Experimental monochromatic light-emitting diode fixture impacts Pekin duck stress and eye development

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ABSTRACT Poultry color perception of artificial light-emitting diode (**LED**) lighting mediates bird physiology and behavior; however, very limited research has focused on how changes in LED light color affect these same parameters in Pekin ducks. Therefore, the objective of this investigation was to determine how four LED bulbs emitting various portions of the visible light spectrum − monochromatic blue (**BLUE**), monochromatic green (**GREEN**), monochromatic red (**RED**), and white (**WHITE**) - impact the stress, fear responses, eye development, and growth of 768 straight run Pekin ducks. Elevated plasma corticosterone concentration and heterophil to lymphocyte ratio was observed in BLUE and RED ducks compared to WHITE and GREEN ducks ($P = 0.005$ and $P = 0.001$, respectively), and asymmetry scores were highest in BLUE ducks ($P < 0.001$), indicating BLUE and RED lighting increase the stress susceptibility of Pekin ducks. Eye weight was lowest in BLUE and RED ducks compared to GREEN and WHITE ducks ($P < 0.01$). No differences were observed in d 35 body weight, FCR, gait score, or fear response parameters ($P > 0.05$). These results indicate BLUE and RED lighting may not be adequate for Pekin duck growout, and Pekin ducks may require artificial light sources containing a broad range of wavelengths, as seen with WHITE and GREEN lights, rather than lights containing more concentrated ranges such as in RED and BLUE lights, but further investigation is needed to understand how eye weight affects duck light perception and welfare. The current findings emphasize that although Pekin ducks and chickens are both sensitive to light color, species-specific nuances in light perception may cause distinct differences in Pekin duck versus broiler physiological responses and must be considered when selecting artificial light color in Pekin duck growout facilities.

Key words: duck, stress, light, welfare

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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 4, rather than 3 as seen in humans and other mammals, cone cell species with peak absorptions at 415 nm, 455 nm, 508 nm, and 571 nm 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 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;

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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. (2021) 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 4 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) with 4 treatments and 8 replicates per trial. Ducks were housed in 2 tunnel ventilated rooms each measuring 6.1 m × 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 4 bulb types used in this investigation is depicted in Figure 1. Two trial replications were

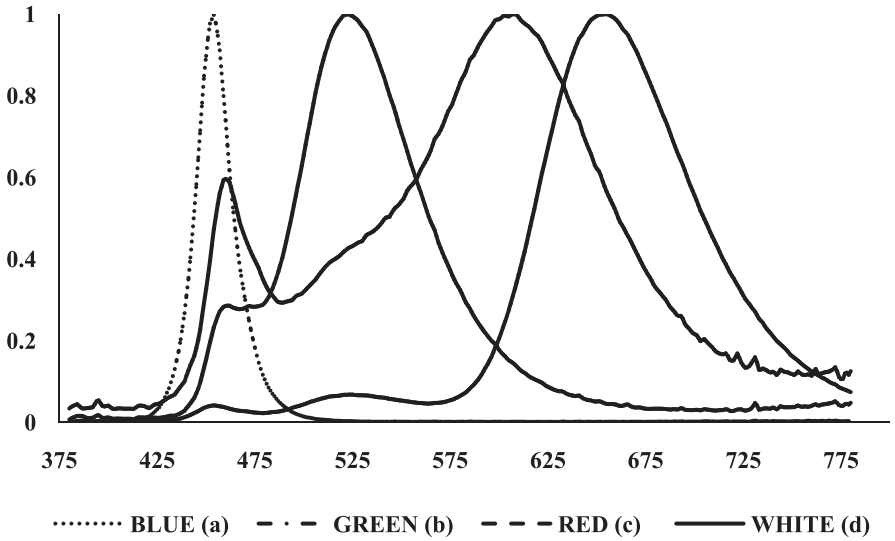

Figure 1. Spectral power readings of BLUE, RED, GREEN, and WHITE experimental prototype LED light fixtures using a spectral flickering irradiance meter. Four treatment groups received exposure to one of the four LED light fixtures. (A) spectral power readings for BLUE LED light fixture, (B) spectral 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.

Table 1. Flicker index readings for four experimental LED bulbs at 20 gallilux and 5 gallilux as measured by a light meter (SFIM-300, Everfine, Hangzhou, China; Hato Lighting Galli-Luxmeter, Hato Lighting, Netherlands) at duck head height.

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

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.

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 to 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 1).

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.

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., 2020). 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, UK). 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 to 8 h and allowed to dry under a chemical hood for 12 h 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 postmortem 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. (2020).

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 min 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 was 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 HL 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 40× 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 3 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: $(|\text{L-R}|_{\text{MTL}}+|\text{L-R}|_{\text{ML}}+|\text{L-R}|_{\text{MW}})/3$ = composite asymmetry score.

Fear Response

Inversion At 5 wk 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; 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 × 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 2. 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 (29980 ± 919.0 g) than WHITE (33789 ± 1218.0 g; $P < 0.05$), and RED and GREEN were intermediates for tibia breaking

Table 2. 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

[a-c]Differences for means in each column are indicated as by superscripts a-c, where $P \leq 0.05$.
[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.

Table 3. Evaluation of Pekin duck stress parameter results under four experimental monochromatic light-emitting diode fixtures.

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

Data analysis of results was conducted using One-way ANOVA.
[a-c]Differences for means in each column are indicated as by superscripts a-c, where $P \leq 0.05$.
[1]N = 160 ducks.
[2]N = 480 ducks.

strength. No differences were observed in FCR, d 35 body weight, or gait score ($P > 0.05$).

Data for CORT, HL, and ASYM are presented in Table 3. 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 ± 1,137.0 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; $P < 0.001$).

Data for eye measurements are presented in Table 4. 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 5. 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 ($P > 0.05$).

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.

Table 4. Evaluation of Pekin duck gross eye development results under four experimental monochromatic light-emitting diode fixtures.

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

Data analysis of results was conducted using One-way ANOVA.
[a-c]Differences for means in each column are indicated as by superscripts a-c, where $P \leq 0.05$.
[1]N = 160 ducks.

Table 5. Evaluation of Pekin duck fear response results under four monochromatic light-emitting diode fixtures.

Treatment	TILatencyto right[1,3]s	TIFirst head Mvmt[1,3]s	TI# Attempts[2,3]	INVFlap duration[1,3]s	INV# Flaps[1,3]	INVIntensity[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

Abbreviations: INV, inversion testing; TI, tonic immobility.
[a-b]Differences for means in each column are indicated as by superscripts a,b, where $P \leq 0.05$.
[1]Data analysis conducted using One-way ANOVA.
[2]Data analysis conducted using Kruskal-Wallis nonparametric test.
[3]N = 480 ducks.

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., 2021). Two other reports indicating differences in duck BW maintained a light intensity of 20 lux (Hassan et al., 2017) and 25 lux (Campbell et al., 2015) respectively. Additionally, Hua et al. (2021) observed differences in duck body weight gain only in the d 35 to 42 phase of growout, 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., 2017). 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 wk of age, and Mohamed et al. (2016) tested 9 ducks per treatment at 13 wk of age, while the current study tested 60 ducks per treatment at 5 wk 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 4 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 4 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. (2021), 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 growout facilities.

DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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