THE EFFECTS OF PRE AND POST HATCH LED LIGHTING ON DEVELOPMENT AND BEHAVIOR IN CHICKENS

A Thesis

by

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ABSTRACT

Lighting is an important factor in raising poultry and has been shown to impact behavior as well as physical aspects of birds. To investigate how light may impact poultry embryos differently depending on egg shell color we conducted an experiment consisting of 4 hatches: 2 using a commercial white leghorn (W-36), and 2 using commercial broiler strains (Cobb 500 and Ross 308) eggs. Each trial consisted of 3 lighted (12L:12D) and 3 dark (0L:24D) incubators containing 288 eggs each. Hatchability and chick quality was measured, and 120 birds from each treatment in the Ross hatch trial were grown to 14 days and tested for behavioral and physical differences. All hatches showed significantly improved ($P < 0.05$) unblemished chicks in the lighted treatments, but only the 2 broiler trials showed greater hatchability when eggs were incubated under lighted conditions (90.12 ± 0.90%) versus dark (85.76 ± 1.58%). The only differences seen in the growout was a significantly lower asymmetry (light/dark: 0.90 ± 0.05 / 1.16 ± 0.07) and heterophil/lymphocyte ratio in the lighted treatments (light/dark: 0.28 ± 0.12 / 0.35 ± 0.11), both of which indicate reduced stress.

A second experiment was conducted to determine how different types of lighting can affect broiler chickens during growth, consisting of 3 lighting treatments: Once LED, NextGen LED, and dimmable CFLs, with 120 broiler chicks in each. Broilers were grown to 45 days of age, and behavioral, welfare, and physical tests were performed throughout. Both LED treatments had lower tonic immobility and asymmetry scores ($P < 0.05$), as well as lower feed conversion ratio. Only the Once LED treatment

had significantly lower H/L ratio and corticosterone concentration, as well as a higher eye height, cornea width, and 14 day bird weight. Spleen weight was lowest in the NextGen treatment and highest in birds under CFLs. Both LED treatments resulted in significantly lower plumage and hock scores than the CFL treatment, with the Once LED treatment also having a lower footpad score, indicating greater perceived welfare. Overall the results of this study show improved performance and reduced fear and stress under LED illumination.

DEDICATION

I dedicate this work to God, through whom all things are done. Without His plan, none of this would have been possible. To my mom, Jaci, who never for a minute lost faith in my ability to complete this step in His plan, I pay homage. She encouraged and supported me from the age of three when I first asked for chickens, homeschooled me from K- highschool, tended my flocks when I was at school and has been my life-sherpa. She kept saying "Someday you will thank me...." I also thank my dad, Tom, who has been my example of the strong work ethic and morals on which our great country was founded. I love you very much and look forward to the continued Journey.

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NOMENCLATURE

CORT	Corticosterone
CFL	Compact Fluorescent Lamp
FCR	Feed conversion ratio
H/L	Heterophil/Lymphocyte
LED	Light Emitting Diode
SE	Standard Error

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CHAPTER I

INTRODUCTION

Efficiency is a trait for which the poultry industry is well known, and is constantly attempting to improve. Ever since the implementation of vertical integration in the 1940s the industry has improved upon housing, feed, management, and even the birds themselves. This has resulted in quality poultry products being produced for a very low cost, and in turn spurred the growth of the industry to become the dominant meat industry in the United States (USDA-NASS, 2013). Notwithstanding the large internal consumption of poultry products, the United States exported 3,171,000 metric tons of poultry meat in 2010 alone, which was approximately 1/3 of the worldwide poultry export market (USDA-NASS, 2012). Of course there is always room for improvement, and the efficacious use of lighting in poultry production could possibly increase efficiency as well as bird welfare (Archer, et al., 2009; Kim, et al., 2013).

One possible management practice to improve efficiency using lighting is to install a light source inside incubators in order to expose the developing embryos to light. Several studies have shown that intermittent lighting during incubation has the potential to accelerate the growth rate of the embryo, result in increased hatchability percent, reduce stress in grown birds, and possibly increase adult bird weights (Lauber and Shutze, 1964; Shafey and Al-Mohsen, 2002; Özkan, et al., 2012b; Archer and Mench, 2013). Implementation of lighting in commercial incubators has previously been somewhat difficult, but with current availability of light emitting diode (LED)

strips the task of adding light to existing incubators has become much easier and cost effective. When utilizing modern LED lights, the incubator can be illuminated without creating excess heat, the spectrum provided can closer match natural daylight, longevity and durability surpasses other lighting available, and there is very little excess electricity usage (Benson, et al., 2013; Morrison, 2013). Using a timer the light and dark periods can be controlled, which will allow circadian rhythms to form in the developing embryo. These rhythms are attributed to the hormonal changes that bring about the differences seen in lighted versus dark incubation (Archer and Mench, 2013).

An additional possibility to improve efficiency is with the use of appropriate lighting in poultry houses. Poultry have a different visual sensitivity than humans, and react quite strongly to diverse lighting (Prescott and Wathes, 1999). Proper spectrum exposure may influence productivity and behavior in poultry, resulting in greater yield and better welfare conditions for the flock. Again, using LEDs for illumination is very cost effective, as they last longer and consume less power than any other alternative on the market today (Watkins, 2014). Current welfare assessments use measurements based on human visual sensitivity to prescribe light levels for poultry rearing. However, since the visual sensitivity of poultry is different than our own, what may be measured as a low or high light level with our current instruments may be perceived in a completely disparate manner by the birds themselves (Prescott, et al., 2003). A proper understanding of the spectrums emitted by each light source used in poultry production, and how the birds themselves perceive the wavelengths, is necessary for creating the optimal lighting environment for the birds.

The study of lighting can also improve our understanding of poultry behavior. It has been previously observed that incubation lighting can cause changes in neural pathways (Isakson, et al., 1970) as well as lowering stress and fear responses (Archer and Mench, 2014b). Further study is needed to be able to identify how this functions, and which kind of responses are changed. The study described herein compares several stress measures along with fear tests on broilers of varying ages, in order to confirm previous studies and lay a groundwork for future research. The effects of different types of modern lighting have not been studied completely, so this project seeks to begin filling in this gap in information by looking at current LED bulbs in comparison to the compact fluorescent lamps (CFLs) which the poultry industry is currently using to replace outdated light sources. Behavioral changes as a result of varying lighting spectrums need to be identified in order to focus future research.

Between the lighted incubation trials and the LED/CFL light source broiler growout comparisons that comprise this study, it is hoped that beneficial data will be produced for both scientific and commercial purposes. The incubation study may provide information to increase hatchability percentages and overall chick quality through the simple addition of low power LED lights. The LED light source comparison study introduces a new technology that may help producers reduce electrical and maintenance costs, while possibly improving bird welfare, behavior, and efficiency due to an improved light spectrum.

CHAPTER II

LITERATURE REVIEW

Lighting and Incubation

The poultry industry has shown consistent growth from 1960 to 2012 with broilers becoming the dominant meat production industry in the United States. The industry surpassed hog production in the 1980s and cattle production in the early 2000s (USDA-NASS, 2013). In 2010 there were approximately 9.06 billion chicks hatched in commercial hatcheries in the United States alone (USDA-NASS, 2012). This growth is the result of a wide diversity of factors such as vertical integration and better feeding practices, which make the poultry industry more efficient, cost effective, and productive than other industries. Efficient incubation and hatching of chickens is therefore an integral part of the industry, and increasing this efficiency can certainly benefit the poultry industry.

The concept of implementing lighting during incubation and hatching has been a subject of study for many years, but only recently has new technology become available to make it feasible for use in commercial hatcheries. The conventional procedure utilized in the commercial poultry industry is to incubate fertilized eggs in complete darkness, with the eggs only being intermittently exposed to light when the incubator is opened (Archer, et al., 2009). There are numerous aspects of light which must be considered when comparing illuminated incubators to the current, mostly dark environments found in many commercial incubators and hatchers. Many aspects of lighting in relation to

incubation have been researched previously. Overall hatchability has been shown to be increased in poultry with the addition of light (Cooper, 1972; Shafey and Al-Mohsen, 2002; Shafey, 2004; Archer and Mench, 2014b), though it seems to vary depending on factors like type of light used or strain of birds. There are some reports of depressed hatchability and increased embryo mortality when light is introduced, which may be attributed to excess heat produced by incandescent bulbs (Tamimie and Fox, 1967; Erwin, et al., 1971). Rate of growth is also affected, with embryos usually showing an accelerated growth rate when exposed to light (Siegel, et al., 1969; Lauber, 1975; Fairchild and Christensen, 2000; Shafey and Al-Mohsen, 2002; Shafey, 2004; Veterany, et al., 2004). Providing light exposure during incubation has been shown to reduce stress and fear levels in broilers post-hatch (Archer and Mench, 2013; Archer and Mench, 2014b). This may be attributed to visual lateralization (Johnston and Rogers, 1999), entrainment of circadian rhythms (Hill, et al., 2004), or changes in hormone levels (Özkan, et al., 2012b). To fully understand the ability of applied lighting to impact incubation, one must first understand some properties of light and how it is sensed by a developing embryo.

Basic principles of light on embryo development

To understand how lighting affects incubation and hatch success as a whole, we must first understand how the light enters the egg, and in what ways the developing embryo is able to sense light. The earliest measurement of an embryo's ability to sense light is at 2 days of incubation, where light exposure stimulates mitosis in neural crest mesoderm (Cooper, et al., 2011). This accelerates the closing of the neural tube , which

in turn differentiates into the precursor of the central nervous system (Isakson, et al., 1970) and is consistent with observations that high intensity light stimulates embryonic cell proliferation (Cooper, et al., 2011). The eye, and more specifically the retina, is the most obvious light sensing organ to consider as it is the primary light sensing organ in an adult bird (Prescott, et al., 2003). It is made up of many photoreceptors, divided into 2 main subgroups- rods and cones - which sense light and relay it to the brain (Witkovsky, 1963). However, light sensing opsins (photoreceptor molecules) were not detected in an embryonic chick until 14 days of development, with development completing on day 18 (Bruhn and Cepko, 1996). The pineal gland, which forms at day 3 of incubation in chickens (Cooper, et al., 2011), is another light sensitive organ possessed by chicks. Aige-Gil and Murillo-Ferrol (1992) directly exposed an embryo's pineal gland to light, which found a significant increase in the number and size of pineal intracytoplasmic lipid droplets in lit versus unlit embryos after 18 days of exposure. Eighteen days is also the time when pineal circadian clocks develop (Cooper, et al., 2011). When light is sensed by the embryonic pineal gland, it triggers the synthesis of melatonin which affects growth rate and development (Archer and Mench, 2014b). Lastly, it has been found that light can penetrate to the cellular levels early in embryogenesis and act on cAMP to regulate cell metabolism, which subsequently leads to DNA synthesis. Thus, light may be able to influence gene expression at a very early stage and accelerate the growth process (Cooper, et al., 2011). The findings of Cooper et al (2011) correlate with a study that showed a difference between the development of lateralization in chicks exposed to light for the first 3 days of incubation vs chicks incubated in the dark or only

lit after 18 days of incubation (Chiandetti, et al., 2013). Furthermore, light exposure during avian embryonic development can cause changes in brain physiological development (discussed below in Visual Asymmetry). All of this information illustrates the importance that light can play during the development of a bird.

Measures of hatchability and chick quality

Hatchability and chick quality are of great importance in determining how productive a hatch will be, so it is imperative to be able to measure and define these terms. Hatchability is simply the number of viable chicks hatched. This can be expressed as a percentage of total eggs set or fertile eggs set, with the latter being more useful for determining the effects of incubator and hatcher conditions on the final product (Shafey, 2004). Chick quality involves several different measurements, including navel development, body weight, leg problems, cull chicks, unhatched egg breakout, and factoring broken eggs into the results. Navel development has been shown to be influenced by light, with the accelerated growth caused by light exposure resulting in improved maturation of the navel over dark trials (Erwin, et al., 1971). This navel maturation is measured by inspecting a newly hatched chick's navel for the presence or absence of navel tags and complete development (Tona, et al., 2005). Scoring the body weight of newly hatched chicks has been shown to be a very good predictor of body weight at slaughter (Willemsen, et al., 2008). Chicks are noted to have leg problems when they show obvious deformities or are otherwise unable to walk. These problems can be due to an old or improperly fed parent flock, genetics, egg handling during first week of incubation, improper egg turning resulting in malposition, low or fluctuating

temperature, or humidity out of optimal range (Tona, et al., 2005; Cobb-Vantress, 2008). Cull chicks are chicks that would be extracted in a commercial hatchery, and include chicks that are dirty, wet, damaged, or otherwise contaminated (Tona, et al., 2005).

Any eggs that are unhatched are often "broken out" and categorized as infertile, early dead, middle dead, late dead, pipped, and broken. Infertile eggs are characterized by complete lack of growth, and are usually removed from hatchability and chick quality calculations. Early, middle, and late dead are categorized as embryos that died on days 1-7, 8-14, and 15-21 respectively (Bungo, et al., 2011). An egg is counted as pipped when the chick begins to crack out of the egg but has not emerged at the time the measurement is taken. Broken eggs are counted when eggs are unintentionally damaged to the extent that it disrupts normal embryogenesis. Early death (0-7 days) can be a result of pre-incubation, rough handling of eggs, improper temperature or humidity, and contaminated eggs (Cobb-Vantress, 2008). Middle death (8-14 days) can occur due to improper turning, inverted eggs, improper humidity or temperature, low ventilation, or contaminated eggs (Cobb-Vantress, 2008). Late dead (day 15-hatch) can result from rough transportation to hatcher, low temperature, improper humidity, inverted eggs, contamination, and inconsistent or wet hatchers (Cobb-Vantress, 2008). Slightly high temperature or small eggs usually results in an early hatch, but too high of a temperature can result in mortality (Cobb-Vantress, 2008). Low humidity or temperature along with large eggs can result in late hatches (Cobb-Vantress, 2008). Unhealed navels can result from high temperature or humidity, and also improper egg storage (Cobb-Vantress, 2008). Most embryo mortality occurs between days 0-7 and 14-21.

Effect of light on development and chick quality

Light has been shown to affect various aspects of chick development, including hatching time, embryonic development, hatchability, visual and physical asymmetry, and stress susceptibility. Lauber and Shutze (1964) conducted a study to determine the effects of light on hatch time, and found that lighted treatments of White Leghorns hatched an average of 20 h before the dark control treatments. Another study using broiler eggs had similar results, finding that light-stimulated eggs hatched an average of 24 h faster than dark controls (Shafey and Al-Mohsen, 2002). Different monochromatic wavelengths show varying degrees of hatch time with yellow, green, and red several hours shorter than dark controls, blue averaging longer than the dark eggs, and regular white still being the shortest overall (Veterany, et al., 2007). This accelerated hatch time has been attributed to an increase in embryonic development (Garwood, et al., 1973). It has been shown that light stimulates embryonic growth and increases daily embryonic weight gain, and decreases time between the stages of embryo development (Shafey, 2004). This growth acceleration has been shown in several strains of chickens (ISA-W, King Saud University Leghorns, Hybro meat breeder and Al-Wadi Pty. Limited meat breeders) (Shafey and Al-Mohsen, 2002; Shafey, 2004), as well as several species of wild birds (*Passer domesticus* and *Columba livia*) (Cooper, et al., 2011). It has also been shown that the first 10 h of incubation are crucial for growth acceleration, and exposure to light for the first 40 h of incubation can increase the growth rate and cell number of a developing embryo (Ghatpande, et al., 1995).

Hatchability has also been shown to be influenced by light exposure, with trials in layers under white florescent light and broilers under green florescent light both showing an average increase of approximately 4-5% over dark trials (Shafey and Al-Mohsen, 2002; Shafey, 2004). Another study compared hatchability under several monochromatic (yellow, blue, green, and red) and white lights exposed for the final week of incubation to dark trials, and found that white light provided the highest hatchability, closely followed by yellow and green, with the dark control having the lowest hatchability (Veterany, et al., 2007). There are several studies that do show negative effects of lighting, for instance Tamimie and Fox (1967) found that lighting an incubator with a 100 watt incandescent bulb resulted in a high percentage of dead embryos, lowered hatchability and chick weight, and deformities in the legs, feet, eyes, and mandibles; this was not seen in dark trials. Isakson, et al. (1970) found that high light intensities (215-430 lux) caused more developmental anomalies than low intensities (54-108 lux), and also stated that temperature increases from the light source may affect development.

Visual asymmetry

Visual asymmetry in the visual pathways has been shown to develop as a result of light stimulation prior to hatching. This is due to the embryo being oriented in the egg such that the left eye is covered and thus only the right eye becomes light stimulated (Rogers and Krebs, 1996). It has been noted that each eye system has unique attributes, with the right eye system (RES) using conspicuous clues to assign stimuli to categories and the left eye system (LES) taking into account all properties of stimuli including

position in space (Andrew, 1988). A test conducted in pigeons showed that dark incubated eggs exhibited no visual asymmetry, while light incubated eggs were right-eye dominant in both tests of visual asymmetry run (Skiba, et al., 2002). Another study that manipulated the embryo to expose the left or right eye showed correlating results. According to Johnston, et al. (1997) a significant amount of asymmetries were seen in several important receptors found in the forebrains of chicks that had their right eye system exposed to light prior to hatching. This corresponded to a similar but reversed pattern found in chicks which had their left eye system exposed to light before hatch. However asymetrical binding of muscimol and AMPA, which was seen in right eye exposed chicks, did not have corresponding asymmetries in left eye exposed chicks. Thus it can be concluded that both brain region and receptor type can play a role in determining neurochemical asymmetries in chick forebrains (Johnston, et al., 1997). Taken together these data show that embryonic light stimulation elicits visual lateralization by differently modulating visuoperceptual and visuomotor systems in both hemispheres (Skiba, et al., 2002). Preference for directional turning in a T-maze has also been observed in broilers exposed to light during incubation (Archer, personal communication).

Eggshell light spectrum filtration

Since it has been observed that different spectrums of light can have an impact on embryogenesis (Veterany, et al., 2007), pigment of the eggshell should be examined, as it can influence which wavelengths of light pass through the shell and reach the embryo. Differences in hatch time were noted in a study by conducting tests of different

florescent lights and were attributed to the eggshell filtering certain light spectrums (Ghatpande et al., 1995). Ghatpande et al. (1995) concluded that only some of the light they were exposing the eggs to was reaching the embryo. A study conducted by Shafey et al (2005) sought to determine the deviations between different eggshell pigment intensities in a lighted incubator. They found that hatchability in lightly pigmented eggs was the highest at ~89% when exposed to low levels (900-1380 lux) of light, as opposed to medium and dark pigmented eggs that only reached ~81% and ~85% hatchability, respectively, when exposed to the same light. When exposed to high intensity (1430-2080 lux) light, the hatchability of lightly and medium pigmented eggs were reduced, while dark pigmented eggs saw no change (Shafey, et al., 2005). Thus they concluded that the shade of brown eggs does indeed impact the light intensity reaching the embryo. It has been hypothesized that eggshell pigmentation can influence the embryo in many ways, including thermo-regulation, UV-B protection, photo-acceleration, lateralization, circadian rhythm, photo-reactivation, and antimicrobial defense (Maurer, et al., 2011). Spectral analysis of pigmented and non-pigmented eggshells shows that on average 99.8% of light will be absorbed by the shell, with absorption in the near-ultraviolet spectrum being higher than the near-infrared. The pigmented eggshells were shown to have a generally higher absorbance than the non-pigmented in each of the wavelengths tested. However, a difference was noted in that the peak absorbance for the pigmented group was around 525 nm while the peak absorbance for the non-pigmented group was 325 nm, indicating that the pigment may have different absorption qualities than the shell itself (Shafey, et al., 2002).

Role of light in development of circadian rhythms

Circadian rhythms are biological rhythms that occur on an approximately 24 h period, and are found in all animals. Circadian melatonin rhythms have been shown to be controlled by the pineal gland, and is entrained by the light:dark cycle (Brainard, et al., 1982). This cycle can be interrupted by exposure to light during the normal dark period, which rapidly reduces pineal melatonin content. Constant exposure to light can cause adverse effects such as hyperopia and flattening of the cornea, which are attributed to the lack of a melatonin rhythm (Li and Howland, 2003). Research has shown that circadian rhythms can be entrained in a prehatch chick by exposing the embryo to light on a 12 h light/12 h dark schedule (Hill, et al., 2004). Hill et al found that embryonic light exposure from day 13 to 18 resulted in circadian rhythms being present in post hatch tonic immobility tests and body temperature measurements. This held true if the embryo was only exposed to light for 12 h on a single day between days 13 to 18. The results of this study also showed that short bouts of light during the usual lighted period can entrain a rhythm, but it is not as strong as a full 12 h cycle. No rhythms were detected in chicks exposed to light before incubation day 13. (Özkan, et al., 2012b), concluded that a 16 h light/8 h dark schedule for the whole incubation period using white florescent lights was also able to entrain a circadian rhythm, due to observed fluctuations in melatonin that was not seen in their dark trials. Contrary to the findings of (Hill, et al., 2004), Özkan did not see as strong of a rhythm in a trial that only exposed embryos to light for the last seven days of incubation. A recent study by Archer, et al. (2009) found that behavior rhythms after hatch were not affected by pre-hatch lighting to a

noticeable degree. Light intensity has also been shown to work alongside the photoperiod in entraining circadian rhythms, with higher light/dark contrasts resulting in more distinct rhythms (Blatchford, et al., 2012). Out of sync circadian rhythms may result in decreased immune responses, depressed growth, or abnormal behavior, so for the sake of efficiency it is important to maintain correct rhythms.

Hormones and behavior

Hormones are commonly known to influence the behavior of many animals, so hormonal changes due to incubation lighting may have an effect on the bird after hatch. As discussed previously, changes in lighting can influence production of the hormone melatonin during dark periods through the pineal gland as well as the retina (Reed and Clark, 2011). Along with circadian rhythms, melatonin is involved in thermoregulation, feeding and digestion, and immune functions in chickens (Özkan, et al., 2012a). Pituitary adenylate cyclase activating polypeptide (PACAP) has been shown to participate in modulation of circadian rhythm and to stimulate melatonin secretion, and induces an increase in cyclic AMP (cAMP) production (Faluhelyi, et al., 2004). The second messenger cAMP has been shown to initiate DNA sequencing and regulate cellular metabolism, though exact behavioral effects in chickens have not been studied (Cooper, et al., 2011). Corticosterone (CORT) is a stress hormone that is produced in chickens during lighted periods and may interact with melatonin to modify the stress response, however it is not known whether early entrainment of embryo circadian rhythms of endogenous melatonin through incubation lighting affects CORT concentration or is directly involved in the oxidative stress status of broiler embryos and

neonatal chicks (Özkan, et al., 2012a). Archer and Mench (2014a) measured melatonin levels in 19 day old embryos, and found that birds incubated under a 12L:12D lighting program had a significantly higher concentration than dark and 1L:23D treatments; at five weeks of age however there were no melatonin differences seen between any of the treatments. However, the light incubated birds were less active at night and fed more vigorously in the morning, which may indicate that light incubation can have long lasting behavioral effects (Archer and Mench, 2014a). It has been shown that an incubation lighting program can influence the hormone production in grown birds. When presented with a stressor, grown birds that had been incubated under a 12 h light/ 12 h dark program showed very little change in their CORT concentration, while dark incubated birds and birds with only one or 6 h of light still showed an increase in CORT (Archer and Mench, 2013). It has been hypothesized that the melatonin rhythms produced due to a lighting period during incubation modifies the HPA axis to change the amount of CORT and melatonin produced, which in turn allows the bird to adapt more easily to stressful situations (Özkan, et al., 2012a; Özkan, et al., 2012b).

Effect of pre-hatch light on bird behavior and stress

Lighting during incubation has been shown to impact several aspects of behavior in post-hatch birds, including imprinting, learning, fear, stress and motility. The previously discussed visual asymmetry that comes about from embryonic light exposure has been shown to affect the ability of the chick to imprint in a study conducted by Johnston and Rogers (1999). By occluding one eye during the final stage of incubation, the researchers were able to control which eye was light stimulated. After hatch, the

chicks were presented with an imprinting stimulus and then tested for their imprinting preference by injection with glutamate. Chicks that had the right eye exposed to light during incubation showed recall of the imprinting stimulus after injection of the left hemisphere but not after injection into the right hemisphere, while the reverse was found for chicks that had the left eye exposed to light.

Sui and Rose (1997) showed that pre-hatch lighting can influence learning and memory retention in chicks, especially if they are exposed at days 19 to 20. Their test involved training 24-72 h old chicks, both dark incubated and light incubated, on a passive avoidance task. Both treatments acquired the behavior normally, but only the light incubated birds retained the avoidance behavior in subsequent tests. Rogers, et al. (2007) found that lateralization through exposure of light to either the left or right eye during incubation is capable of impacting the chick's ability to learn. These results are consistent with previous studies (Vallortigara, 1989; Vallortigara, et al., 1996), which found that visual asymmetries in chicks are capable of enhancing learning ability.

Fear is another behavior influenced by pre-hatch lighting. Dimond (1968) showed that chicks who were exposed to light during incubation would be more inclined to move a further distance away from a moving object than the dark incubated control chicks. While the control chicks displayed no avoidance responses, the lighted chicks showed on average one response after 6 h and 2 responses after 12 h. Dimond and Adam (1972) found that exposing embryos to slowly flickering light (5 min each hour for 12 h, then 0.33 Hz flicker for 24 h) decreased time to approach in an approach test over unlit or embryos exposed to faster flickering light. Deng and Rogers (2002) found

that chicks will show a definite choice in approaching familiar and unfamiliar chicks 3 days after hatch when using their left eye, but only show choice using the right eye after undergoing additional visual/social experience. They did not find any difference in approach behavior when chicks incubated under light for 12 h a day were compared to dark incubated chicks. Increases of fear levels in chicks have been implicated in feather picking, with more fearful birds delivering more pecks (Vestergaard, et al., 1993). This was shown to relate to the fear associated with pre-hatch lighting in a 2004 study, with light exposed chicks having a higher proportion of pecks than dark controls (Riedstra and Groothuis, 2004). They found that the proportion of feather pecks by dark chicks aimed at the familiar individual was lower than that by their light-exposed cagemates. The proportion of pecks at the familiar bird was lower than expected if cagemates had been randomly targeted (expectation = 1/3) in the dark chicks, but did not deviate from random in the light-exposed chicks. Archer and Mench (2014b) recently showed that broilers incubated in lighted conditions for a minimum of the last 2 weeks of incubation exhibited lower fear response in several fear tests when compared to dark incubated controls.

Stress is also impacted by pre-hatch lighting but unlike fear, has been shown to be reduced in light exposed birds. When presented with a stressor, birds who had been incubated in lighted conditions showed a much lower CORT response in relation to dark controls (Archer and Mench, 2013). This indicates that the light incubated birds have an overall lower level of stress, and may not be impacted to the degree that dark incubated birds are during usual industry handling procedures. It has also been noted that light

exposure of eggs during incubation can decrease asymmetry of the birds posthatch, which may indicate that light stimulation can cause a decrease in stress susceptibility later in life (Archer and Mench, 2013). Several tests showed that birds incubated under a 12 h on, 12 h off lighting program showed lower stress hormone levels, lower asymmetry, and less of a stress response to being crated than birds incubated in complete dark or only given light for one or six h a day (Archer and Mench, 2013).

Motility has also been shown to be affected by lighting during incubation. Bradley and Jahng (2003) demonstrated that exposure to light increases the amount of beak clapping, rapid limb movement, and respiratory-like movement in embryos at 18 days of incubation. They stated that the purpose of these movements is to position the chick in the egg in preparation for hatch. Perhaps this is correlated to the increased hatchability exhibited by chicks hatched under lighted incubation conditions.

Lighting and Bird Growth

All poultry need light to live, and modern farming practices usually require artificial lighting to meet this need. Light itself is a complex and varied phenomenon, made up of an entire spectrum of wavelengths and intensities. As such, light affects many aspects of growth and behavior in all manner of living organisms, and must be taken into account when attempting to provide the most efficient controlled environment for poultry production. Poultry have evolved highly specialized visual systems to aid in their survival, and much of poultry behavior is mediated by their vision (Mendes, et al., 2013). If an ideal poultry production environment is to be created, one must understand how the birds will react to different light spectrums and intensities.

For many years the industry has relied on incandescent light bulbs to provide illumination in poultry houses. These bulbs come in a variety of colors and intensities, but are currently being phased out due to their relatively high power consumption. Fluorescent lights, especially the newer compact fluorescent lights (CFLs), offer a significantly lower level of power consumption for a similar light output and are currently favored by the industry (Burrow, 2008). However, CFLs do not all work well on the dimmers needed to set an adequate light level in the house, and those that do, have not standardized their function. They also contain small levels of toxic heavy metals that may cause problems if the bulb is broken. More recently light emitting diodes (LEDs) have been moving into the market and are becoming more affordable. They offer much longer lifespans than the other types of bulbs, decrease power consumption, and provide a different spectrum output which has been described as more realistic by various reviewers (Morrison, 2013). By selecting the optimum light source for a particular flock, one should be able to maximize growth and efficiency while reducing unneeded stress and fostering ideal behavior.

Basic principles of light and poultry vision

Poultry have a wide range of vision as a result of the 4 types of single-cone photoreceptors in their eyes (Osorio, et al., 1999). These provide the birds with the ability to see light in the human visual spectrum as well as the ultraviolet range, meaning they can see light of wavelengths between approximately 350-700 nm with maximum visual sensitivity at 415nm, 455 nm, 508nm, and 571nm (Prescott, et al., 2003). The significance of vision in poultry was substantiated in a test that compared the behavior of

sighted chickens to blind chickens. The blind birds would exhibit an increased amount of time sitting and preening, and were less likely to peck at the environment or engage in group behaviors (Collins, et al., 2011). The blind birds also weighed less, and exhibited unusual behaviors such as air pecking, star gazing, and circle walking. Blindness has been observed in commercial operations, and while the birds can learn their surroundings and survive, they will fail to thrive to the same degree as sighted birds (Cummings, et al., 1986). These results are as expected, but the roles of specific wavelengths are difficult to pinpoint. The role of ultraviolet light has been studied most, and there are several hypotheses about its function. In many birds it can be used for orientation, as there are UV patterns they can see in the sky (Bennett and Cuthill, 1994). Feeding is another theory discussed by Bennett & Cuthill, as certain food sources reflect more UV radiation than their surroundings. Another hypothesis they gave is that it is used in sexual selection, as feathers often reflect UV light in specific patterns that would be visible to other birds. This has been specifically tested in broiler breeders, where one flock was given supplemental UV lighting while the control was not. The UV-enriched environment increased the number of attempted matings over the control as well as resulted in increased locomotion (Jones, et al., 2001).

Discussion of pineal gland and biorhythms

Biorhythms are crucial to the proper function of many animals, and can occur over many different periods of time. The most studied of these are circadian rhythms, which follow roughly a 24 h period and are usually related to the daily light-dark cycle (Kumar, et al., 2004). Other rhythms exist that are as short as a few minutes or as long

as the yearly circannual rhythms. These rhythms are maintained by various tissues known as pacemaker cells and modifications to clock proteins; oscillations in these tissues synchronize with external stimuli to form clocks. There are many clocks working in birds, and the interactions between them form the centralized clocking system. Independent input-pacemaker-output systems are present at a minimum of 3 levels — the retina of the eyes, the pineal gland, and the hypothalamus (Kumar, et al., 2004). The main conductor of these signals to the bodily functions of the bird is the hormone melatonin, which is produced in the retina and pineal gland (Nichelmann, et al., 1999). The pineal gland is directly sensitive to light and is capable of synchronizing its melatonin output to cyclic light input, as well as rapidly inhibiting melatonin release during the entrained normal dark periods if exposed to light (Li and Howland, 2003). It has been shown that a light-dark period is needed to maintain a proper circadian rhythm, as exposure to constant light inhibited expression of circadian rhythms in Japanese quail (Lumineau and Guyomarc'h, 2003). The importance of maintaining proper rhythms is outlined by Kumar, et al. (2004), as the various clocks present in birds are capable of controlling behavior, molt, reproduction, and proper physiological function.

Effect of light spectrum on birds

The spectrum of light a bird is exposed to must be taken into consideration when studying lighting effects. As previously noted, birds perceive light differently than humans, and their vision is often superior to our own in that they can see a wider spectrum of light (Prescott, et al., 2003). There are many varieties of artificial light sources available with many different spectrums, so understanding how the bird is

affected by them is essential. Certain behaviors have been shown to be frequency dependent through trials that exposed birds to specific frequencies. Birds were shown to spend more time sitting or standing under short wavelengths (blue/green), and exhibited more locomotion under longer (red/yellow) wavelengths (Sultana, et al., 2013). The red/yellow treated birds exhibited tonic immobility for longer periods of time, indicating that they were more fearful than the short-wavelength exposed birds. Green light caused the greatest feeding duration of all the trials of the Sultana study, but a different study showed green light to reduce time spent feeding (Huber-Eicher, et al., 2013). Red light has also been shown to increase the speed at which layer hens reach sexual maturity, and increased levels of estradiol in serum samples (Gongruttananun, 2011). Skeletal muscle growth can also be affected by light spectrum, with higher muscle weights being found in birds exposed to green or blue lights (Halevy, et al., 1998). When exposed to ultraviolet light at a young age, birds were seen to have significantly reduced development of rickets and tibial dyschondroplasia (Edwards, 2003). Another study showed that exposure to ultraviolet light significantly increased egg output in broiler breeders, suggesting that the exposure prolongs the laying cycle through a modification of the hormonal control of photorefractoriness (Lewis, et al., 2007). The spectra emitted by various commercial bulbs varies quite a bit by type; incandescent bulbs have an almost linear increase in intensity with very low UV output up to high infrared output, CFLs have a spectrum composed of many highly focused peaks throughout the visual spectrum, and LEDs produce a fairly smooth spectrum with a small peak in the blue range and a larger peak in the red range (Morrison, 2013). Of the 3 spectra, the LED

output most closely matches the spectral sensitivity of birds as outlined in Prescott and Wathes (1999).

Effect of light intensity on birds

Taking into account that ultraviolet radiation is also visible to birds it must be included in any calculations, along with any differences in perceived spectrum (Prescott and Wathes, 1999). This implies that the modern measurement of lux may not accurately depict the light intensity perceived by the fowl. Determining an optimal light intensity is crucial, as a bright environment can result in unwanted behavior and a dim environment can impair the bird's ability to see. Along with ultraviolet light visible to birds, perception of color intensity must be analyzed since birds have different color receptors than humans (Bennett, et al., 1994). Birds have been seen to have several sensitivity peaks in their vision: ~570nm (yellow) is the highest point (Prescott and Wathes, 1999), with other peaks in blue, red, and ultraviolet (Osorio, et al., 1999). These visual differences may result in drastically different perceptions between humans and birds; for instance the linearly increasing wavelength of incandescent bulbs may appear much more red shifted to birds (Prescott and Wathes, 1999), or the drastically peaked output of CFLs (Morrison, 2013) may be much less or more intense than we perceive depending on where the peaks lie. Early studies on light intensity showed that exposure to 2 or 5 foot candles resulted in significantly heavier birds than trials with more intense lights (Skoglund and Palmer, 1962). A later study compared 1, 10, 20, and 40 lux treatments and found that body weight did not change between the trials, but carcass, thigh, and drum yield as a percentage of live weight as well as ulcerative

footpad lesions decreased linearly with increasing light intensity (Deep, et al., 2010). When social behavior of hens was tested in 1, 5, 20, and 100 lux environments, only the 1 lux trial showed any impairment in their behavior (Kristensen, et al., 2009). In a test for intensity preference it was found that broilers and layers preferred the bright (200 lux) environment, but when tested again at 6 weeks preferred the darkest (6 lux) environment (Davis, et al., 1999). This behavior was again seen in another study, and was attributed to broilers preferring higher light intensities when they are active and lower intensities when they are inactive (Alvino, et al., 2009). As the broiler grows it usually becomes less active, hence the preference change from 2 to 6 weeks of age. This same study indicated that higher intensity lights resulted in longer, less interrupted resting bouts during the scotophase, and resulted in greater behavioral synchrony in the flock. An additional study showed that there was no change in melatonin levels of broilers raised under 1 or 40 lux, but the 1 lux birds rested more often and preened less, potentially indicating a reduced welfare state (Deep, et al., 2012).

Effect of light period on birds

Lighting period has been known for quite some time to have a strong impact on poultry. This is most strongly seen in layers, which require an increase and stabilization of their photoperiods from 12 h to around 16 h to stimulate laying (Rozenboim, et al., 1998). Changes in these lighting schedules can cause a reduction or cessation of laying, or potentially induce molt, so alternative lighting periods are not commonly found in commercial laying flocks. Many different lighting schedules have shown various degrees of success in broilers, including 23L:1D, 18L:6D, 8(1L:2D), and

6(1L:2D):1L:5D) (Lewis, et al., 2010). However, exposure to constant light has been found to result in corneal flattening, hyperopia, cataracts, and photoreceptor damage and is generally not seen in the industry (Li, et al., 1995). Hassanzadeh et al. (2003) found intermittent lighting schedules (1L:3D) to result in lower mortality due to heart failure and ascites when compared to a continuous schedule (23L:1D). This fits with an earlier study showing a higher incidence of sudden death syndrome in birds under continuous lighting as compared to those on an intermittent schedule (Ononiwu, et al., 1979). Another study showed that behavior can be enhanced by using a 16L:8D lighting schedule, and birds reared in those conditions exhibited lower fearfulness and a greater degree of sociality than birds under a continuous lighting schedule (Bayram and Ozkan, 2010). Intermittent lighting schedules have also been shown to result in a higher protein content in the breast meat, but showed no difference in growth performance at the end of the experiment (Li, et al., 2010).

Different types of light sources

There are several different types of light sources available to the industry including incandescent bulbs, CFLs, dimmable CFLs, cold-cathode bulbs, tube fluorescents, high pressure sodium (HPS) vapor bulbs, LED bulbs, and LED strip lighting (Burrow, 2008). Incandescent bulbs have previously seen a wide use in the industry, but they do not provide good power efficiency, lifespan, or even the best growth results. A 1990 study compared incandescent lights (IN) to fluorescent (FL) and sodium vapor (SV) sources, and their effects on the growth and reproduction of turkeys. Not only did the study show that IN bulbs had a higher maintenance cost and over 4

times the power consumption as FL or SV sources, but the birds grew better under SV and had better egg production under both SV and FL (Felts, et al., 1990). Durability of the bulbs is another concern, and a group of agricultural engineers has studied how well IN, 2 types of CFL, and LED bulbs can function in long term poultry production conditions. They subjected the bulbs to frequent power cycles to accelerate the failure rate for the test, and found that IN bulbs failed at an average of 1,968 h, the CFLs at an average of 1,640 and 3,312 h (though not all failed), and had no LED failures for the duration of the 416 day test (Benson, et al., 2013). The LEDs did show the most degradation decreasing their output by around 50% over the test, but the researchers state that there were no poultry LED lamps available at the time and the experiment will have to be done again to test the newer bulbs made for those conditions. Recent field observations have shown that the newer LED poultry lamps are maintaining their brightness at 70 to 80% after 2 years, and are resulting in calmer birds due to the lack of the flickering phenomenon found in many CFLs (Watkins, 2014).

Stress and stress measures

Stress occurs when an animal experiences changes in the environment that stimulate body responses aimed at reestablishing the homeostatic condition (Mumma, et al., 2006). The central nervous system perceives these changes as a threat, and develops a biological response to act against the stimulus (Moberg, 2000). Stress is usually considered a negative phenomenon but some studies have indicated the existence of eustress, which is stress that results in a positive outcome (Sherwin, et al., 2013). It is well documented that stress can have a damaging effect on an individual, and can

increase susceptibility to disease, interfere with reproduction, and hamper development (Moberg, 2000). This harmful type of stress is known as distress, and is defined by the animal exceeding its energy reserves and diverting energy away from normal biological functions in order to cope with the stress. Embryonic light exposure has been shown to result in decreased stress levels in birds when compared to birds not exposed to light during incubation (Archer and Mench, 2014b) and it is theorized that birds may be less stressed under LED lighting than other types of lights during growth..

HPA axis

The hypothalamic-pituitary-adrenal axis (HPA axis) is a set of neuroendocrine systems that are primarily involved in metabolic homeostasis and particularly in the regulation of energy fluxes (Mormede, et al., 2007). When external stressful stimuli are detected by an animal, a cascade of events leads to the activation of the sympathetic nervous system which in turn acts on hypothalamic neurons to stimulate the release of corticotropin-releasing hormones (CRH) and vasopressin (VP). CRH and VP then stimulate the release of adrenocorticotropic hormone (ACTH) from anterior pituitary corticotrophs which in turn stimulates glucocorticoid release from the adrenal cortex (Moberg, 2000). The main glucocorticoid hormone of the HPA axis in poultry is corticosterone, and measuring this hormone is the standard approach to the study of stress and welfare in farm animals (Mormede, et al., 2007). Glucocorticoid hormones can be measured in several biological samples, including plasma, saliva, urine and feces. Current methods for the assay of glucocorticoids in biological samples are radioimmunoassay (RIA), enzyme- linked immunoabsorbent assay (ELISA), and high-

pressure liquid chromatography with UV detection (Mormede, et al., 2007). These measures are useful for determining levels of stress in birds by exposing them to a stressor, taking a blood sample, and comparing the corticosterone levels of the various treatments (Archer and Mench, 2013). Archer and Mench used this technique to determine that broilers incubated under 12L:12D lighting had the smallest change in corticosterone levels when compared to birds hatched under darkness or shorter periods of light. While corticosterone measurement has been used often, one must take into account environmental factors, the stress introduced by animal handling and vessel puncture or the rapid oscillations of circulating levels, and the sensitivity of the HPA axis to a large range of stimuli that are not necessarily harmful to the animal (Mormede, et al., 2007). However, compared with other data the HPA axis and corticosterone measurement can provide useful information on the levels of stress exhibited by poultry (Archer and Mench, 2013).

Heterophil/lymphocyte ratio

Heterophil/lymphocyte ratio is another measure of stress in poultry. The number of lymphocytes in chicken blood samples decreased and the number of heterophils increased in response to stressors and to increasing levels of corticosterone in the chicken feed. The ratio of heterophils to lymphocytes was less variable than the number of heterophil or lymphocyte cells, and appears to be a more reliable indicator of levels of corticosterone in the feed and to social stress than were the plasma corticosteroid levels (Gross and Siegel, 1983). To perform this test, one must acquire a blood sample from the bird and create a smear on the slide. After staining the slide to increase visibility, the

heterophils and lymphocytes are counted individually until the total number reaches 100. Then the ratio is simply the number of heterophils to the number of lymphocytes (Campo, et al., 2000). A test of different photoperiods on layer hens (23L:1D, 14L:10D, or 18.5L:5.5D) showed no significant difference in heterophil/lymphocyte ratio, indicating that the photoperiod in this study did not have an effect on the levels of stress in the birds (Campo and Davila, 2002). However, a different study tested the effects of 24 h lighting to a 14L:10D schedule, and found that the heterophil/lymphocyte ratio was significantly higher and tonic immobility duration significantly longer in the continuously lighted birds (Campo, et al., 2007). This indicates that continuous light can stress the birds, and that it correlates with the results of fear tests. The results of a different test suggested that it is possible to select for stress resistance on the basis of heterophil/lymphocyte ratio using the 99% lower confidence limit method of selection, and is positively correlated with several important reproductive traits (Al-Murrani, et al., 2006). Heterophil/lymphocyte ratio was also shown to not be significantly different across different breeds, unlike other stress measures such as physical asymmetry or fear measures like tonic immobility (Campo, et al., 2000).

Physical asymmetry

Physical asymmetry is another measure of stress in poultry. To perform a physical asymmetry assessment, one measures bilateral structures on the chicken and the difference between these 2 structures indicates the amount of asymmetry (Campo, et al., 2008). Composite asymmetry is simply the average of the signed difference of the traits measured. Posthatch lighting has been previously shown to alter the way a bird copes

with stressors (Campo, et al., 2007), and recent findings have found similar changes with pre hatch lighting (Archer, et al., 2009). Continuous lighting has been shown to increase body asymmetry in hens (Møller, et al., 1999; Campo, et al., 2007). Knierim, et al. (2007) suggests that physical asymmetry of this kind may indicate impairment of the bird's ability to cope with stressors throughout its lifetime, and is therefore a decent indicator of animal welfare. The 3 types of asymmetry (antisymmetry, directional symmetry, and fluctuating symmetry) are characterized by a different combination of mean and distribution of left minus right measurements (Yang, et al., 1997). The first 2 types can be caused by either adaptation or detrimental stress effects, whereas the last is most often caused by a developmental instability and therefore the optimal type to measure (Moller and Swaddle, 1997). Since directional symmetry and antisymmetry both have a genetic component, fluctuating asymmetry is most often used as the primary indicator of the effects of developmental stressors (Van Poucke, et al., 2007). However, Graham, et al. (1993) argued that any type of asymmetry may indicate the effect of stress. Lens and Van Dongen (2000) empirically confirmed this by showing that wild birds evidenced a switch from fluctuating to directional asymmetry when faced with increasing levels of habitat disturbance. In a recent review Knierim, et al. (2007) emphasized the importance of measuring a sufficient number of traits and subjects to accurately evaluate differences in asymmetry and notes that effectiveness varies with the types of animals used. Another study found that asymmetry in broilers may only reflect recent growth history, and can be useful for determining the current stress level of the flock instead of lifetime cumulative stress exposure (Kellner and Alford, 2003).

Discussion of fear

Fear has been a popular field of study in many animal behavior and psychology fields, and as such there have been many methods determined to measure fear. Fearfulness has to be considered as a component of personality and we cannot dismiss the validity of fearfulness as an intermediate variable that partly explains the interindividual variability observed in animal behavior (Boissy, 1995). According to Boissy, personality, temperament or individual behavior exists in nonhuman animals, and considerable progress has to be made in the understanding of interindividual differences. In the poultry industry, the fact that chickens are capable of feeling fear, frustration, and pain can be considered a welfare issue (Duncan, 2002). Fearfulness is a partially genetic trait, and has been selected against in modern domestic chickens (Campler, et al., 2009). Campler et al compared the results of 4 different fear tests in the ancestral Red Junglefowl to modern White Leghorn chickens, and found that not only were the White Leghorns less fearful in each test but they also had a shorter latency to feed after being exposed to a fear inducing stimulus than the Red Junglefowl. Fear can result in stress on the bird, which may eventually have negative consequences on production. According to Ratner (1967), fear of predation is a major component in fear behavior of prey animals, and is linked to predator avoidance behavior. Ratner (1967) defines 4 such avoidance behaviors as freezing, fleeing, fighting, and tonic immobility.

Freezing occurs when an animal sees a predator from a distance, and ceases any movement or vocalization in an attempt to camouflage themselves and avoid detection

(Ratner, 1967). This freezing behavior may also assist the prey when already spotted by the predator, in that another moving object may divert its attention away from the prey (Suarez and Gallup, 1983). Freezing is not the same as tonic immobility, and is used in latency to vocalize, open field, and novel object approach tests.

Flight refers to when an animal will attempt to flee an approaching predator, and is most often measured by flight distance (Dwyer, 2004). According to Dwyer (2004), flight distance is the closest proximity a prey will allow a predator to approach before felling, and the radius of this zone is dependent upon the perceived threat and the disposition of the animal. This level of fear can be reduced with acclimatization to the predator (Jones, 1993), and thus it is best to use an unfamiliar human to measure the normal flight distance of birds when performing an approach test. (Miller, et al., 2006) showed that flight distance was a repeatable and predictable measure of fear when tested on Japanese quail.

Fighting occurs when the predator has caught the prey, and consists of the prey struggling in an attempt to break free (Ratner, 1967). This can be measured in poultry through the implementation of an inversion test, described below. Newberry and Blair (1993) state that since inversion is used in transportation of birds it is a very practical measure of fear in the poultry industry, as exhibition of a stronger fear response may result in injured birds during transport. A correlation between inversion and tonic immobility results also strengthens the view that inversion is a good measure of fear (Newberry and Blair, 1993).

Tonic immobility is the last of Ratner's predator-prey reactions, and occurs when the prey is unable to escape the predator. It is characterized by a sustained period of non-responsiveness brought about by physical restraint, (Maser, et al., 1973; Jones, 1986). This is considered to be the final phase of anti-predator behavior in the wild, because if unsuccessful in deterring the predator it will usually end in the prey's death (Ratner, 1967). The length of time a bird will remain under tonic immobility in a controlled environment has been observed to be reduced in birds housed in an environment with distinct day/night cycles when compared to birds housed in constant or near-constant light (Campo and Davila, 2002; Campo, et al., 2007; Onbasilar, et al., 2007).

Fear tests

It is important to be able to measure fear in birds if it is to be studied, and there have been several tests developed to predict how a bird will respond to various fear inducing stimuli. Some of these procedures include the inversion, tonic immobility, isolation, and emergence tests (Archer and Mench, 2014b).

The inversion test involves suspending the bird upside down by its legs for approximately 30 seconds and counting the number of flaps and the duration of flapping (Newberry and Blair, 1993). The counting may be done by a second person, or a video recording may be made and reviewed at a later time. The number of flaps and the time spent flapping are all indicators of the bird's level of fear, with longer and more intense flapping correlating to greater fear. Newberry & Blair also found a correlation between duration of flapping and latency to stand in a tonic immobility test.

The tonic immobility test is a fear test that has been used for many years, and is very sensitive to manipulations which affect fear (Jones and Faure, 1981). Tonic immobility is an unlearned response characterized by a catatonic-like state of reduced responsiveness to stimuli, and is elicited by a brief period of physical restraint (Jones, 1986). To perform the test the bird is placed on its back in a cradle lined with black cloth, just narrow enough to prevent the bird from inadvertently rolling while still being able to right itself consciously. Once in place, the bird will have its head covered for 15 seconds to induce tonic immobility. The number of inductions required to induce tonic immobility is recorded, as well as the amount of time to the first head movement and the duration of tonic immobility (until the bird rights itself) (Jones and Faure, 1981). A longer duration of the bird remaining in the immobile state indicates that the bird is more susceptible to the effects of fear, while a shorter duration indicates that the bird is less fear susceptible and able to more quickly come out of the tonic immobile state (Jones, 1986).

The isolation test involves placing the bird in a 19L bucket that visually and physically isolates it from its flockmates for 3 min, and the bird's vocalizations are counted and recorded (Archer and Mench, 2014b). It has been shown using other livestock that social isolation can induce a strong fearful response, since many domesticated species show a high level of anxiety when separated from a group (Forkman, et al., 2007). For instance, in tests using sheep Forkman, et al. (2007) notes that even isolation from their herd in a familiar pen can induce a strong fear response, indicated by an increase in vocalizations. He also notes that tonic immobility tests

performed in the presence of conspecifics were 4 times sorter than tonic immobility tests performed in isolation, which is another correlation of isolation to fear. This is confirmed by Heiblum, et al. (1998), which found that chickens subjected to isolation generally had a higher TI score.

The emergence test involves placing a bird in a dark container, then measuring its latency to emerge when the container is opened to a lighted area (Jones and Mills, 1983). More measurements can also be taken, including latency to orient toward the door, to move within 2 body lengths and one body length of the door, to extend the head through the door, and the number of times the head or body entered the lighted area (Miller, et al., 2005). The results are interpreted as more fearful birds having longer emergence latencies (Jones and Mills, 1983).

Bird behavior under different lighting

As the industry has advanced over the years, new light sources have become available. These light sources can produce a wide variety of spectra (Manser, 1996; Prayitno, et al., 1997; Evans, et al., 2006; Kristensen, et al., 2007; Deep, et al., 2010; Deep, et al., 2012; Huber-Eicher, et al., 2013; Morrison, 2013), so it is important to know if these differences can cause variations in bird behavior. Kristensen, et al. (2006) demonstrated that broilers exposed to light alternating between 5 and 100 lux were always more active under the more intense light, which is similar to the findings of Downs, et al. (2006). A study comparing incandescent bulbs, Biolux fluorescent tubes, warm white fluorescent tubes, and light made to follow the spectral sensitivity of fowl found that there was no preference for light at one week of age, but at six weeks the

birds preferred the warm white and Biolux illumination (Kristensen, et al., 2007). This was attributed to those light sources having spectra that was closer to natural light than the other sources. A second experiment conducted during that study found that feather-directed behavior (such as pecking and preening) and object manipulation occurred more often under the Biolux light than warm white florescent, which may be due to the Biolux spectrum containing ultraviolet A (UV_A) light that is visible to the birds (Kristensen, et al., 2007). This agrees with results from a previous study which found that birds raised without UV light have higher basal corticosterone and stress levels than birds who were exposed to UV light, and concluded that UV light exposure can increase bird welfare (Lewis, et al., 2000; Maddocks, et al., 2001). A more recent study compared the effects of white LEDs, yellow LEDs, and CFLs on broiler behavior. There was no difference in bird preference of the 2 types of LEDs, but feed intake was significantly higher on days 21, 28, and 35 in the birds exposed to white LEDs (Mendes, et al., 2013). In the second part of that study the researchers compared CFLs to LEDs and found little behavioral differences other than 21 and 28 day old female broilers consuming more feed under CFLs. Some studies have been done on monochromatic light sources, which showed that broilers over one week of age have a preference for blue or green light over red or white (Prayitno, et al., 1997). Light preference in layers has been shown to be affected by what they are exposed to in a pullet house, with adult bird preferences for light being the same as the light they were reared under (Gunnarsson, et al., 2008).

Growth and feed conversion under different lighting

Of all the aspects of raising broiler chickens, the end goal is to create an efficient system that results in inexpensive, high quality meat. Results of early studies have shown that intermittent lighting schedules such as 1L:3D and 1L:5D can enhance carcass quality, improve efficiency of broiler production, decrease FCR, and reduce the incidence of ascites over continuous and extended lighting programs of 23.5L:0.5D (Hooppaw and Goodman, 1976; Cave, 1981; Buyse, et al., 1996; Buys, et al., 1998; Downs, et al., 2006; Olanrewaju H.A., 2006). In a study that compared LEDs to CFLs in broilers, seven day old birds had a better feed conversion under white LEDs but there was no difference in older birds (Mendes, et al., 2013). According to Mendes, et al. (2013), birds raised under LEDs performed better overall than birds raised under CFLs, with males reacting more favorably than females. Another study showed that spot lighting of a broiler house using halogen lamps resulted in greater live weight than normally lit incandescent controls without any reduction in welfare (Bayraktar, et al., 2012). Rozenboim, et al. (1999) found that raising broilers under green and blue light enhanced weight gain over birds raised under white and red light. Later in (Rozenboim, et al., 2004) it was demonstrated that green light best stimulates growth before 10 days of age while blue stimulates growth from 10 to 46 days. Raising layer hens under red LEDs resulted in acceleration of their sexual maturation and showed increased early laying performance (70.6%) over white or green light (52.0 and 40.4%, respectively) (Huber-Eicher, et al., 2013), indicating that there may be differences in how light spectrum impacts birds between strains.

CHAPTER III

EFFECTS OF LIGHTING DURING INCUBATION ON BIRD DEVELOPMENT AND BEHAVIOR

Introduction

Incubation is a crucial step in a chicken's life cycle, and the environment in which an embryo develops can have lasting effects on the bird's wellbeing throughout its life (Archer, et al., 2009). It has been documented that temperature, humidity, sound, and light can all impact development of an embryo (Erwin, et al., 1971; Lauber, 1975; Rogers and Krebs, 1996; Özkan, et al., 2012b; Archer and Mench, 2014b). Providing light during incubation has been shown to have several behavioral effects on birds later in life such as increased social pecking (Riedstra and Groothuis, 2004), greater memory retention (Sui and Rose, 1997) and learning ability (Rogers, et al., 2007), reduction in fear responses (Dimond, 1968; Archer and Mench, 2014b), and a decrease in stress indicators (Archer, et al., 2009; Özkan, et al., 2012b; Archer and Mench, 2013). This reduction in stress may be attributed to visual lateralization (Johnston and Rogers, 1999), entrainment of circadian rhythms (Hill, et al., 2004), and/or changes in hormone levels (Özkan, et al., 2012b). Physical changes in the birds have also been noted, including a reduction in the stress-related hormone corticosterone, reduction in physical asymmetry scores, and increased growth rate in young birds (Archer, et al., 2009; Özkan, et al., 2012b; Archer and Mench, 2013; Archer and Mench, 2014b). Overall hatchability has been shown to be increased in poultry with the addition of light (Cooper, 1972; Shafey

and Al-Mohsen, 2002; Shafey, 2004; Archer and Mench, 2014b), though it seems to vary depending on factors such as type of light used or strain of birds.

While there have been several recent studies that used broiler eggs to test the effects of light upon incubation, there have been none that examined the effects of the same light on broiler and layer eggs. Differences in shell pigment can alter light spectrums, which may have an impact on embryogenesis (Veterany, et al., 2007). Studies have already shown that differences in light intensity through varying shades of brown eggs can have a measureable result (Shafey, et al., 2005). Current management practices for broiler and layer egg incubation are quite similar, and both use dark incubation. Since there are varying properties in layer and broiler eggs and birds, it is important to determine how each reacts to light. Any noted differences must be taken into account, and different lighting programs may be needed for optimum hatching in various strains.

Differences in post-hatch growth as a result of lighted incubation have also been seen in previous studies. Results reported have been inconsistent with some papers reporting differences in growth and weight (Özkan, et al., 2012b; Zhang, et al., 2012), and others reporting no changes in performance (Archer, et al., 2009).

Stress has also been shown to be affected by lighting during incubation. Archer, et al. (2009) showed that light incubated birds had a lower physical asymmetry than their dark counterparts, which suggests that the lack of light during embryogenesis can effect stress responsiveness of adult birds. Archer and Mench (2013) showed that lighting during incubation results in a measurable decrease in apparent bird stress over standard

dark incubation, observing both lowered corticosterone levels after crating and reduced bilateral physical asymmetry. It has been noted that photoperiodic lighting during incubation resulted in birds that adapted more easily to novel environments than their dark incubated counterparts, which may result in better post-hatching development (Özkan, et al. (2012a); Özkan, et al., 2012b). Archer and Mench (2013) also observed a higher immune response in light incubated chicks than dark incubated birds. Overall, these findings show that stress responses can be reduced through exposure of light during incubation.

Fear responses have also been shown to be reduced by lighting during incubation (Archer and Mench, 2014b). Tonic immobility (TI) is the most common measure of fear in poultry, and has been shown to be affected by providing light during incubation. Tonic immobility is an unlearned response characterized by a catatonic-like state of reduced responsiveness to stimuli, and is elicited by a brief period of physical restraint (Jones, 1986). Reduced time to come out of TI indicates reduced fearfulness, which was seen in Archer and Mench (2014b) with light incubated over dark incubated birds. Another test of fear used in poultry is the inversion test, which relies on the antipredator fear response of a bird trying to escape capture (Newberry and Blair, 1993). A longer time spent trying to escape the captor, along with overall flapping intensity, indicates an increased fear response (Archer and Mench, 2014b). The isolation test, while relatively unused in chickens, works on the principle that social domesticated animals express a stronger fear response when separated from familiar conspecifics (Forkman, et al., 2007). Archer and Mench (2014b) recently saw that dark incubated birds vocalized

more frequently than light incubated birds in the isolation test, indicating a lower fear response in the light incubated birds.

As little research has been done on how lighted incubation affects eggs from different chicken breeds; therefore we conducted an experiment to investigate this. The objective of this study was to determine how white LEDs affected white and brown eggs in respect to hatchability, embryo mortality, and chick quality. In addition, data was collected to determine if LED lights had effects on stress, fear, and growth as previously observed in broilers exposed to fluorescent lighting during incubation. It is hypothesized that eggs incubated under lighted conditions will result in greater hatchability and lowered stress susceptibility, with possible differences between the different colors of eggs.

Materials and Methods

Animals and husbandry

There were 4 trials conducted in this experiment. Two used White Leghorn eggs (W-36, N = 3456), and 2 used broiler eggs (broiler trial 1: Cobb 500; broiler trial 2: Ross 308, N = 3456). Unless otherwise noted, all trials followed the same procedure and were performed sequentially. Six GQF 1500 incubators and six GQF 1550 hatchers (GQF Manufacturing, Savannah, GA) were used in each trial, and their front windows were blacked out with cardboard to prevent light intrusion into the machines. Three incubators were operated with the traditional dark method of incubation (0L:24D, Dark), while the other 3 were outfitted with (Superbrightleds WFLS-X3 Saint Louis, MO; Light

LED light strips) on each level, with 2 strips running the length of the racks. The strips were attached to metal frames, which were in turn attached to the bottom of the rack above them. For the top rack, light strips were held up by a metal frame made to rest on the top rack. The lights were operated by a timer, with a 12L:12D light schedule at 250lux at egg level as measured using a light meter (Extech 401027, Extech Instruments, Nashua, NH). Two egg trays were set on each rack with each tray holding 48 eggs, for a total of six trays over 3 levels equaling 288 eggs per incubator. The cleanest and best shaped eggs were selected and set large end up in the trays. None of the eggs underwent any further cleaning. The incubators were maintained at standard temperature and humidity levels of 99.5F and 55% relative humidity. The eggs were incubated for 18 days, at which time they were moved into the hatchers of the same treatment (lighted to lighted, dark to dark). The lights were outfitted similarly to the incubators, except the metal racks rested on top of each hatch tray instead of being attached to the frame above. Again the lights were kept at a 12L:12D schedule. The eggs were transferred with all room lights off to avoid unneeded light exposure. Each egg was candled with a handheld flashlight, and any non-viable eggs were removed and broken out after all eggs were transferred. For each incubator, the number of broken, infertile, early dead, mid dead, and late dead eggs were recorded during the breakout. The remaining eggs were incubated in the hatchers for the remaining 3 days of the incubation period. All of the chicks were weighed and counted at hatch. The quality of the live chicks was assessed, and they were categorized and counted as either good, having an unhealed navel, having leg abnormalities, weak, dirty, having traits a hatchery would cull, or having any other

abnormality. The remaining unhatched eggs were broken out, and counted as pipped, broken, infertile, early dead, mid dead, and late dead.

Fourteen day growout

In the second broiler trial (using eggs from a Ross 308 flock), 120 chicks from each treatment (lighted and dark) were set aside and reared for 14 days. They were housed in pens measuring 1 x 2 meters with 20 birds per pen and placed in a random-block design within the house. They were fed standard starter feed milled at the Texas A&M Poultry Research Center (Table 1). Water was provided through nipple drinkers. The house was illuminated by incandescent bulbs and dimmed to an average of 20 lux at chick level using a light meter (Extech 401027, Extech Instruments, Nashua, NH), and set to a 20L:4D light schedule. All feed was weighed (Ohaus Champ CD-11, Pine Brook, NJ) when added to the feeders, and the residual was weighed and subtracted from the total at the end of the growout to quantify total feed consumed per pen. The chicks were weighed when placed into the pens, at one week of age, and at the end of the growout. Pen weight and feed conversion ratio was calculated using these numbers.

Table 1. Broiler starter feed fed during 14-day growout.

Ingredient name	%
Corn	60.34
Soybean meal	32.75
DL-methionine	0.28
Lysine HCL	0.29
Fat, blended	2.43
Limestone	1.57
Biofos 16/21p	1.57
Salt	0.47
Trace minerals	0.05
Vitamins	0.25

Figure 1. Bucket used for emergence test. At one week of age chicks were placed in the bucket, the door was opened, and time to emerge recorded.

Fear tests

To test the fear response of chicks, 2 fear tests were performed. The first test, the emergence test, was conducted at one week of age. For each pen, 10 birds were

randomly caught from the pen and taken to a separate room. They were kept in a large 133 liter uncovered plastic container, and individually withdrawn for the test to be performed. A lidded 19-liter (5 gallon) bucket was modified to have a sliding door in the side (Figure 1), and the person performing the test was seated at an angle to be able to view the door but not be easily seen by an emerging bird. The birds were individually placed in the bucket through the top with the door closed, and then the top sealed with the lid. After 20 seconds, the door was slid open and a timer was started. The timer was stopped when the bird first stepped out of the container, or at a maximum of 3 min. Afterward, the bird was placed in a separate 133 liter uncovered plastic container. After all 10 birds had been tested they were returned to their pen, and 10 birds from the next pen were collected and tested. Longer latency to emerge was considered to indicate more fearfulness.

The second test, the isolation tests, were performed 2 days after the emergence test by again randomly collecting 10 birds from a pen, bringing them to a separate room, and placing them in a 133 liter uncovered plastic container. The birds were then individually placed in an unlidded 19-liter (5 gallon) bucket. A timer was set for 3 min, and the number of vocalizations produced by the bird during this time were counted. Afterward, the bird was placed in a separate holding container. After all 10 birds had been tested, they were returned to their pen, and 10 birds from the next pen were collected and tested. More vocalizations were considered to indicate more fearfulness.

Stress measures

Heterophil/lymphocyte ratios for growout

At 14 days of age, blood samples were collected from 30 birds per treatment. The area around the wing vein was sanitized with 70% alcohol, and in preparation, the inside of a 3 mL syringe was lined with a small amount of heparin. A small amount (approximately 0.5 mL) of blood was collected from each bird, and a drop was used to prepare a blood smear slide. The blood smear slides were stained using a hematology staining kit (Cat# 25034, Polysciences Inc, Warrington, PA), air dried, and stored in a slide box.

Heterophil/Lymphocyte ratio was measured by taking the blood smear slides prepared earlier and observing them under 1000X magnification (10X eyepiece, 100X oil emersion lens) using an Omax DCE-2 microscope (Kent, WA). An area of the slide that had moderate cell density (no overlapping cells) was chosen, and the numbers of both heterophils and lymphocytes observed were counted until the total observed number reached 100. A keystroke counter was used to accurately keep track of the number of cells observed.

Physical asymmetry

Physical asymmetry of each bird was measured at 14 days immediately after they were euthanized using a CO_2/air mixture, and before rigor mortis began to set in. Using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL), the middle toe length, metatarsal length, and metatarsal width were measured for both the right and left legs (Figures 2, 3, and 4).

Figure 2. Example of how middle toe length measurement was taken during data collection of physical asymmetry. All measurements were taken by a single person to avoid comparison errors.

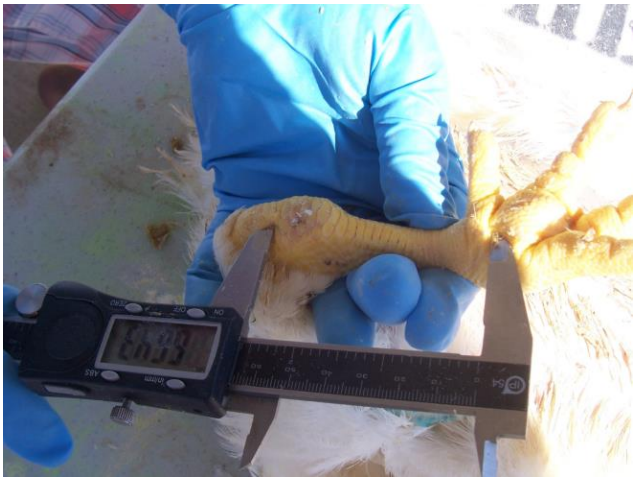


Figure 3. Metatarsal length measurement demonstration. All measurements were taken by a single person to avoid comparison errors.

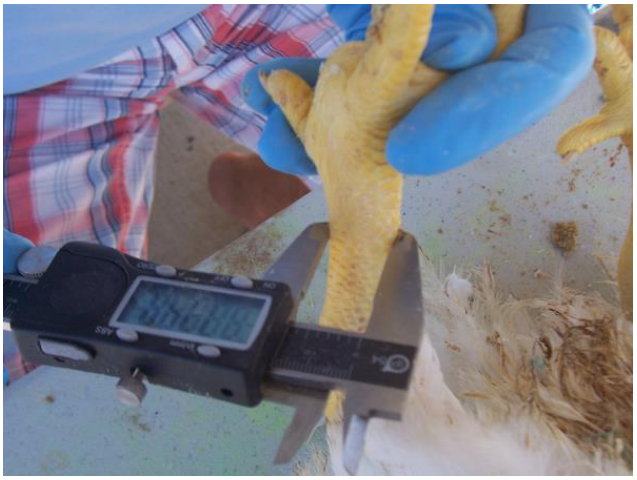


Figure 4. Metatarsal width measurement for physical asymmetry assessment. All measurements were taken by a single person to avoid comparison errors.

The composite asymmetry score was calculated by taking the sum of the absolute value of left minus right of each trait, then dividing by the total number of traits. Thus the formula for this trial would be ($|L\text{-}R|_{MTL}+|L\text{-}R|_{ML}+|L\text{-}R|_{MW}$)/3= composite asymmetry score.

Spectrum analysis

Twenty brown broiler eggs and twenty White Leghorn eggs were obtained and the contents emptied, making sure the large half of the egg remained intact. After the shells air dried for 10 min, they were individually placed over the sensor of an MK350 (UPRTek, Jhunan Taiwan) LED meter and illuminated with a (Superbrightleds WFLS-X3 Saint Louis, MO LED light strip) held 5 cm over the sensor. The spectrum was measured for light passing through all 40 eggs. Then a small flat piece of shell just large enough to cover the sensor was broken off each egg and measured in the same way, in order to test if there was a difference between light passing through a curved shell or a

flat shell segment. A final measurement of unfiltered light was taken as a control, and all duplicated readings were averaged.

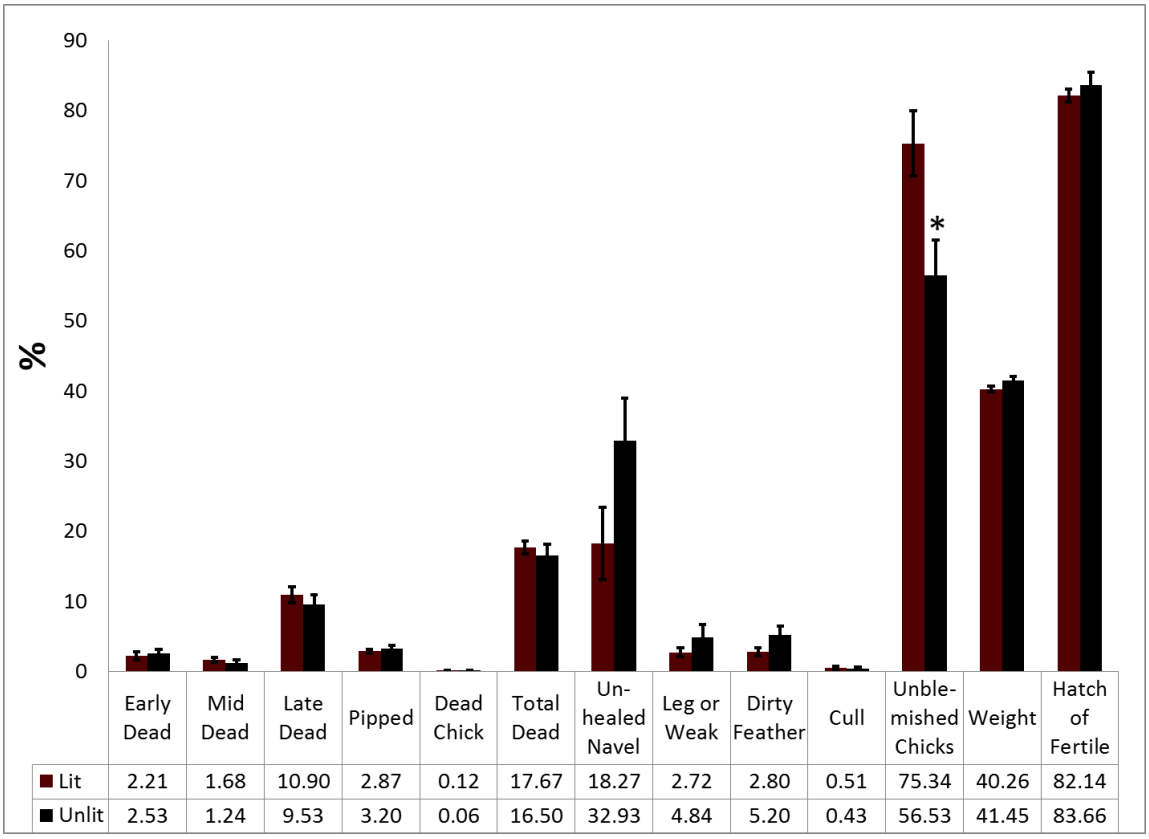
Statistical methods

One-way ANOVAs were used to investigate treatment effects on hatchability, embryo mortality, chick quality, composite asymmetry, corticosterone levels, H/L ratios, fear tests, weight gain, and feed conversion. The least significant difference test was used to test all planned comparisons. All of the assumptions of ANOVA were tested (Shapiro-Wilk test for normality, Levene's test for homogeneity of variance). No transformations were needed to meet assumptions. All analyses were performed using SAS 9.3for Windows (SAS Institute Inc.). The composite asymmetry score was calculated by taking the sum of the absolute value of left minus right of each trait, then dividing by the total number of traits. Thus the formula for this trial would be ($|L-R|_{MTL}+|L-R|_{ML}+|L-R|_{MW})/3$= composite asymmetry score. Significant differences were determined at $P < 0.05$.

Results

In the layer hatch trials (Figure 5) there were no differences between light and dark treatments ($P > 0.05$) in the number of early dead, mid dead, late dead, pipped, dead chick, total dead, chicks with leg problems, dirty chicks, cull chicks, or hatch of fertile. There was however a trend for the light treatment ($P = 0.095$) to have a lower percentage of chicks with unhealed navels than the dark treatment. The total number of unblemished chicks was significantly higher in the lighted treatment ($P = 0.02$), while

the average weight of each chick showed no difference (P > 0.05) between the dark and light treatments.

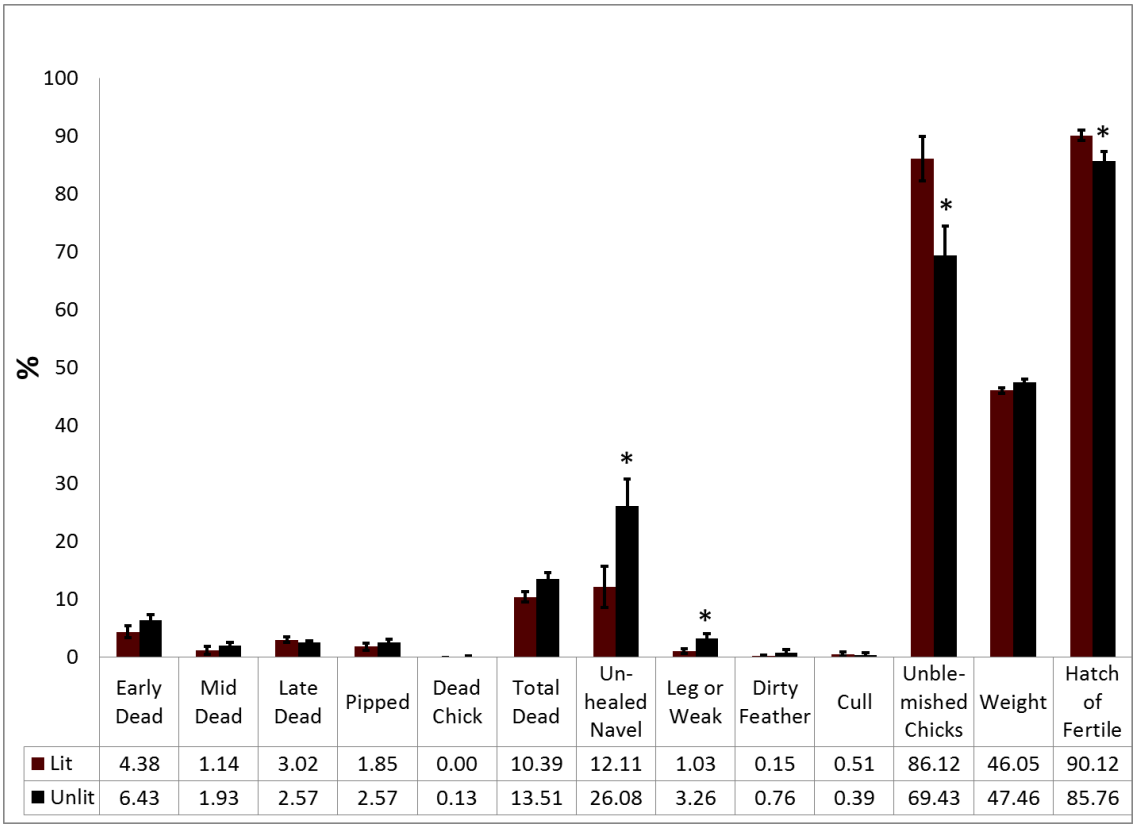


	Early Dead	Mid Dead	Late Dead	Pipped	Dead Chick	Total Dead	Un-healed Navel	Leg or Weak	Dirty Feather	Cull	Unble-mished Chicks	Weight	Hatch of Fertile
Lit	2.21	1.68	10.90	2.87	0.12	17.67	18.27	2.72	2.80	0.51	75.34	40.26	82.14
Unlit	2.53	1.24	9.53	3.20	0.06	16.50	32.93	4.84	5.20	0.43	56.53	41.45	83.66

Figure 5. Layer hatch results, averaged from 2 trials. Comparison of the percent of hatch mortality, navel, leg, dirty, and cull problems, chick weight, overall chick quality, and hatch of fertile eggs between light and dark incubated layer eggs. Significant differences between Light and Dark treatments within measure indicated with * (P < 0.05).

In the broiler hatch trials (Figure 6) there were no differences between treatments (P > 0.05) in the number of embryo mortality, dirty chicks, or cull chicks. Chicks with unhealed navels were significantly higher (P = 0.039) in the dark treatment, as were chicks with leg problems (P = 0.041) when compared to the light treatment. The total

number of unblemished chicks and hatch of fertile was significantly higher in the light treatment (P = 0.026 and P = 0.038, respectively). The average chick weight was not significantly different (P > 0.05) between the 2 treatments.



	Early Dead	Mid Dead	Late Dead	Pipped	Dead Chick	Total Dead	Un-healed Navel	Leg or Weak	Dirty Feather	Cull	Unble-mished Chicks	Weight	Hatch of Fertile
Lit	4.38	1.14	3.02	1.85	0.00	10.39	12.11	1.03	0.15	0.51	86.12	46.05	90.12
Unlit	6.43	1.93	2.57	2.57	0.13	13.51	26.08	3.26	0.76	0.39	69.43	47.46	85.76

Figure 6. Broiler hatch results, averaged from 2 trials. Comparison of the percent of hatch mortality, navel, leg, dirty, and cull problems, chick weight, overall chick quality, and hatch of fertile eggs between light and dark incubated broiler eggs. Significant differences between Light and Dark treatments within measure indicated with * (P < 0.05).

Table 2. Broiler 14 day growout results. Comparison of final bird weight (kg), feed conversion ratio, and composite asymmetry scores (mm). **(**Mean ± SE)

Treatment	14 day weight	FCR	Composite Asymmetry
Light	0.75 ± 0.02^{a}	1.14 ± 0.05^{a}	0.90 ± 0.05^{a}
Dark	0.74 ± 0.03^{a}	1.17 ± 0.13^{a}	1.16 ± 0.07^{b}

Significant differences between Light and Dark treatments of $P < 0.05$ designated by differing superscripts within measure.

For the 14 day growout trial, the composite asymmetry was significantly higher ($P = 0.004$) for birds from the dark treatment compared to the light treatment. Neither overall feed conversion ratio nor ending bird weight showed any ($P > 0.05$) difference at the end of the trial (Table 2). Light treated birds showed a significantly ($P = 0.026$) lower heterophil/lymphocyte ratio than the dark birds (Table 3). There were no differences ($P > 0.05$) in the isolation or emergence tests.

Table 3. Stress and fear measures. Comparison of isolation and emergence scores, along with heterophil/lymphocyte ratios, between light and dark incubated broilers **(**Mean ± SE)

Treatment	Isolation (# of vocalizations)	Emergence (Seconds)	Heterophil/Lymphocyte Ratio
Light	39.83 ± 5.18^{a}	160.6 ± 5.18^{a}	0.279 ± 0.021^{a}
Dark	55.92 ± 8.89^{a}	161.78 ± 4.84^{a}	0.347 ± 0.021^{b}

Significant differences between Light and Dark treatments of $P < 0.05$ designated by differing superscripts within measure.

Discussion

In this study we sought to evaluate the performance of (white) leghorn eggs and were compared to (brown) broiler eggs when they were exposed to LED light. Our hypothesis that the differently pigmented eggs might react differently under lighted incubation conditions. We in fact observed differences between the 2 types of eggs.

The most striking difference was that while broiler hatchability was increased in the lighted trials, there was no difference between the light and dark trials with the leghorn eggs.

Previously, Shafey (2004) did see differences in hatchability among various layer strains, and attributed this to physical dimensions of the eggs allowing different light levels through. While this may play a part, in this study it seems the light spectrum that passes through the eggshell may play a significant part in increasing hatchability. The spectrum readings taken through the shells of each type of egg (Figure 7) are quite different, with the light that passes through the white shell appearing similar to unfiltered light and brown shell producing a much redder spectrum.

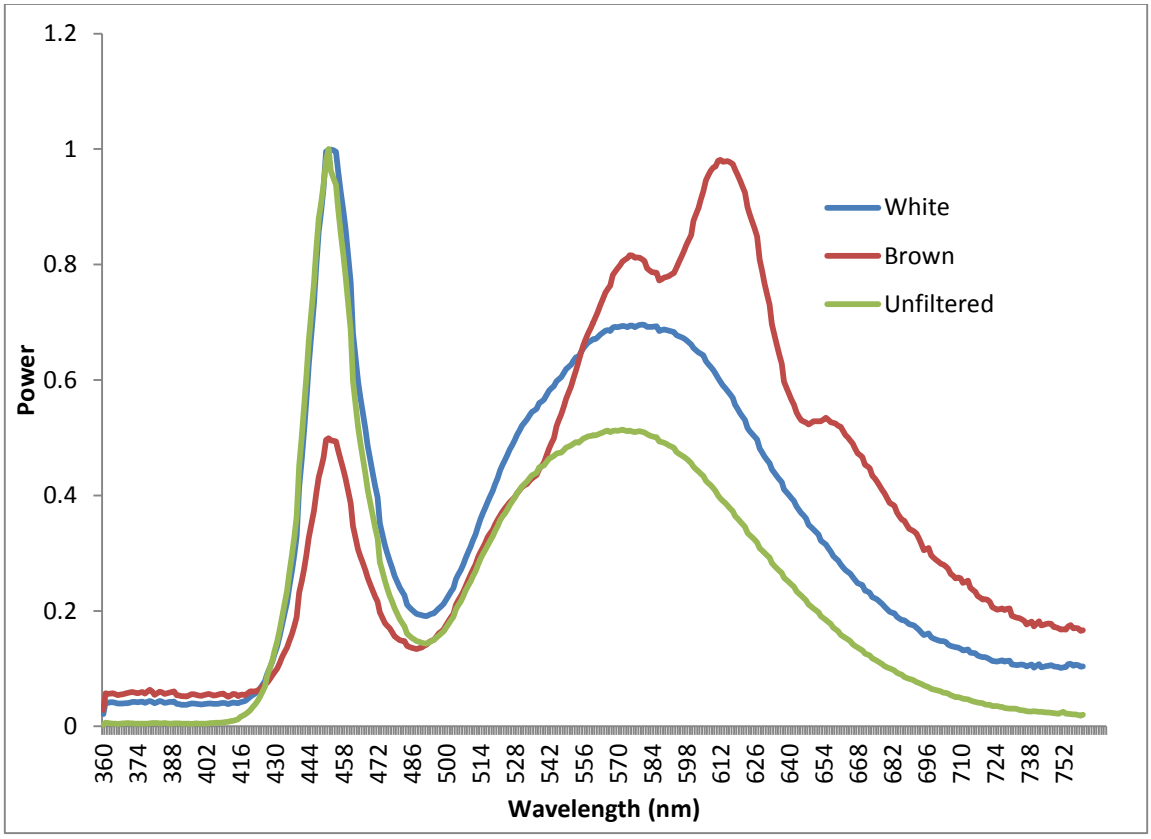


Figure 7. Comparison of spectrum readings through the shells of brown and white eggs to the unfiltered spectrum of the LEDs used in the incubators.

These results correspond to findings by Veterany, et al. (2007), who tested monochromatic lighting during incubation of broiler eggs and found red light produced a higher hatchability than blue, with white light having the highest overall hatchability. It is possible that the higher intensity blue light passing through the white eggs may reduce hatchability, or the brown eggs are able to shift the blue light slightly to the lower energy red wavelength which provides a benefit to the embryo. Further study is needed to determine the exact mechanisms behind the red spectrum shifting phenomenon and the effect of red light on an embryo.

Chick quality, however, does not appear to be strongly affected by the difference in light spectrum, as it was improved in all trials simply by addition of light. This agrees with several previous studies, which found light to increase chick quality as well as growth rate in lighted treatments with dark controls in broilers (Archer, et al., 2009; Özkan, et al., 2012b), layers (Shafey, 2004), turkeys (Fairchild and Christensen, 2000), and wild birds (Cooper, et al., 2011). The largest contributing factor to the difference in chick quality was the number of navel tags seen, with dark treatments having as much as twice the number of navel scores as the lighted treatments. While this difference was only significant in the broiler trials, it did show a trend in the layer trials that may prove to be significant if performed with a larger sample size. This difference in navel tag percentage could be related to the faster growth rate seen in previous lighted incubation experiments (Shafey, 2004; Cooper, et al., 2011), as it may result in the chick internalizing the yolk and healing its navel more quickly than birds incubated in darkness (Shafey and Al-Mohsen, 2002). The lower incidence of leg problems in lighted broiler chicks may also be due to the same principle, with light stimulated growth resulting in better muscle formation or decreased susceptibility to other factors that may lead to deformation. No differences were detected between any of the other measurements of chick quality, but as they only occurred in small numbers it may be beneficial to repeat the experiment with a much larger sample size. There were no differences in chick weight between light and dark treatments, so it does not seem to have been affected by lighting in this trial. However, Shafey and Al-Mohsen (2002) and

Lauber (1975) saw an increase in chick weight in lighted over dark treatments, so further research is required to determine if weight gain is affected by light during incubation.

The results if this study also reproduce and expand upon previous findings in Archer, et al. (2009) and Archer and Mench (2013), which showed that lighting of broiler eggs during incubation resulted in lower stress measures. As seen previously, physical composite asymmetry scores were significantly lower in the light incubated birds than the dark. Since a greater physical asymmetry score indicates the bird underwent some form of stress (Graham, et al., 1993), this suggests that lighting during incubation can reduce the effects of stress on a growing bird. The mechanisms behind this are unclear, but it has been theorized that melatonin rhythms induced during incubation by periodic lighting could alter the HPA axis and make birds more adaptable to stressors (Özkan, et al., 2012a; Özkan, et al., 2012b).

The difference in heterophil/lymphocyte (H/L) ratio observed in this study also suggests that lighted incubation can reduce the effect of stress on birds. Gross and Siegel (1983) demonstrated that the H/L ratio is a good indicator of how much stress the chicken experiences, with a lower value indicating lower stress. The birds in this trial were not intentionally exposed to stressors, so all measures are of baseline stress level. As the results in this study show that birds from lighted treatments have a significantly lower H/L ratio, this again points to the stress reducing effects of incubating under lighted conditions.

The isolation test has not been used often in poultry as a method for determining fear. However, isolation tests done in other animals indicate that decreased vocalization

frequency is a behavior that correlates with reduced fear (Forkman, et al., 2007). Archer and Mench (2014b) saw this same correlation of decreased vocalization to decreased fear in broilers, indicating that it is a viable test of fear in chickens. The results of this study correlate with the stress measures conducted, and show a decrease in fearful behavior in the lighted treatments over the dark treatments. Further study is needed in this area, especially for adult birds, as differentiation between alarm calls and normal vocalizations may be needed (Bayly and Evans, 2003). The other fear test conducted, the emergence test, did not show any significant differences between treatments. This may indicate that lighting can change how chicks perceive different fear-inducing stimuli, but does not universally decrease fear responses. These 2 fear tests observe fear based on separation from familiar conspecifics in the isolation test (Archer and Mench, 2014a) and predator avoidance in the emergence test (Jones and Mills, 1983), so perhaps other antipredator responses such as freezing, fleeing, fighting, or tonic immobility could show differences in future studies (Ratner, 1967).

The feed conversion ratio and overall weight of the birds was not significantly different at the end of the study, as has been seen in other light incubation studies (Zhang, et al., 2012). This indicates that the light acts upon the brain much more than on the physical aspects of the birds. While others have seen increased growth rate in the egg (Shafey and Al-Mohsen, 2002), this is very limited after hatch and does not result in larger or more efficient birds.

Overall, lighting during incubation demonstrated a real potential for benefit to the incubation process. It increases chick quality in both layer and broiler chickens, as

well as increases overall hatchability in broilers. After hatch it reduces the stress susceptibility of the birds, which is beneficial from both a welfare and production standpoint. Fear responses are also reduced, which may reduce damage to the birds and make handling or transportation easier. These results indicate that lighted incubation could not only improve the welfare of the birds but also lead to more profitable production. More research on a larger scale is needed to fully understand the commercial implications, but incubation lighting seems to be a very promising new management practice.

CHAPTER IV

EFFECT OF LED LIGHTING ON BIRD GROWTH AND BEHAVIOR

Introduction

Proper management practices are crucial to improving the efficiency, output, and welfare of commercial poultry operations. While there has been considerable research on feed, temperature, litter, housing, biosecurity, and light periods, there has been relatively little investigation on the best type of light to use. Different light spectrums have been shown to affect bird behavior and even growth, so a proper understanding of the effects of different types of light on poultry could prove to be gainful to the industry. As new technology becomes available, it must be tested to discover positive and negative effects of its implementation. Light emitting diodes (LEDs) have already been shown to be superior to other light sources in terms of power consumption, durability, and longevity (Benson, et al., 2013; Watkins, 2014), but before LEDs can be used in a commercial setting it must be shown that there are no detrimental effects on the birds.

Several factors must be taken into consideration when assessing a lighting program for birds, namely light period, light spectrum, and light intensity. Light period is the most heavily researched aspect of bird lighting, as it is crucial for proper layer management (Rozenboim, et al., 1998) as well as able to increase growth efficiency in broilers (Lewis, et al., 2010). Fear responses have also been shown to be affected by changes in light period, with birds under a 16L:8D lighting schedule showing less fear than birds under continuous light (Bayram and Ozkan, 2010). Light spectrum refers to the amalgamation of different powered wavelengths of electromagnetic radiation emitted

from a light source, and for this paper is limited to the range visible to poultry from 350 to 700nm. The visual range of poultry differs from that of a human in several ways, the most striking being inclusion of the ultraviolet (UV) range due to the addition of a fourth type of single-cone photoreceptor (Osorio, et al., 1999; Prescott and Wathes, 1999). Spectral sensitivity is not even across the spectrum, and birds have been shown to have maximum visual sensitivity at 415nm, 455 nm, 508nm, and 571nm (Prescott, et al., 2003). Certain behaviors have been shown to be frequency dependent through trials that exposed birds to specific frequencies. Birds were shown to spend more time sitting or standing in place under short wavelengths (blue/green), and exhibited more locomotion under longer (red/yellow) wavelengths (Sultana, et al., 2013). The addition of supplemental UV light has been shown to increase mating behaviors, egg output, and locomotion over control birds lit with normal fluorescent lights (Jones, et al., 2001; Lewis, et al., 2007), as well as decreasing the incidence of rickets and tibial dyschondroplasia in developing birds (Edwards, 2003). The spectral output of available light sources can vary drastically: from a direct increase from blue to red in incandescents, to the many narrow peaks seen in CFLs, and finally the 2 or 3 gradual peaks seen in LEDs (Morrison, 2013). Light intensity is related closely to light spectrum, and results in several difficulties in correctly measuring intensity. Since almost all light meters are designed for human sensitivity, they may not be giving a correct approximation of how the bird perceives the light (Prescott and Wathes, 1999). If the peaks in the spectrum do not match the visual sensitivity of the birds, perceived intensity may be much lower than what light meters indicate. Conversely, what a human

perceives as a low intensity may be much more intense to a bird with the inclusion of UV light. While there have been many studies comparing intensity with the same bulb type, it is more difficult to compare light sources with varying spectra. More research is needed to create an accurate model of poultry vision and intensity perception.

Stress has been previously shown to be affected by changes in lighting programs (Özkan, et al., 2012a). Stress occurs when an animal experiences changes in the environment that stimulate responses aimed at reestablishing the homeostatic condition (Mumma, et al., 2006). It is not inherently negative (Sherwin, et al., 2013), but stress is well documented to divert energy away from normal biological functions and interfere with reproduction, immune function, and development (Moberg, 2000). There are several measures of stress used in poultry: physical asymmetry, heterophil/lymphocyte (H/L) ratio, and corticosterone (CORT) concentration. Physical asymmetry is simply a comparison of bilateral structures on a bird; structures on the left and right side of the bird are measured and a larger difference indicates greater asymmetry (Campo, et al., 2008). Physical asymmetry has been strongly correlated to stress in many studies, with greater asymmetry indicating a stronger perception of stress (Graham, et al., 1993; Moller and Swaddle, 1997; Campo, et al., 2007; Knierim, et al., 2007; Archer and Mench, 2013). Heterophil/lymphocyte ratio is another measure of stress in poultry, and involves counting the 2 types of blood cells and comparing their ratio. Gross and Siegel (1983) showed that the number of lymphocytes in chicken blood samples decreased and the number of heterophils increased in response to stressors, but that the ratio of the 2 was a more reliable indicator than individual cell counts. It has been seen that H/L ratio

correlates to other stress measures quite well when measuring constantly lit versus 14L:10D scheduled birds (Campo, et al., 2007). But unlike other stress measures, H/L ratio is not significantly different across different breeds (Campo, et al., 2000). Finally, CORT has been shown to be a reliable indicator of stress in poultry (Archer and Mench, 2013). Corticosterone is a stress hormone that is produced in chickens during lighted periods and may interact with melatonin to modify the stress response, though the mechanism is not fully understood (Özkan, et al., 2012b). Lower CORT concentrations correlate with lower bird stress.

Fear has also been shown to be affected by lighting, with some studies showing that different spectra impact fear responses differently (Sultana, et al., 2013). There are several ways of studying fear in poultry, and can be tied to differences in stress levels and performance. Since poultry are prey animals, fear of predation and predator avoidance are major components of a bird's fear response. Ratner (1967) defines 4 such behaviors as a progression from freezing, to fleeing, to fighting, and finally tonic immobility. The first component, freezing, occurs when an animal sees a distant predator and ceases all movement in an attempt to avoid detection. An animal may still freeze if spotted, relying on other moving objects to distract the predator (Suarez and Gallup, 1983). Fleeing occurs when the predator approaches to a certain distance, known as the flight distance. Once the predator enters the flight distance, the prey will actively attempt to escape and avoid the predator (Dwyer, 2004). If the prey is unable to avoid capture by fleeing, it will attempt to struggle and break free from the predator (Ratner, 1967). This is measured in poultry through the use of an inversion test

described below. Since inversion is used in capture and transport of commercial poultry, Newberry and Blair (1993) state that it is a practical measure of fear for birds used in commercial production. Finally, if the animal is unable to escape, it will enter in to tonic immobility (TI). This response is characterized by a sustained period of non-responsiveness brought about by physical restraint (Maser, et al., 1973; Jones, 1986), and is considered to be the final stage of fear response in wild animals (Ratner, 1967). The length of time a bird will remain under TI in a controlled environment has been observed to be reduced in birds housed under distinct day/night cycles when compared to birds exposed to constant or near-constant light (Campo and Davila, 2002; Campo, et al., 2007; Onbasilar, et al., 2007).

Since there has been little study on the behavioral and physical effects of modern light sources on poultry, an experiment was conducted to elucidate any differences between 3 types of light source. The objective of this study was to compare 2 brands of LED bulbs to the standard CFLs often used in the industry, and to determine if there were any benefits or detriments to using LEDs as a light source. It also compared several stress, fear, and welfare assessments to best determine how changes in lighting affect bird behavior, performance, and efficiency. It is hypothesized that the use of LEDs in place of CFLs will not result in any negative effects on behavior or production, and will act to reduce stress and fear responses in growing and adult birds.

Materials and Methods

Animals and husbandry

This experiment involved 3 treatments: NextGen (AG-PL30-35K, Fayetteville, AR) LEDs, Once Innovations (AC50-662624-12 Plymouth, MN) LEDs, and TCP (TruDim 5012350K Aurora, OH) Dimmable CFLs. Each treatment consisted of 6 pens containing 20 Cobb broiler chicks each in a light tight room outfitted with one of the light sources. Each of the 3 rooms utilized was set up in an identical pattern, with the only difference being the light bulbs in the fixtures. The room measured 8.1x5.8 m, constructed of thick concrete walls, and sealed to prevent any outside light from entering. Ventilation was provided by a single fan on the north end of the room exhausting air, which created negative pressure in the room and drew air in through cooling pads on the south wall. Each of the pens measured 1 m wide, 2 m long and 0.6 m high. The pens were positioned with 3 rows being perpendicular to the east wall and 3 pens perpendicular to the west wall. The pens were constructed of solid black plastic on all but the front side, which was made of mesh wire. The pens were lined with several inches of pine shavings. One feeder and a single row of nipple drinkers were provided per pen, and adjusted for height as the birds grew. All feed was weighed and recorded (Ohaus Champ CD-11, Pine Brook, NJ), and the residual feed at the end of the study was subtracted from the total. There were 6 light fixtures in each room, and 4 of them were directly over the pens 3 meters above the floor. All lights were connected to a single dimmer and timer per room. For the first week, the birds were given 23L:1D at 20 lux of light as measured at bird head height using a light meter (Extech 401027,

Extech Instruments, Nashua, NH). For the rest of the trial the lights were dimmed down to 5 lux and 20L4D. For the first few weeks, heat was provided by a single ceramic heat lamp hung in each pen which gave off no visible light.

For the first 14 days the birds were fed a starter ration (Table 1). After this time the feed was removed from the feeders and weighed. The feeders were then filled with a grower ration (Table 4). Food and water were always available to the birds. In cases of water leak or high humidity, bedding material was added to the pens to keep litter composition consistent across the treatments. Any birds injured or under-developed enough to hamper their access to feed or water were euthanized by cervical dislocation. All mortalities were weighed. The birds in each pen were weighed every 14 days throughout the study. When fear testing began, 10 birds were selected from each pen and marked with a different colored livestock paint on each wing so individual birds could be identified. The color sequences were: (left wing/right wing) pink/pink, green/green, yellow/yellow, black/black, orange/orange, pink/green, green/yellow, yellow/black, black/orange, and orange/green. Upon conclusion of the study, all birds were euthanized with a mixture of air and CO_2.

Table 4. Grower feed fed from day 15 to the end of the study.

Ingredient name	%
Corn	64.73
Soybean meal	29.33
DL-methionine	0.077
Lysine HCL	0.023
Fat, blended	2.00
Limestone	1.46
Biofos 16/21p	1.587
Salt	0.487
Trace minerals	0.05
Vitamins	0.25

Fear tests

Emergence

The emergence test was conducted at 3 weeks of age, modified from methods found in Archer and Mench (2014b). For each pen, the 10 marked birds were taken to a separate room and kept in a large holding container. A lidded 19-liter (5-gallon) bucket was modified to have a sliding door in the side, and the person performing the test was seated at an angle to be able to view the door but not be easily seen by an emerging bird. The birds were individually placed in the bucket with the door and lid closed. After 20 seconds, the door was slid open and a timer was started. The timer was stopped when the bird first stepped out of the container, or at a maximum of 3 min. Afterward the bird was placed in a separate holding container. After all 10 birds had been tested they were returned to their pen, and the 10 marked birds from the next pen were collected and tested. Longer latency to emerge was considered to indicate more fearfulness (Archer and Mench, 2014b).

Isolation

The isolation tests were performed 2 days after the emergence tests by again collecting the 10 marked birds from a pen, bringing them to a separate room, and placing them in a holding container. The birds were then individually placed in an unlidded 19-liter (5 gallon) bucket. A timer was set for 3 min, and the number of vocalizations produced by the bird during this time was counted. Afterward, the bird was placed in a separate holding container. After all 10 birds had been tested, they were returned to their pen, and the 10 marked birds from the next pen were collected and tested. Modified from methods outlined in (Archer and Mench, 2014b), more vocalizations was considered to indicate more fearfulness.

Inversion

The inversion test was performed at 6 weeks of age in the room in which the birds were housed, using methods discussed in Newberry and Blair (1993) and Archer and Mench (2014b). Each marked bird was taken individually from each pen, held upright in front of the camera (Panasonic PV-DV2030, Kadoma, Osaka, Japan) with a hand supporting the breast and the other firmly grasping both legs, and then inverted by removing the hand from the breast and allowing the bird to hang freely upside down. Once the bird ceased flapping for several seconds it was placed back in its pen. After all the birds were inverted and recorded, the video file was transferred to a computer. Using PowerDirector 11 (CyberLink, Taipei, Taiwan) to analyze the video file, the time was found for each bird's duration of flapping (measured from time the hand was removed from the breast to time of last wingbeat), and the number of wingbeats in the time was

counted. Longer and more intense flapping was considered to indicate more fearfulness (Newberry and Blair, 1993).

Tonic immobility

Tonic Immobility (TI) was conducted at 5 weeks of age by again collecting the 10 marked birds from a pen, bringing them to a separate room, and placing them in a holding container. Methods were modified from previous research by Jones (1986) and Archer and Mench (2014b). A 21cm wide by 22cm high by 30cm long wooden cradle with the sides sloping out at a 108 degree angle from the base was obtained, covered in a black cloth and placed on a table. Each bird was individually taken and placed on its back in the cradle. The head of the bird was covered with one hand while the breast was held with the other for approximately 15 s to induce tonic immobility, after which time contact was removed and a timer was started. If the bird righted itself in under 15 s, the timer was reset and the above procedure was performed again. If again the bird righted in under 15 s, it was recorded as a time of 0. Otherwise the time of first head movement and time of righting (or attempting to right) was recorded, with a maximum of 10 min. After all 10 birds had been tested they were returned to their pen, and the 10 marked birds from the next pen were collected and tested. Longer times to first head movement and righting were considered to indicate more fearfulness (Jones, 1986).

Any tests that took multiple days were performed at the same time each day, with equal numbers of birds from each treatment. The lighting and temperature remained constant in the separate room where the emergence, isolation, and TI tests were

performed, and care was taken to transport all the birds to the room in the same low stress manner.

Stress measures

Blood parameters

At 45 days blood samples were collected from 20 birds per treatment, for a total of 60 samples. The area around the wing vein was sanitized with 70% alcohol, and in preparation, the inside of a 3mL syringe was lined with a small amount of heparin. Between 1 to 2 mL of blood were collected from each bird, and a drop was used to prepare a blood-smear slide. The remaining blood was injected into a plasma separation gel and lithium heparin vaccutainer (BD 368056, BD, Franklin Lakes, NJ), which was temporarily stored in an ice bath. Once all samples had been taken, the vaccutainers were spun down in a Beckman GS-6R centrifuge (Beckman Coulter, Brea, CA) for 15 min at 4000 RPM to separate the cells from the plasma. The plasma was poured off into 2mL microcentrifuge tubes and stored in the freezer at -19°C. The blood-smear slides were stained using a hematology staining kit (Cat# 25034, Polysciences Inc, Warrington, PA), air dried, and stored in a slide box.

Corticosterone concentrations were measured using a commercially available ELISA kit (Enzo Life Sciences, ADI-901-097, Farmingdale, NY). The blood plasma samples taken earlier were thawed and spun down in a Beckman GS-6R centrifuge (Beckman Coulter, Brea, CA) for 15 min at 4,000 RPM. Absorbance was read at 405 nm using a Tecan A-5082 Phenix-ST Sunrise (Grödig, Austria) plate absorbance reader (Archer and Mench, 2013). The inter and intra-assay %CV were both under 5%.

Heterophil/Lymphocyte ratio was measured by taking the blood smear slides prepared earlier and observing them under 1000X magnification (10X eyepiece, 100X oil emersion lens) using an Omax DCE-2 microscope (Kent, WA). An area of the slide that had moderate cell density (no overlapping cells) was chosen, and the numbers of both heterophils and lymphocytes observed were counted until the total observed number reached 100 (Campo, et al., 2000). A keystroke counter was used to accurately keep track of the number of cells observed.

Physical asymmetry of each marked bird was measured at 45 days, immediately after each was euthanized using a CO_2/air mixture and before rigor mortis began to set in, following the protocol outlined in Archer and Mench (2013). Using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL), the middle toe length, metatarsal length, and metatarsal width were measured for both the right and left legs. The composite asymmetry score was calculated by taking the sum of the absolute value of left minus right of each trait, then dividing by the total number of traits. Thus the formula for this trial would be $(|L\text{-}R|_{MTL}+|L\text{-}R|_{ML}+|L\text{-}R|_{MW})/3$= composite asymmetry score.

Welfare assessment

A welfare assessment was performed on the marked birds at 5 weeks of age according to procedures outlined in "Welfare Quality: Assessment protocol for poultry" (Butterworth, 2009). The birds were scored on 4 main welfare measures: gait score, plumage cleanliness, foot pad dermatitis, and hock burn.

The gait score was performed by removing individual birds from each pen and encouraging them to walk, and scored using the modified gait scoring system outlined by Garner, et al. (2002). The birds were observed and scored on a 0 to 5 scale. A score of 0 indicates the bird is normal, dexterous and agile with no impairment. A score of 1 indicates a slight abnormality that is difficult to define. A score of 2 indicates that the bird has a definite and definable abnormality. A score of 3 indicates an obvious abnormality that affects the ability of the bird to move. A score of 4 indicates a severe abnormality, with the bird only taking a few steps. A score of 5 indicates that the bird is incapable of walking.

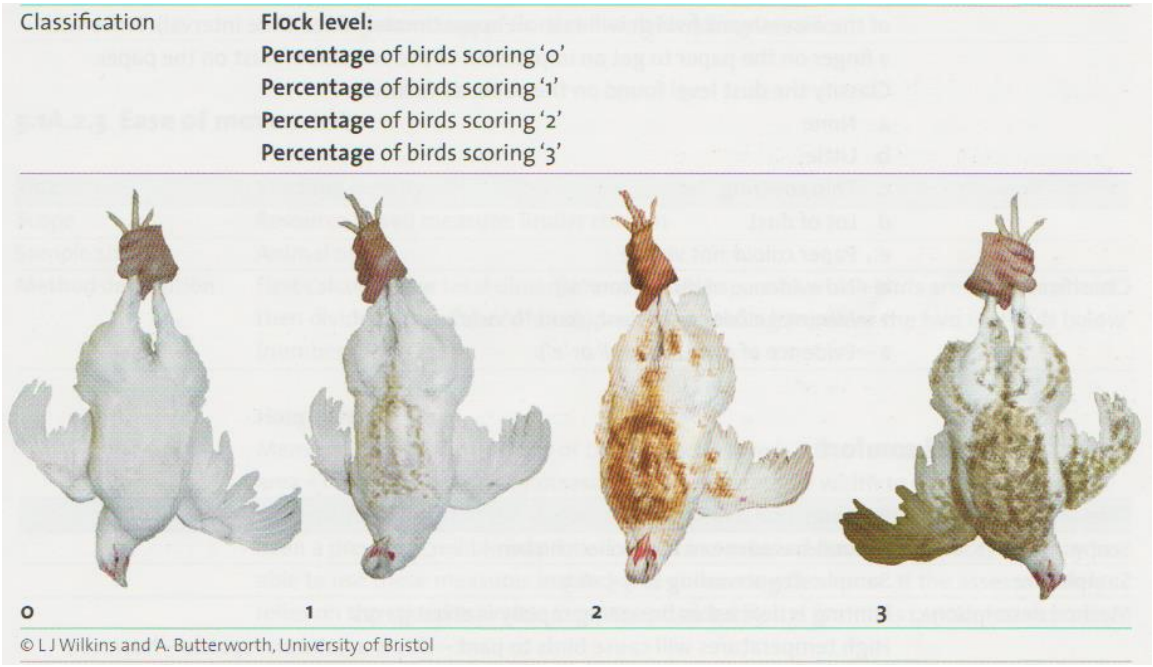


Figure 8. Plumage cleanliness score used as part of the welfare assessment (Butterworth, 2009)

The plumage cleanliness score involved examining individual birds and noting how clean their breasts were. They were scored on a scale of 0 to 3. A score of 0 indicates a clean bird, 1 indicates a bird with slightly dirty feathers, 2 indicates a very noticeably dirty bird, and 3 indicates an almost completely dirty bird (Figure 8).

Foot pad dermatitis scoring involved inspecting the foot pads of individual birds and noting any dark dermatitis lesions present. They were scored on a scale of 0 to 4. A score of 0 indicates no dermatitis is present, 1 and 2 indicate minimal evidence of dermatitis is present, and 3 and 4 indicate that noticeable evidence of dermatitis is present (Figure 9).

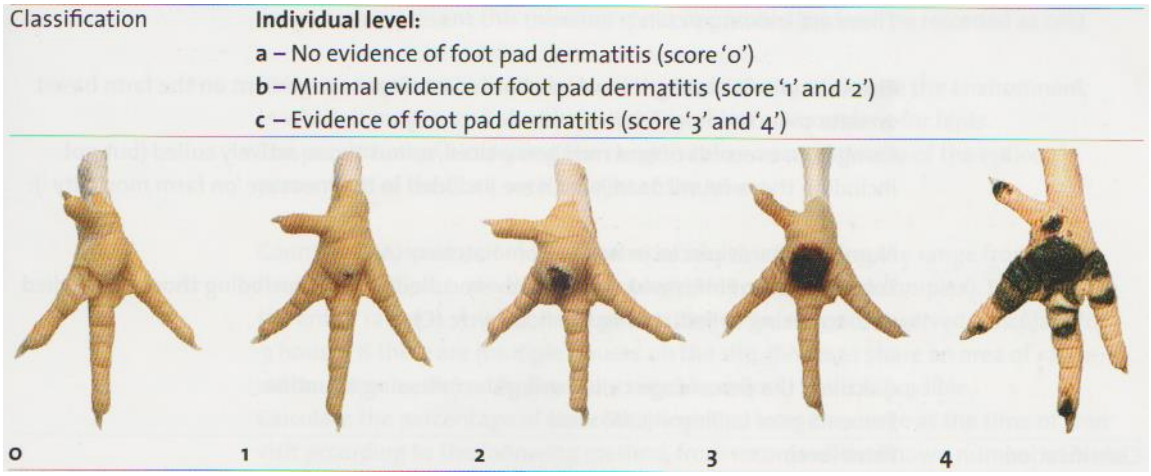


Figure 9. Foot pad dermatitis score used in part for assessment of bird welfare (Butterworth, 2009)

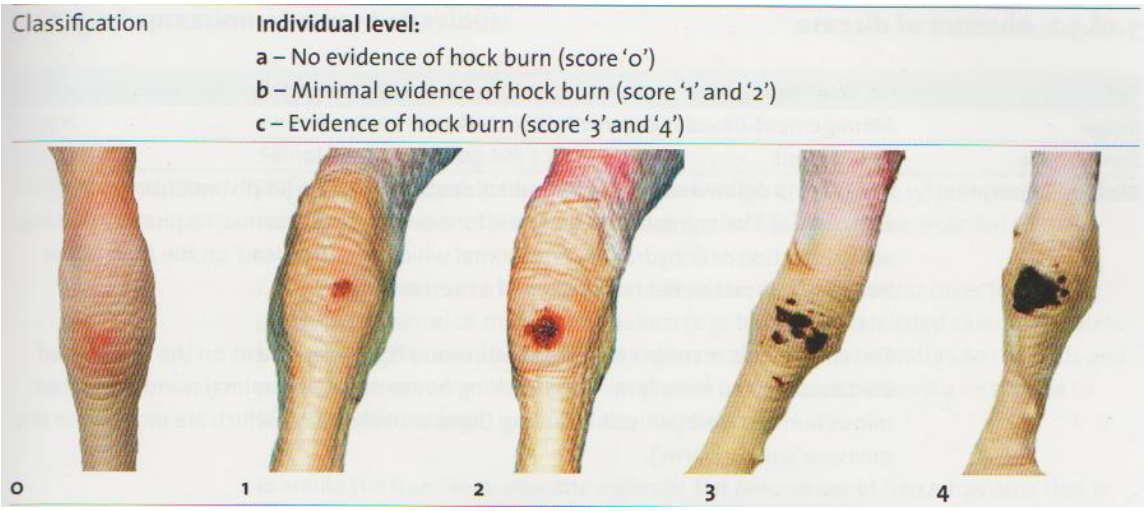


Figure 10. Hock burn score used to determine welfare and environmental effect on birds grown during the study (Butterworth, 2009)

Hock burn scoring involved examining individual birds for the presence of dermatitis on the back of the hock caused by contact with the litter. They were scored on a scale of 0 to 4. A score of 0 indicates no hock burn is present, 1 and 2 indicate minimal evidence of hock burn is present, and 3 and 4 indicate that noticeable evidence of hock burn is present (Figure 10).

Three random homogenized litter samples were taken from each treatment at the end of the trial, and analyzed for percent moisture content. Three 10g subsamples were weighed out from each litter sample using a Mettler PM600 scale (Mettler Instrument Corp, Highstown, New Jersey) and dried at 100C in a Thelco Model 4 (Precision Scientific, Chicago, Illinois) drying oven for 24 hours. The dried litter was reweighed, and the difference in weight used to calculate the percent loss. The 3 subsamples were averaged together to get the overall moisture content for each treatment.

Organ measurements

After they were euthanized, eyes, hearts, and spleens were collected from 20 birds in each treatment. The organs were stored in a refrigerator at 7°C overnight. Each spleen and heart was weighed on an Ohaus Scout Pro (Ohaus SP202, Parsippany, NJ) scale. Both eyes were individually weighed on the same scale, and recorded as left or right. For each eye the cornea width, eye, width, and eye height was measured using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL).

Statistical methods

One-way ANOVAs were used to investigate treatment effects on hatchability, embryo mortality, chick quality, composite asymmetry, corticosterone, isolation, emergence, weight gain, and feed conversion. The least significant difference test was used to test all planned comparisons. All of the assumptions of ANOVA were tested (Shapiro-Wilk test for normality, Levene's test for homogeneity of variance). No transformations were needed to meet assumptions. All analyses were performed using SAS 9.3for Windows (SAS Institute Inc.). The composite asymmetry score was calculated by taking the sum of the absolute value of left minus right of each trait, then dividing by the total number of traits. Thus the formula for this trial would be ($|L-R|_{MTL}+|L-R|_{ML}+|L-R|_{MW})/3$= composite asymmetry score. Significant differences were at $P < 0.05$. Since welfare assessment data were ordinal, they were compared using the Kruskal-Wallis test on the equality of the medians, adjusted for ties. When significant differences were found, the Dwass Steele Critchlow-Fligner method (Hollander and Wolfe, 1999) was used to test for all possible comparisons.

Results

In the tonic immobility tests for the 3 lighting treatments (CFL, Once LED, and NextGen LED) there were no differences ($P > 0.05$) in the time to first head movement. However, time to right was higher for the CFL treatment than for either LED treatments ($P < 0.05$; Figure 11). There was no significant difference between any of the treatments for the time it took the bird to emerge in the emergence test or the number of vocalizations in the isolation test. The inversion test showed no difference in number of flaps, time spent flapping, or overall intensity of flapping (Table 5).

Table 5. Table of isolation, emergence, and inversion test results **(**Mean ± SE)

Treatment	Isolation (# vocalizations)	Emergence (Seconds)	Inversion (# of flaps)	Inversion (flap time)	Inversion (intensity)
Once	39.23 ± 4.60^a	137.10 ± 8.07^a	18.07 ± 1.94^a	3.20 ± 0.30^a	4.22 ± 0.31^a
NextGen	37.20 ± 5.19^a	141.47 ± 7.97^a	16.43 ± 1.90^a	2.97 ± 0.31^a	3.97 ± 0.32^a
CFL	36.03 ± 5.11^a	145.38 ± 7.35^a	17.78 ± 2.16^a	3.20 ± 0.35^a	3.98 ± 0.32^a

Significant differences of $P < 0.05$ designated by differing superscripts.

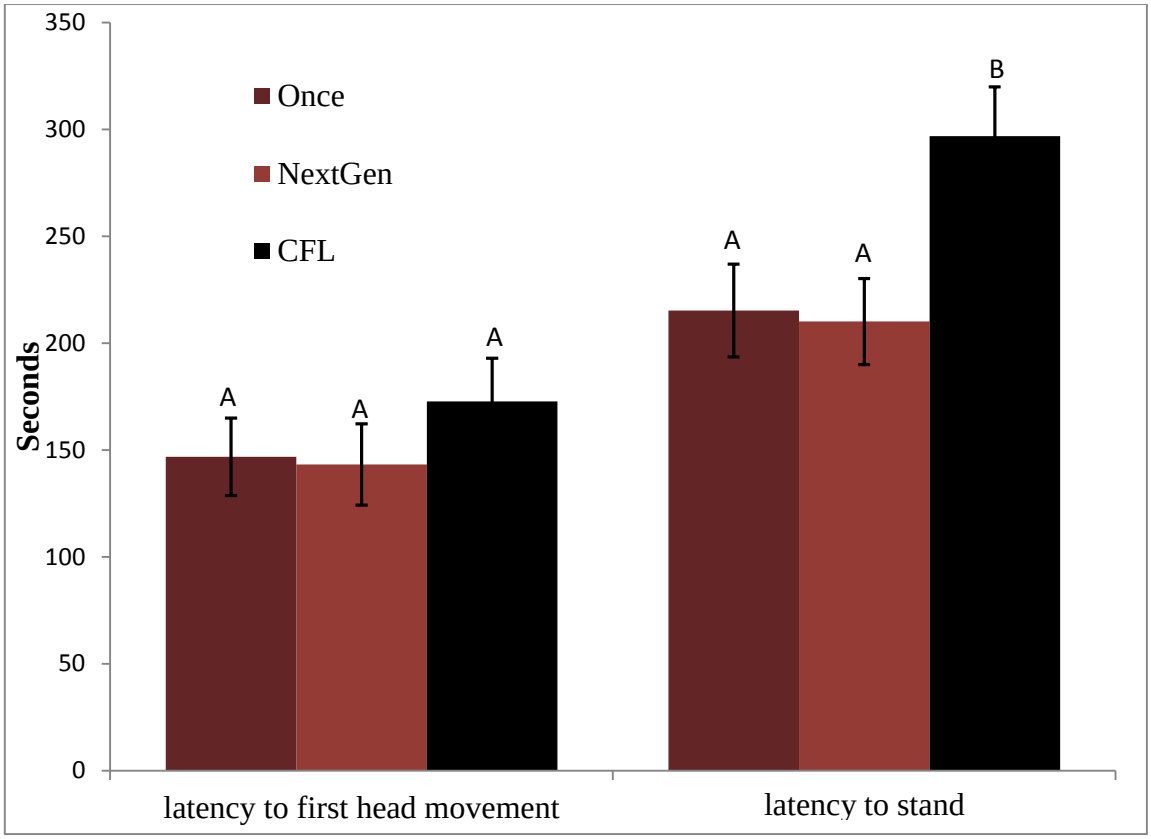


Figure 11. Tonic immobility results between the 3 lighting treatments (seconds). Significant differences within measure indicated with different letters ($P < 0.05$).

Table 6. Comparison of asymmetry and corticosterone measurements, along with heterophil/lymphocyte ratios, between the 3 lighting treatments **(**Mean ± SE)

Treatment	Asymmetry (mm)	Corticosterone (pg/ml)	Heterophil/Lymphocyte Ratio
Once	1.71 ± 0.21^{a}	612 ± 100^{a}	0.22 ± 0.03^{a}
NextGen	1.73 ± 0.17^{a}	2022 ± 423^{b}	0.37 ± 0.04^{b}
CFL	2.34 ± 0.22^{b}	1859 ± 366^{b}	0.35 ± 0.06^{b}

Significant differences between treatments of $P < 0.05$ designated by differing superscripts within measure.

For the asymmetry tests, both LED treatments were the same, but the CFL treatment showed significantly higher asymmetries. Heterophil/lymphocyte ratios were significantly lower in Once bulb treatments than with NextGen or CFLs (Table 6). The

corticosterone test showed that the Once LED treatment had a significantly lower value than the NextGen or CFL treatments (Table 6). Organ measurements (Figure 12) showed no significant differences between heart weight, eye width, or eye weight. Eye height and cornea width were both significantly higher in the Once LED treatment. Spleen weights were only significantly different between NextGen and CFLs, with no differences between CFL and Once or NextGen and Once.

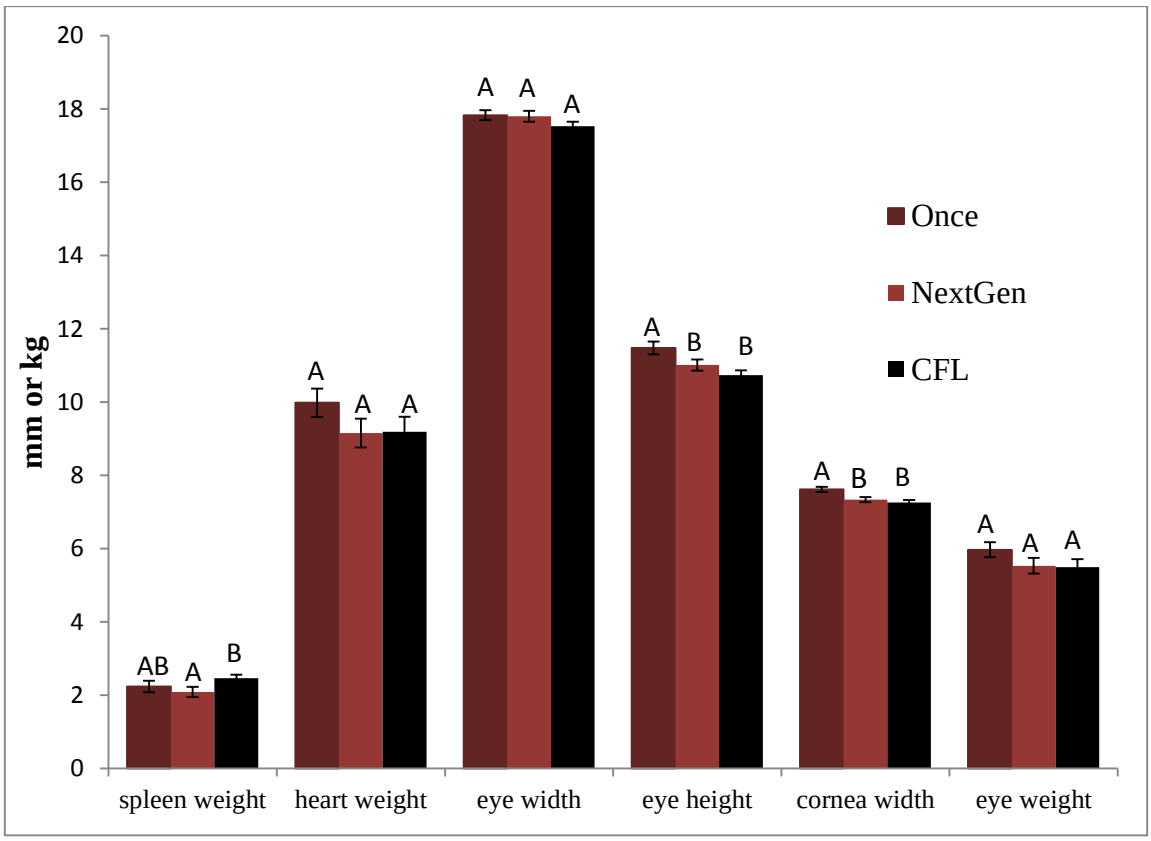


Figure 12. Organ measurement comparison of lighting treatments. Weight in grams, dimensions in millimeters, and significant differences within measure indicated with different letters ($P < 0.05$).

There were no significant differences for the gait score between any of the treatments. Welfare scores did however find differences between various light sources. The plumage and hock scores were significantly higher in the CFL group as compared to either of the LED lights. The footpad scores were shown to be significantly lower in the Once LED treatment than the other 2 treatments (Figure 13).

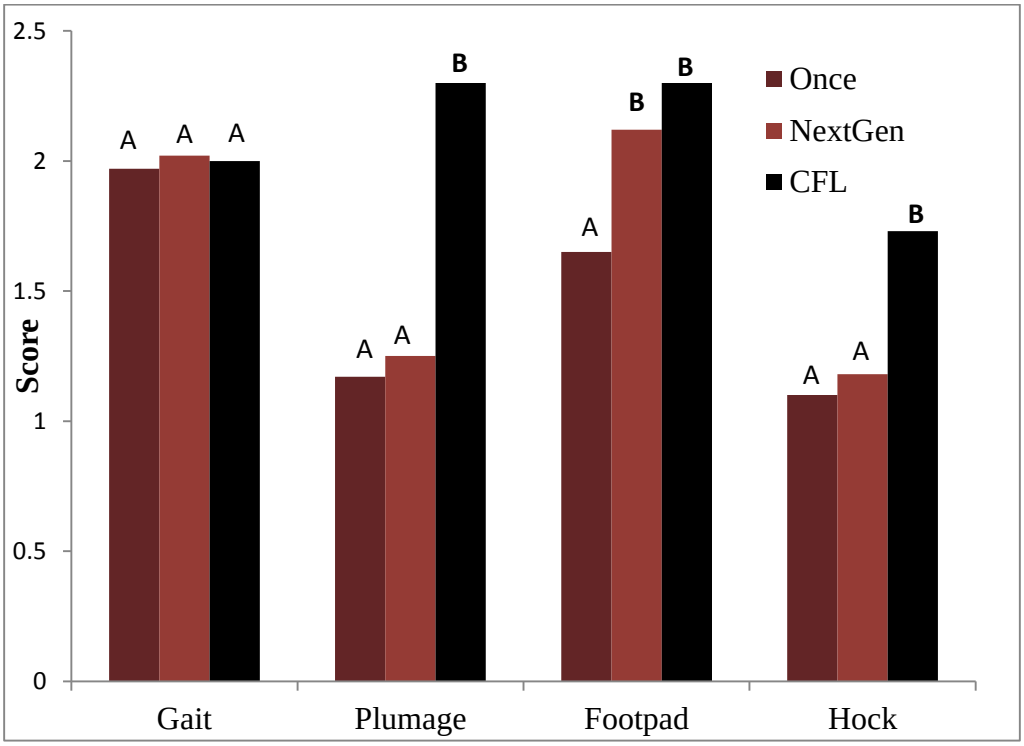


Figure 13. Welfare assessment and gait score comparison of the 3 lighting treatments. Significant differences within measure indicated by different letters ($P < 0.05$).

Table 7. Bird weights and feed conversion ratios of all 3 lighting treatments. **(**Mean ± SE)

Treatment	14 day weight	45 day weight	FCR
Once	0.5 ± 0.01^{a}	2.97 ± 0.03^{a}	1.48 ± 0.04^{a}
NextGen	0.45 ± 0.01^{b}	2.94 ± 0.08^{a}	1.46 ± 0.03^{a}
CFL	0.46 ± 0.01^{b}	2.92 ± 0.02^{a}	1.67 ± 0.02^{b}

Significant differences between Light and Dark treatments of $P < 0.05$ designated by differing superscripts within measure.

The Once LED treatment showed a significantly higher 14 day weight than the CFL or NextGen LED treatment, however at 45 days there was no significant difference in bird weight between the trials. End of trial FCR was significantly higher in the CFL treatment (mean = 1.67) than in Once or NextGen (means = 1.48 and 1.46) (Table 7). There was no significant difference in litter moisture content between trials (pooled mean = 39.829, pooled standard error = 0.505).

Discussion

The results of this study sought to further our overall understanding of the effects of different light sources that are becoming available to the poultry industry. For the purpose of testing new technology, this study compared 2 types of commercially available LED bulbs marketed for poultry production against the standard CFL bulbs used in the industry. A comparison of spectra between these bulbs can be seen in Figure 14. Overall the results seem to indicate a reduction of stress and fear responses in birds raised under LED light, and did not have any adverse effects on growth or feed conversion compared to the CFL.

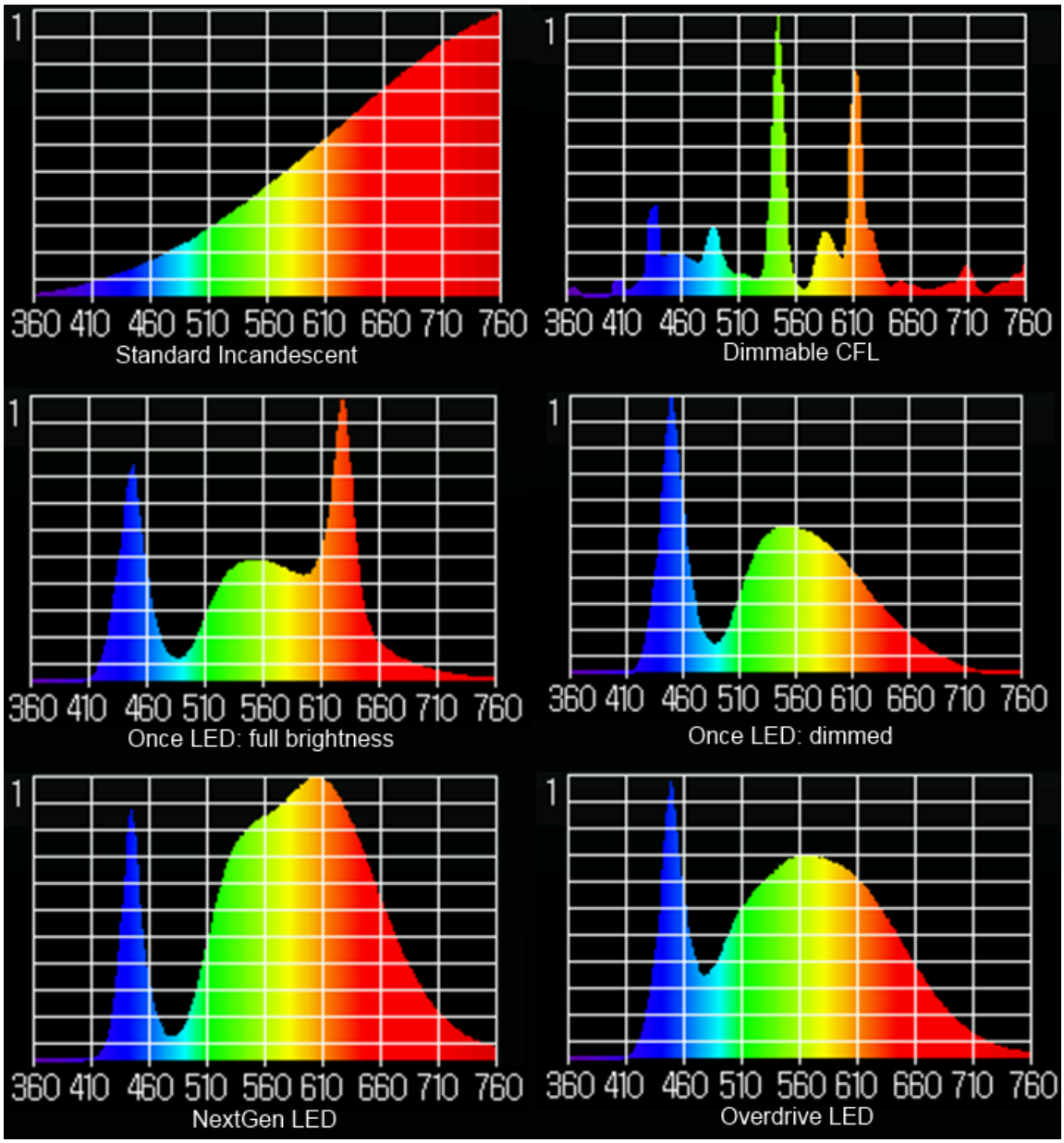


Figure 14. Comparison of spectrum readings of Once, NextGen, and CFLs used in this study. The Overdrive LED and incandescent spectra have been included for further reference. Note, while the spectrum changed in the Once bulbs when dimmed for the study, it did not in the NextGen.

As discussed previously, tonic immobility (TI) is a widely used measure of how animals perceive fear, though there is some debate about measuring latency to first head

movement versus latency to right (Jones and Faure, 1981). During this study there was no significant difference between any of the trials in latency to first head movement, indicating that either LED light does not act upon this response differently than CFLs, or it is simply not as reliable of a fear measure as latency to righting (Jones and Faure, 1981). However, a significantly higher latency to right in CFL birds indicates a greater fear susceptibility than the LED reared birds. The lack of difference between LED trials is evidence of similar fear reducing effects between both brands of bulbs. This is possibly attributed to the wide difference in spectrum between CFLs (many small peaks) and LEDs (two large gradual peaks) (Morrison, 2013), resulting in a more natural, or at least favorable, lighting environment under LED sources. The isolation test did not show any differences between lighting treatments. This may indicate that lighting type only influences certain types of fear. For instance, TI tests for a fear response relating to being caught by a predator (Ratner, 1967), while the isolation test targets fear related to anxiety of separation from flock members (Forkman, et al., 2007). The emergence test also showed no differences between lighting treatments, with mean time to emerge for all treatments at just over 2 min. The final fear test, inversion, also did not show any difference between treatments in any of the factors recorded (time flapping, number of flaps, or overall intensity). These 2 final tests also look at different types of fear: fear of predation (Miller, et al., 2006), and the "fight" response to being captured (Ratner, 1967). Since TI lies at the end of Ratner's sequence of predator avoidance behavior, this may indicate that the lighting has a stronger effect on certain portions of the brain that relate to fear.

Stress has been measured using several methods in poultry, with physical asymmetry being fairly well documented (Graham, et al., 1993; Moller and Swaddle, 1997). The physical asymmetry measures in this study showed that birds raised under CFLs were significantly more asymmetrical than the 2 LED treatments. This indicates that the CFL birds perceived more stress than their LED counterparts, and subsequently grew more asymmetrically. This correlates with the TI scores discussed previously, as an increase in physical asymmetry has been documented to relate to an increase in TI duration (Campo, et al., 2007). The heterophil/lymphocyte (H/L) ratio however showed that only the Once LEDs were significantly lower that the CFLs or NextGen LEDs. This indicates a difference between the 2 LED types, showing that even though LEDs may have similar spectral curves they can still have varying effects on birds. While previous studies using a different stressor saw a correlation between physical asymmetry and H/L ratios, these results indicate that there may be different pathways of stress reduction that can be acted upon by even small changes in wavelengths and spectra. This again is seen in the corticosterone (CORT) results, which again showed Once LEDs to be significantly lower than CFLs and NextGen LEDs. Perhaps blood borne stress indicators are influenced differently from physical growth. More study is needed to discover the cause of this.

Organ measurements showed several differences between treatments. While heart weight, eye width, and eye weight were the same across treatments, eye height and cornea width were both significantly larger in Once LED birds than CFL or NextGen birds. This may indicate that the spectrum of Once bulbs encourages growth in eye

volume. Perhaps the Once spectrum is perceived as a lower intensity by the birds resulting in the need for an increase in light gathering capacity, but this difference was not detected by the lux meter designed around human visual sensitivity. It has been seen previously that a lower light intensity can result in larger and heavier eyes (Deep, et al., 2010). These results do mirror the H/L and CORT scores discussed previously, so it certainly appears that the Once LEDs are interacting with the birds differently than the NextGen LEDs. Further research is required to discover the mechanisms behind this difference. The spleen weight did not differ between the Once LED and the CFLs or NextGen, but the NextGen-CFL relation showed that CFLs resulted in a significantly heavier spleen than the NextGen birds. This difference may be related to the spectrum of each light source, as Xie, et al. (2008) found that birds raised under red light has lower spleen weights than birds under blue light. The lack of difference in heart weight is mirrored by a previous lighting study, which also found no difference in heart weight (Onbasilar, et al., 2007).

The welfare assessment showed several significant differences between treatments. The plumage scores were significantly higher in CFL birds than in either LED treatment. This indicates that the CFL birds were overall dirtier than either LED treatment. Note, there was no significant difference between any of the treatments when litter moisture was measured, so this is not caused by one room having a different pen quality than the others. Thus, the low plumage scores are more likely a result of differing bird behavior under CFLs vs LEDs, with the latter being more active or at least more likely to stand. The hock scores mirror this, with the CFL birds having a higher

score than either of the LED treatments. This strengthens the idea that birds raised under CFL lighting spend more time sitting than LED reared birds, since an increase in hock burns usually indicates longer contact time with the litter. With the footpad scores the Once LED birds showed a significantly lower score than the CFL or NextGen treatments. This is still congruent with the idea that CFLs result in more lethargic birds, since even if the NextGen birds were more active they would still have their footpads exposed to the litter. It is unclear why the Once LED birds still have a lower score, but perhaps it is related to their previously indicated lower stress susceptibility. Perhaps the lower stress levels allow the birds to maintain steadier bodily functions that result in lower incidence of footpad burns, or they simply do not stir up the litter as much to expose themselves to additional ammonia.

The weights of birds were not significantly different between treatments at the end of the trial, but were higher in Once LED birds at 14 days of age than CFL or NextGen treatments. This indicates that Once LED birds may have more rapid growth during their early stages that eventually slows to meet the rate of the other treatments. Ending feed conversion ratio was significantly higher in the CFL treatment than in either of the LED treatments. This may be a result of the lower fear responses and decreased stress found in the LED birds, as they would be using less energy in response to various stressors as compared to CFL birds. This decrease in "waste energy" may increase the amount of energy put towards muscle growth, resulting in a more efficient conversion of feed into muscle. Growth stimulation due to an increase in the red portion of the

spectrum may also be a contributing factor to both the early Once LED bird growth acceleration and overall more efficient feed conversion of both LED treatments.

Overall it appears that LED lighting has many advantages over CFL lights used in commercial poultry farming. No detrimental effects were noted that could have been caused by the LED lights, and overall LED reared birds had reduced stress and fear responses over CFL reared birds. Reduced fear responses may result in a lower incidence of bird damage during handling and transport, while increasing the welfare of the animals. Lower perception of stressors allow the bird to develop more efficiently, and make better use of its energy. Increased welfare of the birds is helpful to public relations, while the mechanisms of this welfare increase result in decreased losses due to ammonia burns. While ending bird weight was not shown to be statistically different, the differences in younger birds may indicate that fine tuning of LED spectral output to the birds is possible to further increase production and efficiency. Finally, a decrease in FCR shows that LEDs can improve efficiency of the poultry growing operation. While there are still differences that need to be studies between brands of LEDs, it appears that LEDs are an overall good investment for poultry producers to consider.

CHAPTER V

CONCLUSION

Lighting will be required for use in any commercial poultry operation, and it is important to consider it in any management plan. Poultry are naturally responsive to light in many ways, including egg production, feeding, growth, and behavior. Thus it is necessary to understand how lighting acts upon poultry in all aspects of growth and production in order to create the most efficient system possible. Researchers have long studied lighting periods on growing and adult birds, and more research is now being conducted on refining lighting techniques and equipment to better match the optimal poultry rearing conditions in every aspect of poultry production. A promising technology, LEDs, offers a wide range of functionality in lighting birds while reducing electrical expenditures and light source replacement frequency. The flexibility of LEDs permits placement in incubators where other light sources are too bulky to fit or put off too much heat, while also being useable in large poultry houses as light sources. The difference in spectrum of LEDs from current standard CFLs acts on the visual and mental pathways of the birds, resulting in decreased stress and fear responses and even more efficient feed conversion.

The first part of this research consisted of 4 incubation trials, each consisting of a light and dark treatment. Two trials used brown broiler eggs, while the other 2 used White Leghorn eggs. Each light incubator utilized LED light strips placed just above the eggs, and set for 12 h light and 12 h dark per day. The hatches were analyzed, looking at embryo mortality, chick weight, chick quality, and hatchability. Both the leghorn and

broiler trials showed a significantly higher chick quality in the lighted treatment over the dark treatment. While other studies have seen increases in growth rate under lighted incubation conditions (Shafey and Al-Mohsen, 2002), the use of LEDs eliminated the detrimental effects seen in trials that used other light sources (Erwin, et al., 1971). Percent hatch of fertile only showed differences in the broiler trials, and was significantly higher in the lighted treatment. This may indicate that development is influenced by light spectrum, as the brown shell pigment changes the spectrum quite drastically from what is seen through the white egg shells.

One of the broiler hatch trials was selected to be grown out for 2 weeks in order to observe growth and behavior. While there were no differences between the treatments in feed consumption or FCR, the dark incubated birds had a significantly higher physical asymmetry score. This indicates that the growth of the embryo under light resulted in changes in how the birds perceived stress, and as a result of this reduced stress developed fewer asymmetries during growth. This stress reduction was also indicated by a heterophil/lymphocyte count. Light incubated birds had a lower H/L ratio, which is another indicator of reduced stress. While 2 fear tests (isolation and inversion) were performed, there was no difference seen between the treatments.

The second phase of this research involved testing the effects of LEDs on growing broilers in order to determine if there are any differences over standard CFLs. Broilers were grown for 45 days under 2 different types of LEDs (Once and NextGen) and standard CFLs. Like the incubation trial, there was no difference seen between the 3 light treatments in the isolation or emergence fear tests. However the tonic immobility

test, which looks at a different type of antiptedator fear response, did show significantly reduced fear in both LED treatments. Perhaps LEDs only influence a specific type of fear response, so future lighting research should expand on the type of fear tests used. Stress responses again mirrored the incubation trials, with LED birds having a significantly lower asymmetry score. Heterophil/lymphocyte ratios were only lower in the Once LED trial, indicating a difference between the 2 LEDs used. A further stress test, corticosterone level, again showed this difference with only the Once LED treatment being lower than the other two. Further differences between the LEDs could be seen in organ measurements, with sizes varying between treatments. The welfare scores indicated that generally the LED birds possessed greater welfare over the CFL birds, but in the footpad scores only the Once LED treatment showed an improvement. At 14 days of age the Once LED birds exhibited a higher weight than the other 2 treatments, however this evened out by the end of the trial where there were no differences in weight seen. This might indicate that at a young age the Once LEDs accelerate growth over the other treatments. Both LED treatments showed a more efficient FCR at the end of the study when compared to the CFL birds.

Overall, light treatments using LEDs proved to be a promising addition to the poultry industry. Use during incubation as well as growth have separately shown to decrease stress and fear responses in birds. Further research is needed to see of a combination of these treatments can further increase this effect, but already it provides a mechanism to produce less fearful birds that are not as strongly impacted by the effects of stress. This behavioral modification, combined with the increase in welfare and feed

conversion efficiency show that LEDs are a promising new technology for use in poultry production. In addition, LEDs have the advantage of long life and low energy consumption. Further research is still needed to refine the precise LED output that is optimal for hatching and raising different types of birds, as well as integration in to current poultry production systems. Lighting research has many paths to follow before it can be fully understood, but in the end should prove to be enlightening to the poultry industry as a whole.

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