality (Newberry, 1990). Panels have been successful in distributing birds as anticipated, but Cornetto and Estevez (2001) also found that panels increased resting behavior and decreased foraging. Bizeray et al. (2002) reported that barrier implementation resulted in perching behavior and decreased time lying on the litter, a positive outcome, but also increased tonic immobility (a measure of fearfulness in birds). This outcome was corroborated by Ventura and others (2010). In summary, perches, platforms, panels, and barriers, which are considered point-source objects of enrichment, provide occupational enrichment; by allowing the animal a choice or a challenge, and physical enrichment; by increasing the complexity of the barn with the addition of a permanent structure.
Bales of straw, foraging materials, dust-bathing substrates

Several forms of destructible foraging materials and substrates have been provided to broilers with the goals of: encouraging natural scratching, food-searching, and dust-bathing behaviors, increasing general activity, and decreasing aggression in broiler breeders (Riber, et al., 2017; Riber, et al., 2018). The incorporation of straw bales as a “freedom food” enrichment have shown success in decreasing time broilers spent sitting and resting, increasing locomotion, and increasing feeding and drinking behavior (Kells, et al., 2001). Addition of wheat onto the floor of pens has been less effective and did not promote foraging behavior or increase activity (Bizeray, et al., 2002). Jordan et al. (2011) found similar ineffective results when scattering whole wheat, but saw an increase in foraging and activity when scattering feed pellets. Unfortunately, body weight gain was sacrificed in feed pellet-treatment birds, finishing at weights 13% lighter than control birds. Pichova and others (2016) likewise showed that whole wheat scattering did not increase activity, alter behavior, or affect measures of fear response in broilers. However, daily scattering of mealworms proved successful in increasing activity, although short-lived and inapplicable to commercial operations.

Sand was provided to broilers as a dust-bathing substrate with the goal to increase activity and natural behavior, but results showed that while broilers preferred sand over wood-shavings, birds in fact spent more time inactive on the sand (Shields, et al., 2005). A greater amount of foraging behavior was, however, seen by Arnould and others (2004) when sand was provided along with wood shavings. An elegant study by Baxter et al. (2018) allowed broilers to choose between five different substrates placed in five rings to determine where birds chose to spend the most time, and which behaviors they preferred to perform in which substrates. Birds spent the greatest amount of time in the peat and wood-shavings rings and performed the most
dust-bathing in the peat and oat hull substrates. Birds foraged the most in the peat, oat hull, and
wood-shaving rings. Hence, this study was successful in stimulating natural foraging and dust-
bathing behaviors by providing what the birds deemed appropriate substrates, but commercial
application of such a system is impractical.

Overall, provision of foraging materials has shown varying levels of success in broilers.
These are point-source objects that have in most cases provided physical enrichment; by the
addition of substrates adding a degree of complexity to the barn, sensory enrichment; by
providing taste and/or tactile stimuli, and nutritional enrichment; by incorporating foraging
opportunities or novel foodstuffs. Very few studies have been successful in stimulating activity
through these forms of enrichment; more research and novel methods to encourage natural
foraging in broilers are warranted (Riber, et al., 2018).

**Novel/pecking objects**

Another form of enrichment in broilers involves the addition of strings or unique items to
the home pen, point-source enrichment objects meant to add some complexity and ultimately
reduce fear-responses, as enrichment is a validated method to reduce fear in poultry (Jones,
1996). Further, broiler chicks have shown motivation to explore novel stimuli (Newberry, 1999).
Hanging strings, incorporated by Arnould and others (2004), received very little attention from
broiler birds, but another study showed significant string pecking activity when strings were
placed by feeders. This behavior peaked at week 3; activity and gait scores were also improved
during week 3 compared to the control. However, interest waned for the remainder of the trial,
and the authors concluded that effects on leg health were minimal (Bailie and O'Connell, 2015).
Altan et al. (2013) added enrichment objects, including colored balls, plastic bottles, toys, and
mirrors to treatment pens to measure their effect on performance and fear response. Results
showed no differences in growth performance or heterophil: lymphocyte ratio, but enriched birds were less fearful, as measured by decreased tonic immobility duration and decreased blood basophils. These objects fall into the category of physical enrichment as accessories to the animal’s environment, and sensory enrichment, as they stimulate a tactile response, and require further inquiries into their true biological relevance to the broiler.

**Auditory and visual enrichment**

Sensory-based auditory and visual enrichment in broilers is arguably the least-researched; and few published studies can be found. Although there is evidence that birds possess some olfactory function (Steiger, et al., 2008), it is well established that birds rely on auditory and visual cues, and have extremely advanced color vision (Ham and Osorio, 2007; Toomey, et al., 2016; Olsson and Kelber, 2017) as well as the ability to differentiate textures (Jones and Osorio, 2004) and shapes (Gibson, et al., 2007). Auditory enrichment in the form of classical music was played to layer-type chicks and decreased stress responses, including tonic immobility duration and heterophil: lymphocyte ratio (Dávila, et al., 2011). Contrastingly, a study in broiler chicks including four different musical treatments showed no differences in feed intake, weight, or stress response due to auditory enrichment (Christensen and Knight, 1975). However, Gvaryahu and others (1989) showed that a combination of filial imprinting and classical music in broiler chicks led to birds that were less fearful and had greater feed intake than the control, particularly while the music was on.

Enrichment utilizing the vision-oriented predatory behavior of birds is lacking in broilers. Newberry and others (1985) have shown that broiler activity can be increased with alternative light to dark hours, but leg condition was not improved. Prayitno and others (1997) successfully used red light to stimulate broiler activity but reported decreased performance. Only one study
has looked at the effects of moving lights on broiler behavior, foraging activity, and movement: work by Bizeray et al. (2002) tested red, blue, green, and yellow moving spotlights on pen floors for four hours/day on a subset of 450 out of 1800 Ross 308 broilers for 44 days. The authors reported no differences in foraging activity, no pecking at the spotlight, and no increase in walking due to this treatment, and concluded that the lights may have been moving too quickly for the broilers to follow.

Auditory enrichment is a form of sensory enrichment, but visual enrichment utilizing moving lights could encompass occupational enrichment (encouraging exercise), physical enrichment (adding a layer of complexity to the environment), and nutritional enrichment (driving birds to express natural foraging/feeding behavior), as well as sensory enrichment. Further work in this research area is necessary to determine enrichment effectiveness using different sizes, colors, and speeds of moving light.

**Broiler Motivation, Attention-Span, Fear, and Habituation**

Broiler bird motivation is an interesting concept; although young chicks show clear motivation to explore novel objects compared to an empty pen, they also show a high proportion of inactive behavior (70%) even while young (Newberry, 1999). This is believed to be a result of selection for weight gain and feed efficiency that allows for less energy partitioned to walk, run, or unnecessary physical behavior (Bizeray, et al., 2000). However, even lame birds will walk the bare minimum to access feeders and waterers (Weeks, et al., 2000), as feeding is consistently considered a highly motivated behavior in broilers and broiler breeders (Bokkers and Koene, 2002; Bokkers and Koene, 2004). Feed restricted birds (50% of *ad libitum* intake provided) paid a greater “price”, measured in number of pecks on a key, than 75% feed restricted birds. Additionally, 50% feed restricted broilers had a shorter latency to feed when food reward was
provided, indicating a strong motivation to feed when food is withheld (Bokkers and Koene, 2004). Further, birds who were not feed-deprived but solely received delayed food rewards walked faster to the end of a runway and showed higher motivation to feed than control birds (Bokkers and Koene, 2002).

Attention-span in broilers has not yet been quantified, but work done using other bird species has shown that ravens pay more attention to conspecifics and to food-related objects than other birds and other objects, an outcome that was not seen in jackdaws in the same study. The authors concluded that these results are logical because ravens (like chickens) are natural foragers and place different values/attention on different objects (Scheid, et al., 2007). Attention-span is somewhat understood in songbirds, where songs last only 3-5 seconds before the bird pauses and repeats the song. It is believed this short length followed by repetition is indicative of a short attention span, although certainly not a short memory, in birds of song (Hartshorne, 1956).

The concept of attention span, defined as “the length of time that a subject will devote to a task”, was evaluated in psittacines (Pepperberg and Funk, 1990). The authors reported that within a small group of four birds (one parrot, one macaw, one parakeet, and one cockatiel) the attention-span varied between and even within birds. For example, one bird completed 10 tasks in one hour, but never moved directly from one task to the another and always avoided attending to the task. Overall, there is a considerable lack of information regarding length of time that a bird is able to pay attention to one specific object/task, and no quantification for broiler chicken attention is available in the literature.

Fear is a complex, natural evolutionary response that drives animals to avoid danger, particularly from predators (Stankowich and Blumstein, 2005). In production, fear can become a
chronic stressor when an animal is unable to react (flee) to frightening or unpleasant circumstances as they would naturally (Jones, 1996). Fear clearly plays a role in poultry as they are prey species that consider humans a threat; red junglefowl selected for low fear of humans are more active, a desirable trait in commercial broilers, than high fear birds (Katajamaa, et al., 2018). Previous experience with people has measurable stress effects and even production outcomes. Hemsworth and others (1986) found that unpleasant handling three times/ week negatively impacted reproductive performance in boars due to chronic stress. Laying hen work has shown that following a 2-week period of positive human handling, hens remained closer to a human during the Avoidance Distance Test and Stationary Person Test, and more hens were able to be touched during the Touch Test, indicating a decreased fear of humans (Graml, et al., 2008).

In broilers, fear and stress are commonly measured using tonic immobility, a validated measure of inducing temporary paralysis (Gallup, et al., 1971), or blood parameters; heterophil: lymphocyte ratio and corticosterone concentration, but certainly more work has been done in this field using laying hens. Kannan and Mench (1996) replicated typical commercial husbandry of broilers before transport and slaughter, and found that inverted (upside-down) carrying of birds versus upright handling, as well as the crating process, increased corticosterone levels. This indicates a significant stress response that the authors believe is likely even more intense in the field. Following transport, fear levels were likewise increased in broilers, as measured by lengthened tonic immobility duration (Cashman, et al., 1989), similar to an increased fear response seen in broilers that been exposed to environmental enrichment and human handling compared to birds who were exposed to enrichment but not handled (Nicol, 1992).

However, human contact is not always negative. Jones (1992) paired rough handling immediately prior to tonic immobility induction and reported an increased fear response
compared to broilers and layers that were gently handled and stroked prior to induction. Further, broilers that received regular human contact showed less avoidance /fear of humans, which was correlated with improved feed conversion, a critical outcome indicating that fear of humans may be hindering production potential of broilers (Hemsworth, et al., 1994). Human-animal interactions are the primary cause of stress in commercial broilers, but mitigation is possible by incorporating positive, regular handling of birds, and by enriching the environment.

Altan and others (2013) introduced novel objects (plastic balls, bottles, toys, and mirrors) and saw that birds were less fearful. Classical music played to layer-type chicks decreased tonic immobility duration and heterophil: lymphocyte ratio (Dávila, et al., 2011). Gvaryahu et al. (1989) showed that a combination of filial imprinting and classical music enrichment resulted in broilers that were less fearful. An interesting study by Reed and others (1993) showed that auditory and visual enrichment as well as experience with humans during rearing in young laying hens reduced fear reactions in adulthood. It has not been established in the literature why enrichment has, at times, had the ability to decrease fear and stress in poultry, but is likely due to the function of enrichment to provide some degree of control or complexity in the home environment.

Habituation is defined as a “relatively permanent, stimulus-specific decrement in response strength that occurs as a consequence of repeated response elicitation” (Nash and Gallup, 1976). For example, captive bird species in zoos show a diminished response to auditory enrichment (“Sounds of the African Rainforest”) over exposure time (Robbins, et al., 2016). Similar habituation may be possible in auditory enrichment provided to broilers. Research has shown that broilers can become habituated to tonic immobility induction within two days of undergoing induction five times/day (Nash and Gallup, 1976). Broiler breeder habituation to
Environmental enrichment in the form of bunches of string was reported by Hocking and Jones (2006), but the length of time it took for birds to become habituated and lose interest is unclear.

Habituation to visual contact with humans for 10 minutes twice/day was established in broilers within 3-6 weeks, and birds showed reduced fearfulness and a greater antibody response to Newcastle vaccine as a result (Zulkifli, et al., 2002). Similarly, broiler and layer chicks became habituated to humans through visual contact for 30 seconds/day for 10 days (Jones, 1995). A different study by Jones and others (2000) incorporated enrichment in the form of hanging strings for 10 minutes/day for five consecutive days, and saw that layer-type birds maintained interest in pecking the strings for up to 14 weeks, a promising result indicating enrichment remained relevant for many weeks. This may be attributed to the limited, not constant, daily exposure. Discussion of habituation is absent from much of the broiler enrichment literature covered here, perhaps because the birds are relatively short lived (typically 6-week production cycles). Regardless, further research is warranted in broiler, and particularly longer-lived broiler breeder, enrichment to ensure that birds are not becoming habituated and that enrichment is truly biologically relevant.

**Bird Vision**

**Eye Biology**

Birds rely on vision more than olfactory or even auditory signals, thus have adapted eyes to suit this very uniquely compared to mammals (Husband and Shimizu, 2001). Bird eyes are up to twice as large in proportion to their skull and vary in shape, a necessity in order to search for food, find mates, orientate, and avoid predation (EverGreen, 2011). A well-established quality in birds is an incredible development of tetrachromatic color vision, compared to only three color
channels in humans. Birds see beyond the primary colors blue, green, and red, into a fourth “UV” spectrum that we cannot see (Finger and Burkhardt, 1994). This means that not only can birds see secondary colors; yellow, blue-green, and violet-ultraviolet, but also secondary hues “from mixing spectral lights: purple (red and violet), “bird's purple” (red and UV), and “green purple” (green and UV)” (Burkhardt, 1989). Unsurprisingly, birds have more than twice the number of cones than humans, with bird cones having maximum spectral sensitivity ranging from 370, 450, 480, and 570 nm (Chen and Goldsmith, 1986; EverGreen, 2011).

Gibson and others proved that birds identify shapes in the same non-accidental manner as humans, using pigeons as a model (2007). Further, laying hen chicks were able to differentiate between visual textures of the same color (Jones and Osorio, 2004). In stark contrast to humans, birds rely more on high-velocity head movements (jerking, bobbing, thrusting) than movement of the eye itself (Pratt, 1982). Dawkins and Woodington (2000) identified an interesting visual pattern in birds; individual laying hens always approached a decorated block using the same path and viewed it at consistent distances with similar head movements/angles from the same eye, even on different occasions. These results indicate that birds use “active vision”, because although the head distance and movements were consistent, the small movement of the actual eye varied. Dawkins (2002) studied laying hen head movements while approaching another hen, food, or a novel object; a tendency to move from lateral to frontal viewing as the bird came closer to the conspecific or object was identified. Birds also showed a huge range of head movements while approaching that are difficult to interpret, but may indicate that hens were identifying the other bird or object of interest with different parts of the eye and completely different eyes.
Vision-Related Behavior

Birds use their advanced color vision to identify plumage colors in conspecifics, and can identify colors in brown or green plumage that may serve as environmental camouflage to the human eye (Finger and Burkhardt, 1994). Different colored bird feathers maintain a vast range of UV reflectance, light-absorbance, iridescence, velvetiness, shininess, etc., only seen by other birds, as humans can see a comparatively small degree of this spectrum (Burkhardt, 1989). The implications of this variety need further research regarding their biological significance. Beyond this, there are three proposed major functions of UV vision in birds: 1) orientation, 2) foraging, and 3) sexual selection (Bennett and Cuthill, 1994).

There is some evidence that birds in the wild may use UV wavelengths to orientate themselves relative to the sun. There is a “yellow field” in pigeon retina that is associated with panoramic visual function, along with evenly dispersed cones, “consistent with a system designed for detecting large-scale gradients across the sky” (Bennett and Cuthill, 1994). However, this may not be reflective of all bird species and more investigation is needed to solidify this theory. The second proposed major function is UV vision-orientated foraging. There is strong evidence for this, considering that fruits, seeds, moths, and butterflies (bird food objects and prey) are UV reflective, while non-foraging materials (e.g., leaves) are not. The last UV function, and most established, is sexual signaling, tied in with the incredible range of feather colors discussed above. Feather color and even ornaments are UV reflective, and are used as a cue for potential mates or competitors within species. In research, birds prefer potential mates seen through UV-transparent plexiglass versus UV-opaque material, indicating the importance of UV vision and feather reflectance on conspecific identification (Bennett and Cuthill, 1994). In light of this broad range of UV light-related natural bird behavior, some researchers have
suggested that incorporating a UV lighting program into commercial poultry barns has the potential to improve welfare (Prescott, et al., 2003).

In domestic poultry, untrained laying hen chicks have shown preference for orange over blue and red over green (Ham and Osorio, 2007). In another study, laying hens showed an initial, untrained preference for novel colors above even food-reward associated colors (Olsson and Kelber, 2017). This preference for novelty is expected in a naturally foraging species, who aim to reduce uncertainty in their environment and are interested in new feeding opportunities (Inglis, et al., 2001). Furthermore, it has been well established in the literature and in this review that birds are visual and social foragers who rely on sight-driven behavior to eat (Fernandez-Juricic, et al., 2004). Young broiler chicks show an equal propensity to peck at small particles, whether they are food or not, when food is otherwise provided. However, if food is withheld, chicks will learn by 3 days of age to peck at food more than non-food particles (Hogan, 1973). The importance of sight in poultry was highlighted by Collins et al. (2011), who compared the behavior of blind versus sighted/control broilers. The authors found that blind birds ate less and were lighter, indicating difficulty in feeding without sight. Blind broilers also showed reduced activity, and carried out abnormal behaviors, including pecking at the air and walking in circles, possibly a presentation of misdirected foraging behaviors.

Summary

This review has covered the current state of broiler chicken welfare and highlighted two primary concerns: leg/skeletal disorders and contact dermatitis. Leg disorders are driven by genetic advancement for a fast rate of growth leading to poor locomotion. Nearly one third of commercially produced broilers are affected by lameness. Evidence shows that these disorders are painful, cause suffering, and ultimately lead to increased inactivity and time spent lying down.
on the litter. This increased litter contact is associated with contact dermatitis, a condition where the breasts, footpads, and hocks of birds can become inflamed, scabbed, or ulcerated, and is believed to be painful. Both conditions are associated with the environmental state (litter moisture, ventilation, stocking density, etc.), and may be mediated with environmental enrichment and exercise. However, environmental enrichment is currently not required by the NCC audit guidelines (U.S.) or the Welfare Quality assessment tool (EU).

Regarding broiler bird environmental enrichment, several options have been explored that fall into the five different categories of enrichment (social, nutritional, occupational, physical, and sensory). Perches, platforms, panels, and barriers have shown some success in reducing litter contact and improving skin condition, but do not serve to increase physical activity and in the case of perches, become consistently less used as the broilers age. Foraging materials and substrates have, in some cases, shown the capability to increase natural foraging behavior and activity, but in others have had no effect (whole wheat scattered), increased inactivity (sand), or reduced performance (feed scattering).

Novel objects used to stimulate pecking behavior such as strings, toys, and plastic bottles have shown inconsistent results. In some cases, they have no effect, and in others they increase activity temporarily or reduce fear responses. These sorts of objects have not been shown to enhance performance. Sensory enrichment has been decidedly less-studied but has shown potential in the form of auditory enrichment to reduce stress and increase feed intake in broilers. Visual enrichment thus far has been less successful and even less studied; an ambient red-light treatment served to increase activity but hindered performance, and only one study has reported using moving spotlights, but saw no changes in broiler behavior. Further, there is lack of quantification of broiler attention span or habituation to enrichment.
Despite the general lack of vision-related enrichment for broiler chickens, it has been well-established that vision drives most bird behaviors and is particularly important in feeding and foraging patterns of the chicken’s red junglefowl ancestor. This review has described the unique tetrachromatic vision of birds, allowing them to see into a fourth, UV, spectrum, and the importance UV vision serves in not only food-finding, but in spatial orientation and identification of conspecifics. Thus, considering UV lighting programs for commercial poultry may be an option to improve captive bird welfare.

Overall, this review has identified a lack of: animal behavior- or enrichment-based measures in welfare guidelines, knowledge on the attention-span of broilers and habituation to environmental enrichment, visual-foraging and moving light-focused enrichment, and UV lighting programs for commercial poultry.

Literature Cited


CHAPTER 3: DEVELOPMENT AND VALIDATION OF BROILER WELFARE ASSESSMENT METHODS FOR RESEARCH AND ON-FARM AUDITS

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Abstract

Required auditing of on-farm broiler welfare in the U.S. has increased; however, a lack of validated tools exists for assessment of enrichment. National Chicken Council (NCC) guidelines were used on a subset of 300 Ross 308 broilers out of 1200 to validate and adapt welfare measures. Half of the broilers were exposed to environmental enrichment, hence these measures were used to evaluate the enrichment within the context of behavior and welfare, although the nature of the enrichment is not described in detail here as the aim is to serve solely as a description and validation of methods using a subset of example data. Birds were recorded in repeated 4-min periods to quantify behavior and walking distance. Outcomes were categorized to improve auditing and make recommendations to producers and researchers. Bone mineral density, content, and breaking strength were successful in determining numerical differences. Quantifying lameness using an enclosed walkway and measuring toe pad dermatitis weekly are recommended on-farm. We recommend including additional measures not required by the NCC: monitoring breast condition in the flock and including a behavior component with a scoring ethogram.
Key words: welfare, broiler, enrichment, lameness, behavior

Introduction

On-farm welfare audit guidelines provide expectations that animal producers must comply with. Guidelines for broiler chickens exist internationally within the Welfare Quality® assessment tool (2009) and European Food Safety Authority Council Directive 2007/43/CE (2007). In the U.S., auditing guidelines currently exist for swine (Common Swine Industry Audit, National Pork Board), dairy cattle (National Dairy FARM Animal Care Program, National Milk Producers Association), beef (Beef Quality Assurance, National Cattlemen’s Beef Association), sheep (Sheep Care Guide, American Sheep Industry Association), turkeys (Animal Care Guidelines, National Turkey Federation) and laying hens (Animal Husbandry Guidelines for U.S. Egg-Laying Flocks, United Egg Producers). Animal welfare audits for cattle, pigs, and chickens that use the Hazard Analysis and Critical Control Point principles with animal-based outcome measures have been developed for use on-farm (Grandin, 2017) and at the slaughter plant (Grandin, 2010). The National Chicken Council (2017) publishes guidelines for U.S. producers that are used nationally to audit personnel training, hatchery operations, grow-out operations (management, nutrition, comfort and shelter, health care monitoring, and flock husbandry), catching and transportation, and processing commercial broilers. Missing from the NCC guidelines is environmental enrichment (EE) and methods to assess EE options. EE is defined as “an improvement in the biological functioning of captive animals resulting from modifications to their environment” (Newberry, 1995). Commercial broiler farms do provide the birds with social enrichment (direct contact with conspecifics), physical enrichment (litter substrate), and nutritional enrichment (*ad libitum* access to feeders and water). Lacking in broiler
barns are occupational enrichment, which includes enrichment that stimulates exercise, and sensory enrichment, including visual stimuli (2010).

Therefore, the objectives were to (1) test and modify broiler welfare measures and (2) make recommendations on adding welfare measures to the NCC guidelines to measure effects of EE during the broilers’ lifespan.

**Materials and Methods**

All live bird procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

**Animals and Housing**

Twelve-hundred straight run Ross 308 broilers were housed in 1.2 by 2.4 m pens with 30 birds/pen (0.10 m²/bird). Birds had *ad libitum* access to feed and water throughout the 42-d growth cycle and were housed on fresh pine shavings over concrete. At placement, birds were exposed to 23h light:1h dark and gradually moved to 20h light: 4h dark over the growth period. Twenty pens were exposed to environmental enrichment in one room of the barn, and the other 20 served as control pens in the opposite room of the barn. The environmental enrichment utilized was a novel device that projected 2 laser dots onto the floor of 2 adjoining pens. The device aimed to stimulate vision-oriented active behavior in the birds, thus was a suitable treatment to validate a variety of welfare measures. Environmental conditions, diet, and management were kept identical between both rooms. A subset of 5 pens from enriched treatment and 5 pens from control each were chosen at random for videography. Within each of those pens, 5 randomly chosen birds (50 focal birds total) were marked with animal-safe food coloring on the back of the head/neck on d0 for behavioral analysis and collection of animal-based welfare outcomes. Focal bird colors included red, green, blue, purple, and black; the red
and blue-colored birds, respectively, were observed for broiler home pen behavior and walking distance measures. Data from the 10 randomly selected video-recorded pens (n=5 pens/treatment) are presented here (n=300 broilers).

**Video Camera Set-up and Training**

One Sony HDR-CX440 Handycam (Sony Corp. of America, New York, New York) was affixed above each video-recorded pen (10 video-recorded pens total) using brackets adjusted to capture the entire pen. Filming occurred in real-time (30 fps) once weekly. Video-watching observers were trained to analyze broiler home pen behavior and walking distance by an individual with previous animal behavior observation experience to 90% agreeability using 4-min video clips used for analysis. Clips analyzed were from four 4-min enrichment periods every 6 hours starting at 05:30 from each day recorded, one day/week, wk 1-6.

**Welfare Measures**

**Broiler bird home pen behavior**

Observers watched the red focal bird during 4-min enrichment periods (4 periods/day; 05:30, 11:30, 17:30, 23:30). Trained observers categorized focal bird behavior continuously during the 4-min clips using a pre-determined behavior ethogram (Table 3-1) on d2, 9, 16, 23, 30, and 37. Behavior data was collected in frequency and duration of time per behavior; duration was converted to percent of time during 4-min periods, and results are expressed as mean percentages by week.

**Walking distance**

The distance in centimeters walked by the blue focal bird was measured over the 4-min enrichment periods one day/week. The observer taped a clear sheet protector over the computer
screen and watched each minute individually. At the beginning of each minute, the video was paused and the observer drew a line at the bird’s beak. Video was resumed, and if the bird moved the video was paused and a line was drawn at the new position of the beak where the bird stopped. Next, the observer used a ruler to draw a line connecting each stopping mark. After drawing the interconnecting line, the observer opened Adobe Photoshop (Adobe Systems Inc, San Jose, California) with a pen template image. The observer used a known length within the pen (58.4 cm between two segments of the water line, measured on-farm) to standardize the custom ruler tool on Photoshop, measured in pixels (58.4 cm= approximately 194 pixels). The tool would then equate x number of pixels to calculate distance walked. The observer placed the clear sheet protector over the template image and used the custom ruler tool to measure the lines. This was repeated for each individual minute and then a sum of all line measurements over the 4-min period was calculated.

**Walking lameness**

Focal birds (n=50) were removed from their home pens once weekly and assessed for lameness. Two researchers conducted the lameness test, with one researcher assigning scores. Birds were placed on a custom-designed plywood runway 1.80 m long and 0.46 m wide, with 0.30 m tall walls on all sides. The runway had 0.15 m start and finish sections, a 1.5 m walking space, and delineations marking every 0.30 m and 2.5 cm. Birds were placed on the runway starting section. Birds either moved the 1.5 m independently or were encouraged to walk with a ping-pong paddle and/or the researcher’s gloved hand waving behind the birds and/or gently tapping on their backs. Scores were assigned using a 0-2 scale adapted from NCC guidelines where 0 indicated the ability to walk 1.5 m with no signs of lameness, 1 indicated the ability to
walk 1.5 m but showed unevenness in steps or stopped and sat down at least once, and 2 indicated a bird that could not walk 1.5 m; distance walked was recorded.

**Human-approach paradigm**

Prior to the Human-approach paradigm (HAP), two researchers determined the optimal bracket angle and camera location for each pen, so that one image captured an entire pen. Colored tape identified bracket location; locations ranged between 47.75-59.00 cm measuring out from the central PVC pipe. The HAP was completed once during wk 1 and once during wk 6 beginning at 09:00, and the pen order was kept the same each week (n=10 pens, all 30 birds/pen). The barn was emptied of personnel apart from the two researchers carrying out HAP. The HAP image was taken with a hand-held camera (Pentax Optio W90, Pentax Imaging Company, Golden, Colorado), and the camera’s focal length was 28 m with a 12.1-megapixel resolution.

Methods were based on swine nursery work completed by Weimer et al. (2014). Briefly, Researcher A was defined as an unfamiliar human in the pen and Researcher B placed the camera/bracket and took the image. Researcher A wore different colored coveralls than the rest of the research and farm crew, but the same boot covers. The researchers approached each pen quietly and recorded the number of birds per pen. Researcher B positioned the bracket on the pen’s side in the pre-determined location and then Researcher A stepped over the front of the pen with a stopwatch in their right hand. Researcher A walked to the side of the pen opposite Researcher B and crouched facing the camera with their body angled towards the birds and both arms held close to the body. Once in position, Researcher A began the stopwatch, avoiding looking at the birds for 15 s. After 15 s, Researcher A stopped the watch and looked up at the birds. Researcher B took an image at the precise moment Researcher A looked up. One student observer, trained using the same methods as video observers but with wk 1 HAP images,
reviewed the images. Within each digital image of individual pens, broilers were classified into two categories: Interacting or Not Interacting. Birds classified as Not Interacting were further categorized into three mutually exclusive behaviors (Table 3-2). Figure 3-1 displays an example image from wk 1 showing relative researcher and camera position, including different bird behavioral classifications. The number of birds in each category was converted to percent for clarity.

**Bird location within home pen.** Using the HAP images, bird location within the home pen relative to the unfamiliar human was determined. Each pen had vertical PVC pipe supports every 0.61 m along the back divider. These supports were used in HAP image analysis to divide the home pen into four equal sized quadrants (0.61 by 1.22 m). Quadrants were identified as 1, 2, 3, and 4, with 1 being the pen quadrant containing Researcher A, and 4 being the furthest from Researcher A. A clear sheet protector was affixed onto the computer screen with tape. Using a ruler and marker, each pen was divided into fourths using the PVC pipe supports to count the birds present in each quadrant. A bird was assigned to the quadrant of the pen that its beak was present in; number of birds is presented as percent in each quadrant.

**Physical Measures**

**Bone parameters**

On d42, focal birds were euthanized using carbon dioxide and the right tibia was collected from each bird and frozen (-20°C). Tibia were thawed overnight, weighed, and scanned using dual energy x-ray absorptiometry (DXA, Hologic, Marlborough, Massachusetts). The bones were scanned in groups of seven using the “rat whole body scan” protocol for bone mineral density and bone mineral content. Bone breaking strength of focal bird tibia was measured using the tensile test and compression method on an Instron 3367 Universal Test
Machine (Norwood, Massachusetts). The machine had a 30 kN load capacity and two platons controlled to fracture the bone between them. Each tibia was kept in a plastic bag and wrapped in cheesecloth to prevent contamination of the machine or slippage due to the bag. Tibia were placed on the bottom platon with the lateral/medial condyle end of the bone intentionally placed over the edge out of reach of the platons and the bend of the tibia facing down. The test was set up so that the top platon moved vertically downwards towards the bone at a rate of 10 mm/min and a 15% rate of load. The machine was stopped at the distinct rapid decline in force visualized on the monitor and simultaneous sound of the bone fracturing. Load (kgf) was recorded at the point of break and divided by area (cm$^2$) of the tibia to calculate breaking strength.

**Breast blisters and footpad dermatitis**

Once weekly focal birds were examined in their home pens for breast blisters and footpad dermatitis. Both examinations took place at the same time and were done by the same researcher. Footpad dermatitis was scored pass/fail using the American Association of Avian Pathologists (AAAP) Paw Scoring system where a normal yellow color or slight discoloration with thickening/hyperkeratosis on an area less than one half of the footpad was scored a pass, and erosions, ulcerations, scabs, hemorrhages, and/or swelling on an area greater than one half of the footpad was scored a fail. Breast blisters were scored on a present/absent basis adapted from Greene, et al. (1985) where blisters were considered present when a blister was equal to or larger than 0.5 cm$^2$, when there were one more breast burns, or when there were scabs on breast skin. A brownish-colored scab was considered “mild” and an ulcer with black exudates was considered “severe”.
Air and litter quality

Ammonia in the air (ppm) was measured at bird height weekly in the front, middle, and back of each room in the barn with a hand-held ammonia reader (GasAlert Extreme, BW Technologies, Schaumburg, Illinois) and ammonia test strips. Litter quality was analyzed weekly according to the NCC Audit Guidelines: litter moisture was evaluated in three different pens in each room of the barn (front, middle, and back). One handful of litter sample was gathered from three sections excluding within 15 cm of the water line of each randomly selected pen. Litter quality was scored pass/fail by the same researcher weekly; to pass litter must be “loosely compacted when squeezed in the hand. If the litter remains in a clump when it is squeezed in the hand, it is too wet” (NCC, 2017).

Statistical Analysis

For the subset of data used to validate methods here, the experiment used a complete randomized design where birds were randomly assigned to pens upon arrival, and where the individual pen was the experimental unit (enrichment applied to pen). All data were analyzed using SAS software version 9.4 (SAS Institute Inc.; 2016). PROC UNIVARIATE was used to assess the distribution of data prior to analysis; this procedure is recommended particularly with behavior data as it often not normally distributed. Home pen behavior, walking distance, and HAP data were skewed to the left (Poisson distribution), thus were analyzed using PROC GLIMMIX. GLIMMIX fits models to data with non-constant variability, correlations, or that are not normally distributed. In this example, the fixed effects were treatment, week, and treatment by week interaction. Walking lameness data were analyzed using PROC FREQUENCY and CHI SQUARE to observe the distribution of lameness score by treatment.
Bone mineral density and content of tibia data from DXA scanning and bone breaking strength data from Instron compression were normally distributed and thus were analyzed using PROC MIXED, a mixed linear model, with treatment as a fixed effect. PROC PRINCOMP was used to test for correlation between bone parameters. Air quality measures (ammonia, ppm) and litter quality (pass or fail) were analyzed using PROC FREQUENCY to determine the distribution of readings by treatment. For all measures, a value of $P \leq 0.05$ was considered significant and $P$-values from the differences of Least-Squares Means were obtained.

**Results and Discussion**

**Welfare Measures**

**Broiler bird home pen behavior**

The NCC grow-out operations guidelines state that “*birds should have space to express normal behaviors such as dust bathing, preening, eating, drinking, etc.*” but do not include specific recommendations on how to measure behavior or further define what is normal for the broiler. Utilizing video recordings and a specific behavior ethogram, we were able to successfully quantify both frequency and duration for natural broiler behaviors. Similar ethograms used in research have been successful in measuring the effects of different lighting, dietary, and enrichment treatments on broiler behavior (Kristensen, et al., 2007, Mahmoud, et al., 2015, Ventura, et al., 2012). In the work used as an example to evaluate methods described here, measuring duration unit, the length of time in seconds the behavior was performed, and converting it to a percentage of bird time engaged in a specific behavior over a 4-min period was determined to be the gold standard. This is comparable to determining time-budget, a method used successfully in broiler research (Weeks, et al., 2000). Results from the broiler home pen behavior measure showed that the week by treatment interaction was highly significant.
(P<0.0001) in all behavior categories. A subset of the behavior data are presented in Figure 3-2A-C to validate efficacy of the ethogram in quantifying and detecting differences between treatments.

Furthermore, simplification of behavioral categories will provide an easier transition from research to commercial application. For example, in this study categories were labelled as “active” versus “inactive” rather than “sitting”, “lying”, “standing”, “walking”, and “running”, (Table 3-1). Video recording allows for accurate and detailed assessment which suits a research environment but may not be practical for commercial producers and auditors to implement unless security cameras are used in the barn and could be analyzed for key behavior outcomes in a simplified manner. Producers with security cameras may be able to adopt or select behavior ethograms specifically of interest to their situation to determine if EE works for their flock, taking measures such as time spent interacting with the enrichment, time spent at feeder, longevity of the enrichment (in the case of straw bales), etc. Therefore, measures need to be objective, robust, and repeatable during a live assessment/audit and across flocks.

Another element validated through this research is that randomly selected focal birds from each pen will give an accurate reflection of the entire population. Thus, auditors should use a randomly selected subset of birds in a barn and live-score using an ethogram to identify deviations from normal activity and behavior. This provides a practical, quick, efficient but accurate way to score welfare on farm.

**Walking distance**

The walking distance method was loosely based on the “walking speed” methodology used by Dawkins, et al. (2013), whereby bird’s walking speed for 10 consecutive seconds was measured using video analysis. Measuring distance walked rather than speed better allowed us to
observe the environmental enrichment effect on physical activity. This novel measurement proved to be effective in further quantifying bird physical activity in their home pen and is strongly recommended to researchers. Results showed that there was a highly significant week by treatment interaction (P<0.0001) on the total distance walked by focal birds during enrichment periods and validated that the novel measure described here was successful in quantifying distance walked by broiler chickens (Figure 3-3).

Not only could this measure be valuable in studying the effects of specific treatments, but it could be applied to assessing lameness in a situation where birds are not disturbed by humans or encouraged to walk but rather are walking of their own volition on commercial farms. Auditors/producers could estimate distance walked in a simplified method using known lengths in the barn and observing unprovoked broilers, or even using precision livestock farming methods of video or audio recording previously used to track growth and age (Fernández et al., 2016, Fontana et al., 2016). Based on the effectiveness of the measure used in this example, a modified assessment on-farm is recommended to producers.

**Walking lameness**

The NCC guidelines require that broiler gait monitoring must be included in each flock’s health and welfare monitoring plan. The guidelines also recommend that auditors gait score 100 birds out of the flock within one week of slaughter, but this measure is not required. The NCC audit lameness scores are assigned using the U.S. Gait Scoring System on a 0-2 scale with 0 being a bird that shows no signs of lameness, 1 being a bird appearing uneven in steps, and 2 being a bird unable to walk 1.5 m without sitting down/obviously lame broiler; only scores of 2 are to be recorded. This method could be improved upon by utilizing a lameness assessment structure like the one described here. The low-distraction enclosure with a specific goal length
for broilers to walk allows for a greater amount of accuracy and consistency between birds. This enclosure would be simple and inexpensive for producers to implement into their gait monitoring plan. Results from the subset of focal birds scored weekly in our study showed that under our research conditions, most birds were not lame (Table 3-3), and the effect of treatment was not significant on lameness score (P=0.272).

If use of a walkway is not preferred, an auditor could modify this measure by walking behind a broiler for a set number of seconds to encourage walking and record lameness score. This method would lack the detail of exactly what distance lame birds ceased walking and could be more prone to distractions, but birds would be free to move in any direction and lame birds could still be identified if unable to walk away. Additionally, lameness scoring by the auditor should be required for every flock considering the significant incidence of this health issue in broilers, affecting up to 30% (Nääs et al., 2018). Depending on the size of the flock, a set 100 birds/flock may not be adequate to quantify the scope of the problem; perhaps NCC guidelines should require up to 30% of the flock to be scored considering the prevalence of lameness. Severe lameness has been reported to be painful and if given the chance birds will self-medicate, choosing to eat an analgesic in their feed (McGeown et al., 1999, Danbury et al., 2000). Monitoring lameness prevalence in commercial flocks is recommended as a strict requirement in welfare-auditing guidelines.

**Human-approach paradigm**

Measuring animals’ responses to an unfamiliar human in the pen following this method has been successfully used in swine research to determine willingness to approach or fearfulness (Weimer, et al., 2014). Our use of this method showed that broilers, unlike pigs, were either too fearful of an unfamiliar person or lacked the motivation to approach or orientate towards a
person within 15 s of the human entering the pen. In our study, the percentage of birds interacting with the unfamiliar human (week by treatment P=0.280), Not interacting (week by treatment P=0.827), nor the percent of birds in each quadrant of the pen (week by treatment P>0.05 for all quadrants) was affected by treatment. Only the fixed effect of week was significant on Interacting behavior and on the location of birds within the home pen (second and fourth quadrants, Table 3-4). The differences by week are largely attributed to the increasing size of birds forcing them to use a greater area of the pen by nature.

The measure proved to be contradictory to normal chicken behavior unless some training is involved. Similar research in cage-free laying hens showed that a 2-wk handling period decreased the avoidance distance from humans and increased the number of birds a human was able to touch during a “touch test” (Graml, et al., 2008). While curious behavior might be natural in pigs, and avoidance behavior can be decreased in hens by handling, based on our research this test was not as successful in unconditioned broilers who normally avoid contact with humans, unfamiliar or not. It has been shown that increasing the number of dark hours can decrease broiler avoidance of humans (Bassler, et al., 2013), but producers are unlikely to increase dark hours/change their typical practices for this reason alone. Thus, this measure is not recommended in commercial broilers but could be applicable to other poultry species such as turkeys with differing normal behaviors, and under certain research conditions.

Physical Measures

Bone quality

Post-slaughter, NCC requires scoring 500 birds/flock for leg injuries (breaks, fractures, hematomas) on a pass/fail basis to record the number of injuries obtained during handling at the farm and processing plant. Guidelines do not recommend quantifying bone quality as a welfare
measure regarding the life of the bird pre-harvest. Measuring bone mineral density and content using post-mortem DXA is not practical for producers but is recommended in a research setting; it has been successfully validated to compare bone quality between treatments and lines of birds (Swennen, et al., 2004, Castro, et al., 2018). Bone mineral density, content, and breaking strength of the tibia from our subset of 50 focal birds are presented in Table 3-5. Our data indicate that the example enrichment treatment used here did not affect bone parameters, although the breaking strength derived from using the compression method of the Instron 3367 was numerically increased in enriched birds.

Bone breaking strength has been validated in research as a leg welfare measure for broilers (Shim et al., 2018), however, compression strength has been used less frequently in poultry. Bone mineralization is the source of compression strength in bone and may be less dependent on bone size than alternative bone quality measures (Rath, et al., 2000). Interestingly, while bone mineral content and density of the tibia were highly correlated in this study (r=0.835), bone breaking strength was weakly negatively correlated with bone mineral density (r=−0.293) and content (r=−0.491). However, in our case bone breaking strength was more successful in detecting numerical treatment differences, although not significant. Thus, bone breaking strength using a compression method is recommended to researchers comparing treatment effects (EE, diet, etc.) on quality of the tibia, but is impractical in a production setting.

**Breast blisters and footpad dermatitis**

The NCC welfare guidelines state that “footpad health must be assessed at the processing plant by the auditor” on a pass/fail basis. The auditor examines feet from 100 randomly-selected birds, or 200 feet, at the processing plant, and 90% of pads must pass. The
pass/fail AAAP paw scoring system is effective and recommended to producers; based on our research additional scoring on farm may also be valuable to track progression of footpad lesions weekly and allow the opportunity to prevent further ulceration and pain for the broiler before slaughter. At least 100 randomly selected birds, following AAAP paw scoring system, caught from different sections of the broiler house should be scored weekly to monitor the prevalence of the condition using an animal-based measure rather than only scoring the litter, although the condition of the feet will likely be reflective of the moisture in the litter. Apart from being an animal welfare concern, downgraded/blemished paws are also an economic loss for producers, as “large, unblemished paws have become a priority to poultry companies all over the world” (Shepherd, et al., 2010).

Interestingly, breast blister or irritation scoring is not included in NCC guidelines, neither at the farm nor plant level, despite the condition leading to downgrades of 15-30% broiler carcasses (Greene, et al., 1985). Breast blisters in poultry are the result of an enlarged sternal bursa, caused by continuous pressure or friction on the keel bone from litter as broilers place 60% of body weight on the keel when lying, causing the bursa to become swollen and filled with fluid (Nielsen, 2004). The condition is likely painful for the animal and can fester anaerobic bacteria, thus is an animal welfare and food safety concern (breast meat) that should be included in NCC guidelines and monitored along with footpad dermatitis on farm. Under our research conditions in this example, the birds were housed on clean, fresh bedding that was not representative of typical commercial litter conditions and lacks common pathogens found in reused litter. Thus, we did not observe breast blisters or footpad dermatitis and grant that these measures may be less useful in clean research conditions than in production, where they would be valuable.
Air and litter quality

The NCC guidelines state that ammonia must never exceed 25 ppm at bird height and that producers must have a “documented ammonia monitoring program” including corrective actions if this level is surpassed. In this trial, utilizing a handheld ammonia monitor and ammonia test strips proved effective in monitoring levels in the barn weekly; strips are recommended for ease of use but the monitor, although more expensive, provides an exact number and is more accurate than the range provided by the strips. Ammonia levels in our barn never exceeded 4 ppm in the enriched room and 8 ppm in the control room using the GasAlert monitor, and never exceeded a range of 15-20 ppm in either room of the barn using the strips. No treatment/room differences were detected.

The NCC litter moisture evaluation methods are recommended commercially as they were easy to follow and quickly identify issues with the litter. However, according to Fernandez, et al., monitoring broiler activity in different parts of the barn using an automated camera method may be adequate to monitor the subsequent litter quality (2016). Litter quality is crucial to audit as footpad lesions and hock burns are higher in flocks with wet, sticky, or crusted litter (Allain, et al., 2009). In our research conditions, litter in both rooms of the barn scored “pass” on all weeks 1-6. In a typical research setting, air and litter quality are important to measure but are generally less of a concern due to the high cleanliness of the research environment.

Conclusions and Recommendations

Environmental enrichment showing consistent success in stimulating active behavior and reducing leg lameness and contact dermatitis in broilers, and reducing aggression in broiler breeders, is lacking. This topic will continue to be important in research and to producers (Riber,
et al., 2017), thus consistent methods for measuring welfare and behavior outcomes are necessary. The National Chicken Council Animal Welfare Guidelines and Audit Checklist is considered the animal welfare standard for broilers in the United States. The guidelines are science-based recommendations that are reviewed and updated on an indefinite 2-yr review cycle to accommodate new scientific findings and changes in the broiler industry. The next review is scheduled for 2019. Measures, outcomes, and recommended applications are summarized in Table 3-6. Specific measures validated here are recommended to either include or adapt within the guidelines (Table 3-7) based on application in a research setting.

A simplified measure of home pen behavior without video recording to quantify deviations from normal is recommended to implement into commercial welfare auditing, but the more in-depth measure used here is recommended for research. If producers utilize security cameras in barns, they may be able to use additional behavioral-based outcomes. Gait scoring for walking lameness is recommended in current NCC guidelines but could be improved by using a walkway described here. This modification, along with making the measure a requirement for every flock, is recommended commercially to unify lameness scoring in a consistent setting. Under our conditions, very few birds ever received a score of 2, likely due to the high cleanliness and health of the research flock. Hence, while the measure is recommended in research when necessary, it was less effective than other measures in detecting differences between treatments and is generally more highly recommended commercially. Breast blister or irritation scoring on live birds is absent in NCC guidelines but should be included due to the prevalence and welfare concerns of this condition. Footpad dermatitis scoring using the AAAP paw scoring guide is currently recommended post-slaughter, but should be modified to include scoring on live birds throughout the production cycle. Air and litter quality measures were shown to be effective in a
research setting with no modifications, but are more vital in a commercial setting where birds are housed on re-used bedding and are generally exposed to higher ammonia levels.

Other measures used in this example are not practical for producer implementation but were useful in measuring the effects of environmental enrichment under our research conditions. Walking distance is an example of a novel measure that is highly effective and recommended for researchers but impractical for producers due to the time and technology required. HAP may be useful in certain research conditions studying fearfulness in broilers, but is not recommended to be included in NCC audit guidelines or used commercially due to the unlikelihood of unconditioned broilers to approach a human. The bone parameters used, including DXA scanning for bone mineral density and bone mineral content of the tibia, and bone breaking strength analysis using an Instron compression test, are valuable measures for researchers to study the differences in bone quality between treatments, but are clearly inapplicable to producers as broiler legs will be processed for human consumption.

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### Table 3-1. Adapted ethogram for focal broiler bird home pen behavior

<table>
<thead>
<tr>
<th>Measure</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active²</td>
<td>Bird legs were in a continuous forward motion (walking or running).</td>
</tr>
<tr>
<td>Inactive</td>
<td>Bird stood in one place or rested its abdomen on the litter, head</td>
</tr>
<tr>
<td></td>
<td>rested against chest, litter, or another bird, or raised [(Kristensen et</td>
</tr>
<tr>
<td></td>
<td>al., 2007)].</td>
</tr>
<tr>
<td>At feeder</td>
<td>Bird head over feeder circle, bird in feeder or bird stood on feeder</td>
</tr>
<tr>
<td></td>
<td>tray.</td>
</tr>
<tr>
<td>At drinker</td>
<td>Bird stood beneath drinker line.</td>
</tr>
<tr>
<td>Other</td>
<td>Dust-bathed or head/beak twisted around in contact with feathers or</td>
</tr>
<tr>
<td></td>
<td>any behavior not otherwise identified.</td>
</tr>
<tr>
<td>Out of view</td>
<td>Bird was obstructed or not observed due to being under the heat lamp or</td>
</tr>
<tr>
<td></td>
<td>inside the feeder and could not be seen.</td>
</tr>
</tbody>
</table>

¹All behaviors were collected as frequency (the number of times the behavior occurred during 4-min periods) and duration (defined as length of time behavior was exhibited in seconds)

²Activity was further quantified by counting the number of 0.6 by 0.6-m gridlines crossed by the focal bird during the 4-min enrichment period

³Bird behavior was measured continuously during 4-minute enrichment periods at 05:30, 11:30, 17:30, and 23:30
Table 3-2. Broiler behavior classification using a digital image analysis upon conclusion of human-approach paradigm (HAP)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interacting</td>
<td>Using a ruler and a clear sheet protector taped to the screen, a line was drawn from the midpoint of the bird’s head to the pen edge. If the line intersected with researcher A, or if any part of the bird was physically contacting Researcher A.</td>
</tr>
<tr>
<td>Not interacting</td>
<td>Birds not exhibiting the above two behavioral classifications.</td>
</tr>
<tr>
<td>Feeder</td>
<td>Bird head over feeder circle, bird in feeder or bird stood on feeder tray.</td>
</tr>
<tr>
<td>Drinker</td>
<td>Bird stood beneath drinker line.</td>
</tr>
<tr>
<td>Other</td>
<td>Laying (rested its abdomen on the litter, head rested or raised [(Kristensen et al., 2007)], preening (dust bathed or head/beak twisted around in contact with feathers), wings stretched out, piling (group of three or more birds pressed against each other and/or on top of each other, all bird heads facing away from the human in the pen and not performing any other discernible behavior [(Campbell et al., 2016)]), or not visible.</td>
</tr>
</tbody>
</table>

1Ethogram based on Weimer et al. 2012
Table 3-3. Ross 308 broiler walking lameness score\(^1\) distribution by treatment over wk 1-6. Values presented as percentages of total scores (n=50 birds each scored 6 times, or 300 scores). A score of 0 indicated no lameness, 1 indicated a bird that showed unevenness in gait or stopped at least once, and a score of 2 indicated a lame bird unable to walk 1.5 m.

<table>
<thead>
<tr>
<th>Lameness Score</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>94.67%</td>
<td>94%</td>
</tr>
<tr>
<td>1</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>2</td>
<td>1.33%</td>
<td>0%</td>
</tr>
</tbody>
</table>

\(^1\)Broilers were gently encouraged to walk 1.5 m on a custom designed, plywood walkway in groups of 5 (wk 1) or 2-3 (wk 2-6)
Table 3-4. Percent±SEM of Ross 308 broilers \((n=300)\)\(^1\) in each behavior category following the Human-Approach Paradigm (HAP)\(^2\)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Week 1</th>
<th>Pooled SEM</th>
<th>Week 6</th>
<th>Pooled SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Enriched</td>
<td>Control</td>
<td>Enriched</td>
<td></td>
</tr>
<tr>
<td>Interacting</td>
<td>5.29</td>
<td>5.06</td>
<td>1.63</td>
<td>2.41</td>
<td>3.84</td>
</tr>
<tr>
<td>Not Interacting</td>
<td>94</td>
<td>94.67</td>
<td>4.34</td>
<td>97.26</td>
<td>95.95</td>
</tr>
<tr>
<td>Quadrants of the pen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>4.75</td>
<td>11.21</td>
<td>2.46</td>
<td>3.93</td>
<td>6.79</td>
</tr>
<tr>
<td>Second</td>
<td>4.29</td>
<td>3.03</td>
<td>0.86</td>
<td>22.18</td>
<td>24.82</td>
</tr>
<tr>
<td>Third</td>
<td>37.04</td>
<td>27.96</td>
<td>4.09</td>
<td>36.84</td>
<td>35.08</td>
</tr>
<tr>
<td>Fourth</td>
<td>59.45</td>
<td>61.36</td>
<td>4.93</td>
<td>39.19</td>
<td>35.84</td>
</tr>
</tbody>
</table>

\(^1\)A subset of 10 pens with 30 birds/pen were used on weeks 1 and 6 for the HAP

\(^2\)HAP behaviors were categorized 15s following an unfamiliar human entering the pen by using digital images and the ethogram described in Table 3-2
Table 3-5. Tibia parameters from a subset of 50 Ross 308 broilers (5 focal birds/10 video-recorded pens). Right tibia were collected on d42 for dual-energy x-ray absorpiometry (DXA) scanning$^1$ and bone breaking strength analysis$^2$. Values presented as LSMeans±SEM with the fixed effect of enrichment treatment.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Enrichment Treatment</th>
<th>Pooled SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density (g/cm$^2$)</td>
<td>0.13</td>
<td>0.13</td>
<td>0.005</td>
<td>0.725</td>
</tr>
<tr>
<td>Bone mineral content (g)</td>
<td>1.01</td>
<td>1.00</td>
<td>0.089</td>
<td>0.970</td>
</tr>
<tr>
<td>Bone breaking strength (kgf/cm$^2$)</td>
<td>9.22</td>
<td>11.08</td>
<td>0.766</td>
<td>0.124</td>
</tr>
</tbody>
</table>

$^1$Focal bird tibia were DXA scanned in groups of 5 using the “rat whole body scan” protocol for bone mineral density and content.

$^2$Bone breaking strength was determined using an Instron 3367 Universal Test Machine Compression Method; Load (kgf) recorded at point of break was divided by area (cm$^2$).
Table 3-6. Broiler welfare measures and applications

<table>
<thead>
<tr>
<th>Measure</th>
<th>Outcomes</th>
<th>Suggested application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler bird home pen behavior</td>
<td>Categorize behavior of birds within home pen</td>
<td>Commercial &amp; Research</td>
</tr>
<tr>
<td>Walking distance</td>
<td>Quantify level of activity in a specific time period</td>
<td>Commercial &amp; Research</td>
</tr>
<tr>
<td>Walking lameness</td>
<td>Quantify number and severity of lameness in the flock</td>
<td>Commercial &amp; Research</td>
</tr>
<tr>
<td>Human-Approach Paradigm</td>
<td>Quantify response to humans in the home pen</td>
<td>Research</td>
</tr>
<tr>
<td>Bone mineral density</td>
<td>Measure quality of material in the tibia</td>
<td>Research</td>
</tr>
<tr>
<td>Bone mineral content</td>
<td>Measure quantity of material in the tibia</td>
<td>Research</td>
</tr>
<tr>
<td>Bone breaking strength</td>
<td>Measure strength of tibia/resistance to fracture</td>
<td>Research</td>
</tr>
<tr>
<td>Breast blisters</td>
<td>Quantify number and severity of blisters in the flock</td>
<td>Commercial &amp; Research</td>
</tr>
<tr>
<td>Footpad dermatitis</td>
<td>Quantify number and severity of dermatitis in the flock</td>
<td>Commercial &amp; Research</td>
</tr>
<tr>
<td>Air and litter quality</td>
<td>Measure ammonia at bird height and moisture in the litter</td>
<td>Commercial &amp; Research</td>
</tr>
</tbody>
</table>
Table 3-7. Recommendations on NCC guidelines to broiler producers

<table>
<thead>
<tr>
<th>Measure</th>
<th>Recommendation</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler bird home pen behavior</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Walking distance</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Walking lameness</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Human-Approach Paradigm&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Breast blisters</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Footpad dermatitis</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Air and litter quality</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bone mineral density</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bone mineral content</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bone breaking strength</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

<sup>1</sup>Measure proved not particularly useful in broilers due to flight zone instincts unless under specific research conditions
Figure 3-1. Digital Human-Approach Paradigm (HAP) image used for evaluation wk 1 example with birds with different behavioral classifications circled and labeled 1-5:

1. Bird 1: Interacting; Bird 2: Not interacting; Bird 3: At drinker; Bird 4: At feeder; Bird 5: Other
Percent of time spent active by focal birds

Percent of time spent inactive

Week
Figure 3-2. Ross 308 broiler focal bird home pen behavior during 4-min enrichment periods in A) Active, B) Inactive, and C) At feeder categories. Results indicated that the methods and ethogram (Table 3-1) validated here were effective in categorizing and detecting differences between the control and example enrichment treatment. Values presented as week by treatment LSMeans± SEM

\(^1\)Video was analyzed one day/week (wk 1-6) during four 4-min enrichment periods. Behavior was recorded continuously during these periods as frequency and duration (s), then converted to percent of focal bird time.

\(^2\)Bars lacking a common superscript are statistically different (P≤0.05)
Figure 3-3. Total distance walked during 4-min enrichment periods by Ross 308 broiler focal birds wk 1-6. Results validate effectiveness of method in detecting treatment differences and quantifying real-life cm walked by broilers from video recording. Values presented as week by treatment LSMeans±SEM

1Video was analyzed one day/week (wk 1-6) during four 4-min enrichment periods

2Bars lacking a common superscript are statistically different (P≤0.05)
CHAPTER 4: A NOVEL ENVIRONMENTAL ENRICHMENT DEVICE IMPROVED BROILER PERFORMANCE WITHOUT SACRIFICING BIRD PHYSIOLOGICAL OR ENVIRONMENTAL QUALITY MEASURES

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Environment, Well-Being, and Behavior

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Abstract

Modern commercial broilers have been genetically selected for fast growth and heavy breast muscling, contributing to a top-heavy phenotype and increased leg lameness. A quick-growing phenotype coupled with poor leg health fosters inactivity. The objective of this study was to stimulate broiler movement using novel environmental enrichment and determine the impact of movement on production, leg health, and environmental parameters. Twelve-hundred Ross 308 broilers were housed in 40 pens with 30 birds/pen for 6wk in 2 separate rooms (laser enrichment or control). Each enrichment device was mounted above 2 adjoining pens, projected 2 independent, randomly-moving laser beams at the floor to stimulate innate predatory behavior, and was active 4 times daily in 4-min periods. Performance outcomes were calculated by pen and averaged per bird for each performance period and overall d0-42. Seventy randomly-selected focal birds were examined for breast blisters and footpad dermatitis each wk and euthanized on d42 for tibia quality measures. Air quality and litter moisture were sampled by wk. Laser-
enriched pens had greater average bird feed intake in starter (P<0.001), grower (P=0.004), finisher periods (P=0.004); and overall d0-42 (0.19 kg/bird; P=0.0003). Average bird weight gain was also increased in enriched pens in each performance period: starter (P=0.043), grower (P=0.001), finisher (P<0.001), and overall d0-42 (0.24 kg/bird; P<0.001). Enriched pens had improved feed conversion ratio (FCR) vs. control with a decrease of 0.03 FCR points in the grower (P=0.031), 0.18 points in the finisher (P<0.001), and 0.07 points overall (P<0.001). Enriched pens had higher ADG during starter (P=0.048), finisher (P<0.001), and overall (5.7 g/bird/ d; P<0.001). No differences were found in breast blister, footpad dermatitis, tibia, air, or litter quality measures (P>0.05). In summary, a novel enrichment device based on bird visual feeding and predatory instincts positively affected performance through decreased FCR and increased ADG without sacrificing external animal-based measures, tibia quality, or air or litter quality.

Key words: broiler, welfare, environmental enrichment, performance, sustainability

Introduction

Due to intense genetic selection, modern broilers weigh 4 to 5 times more than broiler lines from the 1950’s at the same timepoints, and are 2 to 3 times more feed efficient (Havenstein, et al., 2003; Zuidhof, et al., 2014). However, this selection for increased growth rate has contributed to up to 30% of modern commercial broilers being affected by leg lameness or poor locomotion (Knowles, et al., 2008; Bassler, et al., 2013). Lameness leads to increased time spent lying down, which in turn increases litter contact and could result in a higher breast blister occurrence and contact dermatitis (Weeks, et al., 2000; Nääs, et al., 2009; Bassler, et al., 2013). Further, lack of activity compounds lameness by negatively affecting bone strength, mass, and
ability to bear weight properly (Lanyon, 1993; Rath, et al., 2000). Contact dermatitis-driven tissue damage may be caused by urea in the litter generating ammonia, creating a chemical burn effect and is likely painful. The prevalence of this issue, affecting 21.87% of Ross 308 broilers in a recent year-long study, may be reflective of air and litter quality in the house (Haslam, et al., 2006; Dinev, et al., 2019). The National Chicken Council (NCC, 2017) Animal Welfare Guidelines and Audit Checklist for Broilers require that ammonia in the air never exceeds 25 ppm at bird height, and litter must be evaluated for friability and moisture.

Severe lameness hinders birds from accessing feed and water, and negatively impacts the industry economically, necessitating up to 2% culls in a $30 billion industry (Dunkley, 2007; USDA, 2017). In addition, studies have reported that broilers with severe leg lameness eat more analgesic-containing feed than healthy birds, and birds fed an analgesic diet showed improved speed of walking, indicating relief from pain and discomfort caused by leg abnormalities (McGeown, et al., 1999; Danbury, et al., 2000). The 2017 NCC broiler audit guidelines have recognized lameness as a detrimental welfare issue and recommend gait scoring 100 birds per flock to evaluate leg health within 1 wk of slaughter, and footpad scoring 200 paws at slaughter.

Past research in laying hens has shown that restricting exercise had a clear, negative impact on bird skeletal health (Shipov, et al., 2010), and work in broilers has shown that motivating physical activity increased tibia strength and decreased lameness (Reiter and Bessei, 2009). Prayitno and others (1997) concluded that activity driven by red light, early and late in the rearing period, improved locomotion compared to a blue light treatment where broilers were less active. Birds are visual feeders and prefer red and orange colors over green and blue (Ham and Osorio, 2007). Bizeray et al. (2002) studied the effects of red, blue, green, and yellow spotlights moving across the floor but did not see a change in broiler physical activity, and the authors
concluded that the spotlights moved too quickly. Baxter and others (2019) implemented perches and dust baths but did not see an effect of enrichment on foraging, play, or activity, nor, in a separate paper published on the same study, leg health (Bailie et al, 2018). Platform use by broilers in Norring et al. (2016) likewise did not increase overall activity. A study by Jordan and others (2011) showed that broiler activity and foraging was increased by scattering feed in the litter, but broilers in the enriched treatment had 13% lower weights at harvest.

Certainly, some forms of environmental enrichment have been shown to improve broiler welfare outcomes, as in Ventura et al. (2012), where barrier perches stimulated natural perching behavior and reduced aggressive interaction and rest disturbances compared to the control. Recent work by BenSassi and others (2019) showed that increasing environmental complexity was associated with fewer skin concerns, lower mortality, fewer underweight birds, a lower overall rejection rate at harvest, and less welfare problems overall. However, an enrichment option designed to motivate broiler activity and improve physical and performance outcomes is still lacking in the published literature. Thus, we developed a novel form of environmental enrichment designed to motivate physical movement through visual stimulation. The objectives of this work were to determine the impact on bird physiology (leg lameness and footpad and breast condition), performance, and environment (air and litter quality).

**Materials and Methods**

All live bird procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

**Animals**

Twelve hundred and sixty straight-run Ross 308 broiler chicks (day of hatch; BW 47.38± 0.14 g) were obtained from a commercial hatchery and transported to the Poultry Research and
Teaching Unit at Iowa State University (International Poultry Breeders Hatchery, Bancroft, Iowa). Twelve hundred were randomly assigned to treatment groups and the remainder were culled. A subset of 70 birds were randomly assigned upon arrival as focal birds, identified with wing-bands, and marked with unique animal-safe food coloring (red, blue, green, purple, and black; Wilton, Woodridge, Illinois). Half of the focal birds were assigned to laser-enriched pens, and half were assigned to control pens (n=5 focal birds/pen in 14 pens). Food coloring was applied to a cotton ball, rubbed on the back of the chick’s head and neck, and reapplied on an as-needed basis throughout the trial.

**Housing and Feeding**

Birds were housed in 40 floor pens (30 birds/pen) measuring 1.22 by 2.44 m across 2 rooms in the barn (20 pens/room). One room contained 20 enriched pens (exposed to laser device), and the other contained 20 control pens, with an anteroom separating the 2 so no crossover of enrichment device was possible. Approximately 10 cm deep fresh wood shavings provided bedding over the solid concrete floor, and PVC pipe dividers with mesh walls (1.22 m height) separated pens. High and low temperatures and humidity were monitored daily in the enriched and control rooms of the barn. Average temperatures are listed from the starter, grower, and finisher periods respectively from the enriched room: 85.47˚F, 77.39˚F, and 71.71˚F, and the control room: 85.53˚F, 77.46˚F, and 71.50˚F. Average relative humidity is listed from the starter, grower, and finisher periods respectively from the enriched room: 23.86%, 27.21%, and 33.93%, and the control room of the barn: 19.89%, 23.93%, 27.75%.

Birds were gradually adjusted from 24 h light on day 0-7 (30-40 lux) to 20 h light (20-30 lux) from days 8-42. Chicks were brooded with 2 heat lamps/pen (22.9 cm reflectors with porcelain socket) using 125-watt heat bulbs (Sylvania, Wilmington, MA) for the first wk. Birds
were fed an *ad libitum* diet formulated for Ross 308 commercial recommendations (Table 4-1) out of a hanging chicken feeder (BRHF151, Brower Equipment, Houghton, IA) gradually raised to accommodate bird height. Water was provided *ad libitum* from a hanging nipple water line (8 nipples/pen).

**Laser Enrichment Device**

A total of 10 laser enrichment devices designed and built specifically for this research were affixed over 20 pens in 1 room of the broiler barn. Each device was designed and calibrated to cover 2 adjoining pens. The enrichment device consisted of 2 independent red 650 nm lasers contained within a 20.5 by 20.5 cm metal box with a glass bottom mounted on a custom-designed structure made of 3 wooden beams (2.4 m height) raised above the pens. The lasers projected in the direction of the pen floor and moved in a random pattern at a variable speed between 7.6-30.5 cm/s for 4-min “laser periods”: 05:30 to 05:34, 11:30 to 11:34, 17:30 to 17:34, and 23:30 to 23:34 daily for the entirety of the trial. Overhead snapshots of the activated laser in the pens are shown in supplementary Figures 4-1A-D for day 2, 16, 30, and 37. As this device was novel, and there is no explanation of broiler attention span in the current literature, the 4-min length of laser periods was tested with the knowledge that it would need to be validated and may need fine-tuning in future studies. The decision to expose broilers to laser periods 4 times/d was based off work by Jones and others (2000), which showed that laying hens exposed to environmental enrichment in the form of strings for limited daily time periods (10 min), rather than constant exposure, maintained interest in pecking the strings for 14 weeks.
Performance

The 6-wk trial was separated into a starter, grower, and finisher period that were 2-wk in length. All birds in each pen were weighed as a group, and then focal birds were weighed individually at the start of each period to determine weight gain. Feed disappearance/intake (FI) was recorded throughout. Feed conversion ratio (FCR) and ADG were calculated by pen and averaged by number of birds in the pen.

Breast Blisters and Footpad Dermatitis

Focal birds were examined the same day each week of the trial by the same researcher, on a different day than birds were weighed, in their home pens for breast blisters and footpad dermatitis, with all birds examined on d42. Both examinations took place at the same day and time each wk and were done by the same researcher. Footpad dermatitis was scored pass/fail using the American Association of Avian Pathologists Paw Scoring system (2015), where a normal yellow color or slight discoloration with hyperkeratosis on an area less than 1/2 of the footpad was scored a pass, and erosions, ulcerations, scabs, hemorrhages, or swelling on an area greater than 1/2 of the footpad was scored a fail. Breast blisters were scored on a present/absent basis based on methods use by Greene and others (1985) where blisters were considered present when a blister was equal to or larger than 1.27 cm$^2$, when there were 1 or more breast burns, or when there were scabs on breast skin. A brownish-colored scab would be considered “mild” and an ulcer with black exudates was considered “severe”.

Tibia Quality

On day 42, focal birds were euthanized using carbon dioxide and the right tibia was collected from each bird and frozen at -20°C until further analysis. Tibia (n=70) were thawed
overnight, weighed, and scanned using dual energy x-ray absorptiometry (DXA, Hologic, Marlborough, Massachusetts). The bones were scanned in groups of seven using the validated “rat whole body scan” protocol for bone mineral density (BMD) and bone mineral content (BMC).

Bone breaking strength of focal bird tibia was measured using the tensile test and compression method on an Instron 3367 Universal Test Machine (Norwood, Massachusetts). The machine had a 30 kN load capacity and 2 platons controlled to fracture the bone between them. Each tibia was individually fractured in a plastic bag wrapped in cheesecloth to prevent contamination of the machine or slippage due to the bag. Each tibia was placed on the bottom platon with the lateral/medial condyle end of the bone intentionally placed over the edge, out of reach of the platons, and the bend of the tibia facing down. The test was set up so that the top platon moved vertically downwards towards the bone at a rate of 10 mm/min and a 15% rate of load. The machine was stopped at the distinct rapid decline in force (visualized on the monitor) and simultaneous sound of the bone fracturing. Load (kgf) was recorded at the point of break, and divided by area of tibia (cm², obtained from DXA scanning) to calculate bone breaking strength per manufacturer recommendations (Instron; Norwood, MA).

Air and Litter Quality

Ammonia in the air (ppm) was measured at bird height in the front, middle, and back of each room on one d/wk wk 2-6 with a hand-held ammonia monitor (GasAlert Extreme, BW Technologies, Schaumburg, Illinois) and ammonia test strips. The ammonia monitor was titrated every 14 days with an ammonia tank and provided an exact value, while the strips provided a range of 5 ppm. Litter quality was analyzed weekly according to the NCC Audit Guidelines. Litter moisture was evaluated in 3 randomly-selected pens in the front, middle, and back of each
room of the barn. One handful of litter sample was gathered from 3 sections; litter within 15 cm of the water line of each pen was intentionally excluded. Litter quality was scored pass/fail by the same researcher weekly; to pass litter must be “loosely compacted when squeezed in the hand. If the litter remains in a clump when it is squeezed in the hand, it is too wet” (NCC, 2017).

Statistical Analysis

In this experimental design, individual control pens (n=20) were considered the experimental units, and laser-enriched pens were analyzed as a group of 2 pens with a shared laser device (n=10). Room within the barn was confounded by laser treatment, thus was not included in the model, but environmental conditions, management, and feeding were kept as identical as possible between both rooms. All data were analyzed using SAS software version 9.4 (SAS Institute Inc.; 2016). PROC UNIVARIATE was used to assess the distribution of data prior to analysis. Performance and tibia quality data were normally distributed, hence were analyzed using PROC MIXED, a mixed linear model, with treatment as a main effect. Principal Component Analysis (PROC PRIN COMP) was used to test for redundancy and correlation within the bone quality measures, and then Multidimensional Preference Analysis (using PROC PRIN QUAL) was performed to visualize the correlation between variables and reduction to 2 components. Air quality measures were analyzed using PROC FREQUENCY and CHI SQUARE to determine the distribution and association of readings by treatment. For all measures, a value of $P \leq 0.05$ was considered significant and differences between means were detected using PDIFF.
Results

Performance

All performance measures, including FI, weight gain, FCR, and ADG were averaged per bird by each 2-wk performance period and overall (d0-d42). Feed intake was increased in laser-enriched birds in all periods compared to the control: 4% increase in the starter, P<0.001; 3.1% in the grower, P=0.004; 5.1% in the finisher, P=0.004; and 3.9% overall, P=0.003 (Table 4-2). Enriched birds had an increased intake of 5.52 kg/pen overall compared to the control (P=0.006). Weight gain was also increased in laser-enriched birds in each performance period when compared to the control: 2.6% in the starter, P=0.043; 5.5% in the grower, P=0.001; 13.8% in the finisher, P<0.001, and 7.9% overall, P<0.001, (Table 4-2). Enriched pens showed increased gains of 7.19 kg/pen overall compared to the control (P<0.001).

Enriched birds had improved FCRs compared to control birds with a decrease of 0.03 FCR points in the grower (P=0.031), 0.18 points in the finisher (P<0.001), and 0.07 points overall (P<0.001, Table 4-2). When averaged per bird, laser-enriched bird ADG was increased by 2.9% (P=0.048) in the starter period, 13.2% (P<0.001) in the finisher, and 7.9% overall (P<0.001, Table 4-2), and was increased overall on a pen basis when compared to the control (0.17 kg/day; P<0.001).

Breast Blisters and Footpad Dermatitis

Under our research conditions, no control or laser-enriched birds displayed breast blisters or footpad dermatitis.
Tibia Quality

DXA scan results of focal bird tibia showed no changes in BMC or BMD, although the enriched tibia were numerically higher in both categories compared to the control (Table 4-3). BMD and BMC were strongly correlated (Figure 4-1), as were BMC and tibia weight (r=0.720). Interestingly, BMD and tibia weight were only moderately correlated (r=0.479). Bone mineral content of the tibia and bird body weight were strongly correlated (r=0.677), but BMD and bird weight were again only moderately correlated (r=0.456).

Bone breaking strength, determined using the Instron 3367 Universal Test Machine compression method and reported as load (kgf)/area (cm$^2$), was numerically higher in enriched focal bird tibia than control, but the difference was not significant (Table 4-3). Bone breaking strength was moderately negatively correlated with tibia weight (r=-0.486) and bird weight (r=-0.325). The correlation between all bone measures can be visualized in Figure 4-2 using a Multidimensional Preference Analysis.

Air and Litter Quality

The birds started on fresh, dry litter that remained friable throughout, and litter scored “pass” in all pens for all 6 wk the birds were on trial. Ammonia strip readings were identical on both the enriched and control rooms of the barn weekly. Before birds arrived, the baseline ammonia levels were 0 ppm in both rooms of the barn. The averaged readings were wk 2, 5 ppm; wk 3, 5 ppm; wk 4, 10 ppm; wk 5, 10 ppm; and wk 6, 16.67 ppm. Variable readings only occurred on wk 6, with 2 readings of 20 ppm and 1 reading of 10 in each room of the barn.

Means from the ammonia monitor were compared using a simple frequency and chi square distribution with enrichment treatment as a fixed effect. Treatment effect was not
significant (P=0.112). In the control room of the barn, 60% of readings were 0 ppm, 20% were 3 ppm, and 20% were 8 ppm. In the enriched room, 60% of readings were 0 ppm, 20% were 3 ppm, and 20% were 4 ppm. Thus, according to the more accurate GasAlert ammonia monitor, the control room peaked at 8 ppm and the enriched room never surpassed 4 ppm at bird height.

Discussion

At the commercial level, improved FCR is arguably the most valued production trait as it translates to greater weight gain from the same or lesser amount of feed, a cost savings in production, and thus improved sustainability (Stenholm and Waggoner, 1991). Reducing FCR by 0.17 points could equal to more than a 5% decrease in feed costs (Emmerson, 1997), which account for >70% of the costs of broiler production (Banerjee, 1992). The laser enrichment device successfully decreased FCR by 0.18 points in the finisher period of our study vs. control. The increased weight gain of 0.24 kg/bird overall could be translated to between $0.71 to $1.39 more saleable product/bird, using current breast meat prices as an example (USDA, 2019). Improved feed conversion may be attributed to decreased maintenance requirements, or more energy partitioned towards growth (Urdaneta-Rincon and Leeson, 2002).

Laser-enriched birds showed significantly increased physical movement during laser periods (Meyer, et al., unpublished data). Increased physical activity has been reported to reduce leg disorder parameters in broilers (Prayitno, et al., 1997; Reiter and Bessei, 2009) and laying hens (Shipov, et al., 2010), but has also been associated with worse feed conversion (Akbar, et al., 1985), or no change in weight gain or FCR (Prayitno, et al., 1997; Reiter and Bessei, 2009; Ruiz-Feria, et al., 2014). Indeed, feed conversion in chickens may have a behavioral component, as was suggested in laying hen genetics research by Fairfull and Gowe (1979), but whether this
effect may be positive or negative based on exercise has remained unclear. Similar results to ours were seen by Sorensen and others, (2000) where broilers raised at a lower stocking density showed both increased weight gains and improved walking ability. It was hypothesized by Lewis and Hurnik (1990) that there is likely a “locomotory-neutral zone or comfortable upper limit” in broiler movement; meaning that there is an ideal activity level somewhere between the bare minimum distance traveled to access necessary resources and overexertion.

Researchers have successfully forced broilers out of the bare minimum range of movement by increasing distance or introducing barriers between feeders and waterers without compromising performance (Ventura, et al., 2012; Ruiz-Feria, et al., 2014), but other, non-resource-based methods have been less successful. For example, Bizeray and others (2002) tested wheat scattered on the pen floor and colored, moving spotlights but concluded that “forcing animals to exercise more…was more effective for increasing physical activity than was attempting to stimulate foraging activities”. Shields and others (2005) hypothesized that broiler exercise would increase, and leg lameness would decrease, when provided sand bedding, but they were unable to support this as birds rested and displayed more inactive behavior on the sand.

Thus, it appears that the success of the novel laser device in motivating broiler physical activity, while simultaneously improving FCR and ADG, is among the first to accomplish this goal. Further, we may speculate that the 4-min laser periods induced a suitable amount of physical activity without increasing maintenance requirements, hence sacrificing FCR or incurring negative changes to footpad quality, but different lengths of time would need to be tested to validate if this is the most ideal duration. The increased FI observed in laser-enriched pens may be attributed to the laser motivating the broilers to move, thus driving them towards
the feeders. It has been established by Yngvesson and others (2017) that when resting broilers are disturbed by their conspecifics (a common occurrence), they get up and walk away. We hypothesize that a similar effect was driven by laser-enriched birds, who were physically active at a level considerably greater than control birds at the same time during laser periods, triggering other birds in the pen to move and ultimately move towards the feeders, much like the presence of humans walking a commercial barn motivates broilers to rise and head towards feed. Yngvesson et al. did not record feeding behavior post-disturbance for comparison, but this hypothesis is supported by our finding that 71% of laser-enriched focal birds were at the feeder at least once either during or within 5 min following laser periods (Meyer, et al, unpublished data).

It is thought that most skeletal support is established in broiler birds by day 18, following “intensive bone formation to provide rapid mineralization”. However, bone porosity or density changes more slowly over time to support increasing bird weight (Williams, et al., 2000). Further, bone strength and mass are increased with activity (Rath, et al., 2000), and load-bearing bones need to develop bearing weight, or they will immediately fail to do so when given the opportunity (Lanyon, 1993). Thus, we hypothesized that the increased movement, activity, and growth seen in laser-enriched birds may have been reflected in improved tibia quality measures (Meyer, et al, unpublished data). DXA scanning has been used successfully in broilers to measure BMD and BMC (Swennen, et al., 2004; Shim, et al., 2012; Castro, et al., 2019), and bone breaking strength has been used to detect treatment differences as well (Rowland, et al., 1970; McDevitt, et al., 2006; Shim, et al., 2012). Bone mineral density is believed to be reflective of mineral content (Rath, et al., 2000); our highly correlated (r=0.857) BMD and BMC
values agree with this. Further, BMD and BMC of the tibia obtained from DXA scanning were within normal range, similar to values seen in broiler tibia by Shim and others (2012).

Body weight, tibia weight, BMD, and BMC collectively explain 71.25% of the total experimental differences seen in these variables (Figure 4-2). Body weight and tibia weight positively influence overall bone measures and tightly cluster, while BMC and BMD cluster with an overall weak negative influence on the collective bone-related outcomes. Although BMD is commonly used as an indicator of bone strength, as bone mineralization is believed to provide compression strength (Rath, et al., 2000), our data indicate a weak negative correlation between BMD and BMC of tibia with bone breaking strength (Figure 4-2), an outcome that was not expected based on previous work in poultry (Leterrier and Nys, 1992). However, work done in humans (Divittorio, et al., 2006) has indicated that increases in bone density are not consistently correlated with decreased occurrence of fractures, and work in non-human primates (Vahle, et al., 2015) stated that BMD is not always indicative of whether bone will fail in “repetitive loading, as in a stress fracture, or when subjected to high impacts”.

Our bone breaking methods using compression and a constant rate of load would have reflected this repetitive loading, and may contribute to the lack of correlation seen between these bone quality measures. Further work is necessary to validate this unexpected, negative correlation. It is important to note that BMD, BMC, and breaking strength were each numerically higher in laser-enriched birds. These outcomes have previously been seen by Shim and others (2012) in fast growing broilers compared to slow growing, but when analyzed in terms of body weight of the birds, the slow growing birds ultimately had better bone quality. However, in our study the body weight of birds was weakly negatively correlated with bone breaking strength,
(r=−0.325), indicating that this measure was not simply reflective of tibia weight or bird weight, but rather a possible true numerical increase of bone quality in the enriched birds.

Commerically, contact dermatitis including breast blisters and footpad dermatitis necessitates downgrading of 15-30% of broiler carcasses/wk (Greene, et al., 1985). Breast conditions range from “mild” brownish colored scabs to “severe” exudate and litter filled ulcers that are aggravated because broilers rest 60% of their body weight on the keel while lying (Nielsen, 2004). Footpad dermatitis is a similar condition, but on the plantar surface of broiler feet and toes, with symptoms of inflammation and necrotic lesions. This is an obvious animal welfare concern, but also represents a considerable economic loss to the industry, where paws are “the third most important economic part of the chicken behind the breast and wings…accounting for approximately $280 million a year” (Shepherd and Fairchild, 2010). However, in our clean research environment, neither of these conditions occurred. Other researchers have assessed these on commercial broilers at the slaughterhouse to gather a true representation of the issue (Allain, et al., 2009), and considering that our birds were housed on fresh pine shavings with a relatively lower stocking density and number of birds than a commercial broiler house (0.24 m²/bird recommended by the NCC; 0.33 m²/bird provided), we were not expecting a high occurrence in our flock. However, we were able to successfully show that the enrichment device did not negatively influence the birds’ health by worsening breast or feet condition compared to the control.

Tied in with breast blisters and footpad dermatitis are air and litter quality. These conditions may be caused by ammonia, originating from urea in the litter compounded by mixing with leaked water from drinkers, causing a chemical burn effect. Haslam and others (2006) showed that percent of birds with footpad concerns was correlated with ammonia concentrations
in the house and litter moisture, although they did not find this association with breast burns. In the current study, ammonia levels were low, peaking at 8 ppm and 4 ppm on the control and enriched rooms of the barn, respectively, using the GasAlert monitor. This is well within the acceptable range of up to 25 ppm accepted by the NCC audit. Likewise, litter remained dry and friable throughout the 6 wk in both rooms of the house. These outcomes indicate that the larger, more active laser-enriched birds were not generating more moisture in the litter from increased waste or stirring up greater quantities of ammonia over the course of the experiment.

Regarding impact of the laser on bird welfare, data from this study have thus far provided no evidence that animal welfare was negatively impacted by laser enrichment. There was not an increase in lameness, dermatitis, nor mortality; nor did we see a decrease in body weight, tibia quality, or environmental conditions due to laser treatment. In the behavior companion paper (Meyer et al., unpublished data) the Human-Approach Paradigm was utilized as a measure of fearfulness. Results showed that a greater number of laser-enriched birds were closer to the unfamiliar human in the pen during wk 6 of the trial than the control birds, hence an increased fear response was not observed. Future work in this area should include taking physiological stress measures, such as serum or feather corticosterone, to determine if laser enrichment is causing a stress response in broilers. However, it has been shown that broilers are interested in exploring novel objects (Newberry, 1999), and have a propensity to peck at small objects, (Hogan, 1973), so although it is certainly possible that in some cases birds may have been moving away from or unduly stressed by the lasers, thus far our data indicate they were not negatively impacted and were rather interested in the novel nature and small size of the laser dots.
In summary, these data have provided a strong indication of positive performance effects related to this novel environmental enrichment device. Furthermore, the environmental enrichment device did not result in any unintended negative consequences on the birds’ tibia quality, breast and feet condition, or living environment. This unique device improved gains and feed conversion compared to the control, with peak performance results seen in the crucial grower andfinisher periods. Following future validation in research as well as in a commercial setting, this enrichment option may be effective for producer implementation. The device does not come into contact with birds, therefore reducing the potential for disease vectors (as in perches/straw bales/tiles) and can be used across multiple flocks. Further work is needed to refine the device as well as performance outcomes, and this work also needs to be extended to commercial conditions.

Acknowledgments

The authors would like to thank the Poultry Research and Teaching Farm staff for help in animal husbandry and barn management as well as M.S. student Ella Akin, Dr. Samaneh Azarpajouh, and undergraduate students Julianna Jespersen, Maddison Wiersema, and Stephanie Nielsen for help on-farm mixing diets and weighing birds. Support from the Department of Animal Science, College of Agriculture and Life Sciences at Iowa State University and USDA for partial funding of Dr. Johnson’s salary, and from US Poultry and Egg Grant #703 for M.S. student and project funding.

Literature Cited


Table 4-1. Starter, grower, and finisher diets provided *ad libitum* to Ross 308 broilers. Each diet was fed for 14 days: starter diet wk 0-2, grower wk 2-4, and finisher wk 4-6. Analyzed values are presented on as as-fed basis.

<table>
<thead>
<tr>
<th>Ingredients²</th>
<th>Starter (%)</th>
<th>Grower (%)</th>
<th>Finisher (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (%)</td>
<td>55.32</td>
<td>58.69</td>
<td>62.78</td>
</tr>
<tr>
<td>Soybean Meal (%)</td>
<td>37.15</td>
<td>33.40</td>
<td>28.59</td>
</tr>
<tr>
<td>Soy Oil (%)</td>
<td>2.02</td>
<td>2.98</td>
<td>3.97</td>
</tr>
<tr>
<td>Salt (%)</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>DL-Methionine (%)</td>
<td>0.33</td>
<td>0.30</td>
<td>0.27</td>
</tr>
<tr>
<td>Lysine HCl (%)</td>
<td>0.25</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Threonine (%)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Limestone (%)</td>
<td>1.30</td>
<td>1.01</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium Phosphate (%)</td>
<td>2.05</td>
<td>1.81</td>
<td>1.60</td>
</tr>
<tr>
<td>Choline Chloride 60 (%)</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Vitamin Premix¹ (%)</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
</tbody>
</table>

**Calculated Values**

<table>
<thead>
<tr>
<th></th>
<th>Starter (%)</th>
<th>Grower (%)</th>
<th>Finisher (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (%)</td>
<td>23.05</td>
<td>21.50</td>
<td>19.50</td>
</tr>
<tr>
<td>ME (kcal./kg)</td>
<td>3000</td>
<td>3100</td>
<td>3200</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.59</td>
<td>5.59</td>
<td>6.64</td>
</tr>
<tr>
<td>Digestible Lysine (%)</td>
<td>1.30</td>
<td>1.19</td>
<td>1.06</td>
</tr>
<tr>
<td>Digestible Threonine (%)</td>
<td>0.92</td>
<td>0.87</td>
<td>0.80</td>
</tr>
<tr>
<td>Digestible Arginine (%)</td>
<td>1.39</td>
<td>1.28</td>
<td>1.14</td>
</tr>
</tbody>
</table>

**Analyzed Values (As fed)**

<table>
<thead>
<tr>
<th></th>
<th>Starter (%)</th>
<th>Grower (%)</th>
<th>Finisher (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter (%)</td>
<td>89.40</td>
<td>89.81</td>
<td>89.23</td>
</tr>
<tr>
<td>Crude Fat (%)</td>
<td>6.42</td>
<td>7.63</td>
<td>8.74</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>24.17</td>
<td>21.66</td>
<td>19.89</td>
</tr>
</tbody>
</table>

¹Vitamin and mineral premix provided per kg of diet: Selenium 200 μg; Vitamin A 6,600 IU; Vitamin D₃ 2,200 IU; Vitamin E 14.3 IU; Menadione 880 μg; Vitamin B₁₂ 9.4 μg; Biotin 33 μg; Choline 358 mg; Folic acid 1.1 mg; Niacin 33 mg; Pantothenic acid 8.8 mg; Pyridoxine 880 μg; Riboflavin 4.4 mg; Thiamine 1.1 mg; Iron 226 mg; Magnesium 100 mg; Manganese 220 mg; Zinc 220 mg; Copper 22 mg; Iodine 675 μg

²Calculated according to NRC (1994)
Table 4-2. Ross 308 straight run broiler\(^1\) performance outcomes including feed intake, weight gain, feed conversion ratio (FCR), and ADG by each 2-wk performance period and overall. Starter period indicates wk 0-2, grower wk 2-4, and finisher wk 4-6. Values presented as LSMeans (pooled SEM) averaged per bird (apart from FCR) with treatment as the main effect

<table>
<thead>
<tr>
<th>Performance measure</th>
<th>Control(^2)</th>
<th>Laser(^3)</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feed intake (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starter</td>
<td>0.48</td>
<td>0.50</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grower</td>
<td>1.56</td>
<td>1.61</td>
<td>0.012</td>
<td>0.004</td>
</tr>
<tr>
<td>Finisher</td>
<td>2.62</td>
<td>2.76</td>
<td>0.030</td>
<td>0.004</td>
</tr>
<tr>
<td>Overall</td>
<td>4.69</td>
<td>4.88</td>
<td>0.041</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Weight gain (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starter</td>
<td>0.37</td>
<td>0.38</td>
<td>0.004</td>
<td>0.043</td>
</tr>
<tr>
<td>Grower</td>
<td>1.04</td>
<td>1.10</td>
<td>0.013</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Finisher</td>
<td>1.37</td>
<td>1.59</td>
<td>0.018</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overall</td>
<td>2.80</td>
<td>3.04</td>
<td>0.026</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>FCR(^4)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starter</td>
<td>1.29</td>
<td>1.31</td>
<td>0.009</td>
<td>0.119</td>
</tr>
<tr>
<td>Grower</td>
<td>1.49</td>
<td>1.46</td>
<td>0.008</td>
<td>0.031</td>
</tr>
<tr>
<td>Finisher</td>
<td>1.92</td>
<td>1.74</td>
<td>0.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overall</td>
<td>1.68</td>
<td>1.61</td>
<td>0.010</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ADG(^5) (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starter</td>
<td>0.0265</td>
<td>0.0273</td>
<td>0.001</td>
<td>0.048</td>
</tr>
<tr>
<td>Grower</td>
<td>0.0740</td>
<td>0.0756</td>
<td>0.001</td>
<td>0.390</td>
</tr>
<tr>
<td>Finisher</td>
<td>0.0992</td>
<td>0.1143</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overall</td>
<td>0.0666</td>
<td>0.0723</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\)Broiler chicks transported from International Poultry Breeders Hatchery (Bancroft, IA) on day of hatch to Iowa State Poultry Research and Teaching Farm: BW 47.38± 0.14g

\(^2\)Control describes pens not exposed to laser enrichment

\(^3\)Birds exposed to laser enrichment device

\(^4\)FCR calculated by dividing kilogram of feed by kilogram of bird weight gain per pen, averaged by treatment for each performance period and overall

\(^5\)ADG calculated by dividing bird weight gain averaged per bird by number of days in each performance period and overall
Table 4-3. Focal bird (n=70) right tibia quality measures and weight (LSMeans, pooled SEM) using treatment as a fixed effect

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Laser</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density (^3) (g/cm(^2))</td>
<td>0.129</td>
<td>0.138</td>
<td>0.005</td>
<td>0.203</td>
</tr>
<tr>
<td>Bone mineral content (^3) (g)</td>
<td>0.975</td>
<td>1.107</td>
<td>0.078</td>
<td>0.237</td>
</tr>
<tr>
<td>Bone breaking strength (^4) (kgf/cm(^2))</td>
<td>9.941</td>
<td>11.143</td>
<td>0.693</td>
<td>0.225</td>
</tr>
<tr>
<td>Right tibia weight (g)</td>
<td>14.97</td>
<td>15.75</td>
<td>0.470</td>
<td>0.250</td>
</tr>
</tbody>
</table>

\(^1\) Control describes birds from pens not exposed to laser enrichment

\(^2\) Laser describes birds in pens exposed to laser enrichment

\(^3\) Tibia were scanned for bone mineral density and content using the DXA “rat whole body scan” in groups of 7

\(^4\) Bones were fractured individually using the compression method on an Instron 3367 Universal Test Machine at a rate of 10 mm/min and a 15% rate of load. The machine was stopped at the distinct rapid decline in force as visualized on the monitor, and value is presented as load (kgf) divided by area of tibia (g/cm\(^2\))
Figure 4-1. Regression of focal bird tibia (n=70) bone mineral content (BMC, g) and bone mineral density (BMD, g/cm$^2$). Content and density are highly correlated (r=0.857)

1Control describes focal birds not exposed to laser enrichment device

2Laser describes focal birds exposed to enrichment device 4 times daily for 4-min laser periods

3Bone mineral content and density of the tibia were obtained using the DXA “rat whole body scan” in groups of 7

4BMC denotes bone mineral content (g), BMD denotes bone mineral density (g/cm$^2$)
Figure 4-2. Multidimensional preference analysis of all focal bird tibia (n=70) measures\(^1\)-\(^3\): bone breaking strength (BBS), bird weight, tibia weight, bone mineral content (BMC), and bone mineral density (BMD). Abbreviations: C: control bird; and L: laser-enriched bird. Symbols denote individual focal birds.

\(^1\)Right tibia were collected from 70 focal birds on d42, weighed, DXA scanned for BMC and BMD, and fractured using an Instron 3367 Test Machine compression method for bone breaking strength.

\(^2\)The cosine between 2 variables indicates the correlation between the variables, the length of the arrows reflect the variance of the original variables. This is a 2-dimensional approximation of the dimensions.

\(^3\)Original variables have been transformed into new variables (Component 1 and 2) that account for most of the variance.
Supplementary Figure 4-1A-D. Overhead snapshots of the activated laser in the pens A: d2, B: d16, C: d30, and D: d37
Supplementary Figure 4-1A-D. (continued)
Supplementary Figure 4-1A-D. (continued)
Supplementary Figure 4-1A-D. (continued)
CHAPTER 5: A NOVEL ENVIRONMENTAL ENRICHMENT DEVICE INCREASED PHYSICAL ACTIVITY AND WALKING DISTANCE IN BROILERS

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Environment, Well-Being, and Behavior

A manuscript submitted to the Journal of Poultry Science

Abstract

Modern broilers are selected for fast growth and a large proportion of breast tissue, contributing to a top-heavy phenotype, leg disorders, and inactivity as birds reach market weight. Therefore, the objective was to motivate broilers to move through environmental enrichment. Twelve hundred Ross 308 broilers were housed in pens of 30; 600 birds were exposed to a novel laser enrichment device (LASER) and 600 were control. Each device projected 2 randomly moving red laser dots onto the floor 4 times/day for 4-min “laser periods”. Seven LASER and 7 control pens, with 5 focal birds/pen (n=70), were randomly selected to be video-recorded d0-8 and once weekly for the remainder of the trial. Videos were analyzed to measure broiler behavior, latency to feed, and distance walked during laser periods. Focal birds were gait scored weekly on-farm. The Human-Approach Paradigm was carried out on wk 1 and 6 on all pens. Data were assessed for normality using PROC UNIVARIATE and analyzed using PROC GLIMMIX (SAS Version 9.4). LASER birds were more active on d0, 1, 3, 4, 5, 7, and 8, moving 254% more on d7 (P ≤ 0.05). Time spent active was increased in LASER treatment by 114% on wk 2; 157% on wk 3;
90% on wk 4; and 82% on wk 5. LASER birds spent more time at the feeder on d0, 1, 2, 5, 8, and on wk 1 and 5, with 84% more time at feeder than control on d5 (P ≤ 0.05). LASER birds walked further during laser periods on d0-8, reaching 646.5 cm greater (d1), and on wk 2, 3, 4, and 5, with an increase of 367.5 cm on wk 2 (P ≤ 0.05). Over wk 1-6, 60.54 ± 7.4% of focal birds were at the feeder during or within 5 mins following laser periods. The laser enrichment device was successful in stimulating broiler physical activity and feeding, and did not negatively impact walking ability.

Key words: broiler, environmental enrichment, well-being, lameness, behavior

**Introduction**

Today’s commercial broiler is up to 5 times heavier than its 1950’s predecessor at the same age due to genetic selection for 3-fold improved feed efficiency and a 300% increased growth rate, resulting in a bird reaching market weight in as little as 4-6 wk (Havenstein, et al., 2003; Knowles, et al., 2008, Zuidhof, et al., 2014). However, this selection for increased growth rate has contributed to up to 30% of modern commercial broilers suffering from leg lameness or and reduced ability to move (Knowles, et al., 2008; Bassler, et al., 2013). Increased age and lameness both contribute to decreased time standing or walking. Sound birds spend around 76% of their time sitting or lying down, while lame birds spent up to 86% of their daily time budget inactive (Weeks, et al., 2000). Inactivity increases litter contact and could result in a higher occurrence of breast blisters and contact dermatitis (Weeks, et al., 2000; Bassler, et al., 2013; Nääs, et al., 2018), which are likely painful conditions caused by urea in the litter generating ammonia, creating chemical burns (Haslam, et al., 2007).
The National Chicken Council (NCC) guidelines for broilers (2017) have recognized this challenge and recommend gait scoring 100 birds/flock to evaluate leg health within 1 wk of slaughter. This issue hurts the industry economically, necessitating up to 2% culls in a $30 billion industry (USDA, 2017; Dunkley, 2018), and there is also considerable evidence that leg lameness is painful for the broiler. Birds with leg lameness eat more analgesic-containing feed than healthy birds, and birds fed an analgesic diet showed improved speed of walking, indicating relief from pain and discomfort caused by leg abnormalities (McGeown, et al., 1999; Danbury, et al., 2000). Past research has shown that motivating physical activity increased tibia strength and decreased lameness (Reiter and Bessei, 2009), thus may be a viable option to stave off leg issues in swiftly growing broilers. Furthermore, birds are visual feeders and consistently prefer the color red when presented with novel colors (Ham and Osorio, 2007). Bizeray et al. (2002) studied the effects of red, blue, green, and yellow spotlights moving across the floor of the pens but saw no change in broiler physical activity, therefore the authors concluded that that the spotlights moved too quickly for broilers to follow. Light/visual efficacy designed to motivate broiler activity and improve welfare outcomes is absent in the literature. The objectives of this work were to stimulate broilers visually using a novel form of environmental enrichment to motivate physical movement, hence increasing walking distance and improving walking ability.

**Materials and Methods**

All live bird procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

**Animals**

1260 straight-run Ross 308 broiler chicks (day of hatch; BW 47.38± 0.14g) were obtained from a commercial hatchery and transported to the Poultry Research and Teaching Unit at Iowa State University (International Poultry Breeders Hatchery, Bancroft, Iowa) for a 6-wk
grow-out experiment in floor pens. 1200 were randomly assigned to treatments and the remainder were culled following standard operating procedures of the farm. Seventy birds were randomly assigned upon arrival as focal birds (n=5 birds/pen in 14 camera pens), identified with wing-bands, and marked with unique animal-safe food coloring (red, blue, green, purple, and black; Wilton, Woodridge, Illinois). Food coloring was applied to a cotton ball, rubbed on the back of the chick’s head and neck, and reapplied on an as-needed basis throughout the 6-wk trial.

**Housing and Feeding**

Birds were housed in 40 1.22 by 2.44 m pens of 30 across 2 rooms in the barn. One room contained 20 LASER pens (exposed to enrichment device), and the other contained 20 control pens, with an anteroom separating the 2 rooms so no crossover of enrichment device was possible. Approximately 10 cm deep fresh wood shavings provided bedding over the solid concrete floor, and PVC pipe dividers with mesh walls (1.22 m height) separated pens. High and low temperatures and humidity were monitored daily in the LASER and control rooms. Average temperatures are listed from the starter, grower, and finisher periods respectively from the LASER room of the barn: 85.47°F, 77.39°F, and 71.71°F, and the control room: 85.53°F, 77.46°F, and 71.5°F. Average relative humidity is listed from the starter, grower, and finisher periods respectively from the LASER room: 23.86%, 27.21%, and 33.93%, and the control room of the barn: 19.89%, 23.93%, 27.75%.

Birds were gradually adjusted from 24 h light on d0, defined as day of arrival and placement, (30-40 lux) to 20 h light (20-30 lux) from d8-42. Chicks were brooded with 2-heat lamps/pen (22.9 cm reflectors with porcelain socket) using 125-watt heat bulbs (Sylvania, Wilmington, MA) for the first wk. Birds were fed an *ad libitum* diet formulated for Ross 308 commercial recommendations out of a hanging chicken feeder (BRHF151, Brower Equipment,
Houghton, IA) gradually raised to accommodate bird height. Water was provided *ad libitum* from a hanging nipple water line (8 nipples/pen).

**Laser Enrichment Device**

Ten novel laser enrichment devices designed and built specifically for this research were affixed over 20 pens in 1 room of the barn. Each device was designed and calibrated to cover 2 adjoining pens. The enrichment device consisted of 2 independent red 650 nm lasers contained within a 20.5 by 20.5 cm metal box with a glass bottom mounted on a custom-designed structure made of 3 wooden beams (2.4 m height) raised above the pens. The lasers projected in a random pattern at a range of 7.6-30.5 cm/second onto the pen floor for 4-min “laser periods”: 05:30 to 05:34, 11:30 to 11:34, 17:30 to 17:34, and 23:30 to 23:34 daily for the entirety of the trial.

**Video Camera Set-up and Training**

Seventy focal birds (n=5/pen) were randomly assigned to 14 randomly selected pens (7 LASER, 7 control) equipped with 1 Sony HDR-CX440 Handycam (Sony Corp. of America, New York, New York) each. Cameras were affixed above each pen using brackets adjusted to capture the entire pen. Filming occurred in real-time (30 fps) for the first 10 days of the trial (d0-9) and once weekly for the remainder. Video observers were trained by an individual with previous animal behavior observation experience to 90% agreeability using the 4-min laser period video clips from any day recorded (d0-9, 16, 23, 30, and 37). All clips recorded were analyzed for the entirety of the enrichment period in LASER and control pens. Observers were not blinded to treatment; either the lasers or the supporting structure were visible in the videos/images.
Broiler Bird Home Pen Behavior

Trained observers watched the red-colored focal bird during 4-min laser periods and categorized bird behavior continuously throughout the clips using a pre-determined behavior ethogram (Table 5-1) on d0-9, 16, 23, 30, and 37. Behaviors were recorded in frequency and duration; duration was converted to percent of time spent on each behavior per 4-min period.

Latency to Feed

Latency to feed following laser turn-off was measured in LASER pens on d0-9, 16, 23, 30, and 37. A student observer watched laser period video from the 7 LASER pens and identified the red-colored focal bird. At the end of the 4-min period, when the laser dots disappeared, the observer started a timer. The timer was stopped when the focal bird exhibited “at feeder behavior” (Table 5-1) or when 5 mins had passed without the bird feeding. Latency to feed was recorded in seconds. Following data collection, latency to feed measures were categorized into 4 mutually exclusive categories, including: A) at feeder during laser period only (obtained from broiler home pen behavior data), B) at feeder when laser turned off, C) went to feeder <5 min following laser turn off, and D) never went to feeder.

Walking Distance

The distance walked by the blue-colored focal bird was measured over the 4-min laser periods (d0-9, 16, 23, 30, and 37). The observer taped a clear sheet protector over the computer screen and watched each min individually. At the beginning of each min, video was stopped and the observer drew a line at the bird’s beak. Video was resumed, and if the bird moved the video was paused again and a line was drawn at the new position of the beak where the bird stopped. This was repeated each min. Next, the observer used a ruler to draw a line connecting each
stopping mark. After drawing the interconnecting line, the observer opened a pen template image in Adobe Photoshop (Adobe Systems Inc, San Jose, California).

The observer then used a known length within the pen (58.4 cm between 2 segments of the water line, measured on-farm) to standardize the custom ruler tool on Photoshop, measured in pixels (58.4 cm = approximately 194 pixels). The tool would then equate x number of pixels to centimeters. The observer placed the clear sheet protector over the template image and used the custom ruler tool to measure the interconnecting lines drawn from video. This was repeated for each individual min and then a sum of all line measurements, or the total distance walked each period, was calculated.

**Walking Lameness**

Focal birds (n=70) were removed from their home pens once weekly and assessed for lameness. Two researchers conducted the lameness test, with 1 researcher assigning scores. Birds were placed on a custom-designed plywood runway 1.80 m long and 0.46 m wide, with 0.30 m tall walls on all sides. The runway had 0.15 m start and finish sections, a 1.5 m walking space, and delineations marking every 0.30 m and 2.5 cm. Birds were placed on the runway starting section. Birds either walked 1.5 m independently or were encouraged to walk by (1) a researcher slowly moving their hand back and forth directly behind the bird (2) a researcher gently tapping the bird on the vent region with a gloved hand or (3) a researcher both waving behind and gently tapping the bird with a ping-pong paddle. Individual birds were considered to have completed the task when both feet had crossed into the finish section. Scores were assigned using a 0-2 scale adapted from NCC guidelines where 0 indicated the ability to walk 1.5 m with no signs of lameness, 1 indicated the ability to walk 1.5 m but showed unevenness in steps or sat down at least once, and 2 indicated a bird that could not walk 1.5 m.
**Human-Approach Paradigm**

The Human-approach paradigm (HAP) was completed once during wk 1 and once during wk 6 beginning at 09:00; pen order was kept the same each wk (n=40 pens). The barn was emptied of personnel apart from 2 researchers carrying out the HAP. Prior to the HAP, the researchers determined optimal bracket angle and camera location for each pen, so that 1 image captured an entire pen. Colored tape identified bracket location; locations ranged between 47.75-59.00 cm measuring out from the central PVC pipe. The HAP image was taken with a hand-held camera (Pentax Optio W90, Pentax Imaging Company, Golden, Colorado). The camera’s focal length was 28 m with a 12.1-megapixel resolution.

Methods were based on swine nursery work completed by Weimer, et al (2014). Briefly, researcher A was defined as an unfamiliar human in the pen and researcher B placed the camera/bracket and took the image. Researcher A wore different colored coveralls than the rest of the research and farm crew, but the same boot covers. The researchers approached each pen quietly and recorded the number of birds per pen. Researcher B positioned the bracket on the pen’s side in the pre-determined location and then researcher A stepped over the front of the pen with a stopwatch in their right hand. Researcher A walked to the side of the pen opposite Researcher B and crouched facing the camera with their body angled towards the birds and both arms held close to the body. Once in position, Researcher A began the stopwatch, avoiding looking at the birds for 15 s. After 15 s, Researcher A stopped the watch and looked up at the birds. Researcher B took an image at the precise moment Researcher A looked up.

One student observer, trained using the same methods as video observers but with wk 1 HAP images, reviewed the images. Within each digital image of individual pens, broilers were classified into 2 categories: interacting or not interacting. Birds classified as not interacting were further categorized into 3 mutually exclusive behaviors: feeder, drinker, or other (Table 5-2).
Further, the pen images were split into fourths by tracing over PVC pipe supports every 0.6 m in the pen with a clear sheet and a marker. The number of birds present in each quadrant of the pen was counted, with quadrant 1 containing the unfamiliar human.

**Statistical Analysis**

In this experimental design, individual control pens (n=20) were considered experimental units, but LASER pens were analyzed as a group of 2 pens with 1 shared laser device (n=10). All data were analyzed using SAS software version 9.4 (SAS Institute Inc., 2016, Carey, NC). PROC UNIVARIATE was used to assess the distribution of data prior to analysis. Home pen behavior, walking distance, and HAP data were all abnormally distributed (Poisson distribution), thus were analyzed using PROC GLIMMIX. GLIMMIX fits models to data with non-constant variability, correlations, or that are not normally distributed. Home pen behavior and walking distance data were analyzed by day (d0-8) and by wk (d2, 9, 16, 23, 30, and 37). Each model (behavior, walking distance, and HAP) included the fixed effect of treatment (enrichment versus control), wk or day, and the treatment by wk or day interaction, with the random effect of pen (or enriched pair of pens) within treatment, as birds were randomly assigned to pens.

Latency to feed and walking lameness categorical data were analyzed using PROC FREQUENCY and CHI SQUARE. The distribution of latency to feed data were observed by day and wk; only LASER focal birds were analyzed, thus treatment was not included in the model. Lameness score distributions were observed by treatment and the association of score to treatment. For all measures, a value of P ≤ 0.05 was considered significant and differences between means were detected using PDIFF.
Results

Broiler Bird Home Pen Behavior

The day x treatment interaction was significant for all behaviors measured: active, inactive, time at feeder, drinker, and other (P < 0.01). Birds out of view occurred so infrequently that data could not be analyzed. LASER birds spent more time active (walking or running) on d0, 1, 3, 4, 5, 7, and 8 (P ≤ 0.05, Figure 5-1A). The greatest increase in active behavior was observed on d7, where LASER birds moved 17.4 ± 1.6% more, equal to a 253% increase, than their control counterparts. LASER birds were less inactive on d2, 3, 4, 5, 6, and 8, with a peak 29.3 ± 3.3% decrease on d2 (P ≤ 0.05, Figure 5-1B).

LASER birds spent more time at feeder on d0, 1, 2, 5, 8 (P ≤ 0.05, Figure 5-1C). On d5, LASER birds were at the feeder 10.2 ± 2.4% more than control birds, equal to an 83.7% increase. Control birds spent a greater percent of time at drinker on d0, 1, 5, 7, and 8, but LASER birds spent more time at drinker on d2 and 6 (P ≤ 0.05, Figure 5-1D). Control birds displayed greater other behaviors on d2, 5, and 8, while LASER birds showed a greater percent of this behavior on d4 (P ≤ 0.05). Other behavior did not contribute heavily to focal bird time budget, with a maximum percent of 4.01 ± 0.59% in LASER birds (d3) and 4.02 ± 0.63% in control birds (d2).

On a weekly basis (1 d analyzed/wk) the wk x treatment interaction was significant in all behavior categories (P ≤ 0.01). LASER birds spent a greater percent of their time active than control birds on wk 2, 12.2 ± 2.8% greater (114% increase); wk 3, 8.2 ± 1.6% greater (157% increase); wk 4, 2.5 ± 0.74% greater (90% increase); and wk 5, 2.9 ± 0.87% greater (82% increase, P ≤ 0.05, Figure 5-2A). LASER birds spent less time inactive on wk 1 and 5 (P ≤ 0.05, Figure 5-2B). Time at feeder was increased in wk 1 by 8.2 ± 3.0%, and in wk 5 by 17.8 ± 3.0%,
a 247% increase, in LASER birds (P ≤ 0.05, Figure 5-2C). Time spent at drinker was increased in LASER birds by 6.5 ± 1.7% in wk 1 and 7.2 ± 1.43% on wk 6, and was 7.9 ± 1.4% higher in control birds on wk 3 (P ≤ 0.05, Figure 5-2D). Other behavior showed no differences by treatment within individual weeks.

**Latency to Feed**

Latency to feed categorical distributions were affected by day (d0-8, P < 0.01) and by wk (wk 1-6, P = 0.03). Over days 0-8, 15.34 ± 0.40% of LASER focal birds were at the feeder during laser periods (but not in 5 mins following laser turning off), 33.33 ± 0.73% went to the feeder in <5 mins following laser turn off, 22.22 ± 0.48% were already at the feeder when laser periods ended, and 29.1 ± 0.77% never went to the feeder during or in 5 mins following laser period. Individual daily proportions are presented in Figure 5-3A. Over wk 1-6, 5.44 ± 0.37% of birds were at the feeder during laser periods only, 28.57 ± 0.76% went to feeder within 5 min of laser turn off, 26.53 ± 0.62% were already at the feeder when the laser turned off, and 39.46 ± 0.96% were never at the feeder. Weekly breakdowns are presented in Figure 5-3B.

**Walking Distance**

For the first 9 days on trial, the day x treatment interaction was significant for each min individually and total distance walked (P ≤ 0.01). LASER birds walked further in the first min on all days, with increases up to 151.1 ± 12.9 cm on d1, a 452% increase, and 107.5 ± 11 cm on d7, greater than a 228% increase (P ≤ 0.05, Figure 5-4A). Likewise, LASER birds walked more on all days during the second min, with an increase of 236.3 ± 30 cm, or a 237% increase, on d1 (P ≤ 0.05, Figure 5-4B). During min 3, LASER birds walked more than control on d0, 1, 3, 4, 5, 6, and 8, with a peak increase of 139.8 ± 21.2 cm, or a 270% increase, on d1 (P ≤ 0.05, Figure 5-4C). In min 4, LASER birds walked greater distances on d0, 1, 2, 4, 5, 6, 7, and 8, walking 150.9
± 21 cm (287%) more on d0 and 108.4 ± 19.2 cm (183%) more on d1 (P ≤ 0.05, Figure 5-4D). Over the total 4-min laser periods, LASER focal birds walked a greater distance than control on all days, with increases reaching up to 646.45 ± 64.6 cm, a 303% increase (d1, P ≤ 0.05, Figure 5-4E).

Analyzed on a weekly basis (1 d/wk) the wk x treatment interaction was significant for each min individually and total distance walked (P ≤ 0.01). During min 1 of laser periods LASER birds walked more on wk 1, 2, 3, 4, and 5, with increases up to 88.6 ± 15.7 cm (130%) on wk 2 and 51.2 ± 7 cm (215%) on wk 5 (P ≤ 0.05, Figure 5-5A). During min 2, LASER focal bird walking distance was higher on wk 2 and 5 (P ≤ 0.05, Figure 5-5B). In the third min, the LASER birds walked more on wk 2, 3, 4, and 5, with increases up to 80 ± 19.7 cm (108%) on wk 2, P ≤ 0.05, Figure 5-5C. Within min 4 LASER focal birds walked further than the control on wk 2 and 4, with an increase of 83.4 ± 19.1 cm (176%) on wk 2 (P ≤ 0.05, Figure 5-5D). Total distance walked during 4-min laser periods was increased on wk 2-5 in LASER pens, with the greatest increase of 367.5 ± 61.9 cm, or 150%, on wk 2 (P ≤ 0.05, Figure 5-5E).

Walking Lameness

Out of the 420 lameness measures taken (70 focal birds/wk for 6 wk), 400 were scored 0 (no signs of lameness). There were 18 scores of 1 (bird showed unevenness in steps or sat down at least once), and only 2 scores of 2 (bird could not walk 1.5 m). In the control birds, 96.19% of scores were 0, 2.86% were scores of 1, and 0.95% of scores were 2. In the LASER birds, 94.29% of scores were 0, 5.71% were scores of 1, and no scores were 0. The chi square relationship of score by treatment was not significant (P = 0.13).

Human-Approach Paradigm
The wk x treatment interaction was significant for percent of birds interacting (P ≤ 0.01). During wk 1, control birds interacted 2.2 ± 0.73% more (P ≤ 0.05), but on wk 6 there were no differences in birds interacting. Averaged over both treatments and wk 1 and 6, 95.59 ± 2.19% of birds were not interacting and there was not a wk x treatment interaction (P = 0.35, Table 5-3). In the not interacting further classified behavior categories, there were 3.7 ± 1.0% more control birds at the drinker on wk 1 and 1.8 ± 0.59% more LASER birds at the drinker on wk 6, with a wk x treatment interaction (P < 0.01). There were no differences in percent of birds at the feeder wk 1 or 6 (wk x treatment interaction P = 0.62), and no differences in birds exhibiting other behaviors on wk 1 or 6 (wk x treatment P = 0.22, Table 5-3).

Regarding bird location in the home pen during the HAP, the main effect of wk was significant for all quadrants of the pen and the wk x treatment interaction was significant for the first and second quadrants (P ≤ 0.01). There were no differences in percent of birds in the first quadrant on wk 1, but there were 3.3 ± 1.07% greater LASER birds in this quadrant on wk 6, more than double the percent of control birds (P ≤ 0.05). There were 2.8 ± 0.89% more control birds in the second quadrant on wk 1 (P ≤ 0.05), but no differences in this quadrant on wk 6. There were no differences due to enrichment in quadrants 3 or 4 (Table 5-4).

**Discussion**

It is well-established that commercial broilers spend 80-90% of their daily time budget inactive at 5-6 wk of age, with sound birds at harvest weight (approximately 2 kg) spending only 3.3% of their day walking, versus 1.5% in lame birds (Weeks, et al., 2000). Hence, past broiler research has studied physical activity and methods to increase active behavior. Weeks and others hypothesized that fast-growing, more feed efficient broilers are inherently more inactive. Reiter and Bessei (2009) used treadmill training to force broilers to exercise for sessions lasting 20 min
or 100 m wk 1-6 and saw improved locomotion. In an additional test from the same study, when distance was gradually increased over d0-5 from 2 to 12 m between feed and water, locomotion was increased threefold compared to the control, where resources remained 2 m apart throughout. Similar methods have been successfully used to encourage broilers above the minimum range of movement by increasing distance or introducing barriers between feeders and waterers without compromising performance (Ventura, et al., 2012; Ruiz-Feria, et al., 2014), but other, non-resource-based methods have been less successful.

Prayitno and others (1997) used ambient red light to stimulate activity in broilers but saw birds with a mean final body weight 47-79 ± 12.4 g lighter than birds in a blue light treatment. However, broilers in the blue light were significantly less active. Bizeray and colleagues tested different forms of enrichment by scattering wheat on the floor of the pen in one treatment, colored, moving spotlights in another, and barriers between feed and water in a third, but concluded that “forcing animals to exercise more…was more effective for increasing physical activity than was attempting to stimulate foraging activities” (Bizeray, et al., 2002). Shields and others (2005) hypothesized that broiler exercise would increase and leg lameness would decrease when provided sand bedding, however, birds rested and displayed more inactive behavior on the sand. Ventura et al. (2012) implemented barrier perches as environmental enrichment, but saw no increase in walking. Straw bales as a form of “freedom food” were successful in increasing broiler activity, but performance or walking ability data were not reported. Performance may have been negatively impacted in the straw bale treatment due to increased fiber in the gut, indicated by the increased drinking behavior (Kells, et al., 2001).

Motivation is understood to be a process driven by both external/environmental and internal/physiological factors resulting in goal-oriented behavior or action (Toates, 1986).
Motivation in broiler birds, however, is not well understood. It has been shown that food and exploring novel objects motivates broilers (Newberry, 1999; Bokkers, 2002; Bokkers, 2004). In the current study, LASER birds spent 17.4% more time active during laser periods on d7 and displayed 12.2% more activity on wk 2. LASER broiler active behavior in this study was increased every day in the first 9 d except d2 and 6 (Figure 5-1A). One d/wk (Thursday) was analyzed for weekly measures, which included d2. Further, the performance of LASER birds was not compromised but was improved, with an overall decrease of 0.07 FCR points and total increased weight gain of 0.24 kg/bird (Meyer et al., 2019). Thus, the success of the novel laser device tested here in motivating, not forcing, an increase in active behavior and walking distance, is among the first to accomplish increased movement in combination with improved performance.

LASER birds were 114% more active during laser periods on wk 2 and 90% more active on wk 5 than control birds. We hypothesize that this success may be due in part to the laser dots stimulating pecking behavior, a documented response to small particles in broilers (Hogan, 1973), or to visual-based foraging and predatory behavior natural to the chicken’s junglefowl ancestors (Fernandez-Juricic, 2004). Junglefowl continuously move while foraging (Arshad, 2000), a behavior that seems to have been replicated in broilers choosing to follow lasers around the pen in this study. However, during wk 6, there were no differences in proportion of active/inactive behavior due to enrichment. This is likely a result of maximal body weight overriding motivation to move as birds neared the end of the grow-out, rather than habituation to the lasers, as a similar pattern is observed in declined use of perches after wk 5 in broilers (Bokkers, 2003). Further, work in laying hens when exposed to environmental enrichment in the
form of strings for limited daily time periods (10 min), rather than constant exposure, maintained
interest in pecking at the strings for 14 weeks (Jones, et al., 2000).

On d0-8, total distance walked during laser periods was increased compared to the
control daily. Minutes 1 and 2 showed an increase in broiler walking distance every day, 0-8,
compared to controls, with peak distance walked during d2, min 2. However, distance walked
during min 3 and 4 was still significantly increased 7 and 8 d out of 9, respectively. Over wk 1-6,
min 1 stimulated the most consistent increase in LASER bird distance walked compared to the
control, with increases on wk 1-5 (maximum distance on wk 2, Figure 5-5A). However, an
interesting behavior pattern was detected in the weekly analysis where min 3 more closely
followed min 1, and LASER birds walked more than the control wk 2-5 during this min (Figure
5-5C). Distance walked was increased in min 2 on wk 2 and 5 only (Figure 5-5B), and min 4
showed increased distances wk 2 and 4 (Figure 5-5D). Hence, it appears that recording the
distance walked during each min of the 4-min laser period is necessary, as over time birds moved
more in min 1 and 3 than 2 and 4. This likely contributed to the increased total distance walked
during laser periods wk 2-5 by LASER focal birds (Figure 5-5E), resulting in a 215% increase in
walking distance on wk 5, a notable outcome in birds nearing-market weight.

As the device was entirely novel, a 4-min laser period was used with the intention to
determine if this length of time was successful in stimulating bird activity and walking distance.
Our data indicate that within the first 9 days, a 4-min period was effective in promoting walking
up to and including min 4. When viewing the entire 6-wk grow-out, following wk 4 the increase
in walking distance and active behavior declined after min 3. Thus, it is possible that a 3-min
laser period would suffice for broilers older than 4 wk, but for birds 4 wk or younger, 4-min
periods were effective. Although no difference in activity was seen during wk 6 (d35-42), weight
gain was increased by 0.22 kg and FCR was decreased by 0.18 points in the critical finisher period (Meyer, et al. 2019). The increased weight gain of 0.24 kg/bird overall could be translated to the range of $0.71-$1.39 more saleable breast meat/bird, using current prices (USDA, 2019).

The increased distance walked in proportion to the increase in activity (walking or running) makes it likely that LASER birds were moving at an increased speed to account for this increased distance. Future studies using this device that incorporate a walking speed measure could validate this hypothesis. In work by Dawkins and others (2009) an increased walking speed, along with increased time spent walking, resulted in decreased lameness. However, in our lameness scoring measure, no LASER birds received a score of 2, hence we did not detect a difference due to laser enrichment. Taking into account the increased weight gain and improved FCR in LASER birds (Meyer et al., 2019), no detection of lameness is a positive outcome. These data indicate that the paradigm postulated by Weeks et al. (2000) that selection for improved FCR leads to less active animals, and thus more lameness, may have been counteracted by the increased exercise stimulated by the laser device. It is important to note that increased movement in LASER birds did not cause lameness as birds reached market weight.

The increase in feeder behavior seen in LASER birds on proportionally more days/weeks is logical, as LASER birds had 0.02 kg greater feed intake in the starter period, 0.05 kg greater in the grower, and 0.14 kg greater in the finisher (Meyer, et al. 2019). Further, feeding latency showed that d0-8, approximately 71% of LASER focal birds were at the feeder at least once either during or within 5 min following laser periods. Over wk 1-6, 60.5% of LASER focal birds were at the feeder either during or shortly following laser periods. This is a positive result indicating that the device increased bird movement but also encouraged feeding, perhaps by stimulating natural foraging or predatory behavior. In 3 4-min timepoints selected for behavior
and walking distance analysis that were not during scheduled laser periods (06:30, 18:30, and 22:30), LASER birds walked further than control birds on d1 and wk 6, spent more time active on d1 and 3, and were at the feeder more on wk 4 and 5 (P < 0.05, unpublished data). These data indicate a maintenance of activity and feeder behavior outside of the laser periods.

The HAP was used here to measure fearfulness in the flock, a measure validated in swine by Weimer and others (2014), based on methods used in pigs and cattle by Hemsworth et al. (1996). Results in these species have shown that animals with positive, regular interactions with humans were quicker to approach, indicative of decreased fear. Environmental enrichment has been shown to decrease fear responses in poultry, for example reducing freezing, avoiding novel objects, and latency to enter an unfamiliar environment (Jones and Waddington, 1992). The “touch test” and “avoidance distance test” have been validated in laying hens, where response to humans was positively influenced by more than minimal human contact (Graml, et al., 2008). Within this study, the overall proportion of control birds interacting with the human decreased from wk 1 to 6, while the proportion of LASER birds interacting increased.

A greater number of LASER birds were counted in quadrant 1, closest to the unfamiliar human, during wk 6 than control birds. Importantly, there was no evidence of piling, a negative behavior associated with fear in poultry (Campbell, et al., 2016). This method requires further research in broilers and may be more applicable in poultry species that naturally tend to approach people, such as commercial turkeys. Other stress markers, including measuring corticosterone concentrations from the serum or feathers, have been validated in broilers and may be an alternative methodology for determining stress (Weimer, et al., 2018).

In summary, these data have provided strong evidence that this novel environmental enrichment device positively increased broiler bird physical activity without negatively
impacting lameness. This unique work motivated broilers to move of their own volition by stimulating them visually, encompassing physical, occupational, sensory, and nutritional enrichment. The laser device is practical and applicable to commercial barns without changing grow-out procedures or negatively impacting bird welfare or performance.

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Literature Cited


Table 5-1. Broiler bird home pen behavior ethogram; focal bird behavior was measured continuously during 4-min laser periods, 4 times daily at 05:30, 11:30, 17:30, and 23:30 for d0-9, 16, 23, 30, and 37

<table>
<thead>
<tr>
<th>Measure</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>Bird legs were in a continuous forward motion (walking or running).</td>
</tr>
<tr>
<td>Inactive</td>
<td>Bird stood in one place or rested its abdomen on the litter, head rested or raised while any part of its body was or was not in contact with another bird.</td>
</tr>
<tr>
<td>At feeder</td>
<td>Bird head over feeder circle, bird in feeder or bird stood on feeder tray.</td>
</tr>
<tr>
<td>At drinker</td>
<td>Bird stood beneath drinker line.</td>
</tr>
<tr>
<td>Other</td>
<td>Dust-bathed, preened (head/beak twisted around in contact with feathers), or any behavior not otherwise identified.</td>
</tr>
<tr>
<td>Out of view</td>
<td>Bird was obstructed or not observed due to being under the heat lamp or inside the feeder and could not be seen.</td>
</tr>
</tbody>
</table>

1 All behaviors were collected as frequency, defined as the number of times the behavior occurred during 4-min periods, and duration, defined as length of time behavior was exhibited in seconds.

2 Behaviors categorized as “Out of view” were so infrequent that the data could not be analyzed; relaxed convergence criteria was attempted to $10^{-4}$
Table 5-2. Broiler behavior classification using a digital image analysis upon conclusion of human-approach paradigm (HAP\(^1\)). Birds were first categorized as interacting or not interacting, then not interacting birds were separated into 3 mutually exclusive categories\(^2\): feeder, drinker, or other

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classification at 15 s using digital image evaluation</strong></td>
<td></td>
</tr>
<tr>
<td>Interacting</td>
<td>Using a ruler and a clear sheet protector taped to the screen, a line was drawn from the midpoint of the bird’s head to the pen edge. If the line intersected with researcher A, or if any part of the bird was physically contacting researcher A, the bird was classified as interacting.</td>
</tr>
<tr>
<td>Not interacting</td>
<td>Birds not exhibiting the above two behavioral classifications.</td>
</tr>
<tr>
<td><strong>Further classification of not interacting using digital image evaluation</strong></td>
<td></td>
</tr>
<tr>
<td>Feeder</td>
<td>Bird head over feeder circle, bird in feeder or bird stood on feeder tray.</td>
</tr>
<tr>
<td>Drinker</td>
<td>Bird stood beneath drinker line.</td>
</tr>
<tr>
<td>Other</td>
<td>Laying (rested its abdomen on the litter, head rested or raised(^3), preening (dust bathed or head/beak twisted around in contact with feathers), wings stretched out, piling (group of three or more birds pressed against each other and/or on top of each other, all bird heads facing away from the human in the pen and not performing any other discernible behavior(^4)), or not visible.</td>
</tr>
</tbody>
</table>

\(^1\)HAP was carried out on all pens once on wk1 and once on wk 6. An unfamiliar human entered the pen and after 15 s, a photograph was taken to measure the birds’ response

\(^2\)Ethogram adapted from Weimer et al. 2014

\(^3\) Kristensen, et al., 2007

\(^4\) Campbell, et al., 2016
Table 5-3. Human-approach paradigm (HAP) results; percent of Ross 308 broilers Interacting vs Not Interacting and Not Interacting behavior further classified using digital image evaluation. Values presented as wk x treatment LSMeans (pooled SEM) on wk 1 and 6. N=80 observations

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Week 1 (%)</th>
<th>Pooled SEM</th>
<th>Week 6 (%)</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control1</td>
<td>Laser1</td>
<td>Control</td>
<td>Laser</td>
<td></td>
</tr>
<tr>
<td>Interacting</td>
<td>5.46</td>
<td>3.22</td>
<td>0.74</td>
<td>2.80</td>
<td>4.31</td>
</tr>
<tr>
<td>Not interacting</td>
<td>94.00</td>
<td>96.33</td>
<td>2.18</td>
<td>96.93</td>
<td>95.09</td>
</tr>
<tr>
<td>Not interacting further</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder</td>
<td>4.55</td>
<td>4.67</td>
<td>0.87</td>
<td>3.81</td>
<td>3.53</td>
</tr>
<tr>
<td>Drinker</td>
<td>10.62</td>
<td>6.94</td>
<td>1.03</td>
<td>3.35</td>
<td>5.13</td>
</tr>
<tr>
<td>Other</td>
<td>83.62</td>
<td>86.93</td>
<td>2.07</td>
<td>92.11</td>
<td>90.24</td>
</tr>
</tbody>
</table>

1C=control, L=laser enrichment

2HAP was carried out on all pens once on wk1 and once on wk 6. An unfamiliar human entered the pen and after 15 s, a photograph was taken to analyze the birds’ response

3Data was collected in bird counts, converted to percent of birds in the pen exhibiting each behavior
Table 5-4. Human-approach paradigm (HAP) results; percent of Ross 308 broilers present in each quadrant of the pen. Values presented as wk x treatment LSMeans (pooled SEM) on wk 1 and 6. N=80 observations

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Week 1 (%)</th>
<th>Pooled SEM</th>
<th>Week 6 (%)</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Laser</td>
<td>Control</td>
<td>Laser</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.86</td>
<td>5.57</td>
<td>1.41</td>
<td>3.00</td>
<td>6.34</td>
</tr>
<tr>
<td>2</td>
<td>8.95</td>
<td>6.18</td>
<td>0.89</td>
<td>20.44</td>
<td>22.18</td>
</tr>
<tr>
<td>3</td>
<td>26.00</td>
<td>26.62</td>
<td>1.46</td>
<td>38.38</td>
<td>36.07</td>
</tr>
<tr>
<td>4</td>
<td>58.68</td>
<td>62.08</td>
<td>4.03</td>
<td>36.98</td>
<td>36.83</td>
</tr>
</tbody>
</table>

1^C=control, L=laser enrichment

2^HAP was carried out on all pens once on wk1 and once on wk 6. An unfamiliar human entered the pen and after 15 s, a photograph was taken to analyze the birds’ response

3^Data was collected in bird counts, converted to percent of birds in the pen exhibiting each behavior
Figure 5-1A-D. Ross 308 broiler home pen behavior results of focal bird during 4-min laser periods: day x treatment LSMeans (±SEM)\(^1\) percent of time spent: (A) active; (B) inactive; (C) at feeder\(^2\); and (D) at drinker; with day and treatment as main effects, d0-8

\(^1\)Values lacking a common superscript are significantly different (P ≤ 0.05)

\(^2\)At feeder convergence criteria relaxed to \(10^{-6}\)
Figure 5-2A-D. Ross 308 broiler home pen behavior results of focal bird during 4-min laser periods: week by treatment LSMeans (±SEM)\(^1\) percent of time spent: (A) active; (B) inactive; (C) at feeder; and (D) at drinker; with day and treatment as main effects, wk 1-6

\(^1\)Values lacking a common superscript are significantly different (P ≤ 0.05)
Figure 5-3A-B. Ross 308 broiler latency to feed of LASER focal birds (n=7) by: (A) day, $P = 0.0014$; and (B) week, $P = 0.03$. Latency was recorded in seconds using video recordings from laser periods and for 5 min following, then separated into 4 mutually exclusive categories.
Figure 5-4A-E. Mean walking distance (cm) of focal bird during 4-min laser periods day x treatment LSMeans (±SEM)\(^1\) during: (A) min 1; (B) min 2; (C) min 3; (D) min 4; and (E) total distance walked\(^2\) (4 min), with day and treatment as main effects, d0-8

\(^1\)Values lacking a common superscript are significantly different (P ≤ 0.05)

\(^2\)Total distance convergence criteria relaxed to 10\(^{-6}\)
Figure 5-A-E. Mean walking distance (cm) of focal bird during 4-min laser periods: week by treatment LSMeans (±SEM)\(^1\) during: A) min 1; B) min 2\(^2\); C) min 3; D) min 4; and E) total distance walked; with week and treatment as main effects, wk 1-6

\(^1\)Values lacking a common superscript are significantly different (P ≤ 0.05)

\(^2\) Min 2 convergence criteria relaxed to 10\(^{-5}\)
CHAPTER 6. GENERAL CONCLUSIONS

Commercial broiler chickens are genetically-selected for feed efficiency and growth that culminates in a market-weight bird at six weeks. This fast rate of growth leads to birds that are 4-5 times heavier and 1.5-3 times more feed efficient than broilers 50 years ago (Havenstein, et al., 2003; Zuidhof, et al., 2014). Up to 30% of today’s heavier birds are prone to leg lameness, a result of skeletal disorders (tibial dyschondroplasia, osteodystrophy, femoral head separation) caused by an out-of-synch bone development in the legs to match rapid muscle mass accumulation (Bessei, 2006; Knowles, et al., 2008; Bassler, et al., 2013). Contact dermatitis (breast blisters, hock burns, and footpad dermatitis) develops due to extended periods of time spent in litter contact (Weeks, et al., 2000; Nääs, et al., 2009). Research suggests leg disorders are painful (McGeown, et al., 1999; Danbury, et al., 2000).

Consumer concerns have arisen in light of these negative welfare issues (Hall and Sandilands, 2007). A viable method to improve leg condition in broilers is to increase physical activity (Reiter and Bessei, 2009). The use of environmental enrichment, particularly utilizing the natural vision-oriented foraging bird behavior, is an option to tackle this without sacrificing performance outcomes but is definitively underused in current production systems (Bessei, 2006; Riber, et al., 2017). The goal of this thesis was to incorporate environmental enrichment to stimulate broilers to voluntarily move and feed, whilst maintaining leg health and performance. A secondary goal was to validate broiler welfare measures recommended by the NCC to understand and improve broiler welfare auditing. Three research chapters (3-5) focused on the following objectives to meet these goals:

1) Validate and modify measures of broiler welfare to determine the effects of enrichment and make recommendations to broiler producers and researchers.
2) Measure the impact of enrichment on broiler production, leg health, and environmental parameters.

3) Study the effects of enrichment on broiler behavior and walking distance within the home pen.

In Chapter 3, the objective was to test and modify broiler welfare measures in-order-to make recommendations regarding measures to include, remove, or adapt within the National Chicken Council (NCC) guidelines, followed by 95% of U.S. chicken producers. The hypothesis was that examining novel and adapted measures of broiler behavior, walking distance, Human-Approach Paradigm (HAP) response, breast and footpad condition, tibia quality, walking ability, and barn air and litter quality would provide valuable additions to the NCC audit welfare audit. In support of our hypothesis, we determined that scoring broiler home pen behavior, walking distance, and breast condition were effective and are recommended for inclusion in the commercial audit. Additionally, we confirmed that adaptations to the gait scoring method used and including footpad dermatitis scoring on-farm are recommended. Tibia quality measures are recommended only in research. Contrary to our hypothesis, the Human-Approach Paradigm proved not to be as effective in broilers as it is in swine (Weimer, et al., 2014) and would only be recommended under specific research conditions or with different poultry species, as broilers do not naturally approach humans. Further validation on a commercial scale of all measures of welfare tested in our study is warranted.

The objective of Chapter 4 was to determine that impact of a laser enrichment device on bird physiology (leg lameness, footpad and breast condition), performance, and environment (air and litter quality). The hypothesis of the work presented in this chapter was that the implementation of the laser device would improve tibia quality and decrease the occurrence of
contact dermatitis while enhancing performance. In support of our hypothesis, performance outcomes were significantly improved in broilers reared in laser-enriched pens. Feed intake and weight gains were increased in each performance period and overall compared to the control, with an overall increased weight of 0.24 kg/bird. Average daily gain was increased in the starter and finisher periods, and overall (5.7 g/bird/d), and FCR was improved in the grower and finisher periods, as well as overall, with a 0.07 point decrease compared to the control. Improving feed conversion and ADG in the same performance period without a change in diet is a notable outcome stemming from this work that is unique compared to other forms of enrichment aimed to stimulate activity (Bizeray, et al, 2002; Jordan, et al., 2011; Altan, et al., 2013).

Additionally, measures of air and litter quality were not affected by enrichment treatment. In contrast to our hypothesis, tibia quality and animal-based measures of contact dermatitis were not affected by the laser enrichment, an outcome corroborated in other studies implementing enrichment in the form of perches (Su, et al., 2000; Bailie and O’Connell, 2015). However, this may be viewed as a positive outcome indicating that no ill-effects of the laser were measured. Future work in this area should look at the repeatability of performance outcomes seen under our research conditions. Additionally, testing the efficacy of the device when implemented for the starter period only (first two weeks of life) would be valuable, as the bulk of bone formation and mineralization take place before day 18 of life in broilers (Williams et al., 2000). Measuring the effects in other species of poultry might also be valuable.

In Chapter 5, our objectives were to stimulate broilers visually using the laser enrichment device to increase walking distance and percent of time active spent active, hence improving walking ability. In support of our hypothesis, our data showed that time spent active was
increased by up to 274% (d7) within the first week compared to the control, and up to 114% (wk 2) over weeks 2-5. Utilizing the walking distance measure, enriched birds walked further than the control on d0-8 and wk 2-5, reaching increases up to 367.5 cm (d1). For a line of bird that spends between 76-86% of its time lying down (Weeks et al., 2000), or 51.6-72.2% of its time inactive over wk 1-6 (control birds from this study, Meyer, et al, 2019; Chapter 5) successfully stimulating the birds to get up and move is noteworthy. This outcome was unique when compared to other forms of enrichment that aimed to stimulate physical activity, but did not see differences (Bizeray, et al., 2002; Shields, et al., 2005; Jordan, et al., 2011; Pichova, et al., 2016).

Further, laser-enriched birds spent more time at the feeder on wk 1 and 5, with up to 84% (d5) more time at the feeder than control birds. The implemented device provided occupational enrichment by encouraging exercise, physical enrichment by increasing complexity in the birds’ home pen, and sensory enrichment by stimulating bird vision (Ag Guide, 2010).

In contrast to our hypothesis, walking ability of laser-enriched birds was not improved above the control, but it was successfully maintained, another notable outcome considering the increased weight gain of enriched birds discussed in Chapter 4. Future work studying the effects of the laser device on broiler behavior and physical movement should be repeated in a research setting, to validate the daily laser time length, and should be carried out in a commercial environment. This could aid in answering the question, would the laser device continue to stimulate movement when birds had significantly more space to roam, and a higher stocking density? Furthermore, determining the minimum number of devices needed per square meter to see the improved performance and increased activity seen here would be important.

The overarching goal of this thesis was to study the effects of a novel laser environmental enrichment device on broiler bird behavior and performance through three objectives: 1)
validation and modification of broiler welfare measures to study the effects of environmental enrichment and make recommendations to broiler producers and researchers, 2) measurement of enrichment effects on broiler production, leg health, and environmental parameters, and 3) quantification of enrichment outcomes on broiler behavior and walking distance within the home pen. The results of this work identify the capability of the laser device to stimulate broiler bird movement and increase time spent at feeder, thus improving feed conversion and weight gain without sacrificing leg health, skin condition, walking ability, or environmental measures.

**Literature Cited**


APPENDIX A. SUPPLEMENTARY FOCAL BIRD TIBIA DATA

Tibia Quality: Multidimensional Preference Analysis Split by Treatment

A.

Figure A1. Multidimensional preference analysis of: (A) control bird tibia (n=35) and (B) laser-enriched bird tibia (n=35) measures¹: bone breaking strength (BBS), bird weight, tibia weight, bone mineral content (BMC), and bone mineral density (BMD)

¹Right tibia were collected from 70 focal birds (35 control, 35 laser-enriched) on d42, weighed, DXA scanned for BMC and BMD, and fractured using an Instron 3367 Test Machine compression method for BBS

²The cosine between two variables indicates the correlation between the variables, the length of the arrows reflect the variance of the original variables. This is a two-dimensional approximation of the dimensions

³Differing colors/symbols represent different birds

⁴Original variables have been transformed into new variables (Component 1 and 2) that account for most of the variance
Figure A1. (continued)
## APPENDIX B. SUPPLEMENTARY BEHAVIOR DATA

### Broiler Home Pen Behavior: Frequency

Table B1. Frequency of focal bird behaviors during 4-min laser periods: day by treatment LSMeans ± SEM d0-8

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Day</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Inactive</td>
<td>C1</td>
<td>L1</td>
<td>C</td>
</tr>
<tr>
<td>Frequency</td>
<td>2.68</td>
<td>3.44</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>fgh</td>
<td>defg</td>
<td>fgh</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>d</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>d</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
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<td>4.39</td>
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<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Active</td>
<td>1.95</td>
<td>3.44</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>i</td>
<td>fgh</td>
<td>hi</td>
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<tr>
<td></td>
<td>a</td>
<td>f</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>4.33</td>
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<tr>
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<td>abc</td>
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<tr>
<td>At feeder</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At drinker</td>
<td>0.48</td>
<td>0.21</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>dfgh</td>
<td>g</td>
<td>abcd</td>
</tr>
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<tr>
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<td>0.65</td>
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<td>Other</td>
<td>0.21</td>
<td>0.35</td>
<td>0.56</td>
</tr>
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<td></td>
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<td>defgh</td>
<td>abcde</td>
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<td>ab</td>
<td>bcd</td>
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<tr>
<td></td>
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<td>ab</td>
<td>abed</td>
</tr>
<tr>
<td></td>
<td>0.83</td>
<td>1.04</td>
<td>0.91</td>
</tr>
</tbody>
</table>

1C=control, L=laser enrichment

2Behaviors categorized as “Out of view” were so infrequent that the data could not be analyzed, relaxed convergence criteria was attempted to $10^{-4}$. 
Table B2. Frequency of focal bird behaviors during laser periods: week by treatment LSMeans ± SEM, wk 1-6

<table>
<thead>
<tr>
<th>Behavior</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>SEM</th>
<th>Week</th>
<th>Trt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>4.37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;deg&lt;/sup&gt;</td>
<td>3.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.53&lt;sup.efg&lt;/sup&gt;</td>
<td>2.54&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.35&lt;sup.efg&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>1.35&lt;sup&gt;efg&lt;/sup&gt;</td>
</tr>
<tr>
<td>Active</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>4.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;deg&lt;/sup&gt;</td>
<td>2.96&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.38&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>2.63&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.71&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>1.28&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>0.92&lt;sup&gt;efg&lt;/sup&gt;</td>
</tr>
<tr>
<td>At feeder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.66&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.63&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.64&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>At drinker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

<sup>1</sup>C=control, L=laser enrichment

<sup>2</sup>Behaviors categorized as “Out of view” were so infrequent that the data could not be analyzed, relaxed convergence criteria was attempted to 10<sup>-4</sup>
Table B3. Broiler behavior during 4-min laser periods: day by treatment LSMeans ± SEM, with day and treatment effects d0-8

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</table>

1C=control, L=laser enrichment

2Values lacking a common superscript within the same row are significantly different (P<0.05)

3 Behaviors categorized as “Out of view” were so infrequent that the data could not be analyzed; relaxed convergence criteria was attempted to 10^-4
Table B4. Broiler home pen behavior during laser periods: week by treatment LSMeans ± SEM, wk 1-6 with week and treatment effects

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<th>6</th>
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<th>Week</th>
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<td>C&lt;sup&gt;1&lt;/sup&gt;</td>
<td>L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>C&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>0.52&lt;sup&gt;eg&lt;/sup&gt;</td>
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</table>

<sup>1</sup>C=control, L=laser enrichment

<sup>2</sup>Values lacking a common superscript within the same row are significantly different (P≤0.05)

<sup>3</sup>Convergence criteria relaxed to 10<sup>-6</sup>

<sup>4</sup>Behaviors categorized as “Out of view” were so infrequent that the data could not be analyzed; relaxed convergence criteria was attempted to 10<sup>-4</sup>
## Walking Distance: Main Effects

Table B5. Walking distance (cm) during laser periods: day by treatment LSMeans ± SEM, d0-8 with day and treatment effects

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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<th>SEM</th>
<th>Day</th>
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<tr>
<td></td>
<td>C(^1)</td>
<td>L(^2)</td>
<td>C</td>
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<td>C</td>
<td>L</td>
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<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>
| 1      | 30.61      | 81.90      | 33.40      | 184.53     | 42.63      | 67.30      | 29.38      | 93.13      | 50.72      | 115.04 | 47.55 | 113.5
|         | 57.25      | 84.11      | 47.21      | 154.74     | 57.05      | 89.59      | 8.61       | <0.01      | <0.01      |     |     |     |
| 2      | 45.18      | 109.98     | 61.68      | 298.37     | 92.04      | 43.74      | 105.6      | 42.24      | 87.26      | 24.98 | 79.03 | 24.76|
|         | 53.98      | 41.29      | 42.22      | 80.77      | 45.86      | 88.86      | 12.69      | <0.01      | <0.01      |     |     |     |
| 3      | 57.13      | 161.16     | 51.85      | 191.68     | 70.56      | 62.52      | 42.02      | 94.97      | 30.59      | 122.97 | 24.87 | 45.31|
|         | 50.86      | 42.91      | 59.29      | 213.17     | 570.61     | 513.17     | 191.58     | 570.61     | 513.17     | 859.66 | 811.28 | 251.12|
| 4      | 52.62      | 203.50     | 59.29      | 167.64     | 42.22      | 68.30      | 46.87      | 49.84      | 21.06      | 76.17  | 30.70 | 68.05|
|         | 45.99      | 410.34     | 127.65     | 391.08     | 15.64      | 59.04      | 187.62     | 356.84     | 91.08      | 35.97  | <0.01 | <0.01|
| Total\(^3\) | 191.58   | 570.61    | 859.66    | 211.28    | 296.14    | 167.17    | 350.65    | 410.34    | 127.65    | 307.1  | 156.0 | 286.4|

\(^1\)C=control, L=laser enrichment

\(^2\)Values lacking a common superscript within the same row are significantly different (P≤0.05)

\(^3\)Convergence criteria relaxed to 10^-6
Table B6. Walking distance (cm) during laser periods: week by treatment LSMeans ± SEM of each minute and total distance, wk 1-6 with week and treatment effects

<table>
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<tr>
<th>Minute</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>C(^1)</td>
<td>L(^1)</td>
<td>C</td>
<td>L</td>
<td>C</td>
<td>L</td>
<td>C</td>
</tr>
<tr>
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<td>40.86(^e)</td>
<td>68.23(^c)</td>
<td>67.65(^{bcd})</td>
<td>156.25(^a)</td>
<td>61.82(^{cd})</td>
<td>92.57(^b)</td>
<td>20.22(^f)</td>
</tr>
<tr>
<td>2(^3)</td>
<td>53.31(^{deg})</td>
<td>83.49(^{cd})</td>
<td>48.48(^{eg})</td>
<td>147.76(^a)</td>
<td>71.64(^{bce})</td>
<td>94.41(^b)</td>
<td>35.67(^i)</td>
</tr>
<tr>
<td>3</td>
<td>71.74(^{bcd})</td>
<td>62.64(^{def})</td>
<td>74.41(^{bcd})</td>
<td>154.42(^a)</td>
<td>41.48(^{cde})</td>
<td>92.52(^b)</td>
<td>28.39(^i)</td>
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<td>4</td>
<td>42.83(^{bce})</td>
<td>63.07(^{cd})</td>
<td>47.27(^{bce})</td>
<td>130.69(^a)</td>
<td>42.78(^{bce})</td>
<td>70.61(^b)</td>
<td>13.14(^e)</td>
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<tr>
<td>Total</td>
<td>212.61(^{df})</td>
<td>289.33(^{cd})</td>
<td>242.47(^{bce})</td>
<td>610.21(^a)</td>
<td>221.03(^{df})</td>
<td>363.02(^b)</td>
<td>98.83(^{b})</td>
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</table>

\(^1\)C=control, L=laser enrichment

\(^2\)Values lacking a common superscript within the same row are significantly different (P≤0.05)

\(^3\)Convergence criteria relaxed to 10\(^{-5}\)
Figure B1. Mean number of 0.6 by 0.6 m gridlines\(^1\) crossed by the red-colored focal bird during 4-min laser periods: (A) d0-8; and (B) wk 1-6; day or wk by treatment LSMeans ± SEM\(^2\)

\(^1\)Video observer placed a clear sheet over the computer screen and used a ruler and marker to divide the pen into eight 0.6 by 0.6 m squares, following the PVC pipe supports present every 0.6 m on the sides of the pen

\(^2\)Values lacking a common superscript are significantly different (P\(\leq\)0.05)
APPENDIX C. BETWEEN LASER PERIODS

Between Laser Periods: Broiler Home Pen Behavior

A. Percent of time active during non-laser periods

B. Percent of time inactive during non-laser periods

Figure C1. Ross 308 broiler home pen behavior results of focal bird during 4-min non-laser periods\(^1\): day x treatment LSMeans (±SEM)\(^2\) percent of time spent: (A) active; (B) inactive\(^3\); with day and treatment as main effects, d0-3\(^4\)

\(^1\)Three 4-min time periods (06:30, 18:30, and 22:30) selected when the laser was not on were analyzed for focal bird behavior

\(^2\)Values lacking a common superscript are significantly different (P \(\leq 0.05\))

\(^3\)Inactive convergence criteria relaxed to \(10^{-7}\)

\(^4\)At feeder and drinker behavior between laser periods occurred so infrequently that data could not be analyzed
Figure C2. Ross 308 broiler home pen behavior results of focal bird during 4-min non-laser periods: week by treatment LSMeans (±SEM) percent of time spent: (A) active; (B) inactive; (C) at feeder; and (D) at drinker; with day and treatment as main effects, wk 1-6

1Three 4-min time periods (06:30, 18:30, and 22:30) selected when the laser was not on were analyzed for focal bird behavior

2Values lacking a common superscript are significantly different (P ≤ 0.05)
Between Laser Periods: Walking Distance

Figure C3. Mean walking distance (cm) of focal bird during 4-min non-laser periods\(^1\) day x treatment LSMeans (±SEM)\(^2\) during: (A) min 1; (B) min 2; (C) min 3; (D) min 4; and (E) total distance walked (4 min), with day and treatment as main effects, d0-8

\(^1\)Three 4-min time periods (06:30, 18:30, and 22:30) selected when the laser was not on were analyzed for focal bird behavior

\(^2\)Values lacking a common superscript are significantly different (P ≤ 0.05)
Figure C4. Mean walking distance (cm) of focal bird during 4-min non-laser periods\(^1\): week by treatment LSMeans (±SEM)\(^2\) during: A) min 1; B) min 2; C) min 3; D) min 4; and E) total distance walked; with week and treatment as main effects, wk 1-6

\(^1\)Three 4-min time periods (06:30, 18:30, and 22:30) selected when the laser was not on were analyzed for focal bird behavior

\(^2\)Values lacking a common superscript are significantly different (P ≤ 0.05)