EVALUATION OF LIGHT-EMITTING DIODE SPECTRAL OUTPUT AND PHOTOPERIOD DURATION ON PEKIN DUCK PERFORMANCE, STRESS, AND WELFARE

A Dissertation

by

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ABSTRACT

As duck production becomes more prevalent on a global scale, modern duck housing systems must maximize duck productivity. The welfare of an animal influences its productivity, and is therefore useful in evaluating which environmental factors, such as artificial lighting, are more favorable in duck production systems. However, limited research has examined the manipulation of photoperiod and light spectrum on Pekin duck welfare and growth. Four experiments were conducted to determine the effects of (i) two photoperiods, (ii) two commercially available LED fixtures, (iii) ultraviolet (UV) light supplementation, and (iv) four experimental monochromatic LED fixtures on Pekin duck growth, stress, and fear responses during a 35 d grow-out period. Ducks reared under a 20L:4D photoperiod had more efficient nutrient metabolism and stronger humoral immune response to Newcastle Disease Virus vaccine due to improved FCR and decreased stress and the effects of stress compared to ducks reared under 16L:8D. Ducks subjected to white/red LED lighting had lower stress susceptibility and fear responses compared to those subjected to white/blue LED lighting, indicating duck welfare may be compromised by blue LED light exposure, even at supplemental levels. Ducks exposed to supplemental UV light had narrower and lighter eyes and lower acute and chronic stress susceptibility compared to ducks not subjected to UV light (control). UV ducks also had a faster latency to first head movement during tonic immobility (TI) and required more attempts to induce TI than in the control ducks. These results indicate supplemental UV lighting can lower stress and fear responses in Pekin ducks and

increase duck welfare. Exposing ducks to monochromatic red and blue LED lighting elevated the stress response of ducks and decreased eye weight compared to white and monochromatic green LED light. This indicates blue and red lighting may not be adequate for Pekin duck grow-out, and Pekin ducks may require artificial light sources containing a broad range of wavelengths, as seen with white and green lights. The current findings indicate Pekin duck welfare and performance can be influenced by artificial lighting duration and spectrum and emphasize the importance of choosing correct artificial lighting for Pekin ducks.

DEDICATION

This dissertation is dedicated to my mom, Angie Helminiak, and my dad, Matt House. No words can describe how much you mean to me. Thank you for your endless love and support.

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NOMENCLATURE

ACTH	Adrenocorticotropic Hormone
ASYM	Asymmetry
BW	Body Weight
CORT	Corticosterone
FCR	Feed Conversion Ratio
GS	Gait Score
H/L	Heterophil to Lymphocyte Ratio
IL	Interleukin
INV	Inversion
KLH	Keyhole Limpet Hemocyanin
LED	Light-Emitting Diode
ML	Metatarsal Length
MTL	Middle Toe Length
MW	Metatarsal Width
NDV	Newcastle Disease Virus Vaccine
TI	Tonic Immobility
UV	Ultraviolet

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CHAPTER 1: INTRODUCTION

Pekin ducks (*Anas platyrhynchos domesticus*) are globally one of the most popular breeds of duck used for meat. Like their predecessor, the Mallard, Pekin ducks are highly social flock animals, but are less fearful and less aggressive than their wild counterparts. However, stress and fearfulness persist as primary welfare concerns in commercial group housing environments due to potential piling, trampling, and consequent reductions in performance and profitability (Chen et al., 2021). Pekin ducks are housed under a variety of light sources, including light-emitting diode (LED), incandescent, fluorescent, and kerosene lanterns in commercial settings (Cherry and Morris, 2008). Although it has been demonstrated that Pekin ducks are highly photosensitive to lighting, lighting choice for commercial facilities is primarily economic, not a matter of animal welfare (Olanrewaju et al., 2016). Surprisingly limited research has focused on the influence of environmental conditions such as lighting on duck welfare and performance in commercial settings.

Light stimuli modulate poultry endocrine processes, circadian rhythmicity, behavioral processes, and photoperiodism in addition to visually perceiving the photic environment (Nyce and Binkley, 1977; Menaker, 1989; Lewis and Morris, 2000). The tetrachromatic retinal cone and cone pigment populations of birds can perceive an entirely different dimension of color that the human trichromatic retinal cannot visualize, resulting in vastly different perceptions of the world between birds and our own species (Goldsmith, 2006). Light perception is also used to maintain biorhythms in birds and other multicellular organisms. The light/dark circadian rhythm mediates an array of

functions over time through specialized clock cells located in endogenous pace keeping oscillators (Brandstätter, 2002). Avian circadian clocks are highly complex and involve several substructures not involved in mammalian light perception, such as deep brain and pineal photoreceptors (Cassone and Menaker, 1984). The relationship between light perception and neural and hormonal input controls the secretion and regulation of retinal and pineal melatonin (Hamm and Menaker, 1980; Ziółkowska et al., 2015), consequently mediating the circadian behaviors of birds, from sleep/wake cycles to visual sensitivity, metabolism, and social interaction (Cassone, 2014). Together, the sensitivity of poultry to circadian rhythms and artificial light spectral output can directly and indirectly influence the biology, behavior, and ultimately, the profitability of a bird in commercial grow-out settings. It is therefore imperative to consider these effects and how they may be used to optimize the well-being of poultry.

Distress causes significant biological damage through the initiation of behavioral, physiological, and immunological events that divert energy away from normal biological functions to re-establish homeostasis (Puvadolpirod and Thaxton, 2000; Cockrem, 2007; Lambert, 2009). This cascade of events compromises performance (Elsasser et al., 2000; Huth and Archer, 2015), immune function (Xie et al., 2008; Yang et al., 2011), and ultimately welfare. Several reliable measures of stress can be used to assess and compare the stress and immune responses of poultry under various conditions including plasma corticosterone concentration (CORT), heterophil to lymphocyte ratio (H/L), physical asymmetry (ASYM), and antibody titer against nonpathogenic immunogens or vaccines.

The impacts of stress on the physiological and immune responses of animals are reflected in bird performance and feed utilization (Lewis et al., 1996), making growth measures such as body weight and feed conversion ratio (FCR) another essential aspect of welfare evaluation. In addition to FCR and body weight, bone characteristics are indicators of activity and skeletal health during growth, especially in poultry genetically selected for rapid growth. Poultry activity, which is heavily influenced by skeletal health, can be affected by the duration and spectral output of artificial lighting (Jones et al., 2001; Maddocks et al., 2001).

Light spectrum and duration are critical environmental factors for commercially reared poultry and can significantly influence their natural responses to changes in their surroundings. Tonic immobility (TI) and inversion (INV) are two practical assessments of poultry fear response. Tonic immobility measures a bird's fear response during captive restraint by a "predator" (the human handler) while the bird attempts to take advantage to escape captivity when the predator regards the bird (the "prey") as dead and motionless (Ratner and Thompson, 1960). Inversion mimics the motions and human interaction involved in pre-slaughter handling, such as line shackling in processing facilities (Newberry and Blair, 1993).

The effects of artificial lighting on the stress, growth, and fear responses of broiler chickens and laying hens has been studied extensively in recent years. There has been very little research performed to understand how these same artificial light programs affect other poultry species, such as Pekin ducks. Although the United States Pekin duck industry is not as large as the broiler and laying hen industries, the global

market for Pekin duck is expansive, and continues to grow in popularity in the United States, along with consumer demands for improved welfare across all poultry industries. It is therefore the objective of this research to investigate how photoperiod duration and artificial light spectral output affect the stress physiology, immune function, fear response, and growth performance of Pekin ducks.

CHAPTER 2: LITERATURE REVIEW

Overview of Duck Production

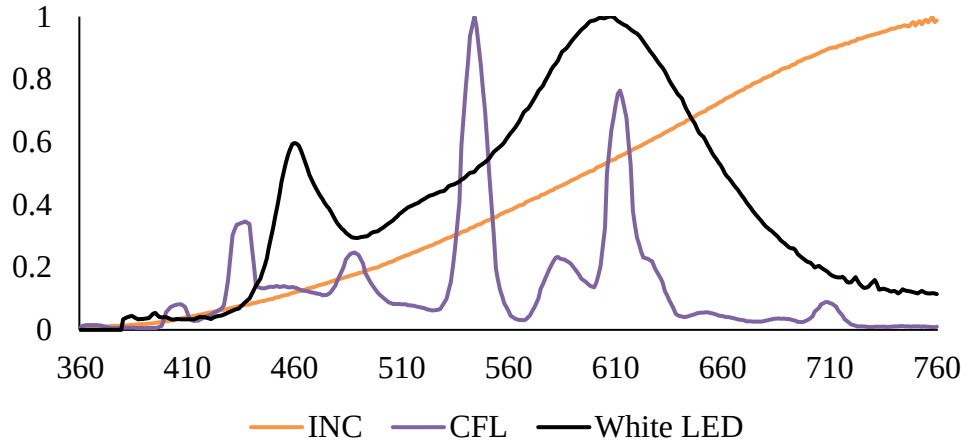
Approximately 2000 years ago, wild Mallards (*Anas platyrhynchos*) were domesticated to develop several breeds of domestic duck still utilized in the modern poultry industry. Asian markets continue to be the leading producers of meat ducks (reviewed in Chen et al., 2021); however, the United States produces 31 million ducks annually, and in 2018 a total of 7.2 million tons of duck products were produced in the United States alone (Chen et al., 2021).

Globally, the most popular domestic duck breed for meat production is the Pekin duck (*Anas platyrhynchos domesticus*). Although domestic Pekin ducks are highly divergent in color and body size from Mallards, both species perform similar complex behaviors that can affect their welfare in commercial or wild environments (Jones and Dawkins, 2010). Pekin ducks are highly social animals that engage in more social interaction with conspecifics, are less fearful, and are less aggressive than Mallards. Although domestic ducks have reduced fear responses compared to their wild counterparts, Pekin duck fear responses remain a concern in group housing environments due to the potential for piling and trampling, which can result in duck injury or mortality (Chen et al., 2021).

Despite the continual growth of Pekin duck production in the United States and abroad, there has been very little research on the influence of environmental conditions such as lighting on duck welfare and performance in commercial settings. Pekin ducks are housed under a variety of light sources, including light-emitting diode (LED),

incandescent, fluorescent, and kerosene lanterns in commercial settings (Cherry and Morris, 2008) as shown in Figure 1; however, lighting choice is primarily economic and not a matter of welfare (Olanrewaju et al., 2016). Light-emitting diode bulbs have many superior qualities compared to other light sources including energy savings, durability, longevity, and overall maintenance (Tracy and Mills, 2011; Benson et al., 2013). Poultry are extremely photosensitive and choosing the correct lighting source can have significant impacts on bird growth, behavior, and well-being. It is therefore necessary to further investigate the effects of artificial light duration and spectrum to elucidate how LED light fixtures may be used to optimize welfare and performance parameters within the meat duck industry.

Figure 1. Comparative spectral output of incandescent (INC), compact fluorescent lamp (CFL), and white light emitting diode (White LED) bulbs.



Overview of Stress

Stress occurs as a result the brain's perception of a stimuli as a threat to an animal's homeostatic balance (Moberg, 2000). Prolonged, severe stress (also known as distress) causes significant biological damage to individuals by initiating a cascade of

behavioral, physiological, and immunological events that divert energy away from normal biological functions to re-establish homeostasis (Puvadolpirod and Thaxton, 2000; Cockrem, 2007; Lambert, 2009). If homeostasis cannot be recovered, the stress response can impair growth (Elsasser et al., 2000; Huth and Archer, 2015), immune function (Xie et al., 2008; Yang et al., 2011), and ultimately welfare. Reliable measures of stress commonly used to evaluate the welfare of ducks and other poultry include plasma corticosterone concentration (CORT) (Siegel, 1995; Scanes, 2016), heterophil to lymphocyte ratio (H/L) (Gross and Siegel, 1983; Scanes, 2016), the physical asymmetry of bilateral traits (ASYM) (Knierim et al., 2007; Archer et al., 2009), and plasma anti-keyhole limpet hemocyanin (KLH) or anti-Newcastle disease virus vaccine (NDV) titer (House et al., 2020a,b; Xie et al., 2008; Onbasilar et al., 2007). To correctly evaluate these measures, it is essential to understand how environmental stimuli such as lighting can activate and modulate the non-specific stress response of poultry.

The stress response begins with hypothalamic-pituitary-adrenal (HPA) axis activation, which will initiate a complex chain of endocrine, immune, and behavioral responses. Hypothalamic release of corticotropin releasing hormone (CRH) and arginine vasotosin (AVT), stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland. The cortex of the adrenal gland will synthesize and release corticosterone (CORT), a primary stress hormone in birds. Biologically active CORT, or that which is not bound to corticotrophin binding proteins (CBG) (Thompson and Lippman, 1974), is circulated and distributed to target tissues where CORT mediates the biological consequences of stress.

Prolonged glucocorticoid release during stress causes immunosuppression of both cell-mediated and humoral immune responses (Siegel, 1995). Corticosterone stimulates lymphoid tissue regression (Glick, 1967; Maurice et al., 2007) and a depletion of circulating lymphocytes while heterophil numbers continue to increase over time during the stress response (Siegel, 1968; Gross and Siegel, 1983). Immune function is further mediated by CORT through the inhibition of integral cytokines such as interleukin 12 (IL-12) production, and interferon gamma (IFN-γ) and tumor necrosis factor synthesis. The mechanisms involved in glucocorticoid modulation of pro-inflammatory cytokines including IL-12 remain largely unknown. Previous studies report conflicting results demonstrating both elevated (Ohtsu et al., 2015) and suppressed (Elftman et al., 2007) IL-12 production during periods of elevated glucocorticoid secretion.

The impacts of stress on the physiological and immune responses of animals are reflected in bird performance and feed utilization (Lewis et al., 1996). As endogenous glucocorticoids are released, increased metabolic energy requirements redirect nutrient utilization, consequently stimulating glycogenolysis, gluconeogenesis, and protein catabolism (Lin et al., 2004) and allowing less energy to be utilized specifically for systems contributing to growth, such as protein accretion. The gastrointestinal (GI) barrier, a structure critical for the restriction of pathogen entry into the body, is composed of tight junctions between endothelial cells, mucous membranes, and tissue macrophages (Lambert, 2009). The release of ACTH, CRF, and CORT are associated with damage to the endothelial layer and tight junctions of the GI barrier, resulting in

compromised membrane integrity, inflammatory immune responses, feed efficiency, and growth (Lambert, 2009).

Measures of Stress

Plasma Corticosterone

Corticosterone, a major glucocorticoid released from the adrenal gland during the stress response, is considered the primary stress hormone in birds (Scanes, 2016). During distress, corticosterone has widespread and detrimental effects on various biological systems including the decreased antibody formation (Post et al., 2003), glucose and mineral metabolism, and the development of gastrointestinal lesions (Siegel, 1995). Chronic stress can additionally result in abnormal bird behavior, including increased feather pecking, cannibalism (Rosales, 1994), and activity (Campbell et al., 2015), which may compromise bird welfare. Artificial photoperiod and light spectra are associated with fluctuations in plasma CORT. Abbas et al. (2008) reported elevated plasma CORT in broilers reared with a non-intermittent restricted photoperiod of 12L:12D compared to intermittent (2L:2D) and continuous photoperiods. However, other studies have observed no photoperiodic effect on broiler plasma CORT (Renden et al., 1994; Olanrewaju et al., 2016), and no previous investigations have studied the effects of photoperiod on Pekin duck plasma CORT concentration. Poultry perception of light color can also influence their physiological response to stress. Several studies have reported the effects of artificial light color on broilers (Riber, 2015; Archer, 2018a,b; House et al., 2020b; Nelson et al., 2020) and laying hens (Parvin et al., 2014; Sobotik et al., 2020), but relatively few studies by comparison have investigated duck responses to

artificial light color. Mohamed et al. (2016) reported elevated plasma CORT in Mulard ducks reared under white or red monochromatic LED compared to those exposed to blue or green monochromatic LED light, which was attributed to the calming effect of short wavelength light often reported in broiler chickens (Rozenboim et al., 1999; Rozenboim et al., 2004a). Conversely, Campbell et al. (2015) observed elevated plasma CORT and depressed carcass quality, body weight, and growth hormone in Pekin ducks exposed to blue CFL lighting compared to those reared under red or white CFL light.

Heterophil to Lymphocyte Ratio

Heterophil to lymphocyte ratio (H/L) is another proven measure of stress response in poultry. While chronic elevated CORT can induce lymphoid tissue regression (Glick, 1967; Maurice et al., 2007) in addition to numerical depletion of circulating lymphocytes, heterophil populations will continue to increase during stress (Siegel, 1968; Gross and Siegel, 1983). Because this ratio increases during stress, H/L is a reliable indicator of HPA axis reactivity to stressors (Gross and Siegel, 1983). One study reported reduced H/L in ducks exposed to intermittent (4L:2D) photoperiods compared to the control group reared under a 16L:8D photoperiod (EI-Badry et al., 2015). Previously published reports concerning the effects of photoperiod on broiler H/L are sparse and contradictory. Campo et al. (2007) found chickens of various breeds subjected to 14L:10D photoperiod had lower H/L than chickens exposed to a continuous (24L:0D) photoperiod. However, several other reports did not observe any physiological changes in H/L to various other photoperiods (Blair et al., 1993; Lien et al., 2007;

Onbasilar et al., 2007). No previous research has investigated the effects of artificial light color on duck H/L.

Physical Asymmetry of Three Bilateral Traits

In addition to affecting the inner biological function of organisms, stress can also irreversibly change body axes symmetry (Leung et al., 2000). Although most animals are not completely symmetrical, bilateral traits can be used as bioindicators for fluctuations from symmetry in birds and other species to determine if genetic or environmental stressors, such as lighting (Yang et al., 1997) affect animal growth through energy redirection or depletion (Sommer, 1996). For this reason, asymmetry is considered a reliable and propitious indicator of long-term stress and welfare in poultry (Knierim et al., 2007; Archer et al., 2009). Three asymmetry categories (fluctuating asymmetry, directional asymmetry, and antisymmetry) have been identified for left minus right (L-R) bilateral differences, each with different distributions around mean zero (Yang et al., 1997). Although these measures are commonly used to determine stress in poultry, the empirical relationships between stress and asymmetry of a single trait are often weak and unreliable, whereas a composite asymmetry value using multiple traits is a more reliable indicator of stress because of increases in statistical power and decreases in comparison standard errors (Leung et al., 2000; Archer et al., 2009). Composite asymmetry measurements have been performed on broilers subjected to various light spectra in previous studies (House et al., 2020b); however, no previous investigations have performed these same measurements on ducks exposed to various lighting conditions.

Humoral Immune Response

The avian immune response contains innate and adaptive branches that are closely related and functionally intertwined (Berghman, 2016). Mucus membranes, leukocytes such as heterophils, and natural killer cells have roles in the innate response. Adaptive immune responses can be cell-mediated or humoral, involving T lymphocytes or B lymphocytes respectively, along with associated cytokines.

The humoral immune response begins with antigen engulfment and digestion by an antigen presenting cell such as a macrophage, which then presents an MHC-II-associated antigen peptide to a T cell receptor (Mashaly et al., 1998; Yang et al., 2011). T lymphocytes then produce cytokines to initiate B lymphocyte proliferation and differentiation to plasma cells capable of producing antibodies to the antigen (Scott, 2004). The first immunoglobulin (Ig) type produced during the adaptive response is IgM; however, upon secondary exposure to the antigen a class switch in Ig class occurs, and IgY antibodies will be produced. Antibodies bind to the antigen and can present it to B or T lymphocytes or direct the destruction and removal of the antigen through complement proteins in the bloodstream.

Immune stress, or exogenous immunostimulation, is an immune response generated by an animal's body through the injection of an external antigen (Liu et al., 2015). In addition to disease, vaccinations and rearing environment can affect bird immune status and trigger indirect or direct immune responses in commercial poultry flocks (Liu et al., 2015). Responses of the HPA and the immune system form a bidirectional network in which HPA hormones affect immune responses, and immune

responses are in turn reflected in neuroendocrine changes (Gaillard, 2001). Elevated plasma glucocorticoid concentrations associated with chronic stress or repeated acute stressors decrease antibody production in response to a foreign antigen (Archer and Mench, 2013; Honda et al., 2015). Therefore, antibody titer is a useful measure of a bird's response to stress.

Immune responses to vaccines and other foreign immunogens can be indirectly affected by environmental factors such as lighting by stimulating chronic stress and glucocorticoid release, resulting in decreased antibody production (Xie et al., 2008). Like the HPA, immune responses can negatively impact growth performance, causing significant economic loss for commercial poultry producers (Liu et al., 2015). Newcastle Disease Virus vaccines (NDV) and keyhole limpet hemocyanin (KLH), a harmless, nonpathogenic and immunogenic protein from the keyhole limpet (Archer and Mench, 2013), are both commonly used to elicit poultry humoral immune responses and antibody production in response to lighting (House et al., 2020a,b; Xie et al., 2008; Onbasilar et al., 2007). However, no studies to date have investigated the effects of lighting on NDV or KLH immune challenges in Pekin ducks.

Overview of Fear

An animal's perception of a threat, also known as fear, can negatively affect the psychology, physiology, and ultimately, the welfare of an individual (Boissy, 1995; Huth and Archer, 2015).

Like humans and other higher order vertebrates, poultry demonstrate interindividual variability in their responses to environmental changes due to variation in

genetics and developmental experiences, indicating the presence of basic personality (Boissy, 1995). Although commercially reared flocks do not face the threat of predation, these innate emotions have been redirected from hawks and other carnivores to human handlers and environmental changes in intensive poultry houses (Boissy, 1995). Prolonged fearful behavior can have detrimental effects on the productivity of poultry, therefore it is necessary to understand the effects of potential environmental changes, such as lighting, on the behavior of Pekin ducks in commercial grow-out houses.

Measures of Fear

Ratner (1967) defines the poultry anti-predator fear response in four categories beginning with freezing, then progressing to fleeing, fighting, and lastly tonic immobility (TI) during capture by a predator. Tonic immobility is a reliable (Gallup, 1979) catatonic-like state which, when analyzed, measures the fear of domestic poultry being handled by a predator while the bird attempts to take advantage of opportunities to escape captivity as the predator regards its "prey" as if it were dead and motionless (Ratner and Thompson, 1960). Inversion, another practical measure of fear for poultry reared in commercial facilities, mimics the motions and human interaction involved in pre-slaughter handling, such as line shackling in processing facilities (Newberry and Blair, 1993). As stated in Newberry and Blair (1993), flapping during inversion to be handled or hung from shackles by the legs stimulates violent wing flapping and struggling, which could increase the potential for bird injury, bruising, carcass condemnation, and compromised welfare.

Lighting is a critical environmental factor for commercially reared poultry and can significantly influence their perceptions of their surroundings. Previous investigations have reported differences in the fear response of ducks reared under various monochromatic colored LED bulbs. Reduced TI duration was reported in ducks reared under monochromatic blue and green LED lighting, indicating shorter wavelength light have a calming effect on ducks (Sultana et al., 2013; Mohamed et al., 2016).

No previous studies have determined if photoperiod impacts duck fear response. Furthermore, inversion fear testing has not been utilized to investigate the effects of either light spectral output or photoperiod on Pekin duck performance and welfare. However, these factors have been investigated in previous investigations using broiler chickens. Broilers reared under a 23L:1D photoperiod had longer TI durations compared to broilers reared under an increasing photoperiod on d 10 of grow-out, however this trend was reversed on d 36, which may indicate broilers in the increasing photoperiod treatment could not adjust to longer photoperiods later in life, resulting in elevated fear responses (Wang et al., 2008). However, it has also been reported that broilers reared under photoperiods that gradually reduce light hours until d 26 and then gradually increase light by 2 h daily until a 24L:0D photoperiod is achieved have reduced TI duration compared to birds under continuous (24L) photoperiods for the grow-out duration (Sanotra et al., 2002). Zulkifli et al. (1998) hypothesized the TI response in broilers reared under a continuous photophase may be augmented by the chickens natural desire to be in enclosed, dark areas at night, similar to its predecessor, the Red Jungle Fowl; if this desire cannot be achieved, poultry may become more stressed, which

can then elevate the fear response. Differences light bulb color temperature and spectrum also impact the fear response of poultry. Archer (2018) reported broilers reared under cool LED light (2700 K) had a shorter TI duration and flapped less intensely during INV than broilers under warm LED light (5000 K), indicating reduced fear responses possibly due to increased levels of blue light wavelengths present in cool light. Tonic immobility duration was shorter in broilers reared under blue monochromatic light, further supporting the hypothesis that short wavelength light is calming to broilers (Sultana et al., 2013).

Overview of Lameness and Bone Health

Commercially reared poultry, including ducks, are genetically selected for rapid growth and heavy muscle development; however, the skeletal integrity of poultry cannot always adequately sustain the physical demands of extreme body growth. Pekin ducks are considered non-specialist walkers, which results in the presentation of a sub-optimal walking gait (also known as the waddle) that has a high locomotive cost and alters movement biomechanics compared to specialist walkers such as chickens (Nudds et al., 2010; Robison et al., 2015). As a result of genetic selection and leg morphology, reduced mobility and lameness are common issues in meat ducks, and a relationship between growth selection and skeletal structure likely exists in these birds (Robison et al., 2015).

Lameness is considered a welfare issue in poultry production due to the condition's potentially painful nature (Weeks et al., 2000). Reduced walking ability compromises producer profitability, as ducks with higher (or worse) gait scores generally have lower body weights because they cannot walk as effectively to feed or

water (Robison et al., 2015). The cause and incidence of lameness appears to be multifactorial. Robison et al. (2015) identified associations between shorter leg length, lower tibia bone ash content, and increased right hip angle and higher gait scores of 1 and 2. Furthermore, some environmental factors such as elevated atmospheric ammonia, temperature, and litter moisture have been demonstrated to adversely affect duck leg health and gait (Jones and Dawkins, 2010). However, flooring type does not appear to affect duck gait scores (Karcher et al., 2013). Cui et al. (2019) reported medullary bone formation increased with longer photophase duration in Jinding layer duck pullets and concluded an 8L:16D photoperiod was adequate for layer duck pullets, as this photoperiod schedule increased body weight, cortical bone generation, and bone mineralization compared to 6L:18D, 10L:14D, 12L:12D, and 14L:10D photoperiods. No previous studies have determined if photoperiod duration or artificial light spectral output, two major environmental factors in commercial poultry production, impact Pekin meat duck walking ability or bone quality.

Measures of Bone Health

Bones are comprised of inorganic (hydroxy apatite) and organic (collagen) matrices that provide bone compressional strength and tensile strength respectively (Rath et al., 2000). Several skeletal assessments have been developed to investigate and improve skeletal disorders and abnormalities in fast-growing commercial poultry including bone ashing and bone breaking strength. Bone ashing is a common skeletal assessment used to measure bone mineral (Ca and P) content (Robison et al., 2015). The amount of inorganic ash material present is proportional to the hardness and strength of

the bone (Bonser and Casinos, 2003). Bone strength indicates the toughness to endure stress and is influenced by a variety of factors such as genetics, physical loading, nutrition, and growth in addition to bone characteristics such as collagen architecture, shape, and material properties (Rath et al., 2000). Once muscle and tissue have been removed from the bone, tibia bone breaking strength is typically measured using a three-point vertical hydraulic force applied to the midpoint of the bone shaft (Lewis et al., 2009). Low bone ash mineral content and low breaking strength are both indicative of poor bone mineralization and leg health in poultry (Rath et al., 2000; Shim et al., 2012).

Gait Scoring

A reliable and simple method for the assessment of non-specialist duck waddling is essential for commercial producers and researchers to identify welfare-related impacts of impaired locomotion in Pekin ducks (Makagon et al., 2015). As a result, a three-point gait scoring system for Pekin ducks has been developed (Karcher et al., 2013) and evaluated for accuracy and validity for ducks over the age of 21 d (Makagon et al., 2015). In the three-point rubric, a score of "0" is used for duck with no waddling impediments. A score of "1" is assigned to ducks with a slight limp or labored walk. Ducks who were reluctant to walk receive a score of "2" (Karcher et al., 2013; Makagon et al., 2015).

The Perception of Light Spectrum and Duration

Poultry Photoreception

Light, or visible electromagnetic radiation, is utilized for environmental perception in many organisms. For poultry, as highly photosensitive animals that can use

both retinal and extraretinal photoreception, light is a major environmental stimulus that modulates endocrine processes, circadian rhythmicity, behavioral processes and photoperiodism in addition to visually perceiving the photic environment (Nyce and Binkley, 1977; Menaker, 1989; Lewis and Morris, 2000). The avian retinal cone and cone pigment populations (tetrachromatic) are capable of perceiving an entirely different dimension of color that the human retina (trichromatic) cannot visualize, resulting in color vision so vastly different from humans that we as people cannot picture what birds really see with our own naked eye (Goldsmith, 2006). As Pekin meat duck production continues to shift to the utilization of artificial lighting in enclosed facilities, light color and photoperiod must be investigated to continue optimizing flock productivity and welfare in commercial operations.

Retinal Photoreception: Cones and Color

Retinal rods and cones are responsible for ocular photoreception in vertebrates. All poultry species possess a large quantity of a single class of rod cell that is activated in low intensity lighting and cannot be used color perception (Hart, 2001; Lewis and Morris, 2006). Unlike rods, four classes of retinal cones (less abundant than rods) are present in the avian eye, which allow comparative chromatic information from all four classes to be simultaneously processed in the brain (Hart, 2001; Lewis and Morris, 2006). The comparison of at least two classes of activated cone photoreceptors with different visual pigments is necessary for the brain to distinguish colors, and the presence of more than two cone classes allows enhanced color discrimination (Goldsmith, 2006). The four retinal cone visual pigment classes include long

wavelength-sensitive (LWS), medium wavelength-sensitive (MWS), short wavelength-sensitive (SWS), and extremely short wavelength-sensitive (UVS/VS) cones (Hart et al., 2001). A double cone class is also present in the retina; however, previous investigation indicates this cone type is reserved for achromatic movement rather than color perception (Osorio and Vorobyev, 2005). Each cone type contains photoreceptive iodopsin pigments comprised primarily of opsin proteins covalently bound to the chromophore 11-cis retinal, an aldehyde of Vitamin A, which absorbs light, transforms to the all-trans molecular configuration, and begins a cascade of biochemical events ultimately leading to cone cell excitation and neurotransmitter release from retinal neurons to the brain (reviewed in Hart, 2001; Goldsmith, 2006). Every cone class has a spectrally distinct photoreceptive pigment: LWS are absorb light maximally between 543 - 571 nm, MWS at 497 – 510 nm, SWS at 420-463 nm, and UVS/VS at 362 – 426 nm (Hart, 2001). The lens and the humors of avian eyes are optically clear, allowing perception of UV-A light that is not visible to the human eye (Goldsmith, 2006).

Another distinguishing feature of the avian eye includes the spherical refractile neutral lipid droplets on the distal end of the retinal cone inner segments, also known as oil droplets (reviewed in Hart, 2001). Although cone oil droplets are found in all vertebrate classes, brightly colored red, orange, and yellow oil droplets are unique to diurnal birds and turtles (Walls, 1944). Oil droplet pigmentation depends on the concentration and type of carotenoids present in the droplet, and this consequently determines the spectral absorptance of the droplet itself and the maximum spectral sensitivity of its cone relative to that of the visual pigment (Bowmaker and Knowles,

1977), thereby reducing the spectral overlap of each cone type, improving color constancy, and allowing birds to distinguish a greater range of visible light (reviewed in Hart, 2001). Visual pigments are associated with one of four oil droplet variations: cones with LWS pigments are associated with red (R) – type oil droplets, cones with MWS pigments are associated with yellow (Y) – type oil droplets, cones with SWS pigments are associated with colorless (C) – type droplets, and cones with UVS/VS pigments are associated with transparent (T) – type droplets (Hart et al., 1999). The T-type droplet is truly transparent, and does not filter out extremely short wavelengths of light. As a result of T-type droplets, UVS/VS cone pigments, and transparent ocular media, poultry can perceive UV light (Bowmaker and Hunt, 1999; Hart et al., 1999; Hart, 2001). Amazingly, there is very little variation in the spectral sensitivities of various poultry species; Pekin ducks have a lower sensitivity than turkeys or chickens to UV-A wavelengths between $360 < \lambda > 400$ nm, but a higher sensitivity to visible light between $400 < \lambda > 694$ nm (Barber et al., 2006). Despite the apparent sensitivity of Pekin ducks to light, relatively few studies have demonstrated the effects of colored lighting on duck productivity or welfare, and no previous investigations have reported if ultraviolet light improves these same factors.

Ultraviolet Light Perception

Ultraviolet electromagnetic radiation is divided into three categories based on wavelength: (i) UV-A (315-400 nm; UV), or blacklight, lies immediately below the visible light spectrum to humans, (ii) UV-B (280-315 nm) or erythemal UV, and (iii) UV-C (100-280 nm), otherwise known as germicidal or bactericidal UV (Commission

Internationale de l'Eclairage). Although the sun emits all three types of UV radiation, UV-B and UV-C wavelengths are screened out by the ozone layer, and as a result, UV-A is the primary ultraviolet light to pass through the atmosphere (Lewis et al., 2009). The detrimental and beneficial effects of UV light depend on the light wavelength – UV-A and UV-B light induce cholecalciferol (vitamin D_3) synthesis from 7-dehydroxycholesterol in the skin, however these wavelengths can also cause vitamin A destruction, collagen damage, sunburn, and skin cancer (Lewis and Gous, 2009). Artificially generated UV-C wavelengths are bactericidal and can be used for microbial sterilization, however overexposure can also cause ocular damage and severe sunburn (Lewis and Gous, 2009). Since the initial identification of UV vision in hummingbirds (Bennett and Cuthill, 1994) and pigeons (Wright, 1972), several studies have investigated the functional significance of avian UV_A perception. In poultry, this ocular function contributes to several behaviors including feeding and growth, locomotion, and peer recognition.

Laying hens exposed to continuous incandescent artificial lighting supplemented with 12 h of blacklight-blue lamp lighting (UV-A) had reduced feed intake during UV exposure, although total daily feed intake was not significantly different from hens exposed to only incandescent lighting (Lewis et al., 2000a). This observation was not anticipated, as generally birds utilize UV reflectance of food objects such as berries, seeds, or insects to either identify or locate food sources in the wild (Cuthill et al., 2000); however, the perceived light intensity of the UV light used in this investigation may have been the aversive factor in this particular situation rather than the UV light itself, as

the combination of blacklight-blue and incandescent lamps used for this trial was 4.9 times greater than white light alone (Lewis et al., 2000). This hypothesis is supported by a preference study that reported regardless of the lighting conditions during rearing, young turkeys chose white fluorescent light supplemented with UV over white fluorescent light alone (Moinard and Sherwin, 1999). Laying hens (Widowski et al., 1992) and turkey males (Sherwin, 1999) also preferred white fluorescent lighting (Lewis and Morris, 2006). Other studies investigating UV light supplementation in poultry did not report differences in feed consumption, FCR, or growth in turkeys (Lewis et al., 2000b), laying hens (Sobotik et al., 2020), or broiler chickens (Hogsette and Wilson, 1999; House et al., 2020b) fed nutritionally complete diets. No previous investigations have reported the effects of UV light supplementation on Pekin duck feed consumption, efficiency, or growth.

Common forms of artificial lighting for commercial poultry houses such as incandescent, fluorescent, and LED bulbs do not emit UV light (Lewis and Morris, 1998). Therefore, commercial flocks grown in light-tight, windowless houses experience a UV-deficient environment. Evidence suggests UV light increases exploratory and walking behaviors in poultry (Maddocks et al., 2001; James et al., 2018), promoting mechanical loading and improved leg health in bird strains selected for rapid growth. Many objects both in environments naturally inhabited by wild birds and artificially designed for commercial poultry contain objects and organisms with UV reflectance, and it is hypothesized that birds use UV wavelengths to identify food objects that either strongly reflect or absorb UV light relative to the environmental background (Burkhardt,

1982; Bailie et al., 2013). James et al. (2018) hypothesized improved walking gait score in broilers reared under white LED fixtures supplemented with UV LED light occurred as a result of improved walking ability attributed to higher activity levels. Maddocks et al. (2001) similarly reported a nonsignificant trend for decreased inactivity in broiler chicks reared in full spectrum lighting as opposed to UV-deficient lighting. Jones et al. (2001) observed more locomotion when broiler breeders were exposed to fluorescent lighting supplemented with UV radiation, which consequently allowed birds to meet more potential mates and encouraged more mating interactions compared to birds exposed to fluorescent light alone.

As flock animals, poultry are extremely reliant on social interaction with conspecifics. Chickens (Prescott and Wathes, 1999) and turkeys (Sherwin and Devereux, 1999) have reflective markings that are visible when illuminated with UV light. It is hypothesized that variations in the color and saturation of these reflective feather markings may act as visual cues during peer recognition and mate selection (Lewis and Gous, 2009). Sherwin and Devereux (1999) noted the onset and location of injurious feather pecking in turkeys coincided with the appearance of reflective body markings; for example, injuries from aggressive pecking appeared at the base of the tail between d 15 – 20, which corresponded to the appearance of reflective feather patches in this same location. Exposing birds to artificial light containing supplementary UV such as fluorescent or incandescent white light and providing environmental enrichment minimized injurious pecking in intact male turkeys, possibly in part due to improved visualization of UV-reflective feather markings; however, the interactions between UV

provision, white light intensity, and environmental enrichment discussed in this study were complex, and more research is needed to determine how light intensity, spectrum, photoperiod, and enrichment can be utilized in turkey hens and other poultry species (Lewis et al., 2000b). The studies discussed above underscore the necessity of UV light inclusion in artificial lighting regimes for commercial poultry production and welfare; however, the effects of UV supplementation on Pekin ducks and its impact on commercial duck welfare remains unknown.

Colored Light Perception

The effects of spectral output of artificial lighting on the productivity, stress, fear response, and welfare of poultry have been extensively studied, particularly in broiler chickens and laying hens; however, limited research has studied how light spectrum affects these parameters in Pekin ducks. It would be simple to assume meat ducks in commercial grow-out environments would benefit from the same artificial light spectral output as broilers in commercial grow-out environments, but ducks, as waterfowl, have a different digestive physiology, growth rate, feed conversion ratio, and visual perception than chickens (Siregar and Farrell, 1980; Barber et al., 2006), and these species differences must be considered when commercially rearing Pekin ducks under artificial light.

Previous investigations demonstrated artificial bulb types including compact fluorescent lamps, incandescent bulbs, and LED bulbs can impact the production, growth, fear, and stress in Pekin ducks reared under various spectral outputs; however, the results presented across these reports are inconsistent and, at times, contradictory.

Sultana et al. (2013) first investigated the effects of artificial LED bulb color on cherry valley duck behavior and fear response in a study using monochromatic blue (460 nm), green (560 nm), and yellow (600 nm) LED bulbs and a control white fluorescent bulb (400-700 nm) as light treatments. In this study, longer wavelength light (yellow LED) stimulated more locomotive, drinking, and social behaviors, possibly because longer wavelengths allow greater visual sensitivity in ducks. Short wavelength light (blue and green LEDs) resulted in more inactive behaviors such as sitting and standing, while calming preening behaviors were observed more in ducks under blue LED light. Tonic immobility duration was reduced when ducks were reared under blue or green LED light, indicating birds reared under short wavelength light were both calmer and less fearful than those exposed to white or yellow light (Sultana et al., 2013). These results were supported by Mohamed et al. (2016), who reported Mulard ducks reared under blue (460 nm) or green (560 nm) LED bulbs similarly displayed shorter TI durations compared with ducks reared under red LED (620 nm) or compact white fluorescent bulbs (400 – 770 nm). These conclusions were also attributed to calmer, less active ducks in the blue and green LED treatments (Mohamed et al., 2016).

The hypothesis that short wavelength artificial lighting may promote duck welfare and encourage calming behaviors has been further supported by studies investigating the impacts of lighting on duck physiology and growth. Mohamed et al. (2016) noted rearing Mulard ducks under either blue and green LED bulbs resulted in decreased H/L and CORT in comparison to ducks reared under longer wavelengths, providing physiological evidence for the calming effects of short wavelength LED light

in ducks. Furthermore, Hassan et al. (2016) reported green (530 nm) and greenish blue (500 - 510 nm) LED light improved cherry valley duck body weight and weight gain during the first grow-out phase (d0 - 21) while both blue (460 nm) and green LED improved body weight and weight gain during the second grow-out phase (d 22 – 42), although no differences in FCR were observed between light treatments, suggesting increased feed intake in ducks reared under blue, green, or greenish blue LEDs. This study also measured bone mineral density between light treatments, but reported no differences in this parameter, indicating bone mineralization and health were not adversely affected by light color (Hassan et al., 2016). Studies investigating the effects of broiler *in ovo* exposure to monochromatic green LED light reported increased body weight and pectoralis muscle yield beginning at week 2 of incubation through d 42 of posthatch grow-out (Rozenboim et al., 2004b), indicating green light may have indirect and direct stimulatory effects on the proliferation and differentiation of satellite cells as well as a promoting effect on muscle fiber uniformity during the early posthatch period (Halevy et al., 2006). Two hypotheses for this phenomenon are that (i) green light indirectly affects myoblast proliferation via photic cues from the retinal and pineal photoreceptors and (ii) secretion of extracellular signals from tissues surrounding myoblasts because of indirect (endocrine stimulation) or direct light effects promote myoblast proliferation and growth pre- and post-hatch (Halevy et al., 2006). However, this phenomenon has not been investigated in Pekin ducks, and it is unknown if monochromatic green LED light exposure solely during grow-out has any effect on myoblast proliferation.

Evidence of the apparent benefits of long wavelength light on Pekin duck production and welfare have also been presented in the literature. Although FCR was improved when Pekin ducks were reared under green (510 – 530 nm) LEDs during wk 1 – 3 of grow-out and by blue (450 – 460 nm) LEDs during wk 4 – 6 of grow-out in comparison to red LED (600 – 630 nm), yellow LED (580 – 590 nm) and white INC bulbs (2,600 – 3,200 K), when both growth phases were pooled together, red and green LEDs equally improved FCR (Kim et al., 2014). During wk 1 – 3, duck body weight and body weight gain were most improved in the white INC bulb group, but during wks 4 – 6 and 1 – 6, red and green LED equally increased body weight and body weight gain in Pekin ducks (Kim et al., 2014).

Finally, previous data has indicated bulbs emitting spectral extremes such as blue or red light are perceived by ducks as environmental stressors and have detrimental effects of Pekin duck growth and stress while bulbs emitting a broad spectral range of light, or white light, may be more conducive to duck production and welfare. Blue light has been reported to compromise Pekin duck performance parameters. Campbell et al. (2015) demonstrated reduced duck body weight and breast meat percentage yield in Pekin ducks reared under blue incandescent (peak at 450 nm, range of 395 – 480 nm) lighting compared to ducks under red (peak at 650 nm, range of 600 – 710) or white (range of 390 – 720 nm) incandescent light. Hua et al. (2020) did not see the effects discussed in Campbell et al. (2015) when studying ducks under blue LED (420 – 470 nm) lighting but did report a reduction in duck abdominal fat percentage in ducks under blue LED light compared to those reared under white (390 to 760 nm), red (630 to 780 nm), yellow

(570 to 600 nm), and green (500 to 570 nm) LEDs, possibly as a result of increased activity in ducks under blue light. The inconsistent study findings between Campbell et al. (2015) and Hua et al. (2020) could be a result of light intensity, as the former study reported a light intensity of 25 lux throughout the study while the latter used a light intensity of 5 lux. Furthermore, the spectral outputs of incandescent and LED bulbs are quite different and, although the peak output for light treatments in Hua et al. (2020) were not presented, it is possible ducks perceive the specific spectral nuances of each bulb type, causing differences in duck performance based on artificial lighting used in the grow-out facility. Campbell et al. (2015) concluded blue light elevated duck stress responses, indicated by elevated CORT and duck activity. Red incandescent lights significantly lowered activity levels in ducks during the first 7 days compared to blue and white incandescent treatments and was therefore not considered ideal for Pekin duck artificial lighting (Campbell et al. 2015). Red LED light enlarged eyeball front-to-back and side-to-side diameters, which may increase pressure on the optic nerve and create nerve damage, ultimately leading to hyperalgesia, a painful eye condition (Tracey et al., 1995). White incandescent and white LED light did not negatively impact behavior, growth, stress, or eye development in either of the studies described above, indicating white artificial lighting may be most suitable for Pekin duck grow-out (Campbell et al., 2015; Hua et al., 2020).

Photoperiodic Perception: Photoreceptors and Pacemakers

The perception of light is critical for the maintenance of biorhythms in multicellular organisms. Although the frequency of biorhythms can occur over minutes

or months, some of the most common and most intensively studied rhythms are circadian, or roughly occurring over a 24 h period (Kumar et al., 2004). In photosensitive species, the light/dark circadian rhythm mediates an array of functions over time through specialized clock cells located in endogenous pace keeping oscillators (Brandstätter, 2002).

Unlike mammalian circadian clocks, which direct photic information from the eyes to the central pacemaker, the suprachiasmatic nucleus (SCN), avian circadian clocks are highly complex and involve several substructures not involved in mammalian light perception (Brandstätter, 2002). Birds perceive the photic environment through retinal, pineal, and deep brain photoreceptors (Cassone and Menaker, 1984). Circadian pacemaking is controlled by clock cell groups in three autonomous and anatomically distinct oscillators: the retina, pineal gland, and a hypothalamic oscillator in both the avian SCN and the lateral hypothalamus, also known as the visual SCN (vSCN) (Cassone and Moore, 1987; Brandstätter, 2002; Cassone, 2014). The integrative relationship between light perception and neural and hormonal input controls the secretion and regulation of retinal and pineal melatonin (Hamm and Menaker, 1980; Ziółkowska et al., 2015), consequently mediating the circadian behaviors of birds, from sleep/wake cycles to visual sensitivity, metabolism, and social interaction (Cassone, 2014).

Extraretinal photoreception represents a basal form of light perception that is particularly common among non-mammalian vertebrates such as birds (Pérez et al., 2019). Functionally, extraretinal photoreception is characterized by daily circadian

rhythms and seasonal reproductive cycles (Underwood et al., 1984; Pérez et al., 2019). Hypothalamic deep encephalic photoreceptors in the suprachiasmatic nucleus (SCN) and the pineal gland are major sites of extraretinal photoreception in birds (Lewis and Gous, 2009) Deep encephalic photoreceptors are primarily involved in photosexual stimulation through control of gonadotropin releasing hormone (GnRH) and gonadotrophin inhibiting hormone (GnIH) release (Ciccone et al., 2004). Photostimulation of the deep encephalic photoreceptors is not successful when birds are subjected to short-wavelength light such as UV, blue, or green light, as these wavelengths are mostly absorbed by tissues such as the feathers, skin, and cranial skull and cannot reach the hypothalamus in completely intact birds possibly due to their subobtimal ambient illuminance (reviewed in Lewis and Gous, 2009). Long-wavelength (red) light, however, is not completely absorbed by cranial tissues and effectively stimulates hypothalamic photoreceptors to elicit the photosexual response at an intensity of 4 lux or greater (Benoit, 1964; Hartwig and Van Veen, 1979).

Circadian Pacemakers

The interactions of the retina, pineal gland, and vSCN may indicate these three tissues are part of a neuroendocrine loop used for avian circadian rhythm control (Cassone and Menaker, 1983). Although the functions of the three circadian oscillators are independent, the amplitude and rhythmicity of avian photoperiodic melatonin are maintained by a combination of neural input from circadian oscillators and photic stimulation (Cassone and Menaker, 1983). The pineal gland, located between the cerebral hemispheres and the cerebellum, contains both a circadian oscillator and

functional photoreceptors (Lewis and Morris, 2006), and is essential for self-sustained circadian rhythmicity (Gaston and Menaker, 1968) and determining time of day (Zimmerman and Menaker, 1979) . Pinealocytes, the photosensitive, secretory cells of the pineal gland, absorb and convert tryptophan to serotonin (5-HT), which is synthesized to melatonin, an indolamine hormone regulated and released from the pineal gland to the blood and cerebrospinal fluid at night through the inhibitory effects of light on melatonin secretion (Cassone and Menaker, 1984). The retina and pineal gland act in parallel but have independent functions, as retinal melatonin is also produced and only secreted at night due to photic inhibition of melatonin secretion (Hamm and Menaker, 1980). Melatonin reaches maximum concentration at the midpoint of the scotophase (Lynch, 1971; Takahashi et al., 1980), and concentrates around the hypothalamus, where it inhibits vSCN metabolism and electrical activity, possibly by activating inhibitory 5-HT pathways. Upon light stimulation, retinal and pineal melatonin are inhibited directly by light, and pineal melatonin is additionally inhibited by hypothalamic norepinephrine (NE), which is secreted in response to daylight from the vSCN (Cassone and Menaker, 1984). The pineal gland is innervated by post-ganglionic neurons from the vSCN, and therefore receives daily circadian input based on rhythmic NE concentration (Cassone, 2014).

The flux of melatonin and NE regulate various endocrine processes and circadian rhythms (Baxter et al., 2014). Several endogenous systems outside of the central nervous system operate on a circadian rhythm regulated by melatonin, including the immune system. Functional connections between melatonin and the immune system have been

identified; melatonin receptors exist on several immune organs such as the spleen and thymus of ducks, suggesting pineal melatonin enhances cell-mediated and humoral immune responses and participates in the development and maturation of immunocompetent cells (Skwarlo-Sonta, 1999). Intermittent photoperiods such as 1L:3D and 2L:2D increase peripheral B and T lymphocyte proliferation and activity, and increase antibody, CD4+, CD8+, and CD3+ cell percentages compared to continuous and non-intermittent (12L:12D) photoperiods in broilers because of decreased glucocorticoid secretion and higher consequent release of melatonin in broilers reared under intermittent photoperiods (Kliger et al., 2000; Abbas et al., 2008). These reports highlight the importance of the relationship between lighting regime, melatonin, and stress in host immune function.

Scotophase Duration and Sleep

In addition to mediating endogenous circadian rhythms, complete darkness during the scotophase encourages rest and sleep. Although the true purpose of sleep is unknown, some primary functions of sleep may include energy conservation, tissue restoration, growth, and homeostatic recovery of brain function after a period of wakefulness (Boerema et al., 2003). Some suggest sleep is a behavioral stratagem to optimize organism survival (Meddis, 1975). A previous report recommended Pekin ducks be provided 14-16 h of daily light (Rodenburg et al., 2005), and the industry standard in the United States for rearing Pekin ducks under artificial lighting is to provide a 16L:8D photoperiod, although very limited research has investigated the effects of photophase or scotophase duration on Pekin duck production and welfare. A previous study reported

rearing meat ducks under continuous photoperiods improved bird performance as a result of extended duck feeding opportunities and locomotor activity (Erdem et al., 2015). Implementing continuous or near-continuous photoperiods in broiler chickens is also associated with maximal feed intake and fast growth (North and Bell, 1990). However, several welfare concerns arise with continuous photoperiod implementation, primarily relating to the lack of scotophase, and therefore regular designated resting times, and the consequences of fast growth, including skeletal abnormalities, excessive fat deposition, and sudden death syndrome, associated with continuous light schedules (Olanrewaju et al., 2006). Continuous lighting may therefore compromise flock welfare if ducks, like chickens, are not provided sufficient scotophases during grow-out. The detrimental effects of continuous lighting underscore the necessity of scotophases and the importance of providing hours of rest for commercial poultry (Malleau et al., 2007).

Photoperiod, Poultry Performance and Wellbeing

Photoperiod is highly impactful on poultry performance and wellbeing. Recommended photoperiod schedules for Pekin duck production vary by country. In the United States, 16L:8D is a standard photoperiod for grow-out Pekin ducks; however, other European countries recommend several other regional photoperiod regimes as described in Table 1 (Rodenburg et al., 2005). Studies examining the effects of photoperiod on Pekin ducks are very limited. Erdem et al. (2015) observed improved body weight gain and higher feed intake, abdominal fat, and percentage of breast, wings, and skin with subcutaneous fat percentage in ducks reared under 24L:0D compared to those under 16L:8D. Although duck performance may be improved by providing continuous lighting, several welfare

concerns arise with continuous photoperiod implementation (see previous section), and more research is needed to determine how industry standard photoperiods may affect Pekin duck performance, behavior, and welfare.

Table 1. Photoperiod recommendations for the United States, France, Germany, and the United Kingdom (reviewed in Rodenburg et al., 2015).

Country	Phase (d)	Photoperiod (Light:Dark)
United States	0 - 35	16L:8D
France	0 - 7	24L:0D
	8 - 14	20L:4D
	15 - 21	16L:8D
	22 – 45	12L:12D
Germany	1 - 7	24L:0D
	8 - 14	20L:4D
	15 – 48	16L:8D
United Kingdom	--	18L:6D
	--	23L:1D

The effects of photoperiod on broiler chickens, another fast-growing meat bird often reared under intensive conditions, have been more extensively studied. Several studies focused on continuous and intermittent photophase durations and have contradictory results. When comparing intermittent (2L:2D), non-intermittent restricted (12L:12D; control), and continuous (23L:1D) photoperiods, Abbas et al., (2008) concluded intermittent photoperiods enhance broiler performance. Feed conversion was improved in intermittent and non-intermittent restricted groups, and the intermittent group also weighed more and had reduced mortality compared to control birds. The non-intermittent restricted photoperiod elevated CORT and H/L while the intermittent photoperiod had no effect on these factors, indicating intermittent photoperiods may be more appropriate than continuous photoperiods for broiler houses (Abbas et al., 2008).

Similarly, Charles et al. (1992) reported birds reared under increasing photoperiods were smaller, had shorter shank lengths than those reared in a constant photoperiod at 3 and 5 weeks of age. Birds under increasing photoperiods also had improved feed efficiency until 6 weeks of age, although this study does not provide details on how photoperiods were increased over time (Charles et al., 1992). Studies by Classen et al. (1991) and Classen and Riddell (1989) also found gradually increasing photophases from short to long during grow-out resulted in reduced growth rate, and reduced incidence of skeletal abnormalities and mortality while maintaining or slightly improving bird performance (Classen and Riddell, 1989; Classen et al., 1991). These findings are supported by Brickett et al. (2007) which reported reduced gait abnormalities at d 25 and d 32 of grow-out in broilers housed under intermittent (12L:12D) light compared to a 20L:4D photoperiod possibly because reducing photophase duration allowed the skeletal system to adapt to increases in body mass; however, the authors argued photoperiod did not have a major effect on bird welfare in this investigation, as indicated by overall low gait scores in both treatments, and a low number of birds with high gait scores at d 35. Sorensen et al. (1999) observed lower body weights in birds reared under shorter photophases (8h and 16 h of light rather than 21 h or 23 h of light) possibly due to lack of sufficient feeder space. In the same study, BW-corrected data indicated decreased walking ability associated with shorter photophase duration (Sorenson et al., 1999). The discrepancies and variation within photoperiod research in broilers highlight the necessity for more data in this field to continue optimizing broiler flock performance and welfare within the industry. Furthermore, these studies demonstrate the knowledge gap

in the duck production industry surrounding appropriate artificial lighting duration for meat duck grow-out conditions. It is possible intermittent photoperiods may produce similar growth rate trends with reduced feed intake and mortality in ducks as seen in broilers. However, when tested commercially using a 1L:2D photoperiod, duck grow-out was extended by 1 d to achieve the same body weight at slaughter as ducks reared under 23L:1D (Lewis, 2006). Extending grow-out time increases the percentage of downgrades due to scratching and bruising as well as a reduction of feather yield (Lewis, 2006). Therefore, species specific lighting strategies must be considered for ducks and chickens individually, as broiler lighting regimes may not be conducive to ducks reared under the same intensive conditions due to variations in growth and behavior.

CHAPTER 3: PEKIN DUCK PRODUCTIVITY, PHYSIOLOGICAL STRESS, IMMUNE RESPONSE AND BEHAVIOR UNDER 20L:4D AND 16L:8D PHOTOPERIODS[*]

Introduction

The well-developed circadian system of poultry has the potential to modulate bird physiology and behavior, which can consequently influence growth performance and welfare. Photoperiod, a critical environmental factor used to manipulate the circadian rhythm of poultry, can be artificially controlled in modern poultry houses to provide ideal conditions for growth and production (Cui et al., 2019). Furthermore, photoperiod can influence the metabolic processes impacting feeding and digestion (Erdem et al., 2015), and can facilitate rest and tissue regeneration (Malleau et al., 2007).

Several studies have identified variable effects of photoperiod duration on growth (Olanrewaju et al., 2019), immune response (Kliger et al., 2000; Özkan et al., 2006), stress (Abbas et al., 2006), and fear response (Zulkifli et al., 1998) or broiler chickens; however, there is limited knowledge of the effects of artificial photoperiod on grow-out Pekin ducks. Although Ducks and broiler chickens are both commercially reared as fast-growing meat birds, it is challenging to compare the two species on many levels due to differences in growth curves and behavior. It is therefore necessary to elicit

[*] Reprinted with permission from "Pekin duck productivity, physiological stress, immune response and behavior under 20L:4D and 16L:8D photoperiods" by G. M. House, E. B. Sobotik, J. R. Nelson, and G. S. Archer, 2021. *Appl. Anim. Behav. Sci.,* 240:105351. Copyright 2021 by the Authors.

more information concerning the impacts of artificial lighting on Pekin ducks to optimize photoperiods from maximum growth and welfare of ducks while minimizing stress.

A previous report recommended rearing meat ducks under a continuous photoperiod (Erdem et al., 2015) to increase body weight gain and carcass development. However, several welfare concerns arise with continuous photoperiod implementation. Melatonin secretion from the pineal gland reaches maximum concentration at the midpoint of the scotophase and may enhance overall immune response and counteract immunodeficiencies related to acute stress (Lynch, 1971; Ben-Nathan et al., 1995; Kliger et al., 2000). Integrating scotophases into photoperiod programs may also encourage birds to sleep, which stimulates anabolic processes and general tissue restoration while preventing stress-like symptoms caused by the disruption of sleep (Malleau et al., 2007). Providing house of darkness during photoperiods may therefore be necessary to not only promote growth and production in Pekin ducks, but also to improve welfare.

Although the stress, immune, and fear response of poultry are key factors in assessing the overall welfare of a flock, no previous studies have investigated the effects of photophase/scotophase duration on these parameters in Pekin ducks. Stress is a physiological response to environmental changes to reestablish homeostasis within the body (Odihambo Mumma et al., 2006; Lara and Rostagno, 2013). Plasma corticosterone, heterophil/lymphocyte ratios, the physical asymmetry of bilateral traits, and humoral immune response can be used to determine the stress response of poultry species (Gross

and Siegel, 1983; Campo et al., 2008; Dávila et al., 2011; Archer and Mench, 2013; Huth and Archer, 2015). Plasma corticosterone, a primary stress hormone secreted from the adrenal cortex, is often used to reliably measure stress responses in poultry (Archer and Mench, 2013). Both plasma corticosterone concentrations and heterophil cell counts will increase in response to environmental stressors (Huth and Archer, 2015; Gross and Siegel, 1983). The symmetry of bilateral traits such as metatarsal length, metatarsal width, and middle toe length can be affected by chronic stress in animals; increasing chronic stress levels can increase the amount of asymmetry in these bilateral traits (Huth and Archer, 2015; Gross and Siegel, 1983; Dávila et al., 2011). Photophase length and photo-stimulation also modulate the avian immune response (Xie et al., 2008). Studies with broilers showed elevated antibody titers of anti-NDV (Onbaşilar and Erol, 2007) and greater cellular and humoral immune responses (Moore and Siopes, 2000) in birds reared under daily light-dark cycles compared to continuous light.

Fear response assessments including tonic immobility (TI) and inversion (INV) can be used to evaluate poultry welfare (Gallup et al., 1971; Jones, 1986; Newberry and Blair, 1993; Archer, 2019). Tonic immobility is a catatonic like state in poultry and other species during which individuals are either immobile or less responsive to their surrounding environment (Jones, 1986). Inversion is a practical measure for determining fear responses while inverted, as most poultry species for determining fear responses while inverted, as most poultry species are typically held during handling and transport to processing facilities (Newberry and Blair, 1993). Increased fearfulness is indicated by

a longer latency to right from TI, and by increased wing flap intensity during INV (Gallup et al., 1971; Archer, 2018a,c).

It has been recommended that Pekin ducks should be provided 14-16 h of light daily (reviewed in Rodenburg et al., 2005), and it is currently industry standard to house Pekin ducks under at 16L:8D photoperiod. Although Erdem et al. (2015) previously observed improved duck growth performance under continuous lighting compared to those on a 16L:8D photoperiod possibly due to extended duck feeding and locomotor opportunities, bird welfare mayu be compromised if ducks are not allowed hours of darkness to rest (Malleau et al., 2007). The authors hypothesized that by extending the standard photophase to 20 h to allow more hours of feeding and exploratory behaviors while incorporating a short scotophase of 4 h to encourage resting, duck production could be enhanced while duck stress and fear responses may be minimized to promote Pekin duck well-being in a grow-out setting.

Materials and Methods

Ethical Note

All ducks were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2017-0426).

Overview

A study was conducted using 384 straight run Maple Leaf Farms, Inc. (Leesburg, IN) Pekin ducklings. Two tunnel ventilated rooms each measuring 6.1 m x 9.1 m were

made light tight and furnished with 8 pens (0.9 m wide, 1.8 m long, 0.6 m high) per room for a total of 16 pens per trial. A nipple drinking system with 3 nipples per pen and one tube feeder, both of which could be adjusted to bird height throughout the grow-out period, were added to each pen before bird placement. Each pen was also bedded with approximately 3 in of fresh pine shavings.

The study was designed with two photoperiod treatments: the industry standard of 16 h of light with 8 h of darkness (16L:8D) and 20 h of light with 4 h of dark (20L:4D). Two trial replications were performed to determine the effects of the two photoperiod treatments on growth, stress, fear response, and gait parameters in meat ducks. One of the two described photoperiod treatments were implemented in each room. Six LED light fixtures (Agrishift ® MLB, Once Innovations, Plymouth, MN) were installed in each room, positioned 3 m above the floor, directly above the pens. All 6 LED fixtures in each room were controlled by a single dimmer and timer.

Ducklings were transported to each experimental room on d 0 in covered cardboard chick crates lined with paper. The ducks were then randomly selected, weighed, and allocated to pens on the day of hatch. Twelve ducklings were stocked in each of the 16 pens per trial (N = 32 pens total). Following industry standards, during the first 24 h after placement, ducks were reared under a light intensity of 20 lx with a photoperiod of 24L:0D as measured by a light meter (Hato Lighting Galli-Luxmeter lighting meter, Hato Lighting, Netherlands) at duck head height. From d 1-10, one room of ducks was reared with a 20L:4D photoperiod while the second room was reared with a 16L:8D photoperiod. All lights in both rooms were dimmed to a light intensity of 20 lx

for days 1-10. Spectral flickering irradiance of the bulbs used during the study was determined using a spectral flickering irradiance meter (SFIM-300, Everfine, Hangzhou, china). The bulbs had a flicker index of 0.04 and 150 hz at an intensity of 20 lx. Lights in both treatments were dimmed to 5 lx beginning on day 11, following industry standards. At 5 lx, bulbs had a flicker index of 0.05 and 350 hz. Dawn/dusk periods were not provided during any time point in the study.

All feed was weighed and recorded (Ohaus Champ CD-11, Pink Brook, NJ), and all feed remaining in feeders at trial termination on d 35 was subtracted from the total amount fed. Standard commercial duck starter (d0-14) and grower (d15-35) diets were fed during both trials. All feed was produced by the Texas A&M feed mill. Upon conclusion of the study on d 35 of grow-out, all ducks were euthanized using a mixture of CO_2 gas and air.

Growth Parameters

All day 0 pen weights were subtracted from day 35 pen weights to determine body weight gain (N = 32 pens). Data from feed intake calculations was acquired by weighing all feed before adding it to feeders in each pen and weighing back all residual feed on day 15 at the end of the starter phase and on day 35 upon conclusion of the study. Feed conversion ratio (FCR) was calculated by dividing the total feed intake per pen by the total body weight gain per pen and then corrected from dead birds. All dead birds were weighed and recorded daily. Mortalities and culls found before d 7 were replaced with a duck of the same weight.

Stress Parameters

Physical Asymmetry

Sixty ducks per treatment (N = 240) were randomly selected on d 35 of the study for the analysis of the physical asymmetry of 3 bilateral traits using methods described in Archer (2018). The metatarsal length (ML), metatarsal width (MW), and middle toe length (MTL) of the right and left legs of each duck were measured using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL). The sum of the absolute value of the right measurement was subtracted from the left measurement of each trait, then divided by the total number of traits in order to calculate the composite asymmetry score (Archer, 2018a,c). The equation for ASYM would be ($|L\text{-}R|_{MTL}$ + $|L\text{-}R|_{ML}$ + $|L\text{-}R|_{MW}$) = composite asymmetry score.

Plasma Corticosterone and Heterophil to Lymphocyte Ratios

Twenty ducks per treatment (N = 80) were randomly selected for corticosterone (CORT) and heterophil to lymphocyte ratio (H/L) analysis on day 35 of each trial. Blood (1-2 mL) from the brachial wing vein of each duck was collected for analysis. Upon collection, a small drop of blood from each bird sampled was smeared on a glass plate for HL analysis. A hematology staining kit (Cat# 25034, Polysciences Inc, Warrington, PA) was used for staining blood smear slides used for HL analysis. One layer of stained blood cells on the glass slide was observed under 40X magnification using an oil immersion lens on a microscope (Omax DCE-2, Kent, WA). Heterophils and lymphocytes were observed in an area of the blood smear slide that did not contain

overlapping cells, and were counted using a keystroke counter until a total of 100 cells were recorded (Campo et al., 2000),

The remaining blood collected from the sampled ducks was injected into a plasma separation gel and lithium heparin vacutainer (BD 368056, BD, Franklin Lakes, NJ) and stored on an ice path temporarily. To separate the plasma and blood cells, all vacutainers were spun down using a centrifuge (Eppendorf 5804, Eppendorf North America, Hauppauge, NY) at 4000 RPM for 15 min. Each plasma sample was transferred to a 2 mL microcentrifuge tube and stored at -19ºC until further analysis could be performed. A commercially available ELISA kit (Enzo Life Sciences, ADI-901-097, Farmingdale, NY) was used to determine the plasma corticosterone concentration from each sample. The inter- and intra-assay % CV were both under 5%.

Humoral Immune Response

Newcastle disease virus vaccine (NDV; Combovac-30®, Intervet/Merck Animal Health, Omaha, NE) was administered at 2 wks of age, and again at 3 wks of age. The antibody response to Newcastle vaccine was evaluated using the same blood samples collected for CORT and HL analysis (N = 80). Analysis was performed using a commercially available ELISA kit (Proflok® NDV AB Newcastle Disease Virus Antibody Test Kit, Zoetis, Parsippany, NJ) to determine the antibody titers of anti-NDV in the two groups. The inter- and intra-assay % CV were both under 5%.

Fear Response Parameters

Tonic Immobility

Tonic immobility was conducted when ducks were 5 wks of age as per the methods described in the study conducted by House et al. (2020b). Sixty randomly selected ducks per treatment (N = 240) were individually placed on their backs in a U-shaped, wooden cradle lined with a black cloth. A trained observer then applied light pressure using the palm of their hand to the thoracic cavity of each duck for approximately 25 s, until tonic immobility was achieved. A timer was started immediately following pressure release from the duck's thoracic cavity. Each duck tested had to maintain immobility for 10 s or more in order for data from the TI test to be recorded. If a duck righted itself in under 10 s, a time of 0 s was recorded, and another attempt to induce full tonic immobility in the same duck was performed. Each duck was allowed a maximum of 3 attempts to be successfully tested. If TI could not be achieved in 3 attempts, a final time of 0 s was recorded. The first head movement and each duck's latency to right itself was recorded, with a maximum time of 10 min. A longer latency to right from TI is indicative of greater fear responses in poultry species (Jones, 1986).

Inversion

Inversion measurements were also conducted when ducks were 5 wk of age using methods described in House et al. (2020b). Sixty randomly selected ducks per treatment (N = 240) were individually caught and positioned upright in the standing position with the legs held together by a trained handler, and the duck head facing away from the camera. Each duck was then inverted until wing flapping ceased, or for 30 s.

All inversion tests were recorded on video at 24 frames/second for later observation (Cannon, ZR900, Melville, NY). Beginning with the first wing flap after inversion was initiated, the number of wing flaps and the duration of wing flapping during inversion for each duck was analyzed by a trained video observer, and the number of wing flaps was divided by the duration of wing flapping during inversion was used to obtain the wing flap intensity for each bird. Greater wing flapping intensity was indicative of higher fear responses in poultry species (Newberry and Blair, 1993).

Gait Parameters

Visual gait scores were determined on d 30 using methods described in Makagon et al. (2015). Six ducks per pen (N = 192) were randomly selected and placed in an observation pen constructed on a flat concrete surface. Each duck was observed alone, without other ducks in the observation pen. Two trained observers, each with a clear view of both duck legs, determined a single gait score per duck. A gait score was measured ranging from 0 to 2, where a score of "0" indicated no gait abnormalities, a score of "1" indicated slightly impaired walking or limping, and a score of "2" indicated poor gait or a reluctance to walk.

Quantitative gait parameters including stride length, stride width, and foot angle were also obtained on d 30 of the study. The same 192 ducks used for visual gait score analysis were used for stride and foot angle measurements immediately following visual gait scoring. Following methods conducted by Campbell et al. (2015), non-toxic black ink was applied with a small paint roller to both feet of 6 ducks per pen (N = 192 ducks). These ducks were the same 6 sucks per pen previously selected for visual gait score

analysis. Once black ink was thoroughly applied to both feet, ducks were allowed to walk in a straight line across one sheet of brown paper. If a duck ran rather than walked across the paper, the feet of the same duck were repainted, and the test was conducted again on a new piece of paper. The footprints for each duck were recorded on separate sheets of paper and allowed to dry before further analysis.

To determine differences in stride length, a straight line was drawn beginning at the footpad of one print and ending on the footpad of the next print. A total of three lines were drawn and measured for each duck sampled, and the average stride length per treatment was calculated. Stride width and foot angle were determined using modified methods from Campbell et al. (2015). One straight line was drawn between two left footprints of each duck, and another straight line was drawn between two right footprints of each duck. The average stride width and foot angle was determined using the same four footprints used to calculate stride length. Two straight parallel lines were drawn between both the two right footprints and the two left footprints for each sample. Beginning at the footpads, two horizontal straight lines were drawn between the two sets of footprints, where a set is one left print and one right print. These two lines were then each measured to determine the stride width. Another straight line was drawn through each footprint to the second knuckle of the middle toe, which formed an angle with the vertical straight line drawn through the right and left footprints described above. This angle was then measured to determine the angle of each footprint to obtain average foot angle.

Statistical Analysis

General Linear Models (GLM) were used to investigate the trial, treatment, and trail x treatment effects on FCR, body weight gain (kg), plasma corticosterone concentration (ng/mL), heterophil to lymphocyte ratio, composite asymmetry scores of bilateral traits, tonic immobility, inversion, stride length (mm), stride width (mm), and foot angle. All GLM assumptions were tested using Levene's test for homogeneity of variance and the Shapiro-Wilk test for normality. All planned comparisons were tested using Fisher's least significant difference test, and all assumptions were met without transformations. Windows SAS 9.3 (SAS Institute Inc., Cary, NC) was used to perform described analyses. As there were no effects observed on trial or trail x treatment interaction, only treatment effects were discussed in the current study. All data are expressed as means ± SEM, and $P < 0.05$ was used to determine all significant differences.

Results

All results for production and gait parameters are presented in Table 2. The 20L:4D ducks had a lower FCR (1.34 ± 0.0238 kg/kg, $P = 0.029$) compared to 16L:8D ducks (1.43 ± 0.0312 kg/kg). Ducks reared under 20L:4D had a longer stride length (20.03 ± 0.295 cm, $P = 0.003$) than those reared under 16L:8D (18.87 ± 0.242 cm). No differences in d 35 body weight, gait score, stride width or foot angle were observed ($P > 0.05$). No differences were observed in stride width or foot angle ($P > 0.05$).

Table 2. General Linear Model mean and pooled SEM results for the comparison of production parameters and gait parameters between Pekin ducks reared under 16L:8D and 20L:4D.

Treatment	16L:8D	20L:4D	SEM	P-Value
FCR	1.43	1.34	0.021	0.029
D 35 Body Weight[1] (kg)	3.08	2.99	0.030	0.150
Gait Score[2]	0.188	0.135	0.028	0.250
Stride Length[2] (cm)	18.87	20.03	0.190	0.003
Stride Width[2] (cm)	10.2	9.70	0.200	0.260
Foot Angle[2] °	24.90	29.30	1.980	0.260

Abbreviations: FCR, feed conversion ratio
[1]N = 32 pens
[2]N = 192 birds

Stress and fear parameters measured for the current study are presented in Table 3. Ducks reared under 20L:4D had lower CORT (6,558 ± 697 pg/mL, P = 0.002), HL (0.41 ± 0.028, P = 0.016), and ASYM (1.73 ± 0.0716 mm, P = 0.002) compared to ducks reared under 16L:8D (10,592 ± 1058 pg/mL, 0.55 ± 0.0503, and 2.09 ± 0.093 mm, respectively). Anti-NDV antibody titers were higher in 20L:4D ducks (393.8 ± 88.19 U/mL, P = 0.035) compared to 16L:8D ducks (191.6 ± 33.21 U/mL). Ducks reared under 20L:4D had a higher INV intensity (4.03 ± 0.184 flaps/s, P = 0.019) compared to those reared under 16L:8D (3.46 ± 0.156). No differences were observed in the latency to right from TI, the latency to first head movement during TI, or the number of attempts to induce TI (P > 0.05). All stress parameters were affected by treatment (P < 0.05).

Table 3. General Linear Model mean and pooled SEM results for the comparison of stress and fear response parameters between Pekin ducks reared under 16L:8D and 20L:4D.

Treatment	16L:8D	20L:4D	SEM	P-Value
Corticosterone[1] (pg/ml)	10,592	6,558	669.16	0.002
Heterophil to Lymphocyte Ratio[1]	0.55	0.41	0.03	0.016
Composite Asymmetry Score[2] (mm)	2.09	1.73	0.06	0.002
Anti-NDV Titer[1] (U/ml)	191.60	393.8	48.18	0.035
TI Latency to Right[2] (s)	156.90	125.2	11.48	0.120
TI First Head Movement[2] (s)	9.43	14.9	2.83	0.340
TI # Attempts[2]	1.49	1.43	0.04	0.300
Inversion[2] (flaps/s)	3.46	4.03	0.12	0.019

Abbreviations: TI, Tonic Immobility
[1]N = 80 birds
[2]N = 240 birds

Discussion

As Pekin duck grow-out houses continue to modernize and utilize various forms of artificial lighting, artificial photoperiods are becoming increasingly common within the industry Previous research, namely in broiler chickens, has demonstrated photoperiod programs can be used to significantly manipulate the growth and affect the welfare of meat bird flocks, indicating photoperiod – like other aspects of light such as intensity, color, and flicker – is a critical environmental factor in poultry grow-out facilities. Although it is clear photoperiods impact poultry, very limited research has focused on photoperiod optimization for Pekin duck production and welfare.

In the current study, 20L:4D ducks had more efficient FCR than 16L:8D ducks, but no differences in d 35 BW were observed between the two treatments. Researchers have reported variable results for the effects of 16 L photoperiods on broiler and duck

FCR, some showing increases (Erdem et al., 2015), decreases (Ingram et al., 2000), or no effect (Olanrewaju et al., 2006; Lien et al., 2007) on FCR, however the growth curve of broilers and ducks are not the same (Erdem et al., 2015), making species comparisons in growth difficult. Furthermore, very limited data has been previously published on the effects of photoperiods on Pekin duck growth. Data from one previous study reported increased BW and feed intake in ducks reared under continuous photophases, suggesting providing longer light hours facilitate more feeding opportunities for ducks compared to shorter photophases, although the same study found no differences in feed-to-gain ratio from d 1-42 between 16L:8D and continuous photoperiods (Erdem et al., 2015). The current study does not reflect the results of this previous report; however, in addition to feed consumption, FCR can be heavily influenced by several other factors such as stress and immune health (Elasser et al., 2000). Based on the presented data, the 16L:8D photoperiod compromised endocrine and immune functionality, possibly stimulating metabolic redirection of nutrients and ultimately elevated DCRs in these ducks compared to the 20L:4D photoperiod.

Although the metabolic redistribution of nutrients can occur for a multitude of factors, the stress and immune responses of the body both play critical roles in this process. Together, these three systems form a series of checks and balances that, when disrupted, can have detrimental effects on the growth and welfare of livestock species. Corticosterone, a major glucocorticoid secreted from the adrenal gland, is commonly used to measure poultry stress (Scanes, 2016) and is heavily involved in the inhibition of cellular and humoral immune functions (Scanes, 2016) and the redirection of nutrient

metabolism (Elasser et al., 2000; Hu and Guo, 2008). Excess CORT released from the adrenal gland not only induces an animal's stress response, but also depresses immune function, resulting in lower immune responses (Blecha, 2000). Previous studies in broiler chickens have demonstrated measurable short- and long-term stress parameters such as CORT (Abbas et al., 2008), HL (Zulkifli et al., 1998), and ASYM (Archer and Mench, 2013) can be affected by photoperiod, however there have also been reports indicating CORT and H/L were not affected by photoperiod schedules (Olanrewaju et al., 2013; Fidan et al., 2017; Ozkan et al., 2006). Photoperiods play a critical role in the immune response of poultry as light-dark stimuli provide information necessary for physiological and behavioral changes in poultry vial melatonin secretions from the pineal gland (Abbas et al, Xie et al., 2008). Previous studies indicate rearing broiler chickens under short daylight hours results in better immune function and antibody production compared to broilers reared under continuous or long daylight hours (Abbas et al., 2008; Moore and Siopes, 2000; Kliger et al., 2000), however no studies have determined the effects of photoperiod on Pekin ducks stress or humoral immune response. In the current study, 16L:8D ducks had elevated CORT, H/L, and ASYM compared to 20L:4D ducks, indicating elevated acute and chronic stress responses throughout the 35 d grow-out period. It is likely thtat the elevated stress and compromised immune response of 16L:8D ducks may have contributed to the higher FCR observed in this same treatment, which indicates photoperiod-induced stress and decreased immune response may also change the metabolic distribution of nutrients in 16L:8D birds compared to 20L:4D birds, ultimately resulting in ducks with less efficient

feed conversion when given an 8 h scotophase. Future studies are needed to fully understand the mechanisms involved in the metabolic relationship between artificial photoperiods and stress in Pekin ducks, and howe differences in photo- and scotophase length may affect duck production and immune function.

Duck production and welfare can also be compromised by impaired mobility or lameness, a common condition which can cause eventual pain and product loss in meat ducks (Danbury et al., 2000; Makagon et al., 2015). Photoperiod duration can affect the level of activity and time spent mobile in poultry, which can consequently impact bird leg health (Makagon et al., 2015). However, no behavioral analysis investigating the time budget differences between the two treatments was performed for this study, and future studies are needed to further understand the influence of photoperiod on the activity levels of Pekin ducks.

Previous reports indicate poultry have altered fear responses upon exposure to longer or shorter daulengths and other variations in lighting environment (Campo and Davila, 2002; Gallup et al., 1971; Jones, 1986). In the current study, no differences were observed in the latency to right from TI, however 20L:4D ducks flapped more intensely during INV, indicating ducks reared under longer photophases may attempt to struggle against the restraint of shackles or human handling during pre-slaughter shackling, which can result in carcass bruising and even broken wings (Newberry and Blair, 1993). These results are confounding, as they are not in line with the previously discussed growth, stress, and gait data for this study. It is possible the fear response of ducks is presented differently than that of broilers reared under various photoperiod schedules,

however to the authors' knowledge no previous research has compared the fear responses of ducks and broilers, and more studies are needed to determine whether species differences exist between these two meat birds when reared under various photoperiod schedules.

In comparison to other poultry species, the knowledge concerning optimal photoperiods for Pekin duck production is relatively limited. In the current study, Pekin ducks reared under 20L:4D photoperiods had reduced stress, which likely improved immune function and FCR compared to the industry standard 16L:8D photoperiod. Reducing stress and the effects of stress, such as compromised immune function and FCR, is critical to improving animal welfare. It is therefore suggested a 20L:4D photoperiod can promote Pekin duck welfare during grow-out. Future behavioral studies are required to elucidate more data on the fear response of ducks reared under 16L:8D, 20L:4D, and continuous photoperiods to further improve Pekin duck welfare.

CHAPTER 4: A COMPARISON OF WHITE/RED AND WHITE/BLUE LED LIGHT FIXTURES ON PEKIN DUCK PRODUCTION, STRESS AND BEHAVIOR[**]

Introduction

Pekin ducks, like other domestic poultry species, possess a complex visual system capable of perceiving a much broader portion of the light spectrum and the combination of different wavelengths of electromagnetic radiation emitted from light sources, compared to the human eye (Archer, 2015; Prescott and Wathes, 1999). The human retina is trichromatic, containing three variations of cone photoreceptors, while the avian retina possesses 4 or 5 types of cone photoreceptors containing visual pigments that are long, medium, short, or extremely short wavelength-sensitive (Hart, 2001; Hart and Hunt, 2007). Bird color perception is further enhanced by the presence of retinal cone oil droplets, which filter light entering the cone before it reaches the visual pigments. This reduces the spectral overlap of light entering the cone photoreceptors and allows for more accurate color discrimination within the eye (Goldsmith, 2006; Precott and Wathes, 1999).

The anatomy of the avian eye and its sensitivity to the visible light spectrum can mediate the physiology, behavior, and growth of individuals, making the source, spectrum, and intensity of artificial lighting in commercial poultry houses a key factor in determining flock welfare (Archer, 2015; Campbell et al., 2015; House et al., 2020b).

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Pekin ducks are descendants of the mallard, which live primarily around and on bodies of water in the wild, and forages for vegetation and small vertebrates just under the surface of the water (Hart, 2001; Jane and Bowmaker, 1988). Ducks that forage underwater have relatively few red R-type oil droplets in their cone photoreceptors compared to chickens and other poultry species, possibly due to the rapid absorbance of long wavelengths which occurs on the surface of water (Hart, 2001). Ducks may therefore use this dense population of short wavelength sensitive cones similarly to how chickens and other poultry use long wavelength sensitive cones to perceive their environment and conspecifics (Campbell et al., 2015).

Stress occurs when an animal must reestablish homeostasis in response to changes in the environment around them, during which energy is often diverted away from normal biological functions, which may interfere with development and immune function (Moberg, 2000; Mumma et al., 2006; Ohtsu et al., 2015). Variations in bulb spectral output have been previously demonstrated to affect changes in several stress parameters, such as blood plasma corticosterone concentration (Huth and Archer, 2015), heterophil/lymphocyte ratios (Onbaşılar et al., 2009), immune responses (Xie et al., 2008), and the physical asymmetry of bilateral traits (Campo et al., 2000). In poultry, plasma corticosterone concentration (CORT), heterophil/lymphocyte ratios (HL), and the physical asymmetry of bilateral traits (ASYM) can be used to measure stress susceptibility. Corticosterone, a primary stress hormone, has been shown to be a reliable indicator of stress in poultry (Archer and Mench, 2013), where lower CORT concentrations are indicative of lower stress responses in birds (Huth and Archer, 2015).

In response to stressors, the number of lymphocytes in the blood will decrease and the number of heterophils will increase, therefore making HL ratios a useful parameter for assessing stress susceptibility in birds (Gross and Siegel, 1983). In ASYM, bilateral traits such as the metatarsal length and width and the middle toe length are measured, and the difference between the left and right trait is determined; a larger difference between the bilateral traits is indicative of greater asymmetry, which is strongly correlated with chronic stress during growth (Archer and Mench, 2013; Campo et al., 2008; Knierim et al. 2007).

The spectral output of light has also been shown to affect the fear response of ducks (Sultana et al., 2013; House et al., 2020b). As prey animals, major components of poultry fear responses include the fear of predation and predator avoidance (Archer, 2015). Anti-predator fear responses contain 4 progressive categories, including freezing, fleeing, fighting, and finally tonic immobility (Archer, 2015). It has been demonstrated that these anti-predator fear responses are the most reliable fear measures and are commonly assessed using tonic immobility induction (TI) and inversion (simulating routine human handling at processing facilities; INV) testing (Campo et al., 2008; Huth et al., 2015). Ducks and broilers reared under longer wavelengths such as red and yellow have longer durations of TI, indicating elevated fear response compared to birds reared under short wavelengths such as green and blue (Sultana et al., 2013; Sultana et al., 2020). Furthermore, broilers and laying hens reared under supplemental ultraviolet (UV) light flap less intensely, and are therefore less fearful, than those reared with no supplemental UV light (House et al., 2020b; Sobotik et al., 2020).

Although several studies have investigated the effects of LED light spectrum on broiler chickens (Rozenboim et al., 1999; Xie et al., 2008; Cao et al., 2008), very few have examined the effects of LED light spectrum on Pekin ducks by comparison. An experiment was therefore conducted to elucidate differences in production and welfare parameters between two commercially available LED poultry light fixture treatments. The objective of the study was to determine the effects of these two light fixtures on Pekin duck growth, stress susceptibility, and fear response. The hypothesis was that an LED bulb containing white/blue light would decrease stress susceptibility and fear responses while promoting growth in Pekin ducks.

Materials and Methods

Ethical Note

All ducks were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2017-0426).

Overview

Animals

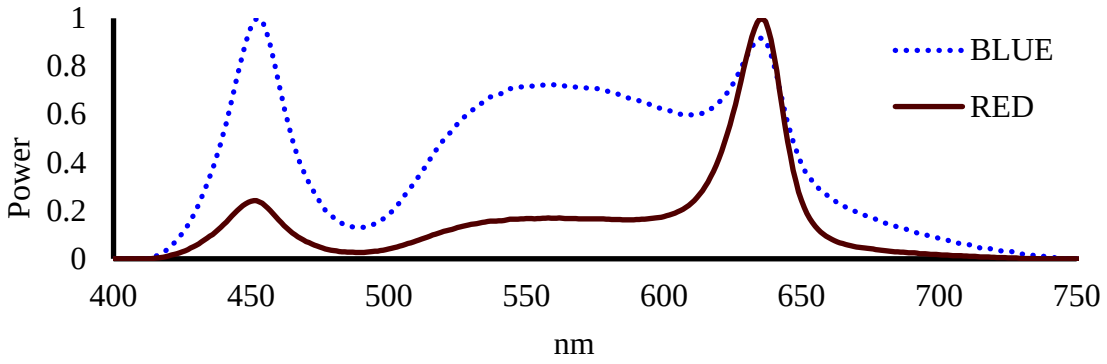
Ducks used during the study were managed according to the guidelines of the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS 2010). The study consisted of two 35 d trials, and in each trial, 192 straight run Pekin ducklings were obtained on day of hatch from Maple Leaf Farms, Inc. (Leesburg, IN). Ducks were weighed and randomly and equally allocated to one of two experimental

lighting treatments. Ducks were housed in two light-tight, tunnel ventilated rooms furnished with 8 pens per room. Twelve ducklings were placed per pen (0.9 m wide, 1.8 m long, and 0.6 m high), where each pen was lined with approximately 3 inches of fresh pine shavings and contained one tube feeder and a nipple drinking system with 3 nipples per pen. Tube feeders and drinking systems were adjusted to bird height throughout the 35 d grow-out period. *Ad libitum* access to feed and water was provided over the entire duration of the study.

Experimental Design

The study design included two commercially available LED poultry light fixture treatments: (i) Once Agrishift ® MLL (WR) bulbs and (ii) Once Agrishift ® MLB (WB) bulbs, which emit white/red light and white/blue light, respectively. A spectral comparison of these two bulbs is presented in the Figure 2. For the first 24 h post-placement, ducks were reared under a light intensity of 20 lux with a photoperiod of 24L:0D as measured at duck head height by a light meter (SFIM-300, Everfine, Hangzhou, China). From d 1-10, the light intensity remained at 20 lux and the photoperiod was changed to 16L:8D. From d 11-35, the light intensity was dimmed to 5 lux with the photoperiod remaining at 16L:8D. The flicker rate of each bulb was measured using a spectral irradiance meter (SFIM-300, Everfine, Hangzhou, China) at a light intensity of 5 lux and 20 lux. The flicker index of the WB bulb was 0.606 at 5 lux, and 0.477 at 20 lux. The flicker index of the WR bulb was 0.582 at 5 lux, and 0.426 at 20 lux. All flicker index readings were also recorded using a spectral irradiance meter (SFIM-300, Everfine, Hangzhou, China).

Figure 2. Differences in spectral power between BLUE and RED LED bulbs using a spectral flickering irradiance meter.



A standard duck starter crumble diet (d 0-14) and grower pellet diet (d 15-35) were provided during both trials (INDUX Meat Duck Management Handbook, Maple Leaf Farms, Inc., Leesburg, IN). All feed was weighed and recorded (Ohaus Champ CD-11, Pink Brook, NJ, USA), and any remaining feed at the end of each dietary phase and upon conclusion of the study was weighed back and subtracted from the total amount of feed fed. All feed was produced by the Texas A&M University feed mill. At the end of each 35 d trial, all ducks were euthanized via a mixture of air and CO_2 gas.

Measures of Production and Growth

All production and growth measures were calculated using methods described in Archer (2018). Feed intake was calculated by weighing all feed before it was added to feeders and weighing back all residual feed upon conclusion of the dietary starter phase on d 14, and again on d 35 upon conclusion of the study. Body weight gain was determined by subtracting d 0 weights from d 35 weights. All mortalities were collected

and weighed daily. Mortalities and culls found before d 7 were replaced with a duck of the same weight. Feed conversion ratio (FCR) was then determined by dividing the pen total feed intake by the total body weight gain per pen and was then corrected for mortality.

Measures of Stress Susceptibility

Blood samples for heterophil to lymphocyte ratios (H/L) and plasma corticosterone (CORT) concentrations were collected on d 35 of each trial between 08.00 and 09.00 AM. Twenty ducks per treatment (N = 80) were randomly selected, and 1-2 mL of blood was drawn from the brachial wing vein of each selected duck for analysis (House et al., 2020b). To minimize bird disturbance in each pen, multiple trained handlers were present, ensuring all selected birds from each pen were caught and sampled within 45 s, as duck CORT and H/L will increase within 1 min after initial handling (Harvey et al., 1980).

Upon collection of each blood sample, a small blood droplet was smeared on a glass microscope slide for HL analysis. All blood slides prepared for HL analysis were stained using a hematology staining kit (Cat# 25034, Polysciences Inc, Warrington, PA, USA). Heterophils and lymphocytes were observed under a 40X magnification oil immersion lens on a light microscope (Omax DCE-2, Kent, WA, USA) in an area of the blood smear slide containing a single layer of non-overlapping cells, and, using a keystroke counter, were counted until 100 cells in total were recorded (Campo et al., 2000).

Plasma separation gel and lithium heparin vacutainers (BD 368056, BD, Franklin Lakes, NJ, USA) were used to store all remaining blood collected from each sampled duck and temporarily stored on an ice bath. Centrifugation (Eppendorf 5804, Eppendorf North America, Hauppauge, NY, USA) at 4000 RPM for 15 minutes was used to separate the blood samples into blood cells and plasma. The plasma from each sample was then removed from vacutainers and transferred to 2 mL microcentrifuge tube and stored at -19°C. Samples were assayed for CORT (Enzo Life Sciences, ADI-901-097, Farmingdale, NY, USA) to determine the effects of lighting spectrum on stress susceptibility of Pekin ducks. The inter- and intra-assay % CV were both under 5%. On d 21, 20 ducks per treatment (N = 80) were randomly selected to receive an intramuscular injection of 0.1 mL of 1 ng keyhole limpet hemocyanin (KLH)/ 0.1 mL saline solution in the thigh. Selected birds were marked with livestock paint and received a second injection of KLH on d 28. On d 35, blood samples were collected from all marked ducks and plasma was stored using identical methods to blood samples collected and stored for CORT and H/L analysis. These blood samples were used to evaluate the secondary immune response of ducks to KLH and blood plasma interleukin-12 (IL-12) concentrations. Anti-KLH IgG titer and IL-12 concentration was analyzed using commercially available ELISA kits (Ch1651, Advanced BioChemicals, Lawrenceville, GA and Ch1651, Advanced BioChemicals, Lawrenceville, GA, respectively). The inter- and intra-assay % CV were both under 5% for both analyses. No ducks used for immune response measures were used in any other stress or fear measurement for the duration of the trial.

On d 35 of the study, 60 ducks per treatment (N = 240) were randomly selected to determine the physical asymmetry of three bilateral traits (ASYM) including metatarsal length (ML), metatarsal width (MW), and middle toe length (MTL) (House et al., 2020b; Nelson et al., 2018). A calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL, USA) was used to measure the ML, MW, and MTL of the left and right leg of each selected duck. The composite asymmetry score was then determined by subtracting the value of the left measurement from the right measurement and adding the absolute values of each trait and dividing this value by the total number of traits measured (Archer, 2015).

Measures of Fear Response

During wk 5 of the study, tonic immobility (TI) and inversion (INV) tests were conducted to determine duck fear response using methods described by (Archer, 2015; House et al., 2020). Sixty ducks (N = 240) were randomly selected for TI and placed in a U-shaped cradle on their back. An observer then applied light manual pressure to the thoracic cavity for 10 s. Once TI was achieved, pressure was released, and a timer was started immediately following the removal of manual pressure from the thoracic cavity. TI test times of 10 s or more were recorded, however if a duck righted itself in under 10 s, the duck was repositioned on its back in the cradle, and another attempt to induce TI was conducted. A final time of 0 was recorded if TI could not be achieved within 3 induction attempts. The time at which each duck first moved its head during TI and the time at which each duck successfully began to right itself from TI were recorded, with a maximum time of 10 min per duck.

Inversion testing was conducted using methods described by Archer (2015). Sixty ducks per treatment (N = 240) were randomly selected, caught, and inverted while being held by the legs for 30 seconds, or until all wing flapping ended. The inversion of all birds tested was recorded at 24 frames/s (Cannon, ZR900, Melville, NY, USA). The duration of wing flapping and the total number of wing flaps per bird was then determined by a trained observer using recorded video. The intensity of wing flapping (number of wing flaps/s) was calculated for each observed bird. More intense wing flapping during inversion and a longer latency to right from TI has been previously determined to be indicative of greater fear responses in poultry species (Jones, 1996; Newberry and Blair, 1993).

Statistical Analysis

Trial, treatment, and treatment x trial effects on FCR, d 35 body weight, H/L, CORT, IL-12, KLH, ASYM, TI, and INV were analyzed using General Linear Models (GLM). Mean separation was performed using Fisher's LSD. Minitab 17.1.0 was used to perform described statistical analyses. Only treatment effects, not trial or treatment x trial effects, were discussed for the current study as no effects were observed on trial or trial x treatment interaction. $P < 0.05$ was used to determine all significant differences.

Results

Production measurements including FCR and d 35 body weight, and all stress susceptibility measurements including H/L, CORT, IL-12, KLH, and ASYM are described in Table 4. No differences in FCR or d 35 body weight were observed ($P > 0.05$). Furthermore, no differences were observed in IL-12, KLH or ASYM ($P > 0.05$).

The WR ducks had lower HL (0.40 ± 0.03, P = 0.029) and CORT (4,498 ± 534 pg/mL, P = 0.038) than WB (0.58 ± 0.08; 6,518 ± 795 pg/mL, respectively).

Table 4. General Linear Model for the comparison of production and stress susceptibility measures between Pekin ducks reared under either Red LED or Blue LED lighting.

Treatment	FCR[1]	D 35 Weight[1] kg	Heterophil to Lymphocyte Ratio[2]	Corticosterone[2] pg/mL	IL-12[2] U/mL	KLH[2] U/mL	Composite Asymmetry Score[3] mm
WR	1.37	2.89	0.40	4,498	126.85	328.87	1.55
WB	1.39	2.88	0.58	6,518	104.67	293.32	1.59
SEM	0.023	0.068	0.04	489.02	14.531	23.104	0.103
P-Value	0.690	0.919	0.03	0.038	0.449	0.503	0.839

Abbreviations: FCR, feed conversion ratio; IL-12, Interleukin-12; KLH, Keyhole Limpet Hemocyanin
[1]n = 32 pens
[2]n = 80 ducks
[3]n = 240 ducks

Fear response parameters including TI measurements and INV intensity are shown in Table 5. No differences were found in the number of attempts to induce TI, or in INV intensity. The WR ducks had a shorter latency to first head movement during TI (9.44 ± 1.22 s, P = 0.06), and had an overall shorter latency to right from TI (25.66 ± 2.99 s, P < 0.001) compared to WB ducks (20.91 ± 6.01 s; 58.76 ± 8.86 s). The duration of time spent in the second stage of TI, where birds observe their environment using head movements while still remaining immobile on their backs, was shorter in WR ducks (16.23 ± 1.73 s, P = 0.001) compared to WB ducks (37.85 ± 6.38 s).

Table 5. General Linear Model results for the comparison of fear response measures between Pekin ducks reared under either Red LED or Blue LED lighting.

	Tonic Immobility[1]				
Treatment	Latency to Right s	First Head Movement s	# Attempts	Latency - Hd Mvmt s	Inversion[1] Flaps/s
WR	25.66	9.44	1.68	16.23	2.11
WB	58.76	20.91	1.78	37.85	2.32
SEM	4.688	3.083	0.047	3.371	0.073
P-Value	<0.001	0.060	0.332	0.001	0.148

Abbreviations: FCR, feed conversion ratio; Latency - Hd Mvmt, the difference between latency to right from tonic immobility and the first head movement during tonic immobility
[1]n = 240 ducks

Discussion

Although several studies have investigated the effects of monochromatic LED lighting, compact fluorescent lamp and incandescent lighting on Pekin duck production, stress, and behavior with varying results (Campbell et al., 2015; House et al., 2020b; Kim et al., 2014; Sultana et al., 2013), no research has previously examined the effects of mixed white/red or white/blue LED lighting on these same parameters. The avian visual system relies on the retinal, pineal, and deep brain photoreceptors for light perception (Lewis and Morris, 2006). The retinal contains cone photoreceptors with 4 to 5 spectrally distinct visual pigments which are sensitive to short wavelengths 1 and 2, medium wavelengths, and long wavelengths (Hart, 2001; Hart and Hunt, 2007). Photons of longer light wavelengths penetrate the skull and stimulate the hypothalamus of poultry more efficiently than photons of shorter wavelengths (Hartwig and Van Veen, 1979). Red light photostimulation of the hypothalamus controls gonadotropin-releasing

hormone (GnRH) secretion which then stimulates luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion from the pituitary gland (Lewis and Morris, 2000). Campbell et al. (2015) found heavier body weights and lower CORT in ducks reared under red or white CFL lighting compared to blue CFLs. Kim et al. (2014) also observed heavier body weights in ducks reared under red LED light compared to white, blue, green, and yellow LED light, and improved FCR under both red and blue LED light. Additionally, ducks reared under longer light wavelengths were more active and performed more feeding and drinking behaviors than those reared under short wavelengths (Sultana et al. 2013), which may encourage body weight gain as seen in previous studies. Unlike ducks, turkeys (Lewis and Morris 1998) and broilers (Rozenboim et al., 2004a; Rozenboim et al., 1999) exposed to short wavelength light had faster growth compared to those exposed to long wavelengths, indicating differences in the spectral sensitivity of various poultry species.

Pekin ducks are descendants of mallards, which live primarily around and on bodies of water where they forage for vegetation and small vertebrates just under the surface of the water (Jane and Bowmaker, 1988; Hart, 2001). Ducks that forage underwater have relatively few red R-type oil droplets compared to chickens and turkeys possibly due to the rapid absorbance of long wavelengths by water and because R-type (red) oil droplets, which cut off wavelengths below 570 nm, reduce the absolute sensitivity of retinal cones by approximately 50% (Hart, 2001; Hart et al., 1999). This increases the relative brightness of downwelling light and therefore increases the conspicuousness of prey in the water (Hart, 2001). Rozenboim et al. (1999) found

broilers perceived LED light wavelengths at 480 nm and 560 nm as brighter than longer wavelengths although all lights in the study had an identical irradiance, and birds reared under 480 nm and 560 nm had heavier body weights than those reared under longer wavelengths of light. As indicated by heavier body weights and gallilux measurements, broiler growth responses appear to be primarily due to light wavelength rather than intensity, thus suggesting broilers perceive blue light as brighter than red or white light even if the lights are at the same intensity (Rozenboim et al., 1999; Lewis and Morris, 2000). Ducks have a smaller proportion of red R-type oil droplets compared to chickens, indicating longer wavelengths may be perceived as brighter compared to shorter wavelengths.

No previous studies have examined the effects of mixed LED lighting on Pekin duck growth. In the present study, no differences were observed in growth or FCR between the two treatments, however this may be attributed to the differences in the proportion of blue or red light provided in this experiment compared to others using strictly monochromatic LED light (Kim et al., 2014). Rearing poultry under LED bulbs has been shown to decrease fear and stress responses compared to CFL bulbs, and variations between different models of LED fixtures emitting similar color spectra has been previously observed (Huth and Archer, 2015; Archer, 2015), therefore artificial light sources appear affect growth and welfare differently depending on the bulb type. It is possible that mixing white LED light, which contains a large portion of the light spectrum, with either red or blue LED light, which contain only long or short wavelengths respectively, may dampen the effects of colored lighting on growth and

FCR, however further investigation is needed to analyze the mechanisms behind mixed lighting perception in ducks.

In the current study, Pekin ducks reared under white/blue LED lighting had elevated CORT concentrations and HL ratios, and longer latencies to right from TI, indicating both higher stress susceptibility and fear responses in this treatment compared to ducks reared under white/red LED lighting. Tonic immobility simulates an antipredator fear response known as “death feigning” (Forkman et al. 2007) and can be divided into two stages; the first involving complete immobility and inhibition of all body movements and the second, beginning at the first head movement, involving head movements possibly for the purpose of environmental exploration before attempting to stand (Jones and Faure, 1981a,b). Prolonged environmental investigation during the second stage of TI under white/blue LED light may indicate more time is required for ducks to sufficiently evaluate their surroundings before righting in a fearful situation, providing additional evidence that ducks may not be able to properly visualize objects in an environment illuminated by blue light compared to an environment illuminated by red light.

Previous studies examining the stress susceptibility and fear responses of ducks under various lighting conditions do not match the results obtained in the present study. Mulard ducks reared under green and blue monochromatic LED lighting have reduced fear responses and lower plasma corticosterone concentrations compared to those reared under red monochromatic or white LED lighting (Mohamed, Abou-Ismail, and Shukry 2017). Similarly, Kim et al. (2014) and Sultana et al. (2013) observed lower fear

responses in ducks reared under green and blue monochromatic LED light compared to white or monochromatic yellow LED lighting. However, Pekin ducks reared under blue CFL lighting had elevated plasma CORT concentrations compared to those reared under white or red CFL lighting (Campbell et al., 2015). As with blue CFL bulbs examined in Campbell et al. (2015), commercially available white/blue LED bulbs may result in decreased environmental perception in Pekin ducks, causing elevated stress susceptibility and fear responses compared to other commercially available bulbs containing longer wavelengths of light. It is speculated that both genotype (Faure et al., 2003) and light bulb type (Huth and Archer, 2015) influence the stress and fear responses of ducks, which may explain differences between studies, however further research is needed to fully understand the interactions between these factors.

Although both broilers and Pekin ducks share many similarities as intensively reared meat birds, several differences, such as the effects of spectral output, exist between these species (Rozenboim et al., 1999; Lewis and Morris, 2020). Broiler growth and performance increases when reared under green monochromatic light during early stages of development, and under blue monochromatic light during later stages, however the effects of blue light on broilers, however no differences in body weight or performance were observed in the current study or in Kim et al. (2014). Light perception in Pekin ducks has not been as extensively studied compared to broilers, and as more duck producers continue to update facilities to artificial LED light sources and become more conscious of flock welfare, the knowledge gap surrounding the effects of artificial lighting in duck houses is becoming more prevalent to the industry. It has been

previously determined that ducks, like other poultry species, are very sensitive to light. The aim of the current study was to contribute more information to global duck industries on the appropriate use of commercially available artificial light sources for Pekin ducks. The results of this study indicate Pekin ducks, unlike broilers and turkeys, have compromised welfare and elevated stress and fear responses when reared under mixed white/blue LED lighting compared to white/red LED lighting. These conclusions demonstrate the importance of identifying appropriate spectral output for each poultry species to improve flock welfare in commercial production settings.

CHAPTER 5: EFFECTS OF ULTRAVIOLET LIGHT SUPPLEMENTATION ON PEKIN DUCK PRODUCTION, BEHAVIOR, AND WELFARE[*,**]

Introduction

Avian species, including ducks, have very sophisticated visual systems that allow discrimination of a broad area of the visible light spectrum (Akyüz and Onbasilar, 2018). Several differences in the perception of color exist between humans and birds, such as the bird's sensitivity to the ultraviolet-A (UV) spectrum (350–400 nm) (Prescott et al., 2003; Lewis et al., 2007). UV light can be perceived by birds because their ocular media is UV transparent whereas human ocular media is opaque, which does not allow UV light transmittance to the retinal cones, and due to the presence of a cone photoreceptor with a peak sensitivity for channeling short wavelengths such as those seen in the UV spectrum (Lewis et al., 2007; Bowmaker and Hunt, 1999). The complexity of avian visual senses provides evidence of the dependence that birds have on visual environmental cues, which consequently influences bird behavior and bird environmental interactions (Cuthill et al., 2000). As poultry production systems with artificial lighting become more refined across the world, the stark differences in visual capabilities between humans and birds, especially in the context of UV light perception,

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must be examined further to determine optimal bird welfare under artificial lighting conditions implemented in the modern poultry industry.

Poultry use UV light to perceive and interact with their environment and conspecifics in the form of visually mediated behaviors including foraging, signaling, and social interactions (Cuthill et al., 2000; Jones et al., 2001; Bennett and Cuthill, 1994; Lewis and Gous, 2009). Pekin ducks, like other poultry species, possess four cone photoreceptors sensitive to specific areas of the perceived visual spectrum. One of these retinal cones is sensitive to very short wavelengths with a maximal sensitivity at 380 nm (Barber et al., 2006). Some poultry species form UV-reflective patches on their plumage that change and develop as the bird ages, which may be used for social signaling, individual recognition, and mate selection among conspecifics within a flock (Sherwin and Devereux, 1999; Maddocks et al., 2001; Jones and Prescott, 1999). Additional UV reflectance has been observed on bedding substrates and feed in modern poultry facilities with high contrast between UV and blue wavebands (Sherwin and Devereux, 1999; Maddocks et al., 2001). Artificial lighting used in modern poultry houses such as fluorescent or light-emitting diode (LED) lighting contains very little UV light, which may make the UV reflective properties of plumage or the environment appear dark (Prescott and Wathes, 1999; Bailie et al., 2013). When UV supplementation is provided in otherwise UV-deficient environments as commonly seen with artificial lighting, turkey poults have been observed to be pecked less by conspecifics (Sherwin et al., 1999). Additionally, a preference for environments with UV light supplementation in turkeys has been reported (Moinard and Sherwin, 1999). Although the spectral

sensitivities of ducks and turkeys are similar, a study performed by Barber et al. (2006) showed that turkeys are more sensitive to UV-A wavebands than ducks. There is evidence that mating frequency of cockerels increases when birds are in an environment supplemented with UV light, possibly due to increased cockerel locomotion, which then leads to more encounters with hens and the birds' ability to visualize the fluorescent plumage patches of their conspecifics under UV light supplementation (Jones et al., 2001).

Stress occurs as a physiological response to environmental changes in order to reestablish homeostasis within the body (Lara and Rostagno, 2013). Several measures of stress including plasma corticosterone, heterophil/lymphocyte ratios, and physical asymmetry scores of bilateral traits (such as the metatarsal length and width, and middle toe length) can be used to determine stress responses of poultry species (Archer and Mench, 2013; Huth and Archer, 2015; Gross and Siegel, 1983; Davila et al., 2011; Campo et al., 2008). Plasma corticosterone is a primary stress hormone released by the adrenal cortex that can be reliably used to measure poultry stress responses (Archer and Mench, 2013), where lower plasma corticosterone levels indicate lower stress responses (Huth and Archer, 2015). Similarly, the number of heterophils in the blood will increase in response to an environmental stressor (Gross and Siegel, 1983). Chronic stress can affect the symmetry of physical bilateral traits (Huth and Archer, 2015; Davila et al., 2011). Consequently, more physical asymmetry in bilaterally expressed traits indicate higher chronic stress in animals (Campo et al., 2008). Poultry welfare can be assessed using tonic immobility (TI) and inversion (INV) tests, which indicate fear responses in

birds (Jones, 1986; Gallup et al., 1971; Newberry and Blair, 1993; Archer, 2018a,c). Tonic immobility is described as a catatonic-like state in which poultry are less responsive to their surrounding environment (Jones et al., 1986). Increased fearfulness is indicated by a longer latency to right from TI (Gallup et al., 1971). Inversion testing can be used for practical application to the commercial poultry industry, as birds are commonly inverted during handling and transport to slaughter facilities (Newberry and Blair, 1993). Increased wing flap intensity during inversion is indicative of higher fear response in poultry (Archer, 2018a,c).

Limited research has been conducted to investigate the effects of artificial UV light supplementation on Pekin duck production and welfare. The aim of this study was to determine the effects of UV supplementation on Pekin duck production parameters, stress susceptibility, and fear response. The working hypothesis was that UV supplementation would increase production parameters such as feed conversion ratio (FCR) and would improve duck welfare by decreasing stress and fear responses.

Materials and Methods

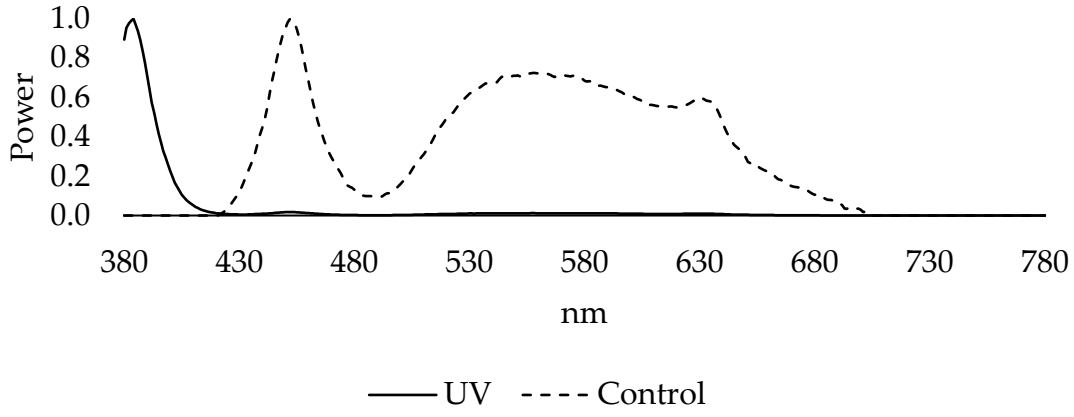
Ethical Note

Ducks were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2017-0426).

Overview

The study was conducted using straight run Pekin ducklings (*N* = 384) obtained from Maple Leaf Farms, Inc. (Leesburg, IN) for 35 days. This experiment consisted of two treatments: control LEDs (Agrishift® MLB, Once Innovations, Plymouth, MN, USA; control) and control LEDs plus ultraviolet (UV) LEDs (Agrishift® HL-UVA, Once Innovations, Plymouth, MN, USA; UV). A spectral comparison of the control LED and UV bulbs are shown in Figure 3. Two replications were conducted to investigate the effects of providing supplementary UV light during the duckling grow-out phase on growth, stress, fear, and eye development in meat ducks.

Figure 3. Differences in spectral power between control and ultraviolet light-emitting diode (LED) bulbs using a spectral flickering irrandiance meter (SFIM-300, Everfine, Hangzhou, China).



Two light tight, tunnel ventilated rooms, each measuring 6.1 m × 9.1 m, were furnished with 8 pens (each measuring 0.9 m wide, 1.8 m long, and 0.6 m high) per room, for a total of 16 pens per trial. All pens were lined with approximately 3 inches of fresh pine shavings. Each pen also contained a nipple drinking system with 3 nipples per

pen and one tube feeder, both of which could be adjusted in height during the grow-out period.

One of the two lighting treatments described above were used in each room. The first treatment consisted of 6 control LED light fixtures per room, which were installed directly over the pens, 3 m above the floor, and were controlled by a single dimmer and timer. The second lighting treatment consisted of (i) two ultraviolet fixtures hung 2.5 m above the floor, 3 m apart, and (ii) 6 control LED light fixtures installed as described in the first lighting treatment. To avoid "room affects", the two lighting treatments were rotated between the two rooms upon conclusion of the first trial.

On the day of hatch, ducklings were randomly selected, weighed, and allocated to pens, where 12 ducklings were stocked in each of the 16 pens used per trial. For the first 24 h after placement, the ducks were reared under a 24L:0D photoperiod at an intensity of 20 lux as measured by light meter (SFIM-300, Everfine, Hangzhou, China) held parallel to the floor at duck head height. From days 1–10, ducks were reared under a 16L:8D photoperiod at an intensity of 20 lux. A spectral flickering irradiance meter (SFIM-300, Everfine, Hangzhou, China) was used to determine the spectral flickering irradiance of each bulb type. At 20 lux, both bulbs had a flicker index of 0.04 and 150 Hz. Beginning on day 11, lights in both treatments were dimmed to an intensity of 5 lux as per industry standards (Maple Leaf Farms Inc., 2018). Both bulbs had a flicker index of 0.05 and 350 Hz at 5 lux. No dawn/dusk period was provided at any time point in the study.

Feed was weighed and recorded (Ohaus Champ CD-11, Pine Brook, NJ, USA), and any remaining feed at the end of each trial was subtracted from the total amount fed. Standard commercial duck starter (d0–14) and grower (d15–35) diets were provided during the course of the study. All administered feed was produced by the Texas A&M feed mill. All ducks were euthanized with a mixture of air and CO_2 gas upon conclusion of the study on day 35 of grow-out.

Growth and Feed Conversion

To determine body weight gain, day 0 weights were subtracted from day 35 weights. Feed was weighed before being added to feeders in each pen, and residual feed was weighed back on day 15 at the end of the starter phase and again on day 35 upon conclusion of the study to allow feed intake calculations. FCR was calculated by dividing the total feed intake per pen by the total body weight gain per pen and was corrected for mortality. Mortalities were weighed and recorded daily. Mortalities and culls found before d 7 were replaced with a duck of the same weight.

Gait Score and Stride Length

Gait scoring was conducted at 5 weeks of age using methods described in Makagon et al. (2015). Six ducks per pen (N = 192) were randomly selected and placed in an observation pen with a flat concrete floor in a well-lit observation room. The gait of each duck was assessed by two trained observers with a clear view of the duck's legs, and a single score was determined between the two observers for every duck assessed at the time of observation. A gait score was measured based on a 3-point rubric ranging from 0 to 2. A score of "0" indicated no visible waddle impediments, a score of "1"

indicated slightly labored walking or limping, and a score of "2" indicated a poor gait or a reluctance to walk.

Stride length was obtained during week 5 of the study by applying black ink to the feet of all ducks assessed for gait scoring (N = 192) and then by allowing each duck to walk in a straight line across brown paper (Campbell et al., 2014). If a duck ran rather than walked across the paper, the test was performed again on a new piece of paper. The footprints of each duck were recorded on separate sheets of paper and allowed to dry. Stride length (cm) was analyzed by drawing a straight line beginning at the footpad of one footprint and ending at the footpad of the next footprint. A total of three lines were drawn for each duck sampled, and the average stride length for each treatment was then calculated and analyzed.

Tibia Bone Ash Mineral Content and Breaking Strength

On day 35, both the left and right tibia were removed from 20 randomly selected birds per treatment (N = 80). Twenty birds per treatment (N = 80) were randomly selected and euthanized via a mixture of air and CO_2 gas on d 35. The heads of all euthanized ducks were removed and placed in bags of deionized water to be stored overnight. The muscle, connective tissue, and fibula were removed from each tibia, and the bones were dried using a Forced Air Oven (VWR 89511-410, Radnor, PA) at 100 °C for 12 h. Left tibias were used to measure bone mineral content. Right tibias were used to measure bone breaking strength. Left tibias were defatted using diethyl ether for 6–8 h and air dried under a chemical hood, allowing all remaining ether to evaporate. Defatted tibias were then dried again at 100 °C for 12 h and then ashed at 600 °C in ceramic

crucibles for 24 h. To determine bone mineral content, all tibias and crucibles were weighed before and after ashing. To minimize moisture content, crucibles were kept at 100 °C for 12 h prior to ashing. Right tibia breaking strength (g) was determined using a QC-SPA system (TSS, York, England) to break each tibia bone at the center point of the tibial shaft.

Eye Development

The physical differences in eye development between treatments were evaluated during the fifth week of grow-out. The same 20 birds per treatment (N = 80) used for tibia bone ash mineral content and breaking strength were used to determine all eye development parameters. After euthanasia on d 35 (described above), the heads of all euthanized ducks were removed and placed in bags of deionized water to be stored overnight. The following day, the left and right eyes from each duck were extracted and measured. A calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL, USA) was used to measure the side-to-side diameter (mm) and back-to-front diameter (mm) of each eye. Additionally, the weight of each eye (g) was recorded. The average eye length, width, weight, and the differences between the left and right eyes for each of these respective measurements was recorded for each treatment.

Stress Susceptibility Measures

Plasma Corticosterone and Heterophil to Lymphocyte Ratio

At 35 days of age, 20 ducks per treatment (N = 80) were randomly selected for plasma corticosterone (CORT) and heterophil to lymphocyte ratio (HL) analysis. Approximately 1–2 mL of blood was collected from the brachial wing vein of each duck.

A small drop of blood from each bird sampled was smeared on a glass plate for heterophil to lymphocyte ratio (HL) analysis (described below). The remaining blood collected was injected into a plasma separation gel and lithium heparin vacutainer (BD 368056, BD, Franklin Lakes, NJ, USA) and temporarily stored on an ice bath. Once all blood samples had been collected, all vacutainers were spun down using a centrifuge (Eppendorf 5804, Eppendorf North America, Hauppauge, NY, USA) at 4000 RPM for 15 min to separate the plasma and blood cells. Each blood plasma sample was then poured into a 2-mL microcentrifuge tube and stored at −19 °C until further analysis was performed. A hematology staining kit (Cat# 25034, Polysciences Inc, Warrington, PA, USA) was used for staining blood smear slides used for H/L.

The plasma corticosterone concentration from each sample was analyzed using a commercially available ELISA kit (Enzo Life Sciences, ADI-901-097, Farmingdale, NY, USA). The inter- and intra-assay %CV were both under 5%.

To determine the heterophil to lymphocyte ratio of collected samples, one layer of stained blood cells on glass slides were observed under 40× magnification using an oil immersion lens on a microscope (Omax DCE-2, Kent, WA, USA). The number of heterophils and lymphocytes observed in an area of the blood smear slide without overlapping cells was counted using a keystroke counter (SEOH B4001-5LC, Navasota, TX, USA) until a total of 100 cells had been recorded (Campo et al., 2000). Increased plasma corticosterone (Cockrem, 2007) and heterophil to lymphocyte ratios (Gross and Siegel, 1983) indicate higher stress susceptibility in poultry.

Physical Asymmetry

On day 35 of the current study, 60 live ducks per treatment (N = 240) were randomly selected to be measured for physical asymmetry of 3 bilateral traits (ASYM) as described in Archer et al. (2009). A calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL) was used to measure the metatarsal length (ML), metatarsal width (MW), and middle toe length (MTL) of the left and right legs of each duck. The composite asymmetry score for each duck was calculated by taking the sum of the absolute value of the left measurement subtracted from the right measurement of each trait and then by dividing by the total number of traits (2017).

Fear Response Measures

Tonic Immobility

Tonic immobility measurements were collected during the fifth week of grow-out using methods described in Archer (2017) and House et al. (2020b). The described methods were designed for the induction of TI in broilers; therefore, the methods used in the current study were slightly modified by lengthening the time for which pressure was applied to the thoracic cavity to induce TI, as described below. Sixty ducks per treatment (N = 240) were randomly selected and were gently placed on their back in a wooden U-shaped cradle lined with black cloth. Slight pressure was placed on the thoracic cavity of the duck for approximately 25 s, until tonic immobility was induced. Contact was then removed, and a timer was started. Each duck must be immobile for at least 10 s in order for its latency to right from TI to be recorded. If the duck righted itself in under 10 s, it was recorded as a time of 0 s. Otherwise the first head movement and the duck's latency

to right itself was recorded, with a maximum time of 10 min. Each duck was allowed a maximum of 3 attempts to be successfully tested. If all 3 attempts were unsuccessful, the duck's final time was recorded as 0 s. A longer latency to right during tonic immobility is indicative of greater fear responses in poultry (Jones, 1986).

Inversion

Inversion measurements were also collected during the fifth week of grow-out. Sixty ducks per treatment (N = 240) were randomly selected and caught and then inverted while holding each duck by its legs until the duck ceased to wing flap or for 30 s (Archer et al., 2009). Each duck inversion was recorded for later observation (Cannon, ZR900, Melville, NY, USA; 24 frames per second). The number of wing flaps and the duration of wing flapping during inversion for each duck was recorded by a trained video observer, and wing flap intensity was determined by dividing the number of wing flaps by the duration of wing flapping during inversion. Greater intensity of wing flapping indicates higher fear responses in poultry species (Newberry and Blair, 1993).

Statistical Analysis

General Linear Models (GLM) were used to investigate the treatment, trial, and treatment × trial effects on feed conversion, weight gain, eye parameters, plasma corticosterone concentration, heterophil to lymphocyte ratio, composite asymmetry scores of bilateral traits, tonic immobility, and inversion. Levene's test for homogeneity of variance and the Shapiro–Wilk test for normality were used to test all GLM assumptions. All assumptions were met without transformations, and all planned comparisons were tested using the least significant difference test. Windows SAS 9.3

(SAS Institute Inc., Cary, NC, USA) was used to perform all analyses. $p < 0.05$ was used to determine all significant differences, and only treatment effects were discussed in the current study, as there were no effects observed on trial or treatment × trial interaction.

Results

All results for FCR, d 35 body weight, gait parameters, tibia bone ash mineral content, and tibia bone breaking strength are shown in Table 5. No differences between lighting treatments were found in body weight or FCR ($p > 0.05$). Additionally, no differences in gait score or stride length ($p > 0.05$) were observed between the two treatments, as shown in Table 6. No differences were found in the percent tibia bone ash mineral content or tibia bone breaking strength ($p > 0.05$).

Table 6. A comparison of results for growth, gait, and tibia bone quality parameters of Pekin ducks reared under normal LED (Control) or Control lighting supplemented with ultraviolet light (UV) in two replicative studies.

Treatment	FCR[1]	D 35 Body Weight[1] kg	Gait Score[2]	Stride Length[2] cm	Tibia Bone Ash[3] %	Tibia Breaking Strength[3] g
UV	1.66	2.70	0.10	18.4	45.7	33,239
Control	1.57	2.62	0.083	18.5	45.8	32,320
SEM	0.038	0.037	0.021	0.225	0.230	1,459
P-Value	0.38	0.32	0.89	0.90	0.79	0.53

Abbreviations: FCR, feed conversion ratio
[1]n = 32 pens
[2]n = 192 birds
[3]n = 80 birds

The UV treatment had lighter eyes (1.46 ± 0.018 g; $p = 0.025$) and narrower eyes (12.3 ± 0.066 mm; $p = 0.010$) than the control treatment (1.53 ± 0.024 g and 12.5 ± 0.056 mm, respectively). However, there was no difference in average eye length between the two treatments ($p > 0.05$). Additionally, the average differences in weight,

width, and length between the left and right eyes of each duck did not differ between the two treatments ($p > 0.05$). All data for eye development parameters are shown in Table 7.

Table 7. A comparison of results for eye development parameters of Pekin ducks reared under normla LED (Control) or Control lighting supplemented with ultraviolet light (UV) in two replicative studies.

Treatment	Eye Weight g	Eye Length mm	Eye Width mm	Abs. Eye Weight g	Abs. Eye Length mm	Abs. Eye Width mm
UV	1.46	8.14	12.3	0.058	0.31	0.36
Control	1.53	8.23	12.5	0.070	0.42	0.32
SEM	0.015	0.028	0.045	0.012	0.030	0.035
P-Value	0.025	0.10	0.010	0.60	0.065	0.57

Abbreviations: Abs., absolute value of the difference between left and right eyes of all ducks sampled
[1]n = 80 birds

All results for stress susceptibility and fear response parameters are shown in Table 8. The UV ducks had lower plasma corticosterone concentrations (6,317 ± 593.790 pg/mL; $p = 0.024$) and lower heterophil to lymphocyte ratios (0.43 ± 0.030; $p = 0.035$) compared to control ducks (9,242 ± 1120.700 pg/mL and 0.54 ± 0.042, respectively), indicating lower stress levels in ducks reared under supplemental UV light. The UV ducks had lower composite asymmetry scores (0.58 ± 0.030; $p = 0.002$) than control ducks (0.76 ± 0.037 mm), indicating lower long-term stress in the UV treatment compared to the control treatment.

Ultraviolet ducks had a faster latency for the first head movement during tonic immobility (61.28 ± 9.486 s, $p = 0.026$) and required more attempts to induce tonic immobility (1.71 ± 0.073, $p = 0.018$) than control ducks (100.7 ± 14.846 s and 1.48 ±

0.065, respectively). There were no differences in inversion intensity or the latency to right during tonic immobility between the two treatments.

Table 8. A comparison of results for stress susceptibility and fear response parameters of Pekin ducks reared under normla LED (Control) or Control lighting supplemented with ultraviolet light (UV) in two replicative studies.

				Tonic Immobility[2]			
Treatment	Corticosterone[1] pg/mL	Heterophil to Lymphocyte Ratio[1]	Composite Asymmetry Score[2] mm	Latency to Right s	First Head Movement s	# Attempts	Inversion[2] Flaps/s
UV	6,317	0.43	0.58	157.69	61.28	1.71	2.85
Control	9,242	0.54	0.76	202.96	100.7	1.48	2.83
SEM	651.240	0.026	0.025	12.487	8.882	0.051	0.076
P-Value	0.024	0.036	0.0002	0.070	0.026	0.018	0.91

[1]n = 80 birds
[2]n = 240 birds

Discussion

In the current study, there were no differences in duck growth performance, feed conversion, gait score, stride length, tibia bone ash mineral content, or tibia bone breaking strength. A study previously conducted by Zhang et al. (2006) found that broiler chickens reared under incandescent light supplemented with UV light had higher body weight, feed conversion, and skeletal development compared to those reared in an ultraviolet deficient environment, which could be attributed to higher levels of phosphorous, calcium, and growth hormone in birds reared under UV supplementation. No differences in feed conversion ratios or body weight were observed in broiler chickens exposed to either UV-deficient lighting or lighting containing UV supplementation (Lewis and Morris, 1998). To our knowledge, no studies have

examined the effects of ultraviolet light on Pekin duck growth and bone development. It has been observed that ducks have a lower spectral sensitivity to the ultraviolet spectrum than other poultry species (Barber et al., 2006), making species comparisons between broiler chickens and Pekin ducks difficult. Therefore, it may be possible that production parameters and bone development of Pekin ducks may not reflect that of broilers when both species are reared under UV light, which may explain why no differences in production parameters or bone development were observed in the current study.

In the current study, ducks reared under supplementary UV light had lighter and narrower eyes than control ducks. No differences were observed in eye width or average differences between the length, width, or weight of the left and right eyes of each duck. Very few studies have examined the effects of ultraviolet light on eye development in poultry. A study conducted by Hogsette et al. (1997) compared the eye pathology of hens exposed to blacklight-blue or blacklight fly trap lamps; however, no differences in eye morphology were observed. To our knowledge, no other studies have focused on poultry eye development under UV light conditions, and further research is needed to determine how changes in eye anatomy and weight affect the welfare of Pekin ducks and other poultry species.

Ducks reared under UV supplementation had lower plasma corticosterone concentrations, lower heterophil to lymphocyte ratios, and grew less asymmetrically than those reared in UV-deficient environments. It has been previously demonstrated that stress parameters such as CORT, H/L, and ASYM can be affected by light (Archer, 2018a,c; Campo et al., 2000; Archer et al., 2009; Onbaşılar and Erol, 2007; Xie et al.,

2008; Sobotik et al., 2019). The biological functions and development of poultry and other animals can be disrupted by stress (Moberg, 2000). A study performed by Huth and Archer (Huth and Archer, 2015) found that two different LED bulbs lowered CORT, H/L, and ASYM in chickens, indicating lower stress susceptibility in chickens reared under two particular LED spectrums compared to a compact fluorescent lamp (CFL) bulb. Furthermore, both broiler (House et al., 2020b) and laying hens (Sobotik et al., 2019) had lower stress susceptibility when reared under LEDs supplemented with UV light, as indicated by lower CORT, H/L, and ASYM than birds reared under just LEDs. The results of this study provide additional evidence, suggesting that UV supplementation can alter both acute and chronic stress susceptibility in ducks.

No differences were found in the latency to right from tonic immobility or in wing flap intensity between UV and control ducks in this study, although UV ducks had a faster latency for first head movements during tonic immobility and also required more attempts to induce tonic immobility. Tonic immobility can be divided into two stages, with the first stage being complete immobility without head movements and the second stage involving head movements (Jones and Faure, 1981a,b), meaning head movement may be associated with the latency to right from TI. A shorter latency to right from TI was observed in broilers subjected to UV light instead of just LED lighting (House et al., 2020b; James et al., 2018). Sobotik et al. (2019) also saw a shorter latency to right in laying hens housed under LED bulbs supplemented with UV light. Laying hens and broiler chickens have also been shown to have lower wing flap intensities when reared under UV light supplementation (House et al., 2020b; Sobotik et al., 2019). The results

from the current study suggest that, although there is no difference in the latency to right from TI or INV wing flap intensity between treatments, there is a possibility that ducks do experience the first stage of tonic immobility (complete immobility without head movements) (Jones and Faure, 1981a,b) for a shorter duration than control ducks. Although UV ducks and control ducks did not differ in the latency to right from TI, it is a possibility that UV ducks are more inquisitive about their environment even while experiencing extreme amounts of fear due to the ability to more effectively perceive the UV reflectivity of objects and may therefore experience shorter durations of the first stage of TI as a result.

Although there is very little knowledge concerning the effects of lighting on Pekin ducks in grow-out settings, as the meat duck industry grows within the United States and abroad, there will continue to be an increasing and persistent need for better welfare in Pekin duck grow-out facilities. Rearing calmer birds will ultimately result in fewer mortalities during transport and a fewer damaged carcasses during processing (Cockram and Dulal, 2018). Based on the results of this study, it can be concluded that ducks reared under UV light supplementation have increased welfare compared to ducks reared in UV-deficient environments, as represented by the behavioral tests and stress susceptibility measures described above.

In comparison to other aspects of poultry production such as rearing parameters, biosecurity, and nutritional requirements, knowledge of poultry light perception and the effects of lighting on poultry behavior and development is relatively limited (Riber, 2015). Similar to other poultry species, ducks utilize the ultraviolet portion of the light

spectrum for visually mediated behaviors, resulting in more effective environmental perception under ultraviolet light supplementation than in environments with UV-deficient lighting. However, the spectral sensitivity of ducks is different from other poultry species (Barber et al., 2006), making it critical to determine the effects of UV exposure on the performance and welfare of Pekin ducks. Based on the results of the current study, Pekin ducks reared under an environment illuminated with both LED bulbs and UV light supplementation have decreased stress and fear responses, indicating better welfare than ducks reared under only LED bulbs, which are deficient in UV light. Eye development can be manipulated by the presence of ultraviolet light; however, the welfare impacts of these changes in development are unknown and present future research opportunities. These results continue to emphasize the need for correct light spectrum supplementation in an artificially illuminated poultry houses.

CHAPTER 6: EXPERIMENTAL MONOCHROMATIC LIGHT-EMITTING DIODE FIXTURE IMPACTS PEKIN DUCK STRESS AND EYE DEVELOPMENT[*,****]

Introduction

Birds possess a highly complex visual system that allows them to discern a vast array of colors beyond the limits of human color perception in electromagnetic radiation wavelengths from sunlight and artificial light sources (Prescott and Wathes, 1999). Pekin ducks and other poultry species have tetrachromatic vision, meaning four, rather than three as seen in humans and other mammals, cone cell species with peak absorptions at 415 nm, 455 nm, 508 nm, and 571 nm (Yoshizawa, 1992) are present in the retina (Hart, 2001; Hart and Hunt, 2007). Bird vision is further enhanced by oil droplets, which filter incident light before it reaches the visual pigments specific to each cone species, thus reducing spectral overlap and elevating color discrimination in the brain (Prescott and Wathes, 1999; Goldsmith, 2006) .

Poultry perception of light source, spectrum, and intensity can mediate physiological and behavioral responses to stress, fear, and growth. Stress occurs as a response to changes in the environment to maintain homeostasis (Moberg, 2000); if environmental stressors persist for an extended period of time, energy may be diverted from normal biological functions, causing deficits in growth and immune function (Gross and Siegel, 1983; Zulkifli et al., 2014; Scanes, 2016). Three common measures of stress

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in poultry, plasma corticosterone concentration (CORT), heterophil to lymphocyte ratio (HL), and the physical asymmetry of bilateral traits (ASYM) can be affected by variations in artificial light bulb spectral output (Campo et al., 2000; Onbaşılar et al., 2009; Campbell et al., 2015; Huth and Archer, 2015; House et al., 2021). Elevated CORT, HL, and asymmetry between bilateral traits are indicative of elevated stress in poultry species (Gross and Siegel, 1983; Campo et al., 2000; Archer and Mench, 2013). Light spectrum can also impact the fear response of ducks (Sultana et al., 2013; House et al., 2021). Tonic immobility (TI), an anti-predator fear response observed in birds and other species, can reliably measure the fearfulness of poultry once this catatonic-like state has been induced by a trained observer (Campo et al., 2008). Inversion testing (INV) simulates routine handling of live ducks and other poultry species in processing facilities; measuring the intensity of wing flapping upon the bird being inverted is used to determine another variation of the fear response of poultry – the desire to escape human handling or a captive situation (Huth and Archer, 2015).

Limited research has explored the effects of light spectrum and LED lighting on Pekin duck production and welfare in comparison to broiler studies (Rozenboim et al., 1999; Cao et al., 2008; Xie et al., 2008). Previous reports indicate Pekin ducks are sensitive to LED light spectra, however results are not consistent across studies - Sultana et al. (2013) observed reduced duck fear responses under blue and green LED lighting, while our previous study indicated ducks reared under white/blue LED bulbs had a greater fear response than those reared under white/red LED bulbs (House et al., 2021). Hua et al. (2020) reported a smaller back-to-front eye diameter for Pekin ducks reared

under blue or white LED bulbs compared to ducks reared under red, yellow, or green LED bulbs, however no other studies have investigated light color-dependent eye development changes in Pekin ducks. The objective of the current investigation was therefore to illustrate how various portions of the light spectrum emitted by four experimental prototype LED fixtures affect the growth, eye development, stress, and fear response of Pekin ducks.

Materials and Methods

Ethical Note

All ducks were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP #2017-0426).

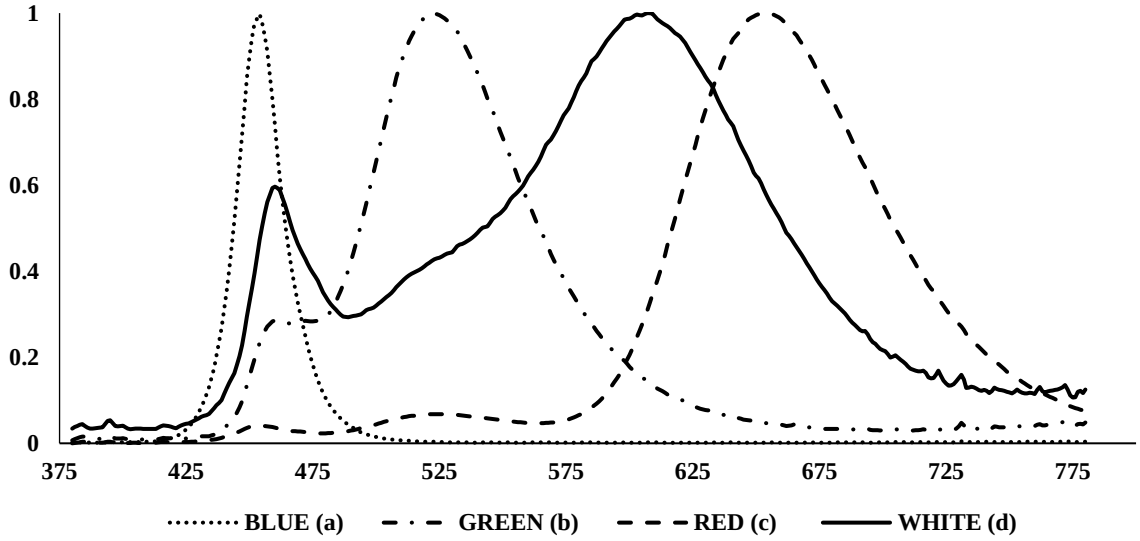
Overview

This investigation was conducted with two identical trials each utilizing 384 Pekin ducklings acquired on the day of hatch from Maple Leaf Farms, Inc. (Leesburg, IN, USA) with 4 treatments and 8 replicates per trial. Ducks were housed in 2 tunnel ventilated rooms each measuring 6.1 m x 9.1 m. Each room was divided in half by a partition to create 4 light-tight sections for each lighting treatment. Each of the 4 room halves was equipped with 8 floor pens (0.9 m wide, 1.8 m long, and 0.6 m high) and all floor pens were furnished with one tube feeder and a water drinking system consisting of 3 nipple drinkers per pen, both of which were adjustable to duck height throughout the

study duration. All pens were bedded with approximately 3 inches of fresh pine shavings.

Ducks were subjected to one of 4 different colored LED light treatments including monochromatic blue LED (B), monochromatic red LED (R), monochromatic green LED (G) or white LED (W) bulbs. The spectral distribution of each of the four bulb types used in this investigation is depicted in Figure 4. Two trial replications were performed to determine the effects of monochromatic LED lighting on Pekin duck growth, performance, and welfare. Each room section was assigned to one of four light treatments (BLUE, RED, GREEN, or WHITE). Three experimental prototype LED fixtures (Ag Lighting Innovations, Madison, TN) per treatment were uniformly installed from the ceiling of each room section directly above the pens for each treatment, 3 m above the floor. One dimmer/timer was used to control all 6 LED fixtures in a single room. To avoid room bias, light treatments were switched between the 2 rooms upon conclusion of the first trial so that in the second trial treatments were in the opposite room.

Figure 4. Spectral power readings of BLUE, RED, GREEN, and WHITE experimental prototype LED light fixtures using a spectral flickering irradiance meter. Four treatment groups received exposure to one of the four LED light fixtures. (a) spectral power readings for BLUE LED light fixture, (b) spectral power readings for GREEN LED light fixture, (c) spectral power readings for RED LED light fixture, (d) spectral power readings for WHITE LED light fixture.



All ducklings were randomly selected, weighed, and allocated to floor pens on the day of hatch. All 32 pens per trial were stocked with 12 ducks in each pen, and pen weights were recorded before placement. During the first 24 h post-placement, all treatment groups were subjected to a 24L:0D photoperiod and a light intensity of 20 gallilux as measured by a light meter (Hato Lighting Galli-Luxmeter, Hato Lighting, Netherlands) at duck head height. Light intensity was adjusted to head height during growth throughout the study. From d 1 – 10, all ducks were reared with a 16L:8D photoperiod. Beginning on d 11, all light fixtures were dimmed to 5 gallilux; this light intensity was maintained until trial termination on d 35. A spectral flickering irradiance meter was used to determine the flicker of each bulb type at 5 and 20 gallilux (Table 9).

Table 9. Flicker index readings for four experimental LED bulbs at 20 gallilux and 5 gallilux as measured by a light meter 9SFIM-300, Everfine, Hangzhou, China; Hato Lighting Galli-Luxmeter, Hato Lighting, Netherlands) at duck head height.

Treatment	Flicker Index	
	20 Gallilux	5 Gallilux
Blue	0.120	0.000
Green	0.066	0.035
Red	0.142	0.171
White	0.120	0.000

Feed for starter (d 0 – 14) and grower (d 15 – 35) phase diets was weighed (Ohaus Champ CD-11, Pink Brook, NJ) and recorded. All feed not consumed at the end of each phase was weighed and subtracted from the total amount fed. Standard duck starter and grower diet formulations were fed during both trials. All feed was produced by the Texas A&M University feed mill.

Growth and Feed Conversion Ratio

Prior to placement on d 0 and again on d 35, bird weights were recorded in pen groups (N = 64). Body weight gain (kg) was then determined by subtracting d 0 pen weights from d 35 pen weights. All feed was weighed before adding to pen feeders, and any residual feed was weighed back at the end of the starter (d 15) and grower (d 35) phases to calculate feed intake calculations. FCR was determined by dividing the total feed intake per pen by the total body weight gain per pen and was corrected for mortality. All mortalities were collected, weighed, and recorded daily. Mortalities and culls found before d 7 were replaced with a duck of the same weight.

Gait Score

Visual assessment of duck gait was conducted using methods described in Makagon et al. (2015). A total of 6 randomly selected ducks per pen (N = 192) were utilized in these measures. Each selected duck was individually placed on a flat, concrete surface in an observation pen which allowed a clear view of both duck legs. Two trained observers then determined a single gait score per duck, where scores ranged from 0 to 2. A "0" score indicated no gait abnormalities, a "1" score indicated slightly impaired walking or limping, and a "2" score indicated reluctance to walk or poor gait.

Tibia Bone Breaking Strength and Ash Mineral Content

Tibia bone breaking strength and ash mineral content were analyzed using the left and right tibias of 20 randomly selected birds per treatment (N = 160) respectively on d 35 (House et al., 2020a). All ducks were euthanized in airtight chambers using a mixture of CO2 gas and air. All connective tissue, muscle, and fibulas were removed from each collected tibia before analysis. Breaking strength (g) at the center point of the right tibial shaft was determined using the QC-SPA system (TSS, York, England). Left tibias were dried in a Forced Air Oven (VWR 89511-410, Radnor, PA) for 12 h at 100 °C. The dried tibias were then defatted in diethyl ether for 6-8 h and allowed to dry under a chemical hood for 12 hours upon the completion of defatting procedures so all ether could evaporate from the bones. Defatted tibias were dried again at 100 °C for 12

h, then ashed at 600 °C in ceramic crucibles for 24 h. All crucibles and tibias were weighed before and after ashing to determine tibia mineral content.

Eye Development

The same 20 randomly selected ducks sampled for described tibia measurements (N = 160) were also used for the evaluation of optic weight and dimensions. The heads of all euthanized ducks were removed post-mortem and stored overnight in bags of deionized water. After 24 h, the left and right eyes of each duck were enucleated, cleaned of any muscle and connective tissues, and measured. The side-to-side (mm) and back-to-front (mm) diameters of each eye were recorded using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL). Individual weights of each eye (g) were recorded. The average eye length, width, weight and the differences in each measurement between the left and right eyes of each sampled duck was calculated as in House et al. (2020a).

Stress Susceptibility

Plasma Corticosterone and Heterophil to Lymphocyte Ratios

On d 35, blood samples (1-2 mL) were collected from the brachial vein of 20 randomly selected ducks (N = 160) for plasma corticosterone and heterophil to lymphocyte ratio analysis between 8:00 and 9:00 AM for each trial. Blood samples were temporarily stored on an ice bath in plasma separation gel and lithium heparin vacutainers (BD 368056, BD, Franklin Lakes, NJ). All blood samples were centrifugated (Eppendorf 5804, Eppendorf North America, Hauppauge, NY) for 15 minutes at 4000 RPM to separate plasma and blood cells. Blood plasma samples were transferred to 2-

mL microcentrifuge tubes and stored at -19 °C. The concentration of plasma corticosterone from each sample was analyzed using a commercially available ELISA kit (Enzo Life Sciences, ADI-901-097, Farmingdale, NY). The inter- and intra-assay %CV were both under 5%. Plasma corticosterone concentration is indicative of the stress response in poultry where more stressful environmental conditions result in increased plasma corticosterone concentrations (Cockrem, 2007).

Multiple trained handlers were present to minimize bird disturbance during blood collection, ensuring all selected birds from each pen were caught and sampled within 45 s, as duck CORT and H/L will increase within 1 min after initial handling (Harvey et al., 1980). A small drop of blood per bird was smeared on a glass microscope slide and stained using a hematology staining kit (Cat# 25034, Polysciences Inc, Warrington, PA). Stained cells were observed under 40x magnification using an oil immersion lens on a standard microscope (Omax DCE-2, Kent, WA). A keystroke counter was used to count heterophil and lymphocyte cells until a total of 100 cells were recorded (Campo et al., 2000). Under chronically stressful conditions, the number of heterophils in blood will increase while the number of lymphocytes will decrease (Gross and Siegel, 1983).

Physical Asymmetry of Bilateral Traits

At 35 d of age, 60 randomly selected live ducks per treatment (N = 480) were measured for differences in the composite asymmetry of the middle toe length and metatarsi length and width using calibrated Craftsman IP54 Digital Calipers (Sears Holdings, Hoffman Estates, IL). A composite asymmetry score for the three traits was determined using methods described in Huth and Archer (2015). The sum of the absolute

value of the left minus right value of each trait was calculated, then divided by the total number of traits, thus following the formula: $(|L\text{-}R|_{MTL}+|L\text{-}R|_{ML}+|L\text{-}R|_{MW})/3$ = composite asymmetry score.

Fear Response

Inversion

At 5 weeks of age, 60 ducks per treatment (N = 480) were randomly selected for inversion testing using protocols described by (Archer and Mench, 2014). Each duck was held by the legs in the upright position, and then flipped upside-down. Inversion tests for all ducks were video recorded (Cannon, ZR900, Melville, NY, USA; 24 frames per second). Video analysis of each inverted duck included the number of wing flaps and the duration of wing flapping (s) to determine the wing flapping intensity (number of wing flaps/duration of wing flapping). More intense wing flapping intensity may indicate elevated fear responses in poultry during human handling and transport (Newberry and Blair, 1993).

Tonic Immobility

Another 60 ducks per treatment (N = 480) were randomly selected during week 5 for tonic immobility testing using adapted methods from Archer (2018). Ducks were placed on their backs in a U-shaped wooden cradle lined with black cotton fabric, and slight pressure was applied to the thoracic cavity of each duck for 30 s, after which pressure and contact were removed and a timer was started. If TI was achieved, each duck was required to remain in TI for at least 10 s before they attempted to escape the observer. If the TI duration was longer than 10 s, the time of first head movement during

TI (s), the overall latency to right from TI (s), and number of attempts required to induce TI were recorded. All ducks were allowed three attempts to remain in TI for 10 s, and if the required time was not reached, a time of 0 s was recorded for the latency to right from TI. A longer latency to right from TI indicates greater fear responses in avian species (Gallup, 1979).

Statistical Analysis

General Linear Models (GLM) were used to determine treatment, trial, and treatment x trial effects on FCR, d 35 weights, eye parameters, CORT, H/L, ASYM, TI, and INV. GLM assumptions were evaluated using Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. GLM procedures were followed with mean separation using Fisher's least significant difference test. Gait score and the number of attempts needed to induce TI were ordinal and evaluated using the Kruskal-Wallis test on the equality of means, not adjusted for ties. Absolute value differences of eye parameters were evaluated using a 1-way ANOVA. All analyses were performed using Minitab 17.1.0 (Minitab, LLC, State College, PA). $P \leq 0.05$ was defined as a significant difference.

Results

Data for FCR, d 35 body weight, tibia bone ash, tibia bone breaking strength, and gait score are presented in Table 10. The BLUE treatment had a lower tibia bone ash mineral content (43.53 ± 0.431 %) than the RED (46.10 ± 0.449 %) and WHITE treatments (44.95 ± 0.533 %; $P = 0.001$), and both GREEN (44.35 ± 0.399 %) and WHITE had a lower tibia bone ash mineral content than RED ($P < 0.05$). The BLUE

treatment also had a lower tibia bone breaking strength (29,980 ± 919.0 g) than WHITE (33,789 ± 1218.0 g; $P < 0.05$), and RED and GREEN were intermediates for tibia breaking strength. No differences were observed in FCR, d 35 body weight, or gait score ($P > 0.05$).

Table 10. Evaluation of Pekin duck production[1], tibia[1], and gait score[2] parameter results under four experimental monochromatic light-emitting diode fixtures.

Treatment	FCR[3]	D 35 Body Weight[3] kg	Tibia Bone Ash[4] %	Tibia Bone Breaking Strength[4] g	Gait Score[5]
Blue	1.49	2.75	43.53[c]	29,980[b]	0.17
Green	1.50	2.73	44.35[bc]	32,790[ab]	0.18
Red	1.51	2.69	46.10[a]	30,874[ab]	0.19
White	1.46	2.79	44.95[ab]	33,789[a]	0.15
SEM	0.031	0.030	0.228	529.0	0.019
P-Value	0.919	0.705	0.001	0.047	0.965

[1]Data analysis conducted using One-way ANOVA
[2]Data analysis conducted using Kruskal Wallis nonparametric test
[3]N=64 pens
[4]N=160 ducks
[5]N=192 ducks

Data for CORT, H/L, and ASYM are presented in Table 11. Plasma corticosterone concentrations and H/L were elevated in the BLUE (9,005 ± 962 pg/mL and 0.58 ± 0.061 respectively) and RED (8,965 ± 1137.0 pg/mL and 0.55 ± 0.054 respectively) treatments compared to WHITE (5,578 ± 556.0 pg/mL and 0.35 ± 0.030 respectively) and GREEN (6,058 ± 708.0 pg/mL and 0.40 ± 0.031 respectively; $P = 0.005$ and $P = 0.001$ respectively). Asymmetry scores were highest in the BLUE treatment (2.55 ± 0.326), and lowest in GREEN (0.69 ± 0.043) and WHITE ducks (0.73 ± 0.090; $P < 0.001$).

Table 11. Evaluation of Pekin duck stress parameter results under four experimental monochromatic light-emitting diode fixtures. Data analysis of results was conducted using One-way ANOVA.

Treatment	Corticosterone[1] pg/mL	Heterophil to Lymphocyte Ratio[1]	Asymmetry Score[2]
Blue	9,005[a]	0.58[a]	2.55[a]
Green	6,058[b]	0.40[b]	0.69[c]
Red	8,965[a]	0.55[a]	1.49[b]
White	5,578[b]	0.35[b]	0.73[c]
SEM	435.0	0.023	0.098
P-Value	0.005	0.001	0.000

[1]N=160 ducks
[2]N=480 ducks

Data for eye measurements are presented in Table 12. The WHITE and GREEN treatments had heavier eyes (1.56 ± 0.018 g and 1.54 ± 0.016 g respectively) than RED and BLUE treatments (1.49 ± 0.019 g and 1.48 ± 0.022 g respectively; $P < 0.01$). The average difference in weight between the left and right eyes was greater in GREEN (0.080 ± 0.012 g) and WHITE (0.06 ± 0.012 g) treatments compared to the BLUE (0.05 ± 0.009 g) and RED treatments (0.04 ± 0.012 g; $P < 0.05$). The WHITE treatment had wider eyes (9.72 ± 0.065 mm) than the RED (9.44 ± 0.073 mm, $P < 0.01$) and GREEN (9.50 ± 0.078 mm, $P < 0.05$) treatments. The BLUE treatment had wider eyes (9.69 ± 0.080 mm) than the RED treatment ($P < 0.02$). No differences were observed in eye length or the average difference in length and width between the four treatments ($P > 0.05$). Data for fear measurements are presented in Table 13. Lighting treatments did not have an effect on TI latency to right, the number of attempts to induce TI, the latency to first head movement during TI or INV intensity ($P > 0.05$).

Table 12. Evaluation of Pekin duck gross eye development results under four experimental monochromatic light-emitting diode fixtures. Data analysis of results was conducted using One-way ANOVA.

Treatment	Eye Weight[1] g	Eye Length[1] mm	Eye Width[1] mm	Abs. Eye Weight[1] g	Abs. Eye Length[1] mm	Abs. Eye Width[1] mm
Blue	1.48[b]	14.82	9.69[ab]	0.05[b]	0.26	0.30
Green	1.54[a]	14.88	9.50[bc]	0.08[a]	0.36	0.41
Red	1.49[b]	14.90	9.44[c]	0.04[b]	0.31	0.38
White	1.56[a]	14.78	9.72[a]	0.06[ab]	0.34	0.31
SEM	0.009	0.033	0.037	0.006	0.022	0.021
P-Value	0.008	0.586	0.015	0.028	0.417	0.170

[1]N=160 ducks

Table 13. Evaluation of Pekin duck fear response results under four monochromatic light-emitting diode fixtures.

Treatment	TI Latency to Right[1,3] s	TI First Head Mvmt[1,3] s	TI # Attempts[2,3]	INV Flap Duration[1,3] s	INV # Flaps[1,3]	INV Intensity[1,3] Flaps/s
Blue	137.80	71.40	1.55	2.07[a]	5.65[a]	2.77
Green	185.00	93.90	1.40	1.73[ab]	5.06[ab]	2.92
Red	171.00	73.20	1.55	1.44[b]	4.15[b]	2.63
White	160.80	68.10	1.43	1.65[b]	4.67[b]	2.68
SEM	7.991	6.411	0.032	0.115	0.286	0.106
P-Value	0.203	0.464	0.405	0.009	0.012	0.347

[1]Data analysis conducted using One-way ANOVA
[2]Data analysis conducted using Kruskal Wallis nonparametric test
[3]N=480 ducks

Discussion

As Pekin duck production and welfare continue to become more prevalent both in the United States and abroad, the effects of duck rearing environments must be evaluated to reduce fear and stress and to promote growth in commercial meat duck grow out facilities. Like modern broiler and turkey grow out houses, many producers

utilize LED fixtures for artificial lighting in Pekin duck facilities; however, the effects of LED light on Pekin meat ducks are relatively unknown. The purpose of this investigation was therefore to understand the effects of various LED spectral outputs on Pekin duck growth, stress susceptibility, and fear response to identify modern lighting sources conducive to improving duck welfare.

Duck d 35 BW and FCR were not affected by lighting treatment in the current study. These results are similar to those reported in a previous study which hypothesized duck performance may not be affected by colored LEDs at low light intensities such as the 5 lux used in the reported study and the 5 gallilux used in the current study (Hua et al., 2020). Two other reports indicating differences in duck BW maintained a light intensity of 20 lux (Hassan et al., 2016) and 25 lux (Campbell et al., 2015) respectively. Additionally, Hua et al. (2020) observed differences in duck body weight gain only in the d 35 – 42 phase of grow-out, suggesting that growth performance may be impacted more during later phases of growth outside scope of the current investigation. Future studies focusing on the interactions of light color, intensity, and age are needed to provide more comparative data for Pekin duck performance parameters.

Due to the rapid growth of Pekin ducks, lameness and other leg deformities are common and can be potentially painful (McGeown et al., 1999; Rodenburg et al., 2005), emphasizing the importance of skeletal development in ducks. In the current study, tibia bone ash mineral content and breaking strength values were numerically lowest in BLUE ducks, suggesting blue light has a negative effect on duck tibia development. However, because these results were not statistically significant, more research is needed

with possibly greater numbers of subjects to determine conclusively if blue light is detrimental to bone development. Furthermore, limited research has studied the effects of monochromatic LED lighting on tibia bone strength and mineral ash content in broiler chickens (Prayitno et al., 1997), and only one previous study reported various monochromatic LED lights did not affect tibia bone mineral density (Hassan et al., 2016). The authors hypothesize that blue LED lighting may decrease locomotor activity in ducks, consequently resulting in less tibia bone ossification and poor leg health (Bessei, 2006; Sultana et al., 2013). Gait scores did not significantly differ between treatments, which may be attributed to the tibia parameter results analyzed in this investigation. Future research is required to identify the differences in bone ossification rate between GREEN, RED, and WHITE light treatments used in the current study to determine the most appropriate light source for leg health in Pekin ducks.

Lighting is considered a major environmental stimulus for poultry due to their natural sensitivity to light intensity, duration, and wavelength (Siegel, 1995; Parvin et al., 2014), and lighting has been previously demonstrated to affect stress physiology and immune function of birds (Xie et al., 2008; Archer, 2019; House et al., 2021). Plasma CORT is a useful measure of acute stress responses, while HL and ASYM measures are commonly used to determine chronic stress responses (Gross and Siegel, 1983; Siegel, 1995; Archer, 2019) in poultry. The current investigation found ducks reared under LED fixtures emitting monochromatic red (long wavelength) and blue (short wavelength) light had higher plasma CORT, HL, and ASYM compared to WHITE and GREEN ducks, indicating elevated stress responses in the former two treatments.

Tonic immobility is a common and reliable measure of avian fear responses (Gallup, 1979), but limited research on the impact of lighting on Pekin duck fear responses is available. White LED light (Sultana et al., 2013) and red light (Mohamed et al., 2016) as previously been found to elevate fear responses during TI in ducks compared to blue and green light. However, our lab has observed elevated fear during TI in ducks reared under white/blue LED light compared to ducks reared under white/red LED light (House et al., 2021). Interestingly, there were no differences in either TI or INV between the four light treatments for the current study, meaning these results are not in line with previously published data. It is possible that differences between this study and previous reports occurred due to variations in sample size or age; Sultana et al. (2013) tested 10 ducks per treatment at both 3 and 6 wks of age, and Mohamed et al.(2016) tested 9 ducks per treatment at 13 wks of age, while the current study tested 60 ducks per treatment at 5 wks of age. Ducks become more fearful as they age (Sultana et al., 2013), so it is likely this is reflected in the varied results seen in the literature.

Color discrimination is a key aspect of bird vision due to the presence of four distinct retinal cone pigments and carotenoid oil droplets which act to filter photons of light bombarding the retina (Prescott and Wathes, 1999; Goldsmith, 2006) . Each type of cone pigment maximally absorbs light at one of four ranges in the visible light spectrum and restricts the activation of their specific cone type to this range of light, further stimulating light color discrimination in the brain (Hart, 2001). In addition to retinal pigmentation and oil droplets, the ecology and evolution of birds can influence the proportion of various types of cones to most effectively visualize the species' original

habitat (Hart and Hunt, 2007). Pekin ducks are descendants of the wild Mallard duck, which often forage for food by dabbling on the surfaces of bodies of water. Ducks and other shorebirds have a larger proportion of short wavelength-sensitive photoreceptors (blue light sensing) compared to chickens and other Galliformes, which have a larger proportion of long wavelength-sensitive photoreceptors (red light sensing) (Hart et al., 1999); Campbell et al. (2015) hypothesized that because Pekin ducks, like their wild counterparts, may utilize this larger proportion of blue light photoreceptors as an aid for object recognition, and artificial blue lighting in duck houses may cause visual deprivation for duck flocks, resulting in stress and compromised welfare compared to ducks reared under red or white compact fluorescent lighting. Eye development may in part mediate duck welfare in addition to light perception; lighting extremes in photoperiod and intensity have been shown to induce ocular abnormalities in avian species such as buphthalmia, or ocular enlargement, and even blindness (Whitley et al., 1984). However, very limited research has explored the effects of light color on gross eye measures and development in ducks or other poultry species. In the current investigation, eye weight was greater in WHITE and GREEN ducks than in RED and BLUE ducks. These results are not aligned with Hua et al. (2020), which reported increased eyeball length (front-to-back) and width (side-to-side) in ducks reared under longer wavelengths such as yellow, red, and green light compared to blue light, and eye weight was not affected. The authors speculate eye weight differences in the current study are attributed to variations in perceived light fixture intensity between the four treatments. Although all treatment light intensity measurements were equated for the

duration of the study, previous research indicates luminescence meters may not be completely representative of the perceived intensity of colored lights by chickens (Prayitno and Phillips, 1997). Rozenboim et al. (1999) reported wavelengths between 480 nm and 560 nm were perceived as brighter by broilers than longer wavelengths although all light treatment intensities were identical, and broilers reared under 480 nm and 560 nm light treatments had heavier body weights than broilers under long wavelength light. This indicates broiler growth responses were primarily due to light wavelength rather than intensity, suggesting broilers perceived blue light as brighter than red or white light even if light intensities were equalized (Rozenboim et al., 1999; Lewis and Morris, 2000). In the current study, eye sizes were not significantly affected between the RED and BLUE light treatments, which may be a result in species-specific differences in spectral sensitivity (Campbell et al., 2015). Furthermore, the anatomical structure of avian eyeballs can be altered by low light intensities (1 lux) in chickens, resulting in enlarged and heavier eyes compared to chickens exposed to bright light (10, 20, or 40 lux) (Deep et al., 2010). It is hypothesized ducks in the current study perceived GREEN and WHITE fixtures as dimmer than RED and BLUE fixtures, resulting in heavier eye weights; however, further investigation is required.

In the current study, Pekin duck stress susceptibility was compromised by both extremes of the visible light spectrum (RED and BLUE), but not by mid-length GREEN light or WHITE light. The authors hypothesize these results can be attributed to the range of wavelengths emitted by each of respective bulb type used in this investigation. RED and BLUE light fixtures emitted a narrower range of light wavelengths compared

to WHITE and GREEN fixtures. If the avian eye is subjected to a light fixture emitting a broad range of wavelengths, as seen in WHITE and GREEN treatments, more cone types may be stimulated, possibly allowing the brain to discriminate more color variations of the bird's environment. Likewise, subjecting birds to narrower portions of the light spectrum as in RED and BLUE treatments may restrict the number of activated retinal cone types, creating the perception of diluted or "washed-out" object color cues that may not reflect the true object color. Color cues have been demonstrated to be an integral aid in environmental perception, object recognition, and identification of conspecific intent in avian species (Moura et al., 2006; Mohammed, 2019), and providing artificial light which removes these cues, such as RED and BLUE fixtures, could be detrimental to Pekin duck wellbeing. Interestingly, the detrimental effects of red light were not observed in House et al. (2021), which concluded a combination white/red LED bulb decreased stress susceptibility compared to a combination white/blue LED bulb, indicating mixed red LED lighting may be more suitable for Pekin ducks than monochromatic red LED lighting. These results further support the current hypothesis that LED light fixtures emitting a broad spectral range may provide the most beneficial artificial lighting environment for Pekin ducks, and that blue LED fixtures, like blue fluorescent bulbs (Campbell et al., 2015), should not be utilized in duck grow-out facilities.

In conclusion, chronic and acute stress responses of Pekin ducks were detrimentally affected by BLUE and RED lighting. No differences were observed in FCR, BW, gait score, or fear response parameters. Based on the results of this study, the

authors speculate monochromatic lights emitting wavelengths at the extremes of the visible light spectrum (BLUE and RED) do not provide sufficient duck retinal cone stimulation for the visualization of environmental color cues and may therefore deprive ducks of adequate sensory input and consequently elevate stress. Light fixtures emitting a broad spectral output, such as GREEN and WHITE LED fixtures facilitate lower stress responses in Pekin duck flocks and may serve as adequate artificial lighting sources for Pekin duck grow-out facilities.

CHAPTER 7: CONCLUSIONS

As environmental management for the purpose of improving animal welfare becomes more prevalent across the commercial poultry industry, it is important to consider how artificial lighting, one of the most impactful environmental factors in poultry rearing facilities, impacts bird behavior, physiology, and performance. Ducks, like other poultry species, are extremely photosensitive, and changes in light spectrum and photoperiod duration must be considered when evaluating artificial light protocols for grow-out facilities. However, very limited research has investigated the effects of artificial LED light parameters such as duration and spectrum on Pekin duck welfare and growth. The objective of this research, therefore, was to evaluate the effects of light duration and commercially available and experimental LED light spectral outputs on Pekin duck stress, fear response, and growth.

In Experiment 1, increasing grow-out photophase duration from 16 h of light to 20 h of light impacted duck performance, stress, fear response, and well-being. The results of this study demonstrated ducks reared under 20L:4D had lower chronic and acute stress during grow-out compared to ducks reared under the control (16L:8D) photoperiod. 20L:4D ducks also displayed a more robust immune response to NDV than the control ducks, possibly because 20L:4D duck immunity to foreign immunogens was not compromised by excessive stress. Stress and immune function are highly interrelated with the efficiency of nutrient metabolism. Nutrient utilization can be redirected during elevated stress and compromised immune function, resulting in lower feed efficiency.

The current study demonstrated control ducks, which had both elevated stress responses and compromised immune function compared to the 20L:4D ducks, also had a higher FCR compared to 20L:4D ducks. Therefore, it is likely the elevated stress and compromised immune function of the 16L:8D photoperiod contributed to the higher FCR observed in this same treatment, indicating photoperiod-induced stress and compromised immunity may also change the metabolic distribution of nutrients in 16L:8D birds compared to 20L:4D birds, ultimately resulting in ducks with less efficient feed conversion when subjected to an 8 h scotophase. Additionally, ducks in the 20L:4D treatment had longer stride lengths than ducks in the control treatment, indicating prolonged photophases may encourage duck mobility and leg health, although future studies are needed to elucidate more information on duck activity under various photoperiod schedules.

Experiments 2, 3, and 4 examined the effects of LED spectral output on Pekin duck stress, fear, and performance. The results of Experiment 4 indicated that although performance data was not affected by light color, tibia bone ash mineral content and breaking strength values were numerically lowest in ducks subjected to monochromatic blue LED light, suggesting blue light has a negative effect on duck tibia development; however, more research with larger samples sizes is needed to determine if these results could be statistically significant. Ducks reared under BLUE and RED light had elevated chronic and acute stress responses compared to WHITE and GREEN treatments. The BLUE and RED light treatments, both of which emitted wavelengths at the extremes of the visible light spectrum, did not provide sufficient duck retinal cone stimulation for the

visualization of environmental color cues and may therefore deprive ducks of adequate sensory input and, consequently, elevate stress. Eye weights were lower in ducks subjected to BLUE and RED light than ducks subjected to WHITE or GREEN light, which may be attributed to variations in perceived light fixture intensity between the four light treatments, where BLUE and RED were perceived as brighter than WHITE and GREEN. Interestingly, in Experiment 3, ducks reared under the UV treatment had lighter eyes, but also had lower stress responses than ducks subjected to the control white LED light. These results indicate that while it is likely ducks perceived the UV treatment as brighter than the control light treatment, supplemental UV light did not adversely affect stress as in Experiment 4. Consequently, it can be concluded that while some LED light intensities may be perceived as brighter by Pekin ducks, this perceived brightness is not always detrimental to bird welfare, unlike spectral output.

Based on Experiment 4 results, the extremes of visible light should be avoided when selecting artificial lighting for Pekin ducks. Ultraviolet light is considered a spectral extreme, as the wavelengths within this region of the spectrum are even shorter than those of blue light; however, UV light is essential for environmental perception in ducks and other bird species and can alleviate stress in Pekin ducks as discussed above. Given the results of these three experiments, it is advised that LED bulbs selected for artificial lighting in Pekin duck grow-out houses emit broad spectral outputs, such as WHITE or GREEN light. Additionally, supplementing white LED light with UV light or red light is beneficial to duck welfare. Blue light, as demonstrated in Experiment 2 and 4,

compromises the welfare of Pekin ducks and should not be utilized as an artificial light source in duck grow-out houses.

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