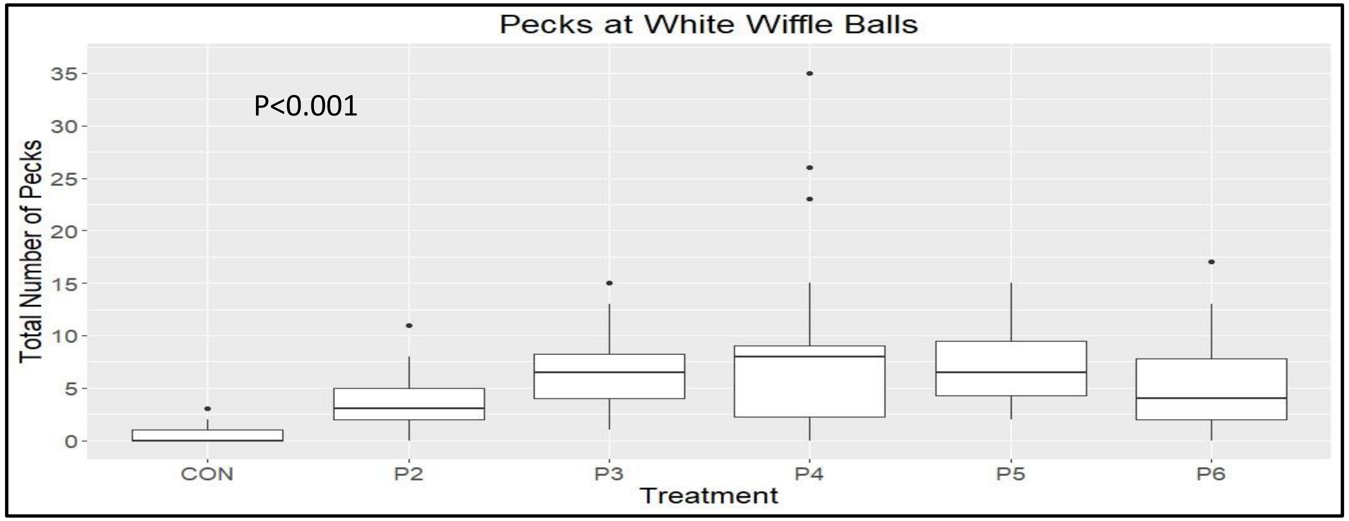
CSU Study on poultry behavior

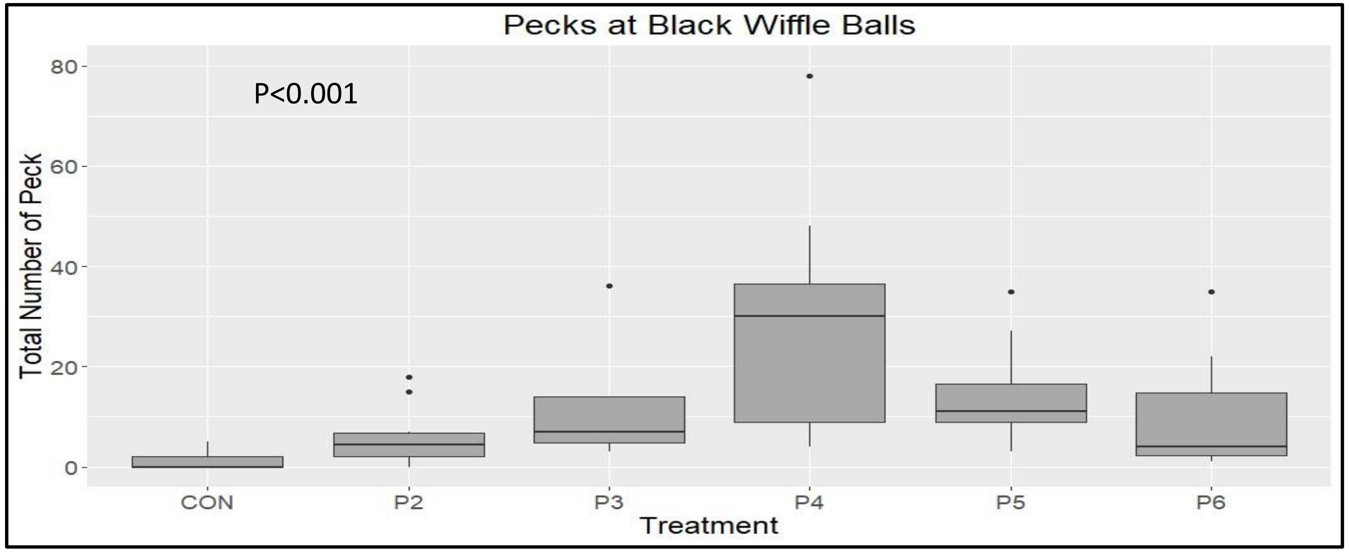
Birds: Hy-Line W36

Lighting:
XTI – 24hrs & Ramping Timer
Control – Ramping Timer

Feed: Ranchway Lay Hen 18%

- Birds under XTI lighting were more likely to interact with both the white and black wiffle ball. This is an indication that XTI lighting may reduce stress and anxiety in the birds.





Pilot Study: Behavioral response to novel objects in pullets housed in pulsed alternating wavelength system (PAWS) environments

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Abstract

A new light-emitting diode (LED) lighting strategy, known as pulsed alternating wavelength systems (PAWS), has been implemented to improve the production capacity of laying hens. Our objective was to determine the impact of PAWS lighting environments on the behavioral reaction of pullets to a novel object. During this pilot study, two hundred and ninety-nine Bovan pullets were randomly assigned to housing enclosures in groups of 16-18 birds. Each tent housing enclosure was randomly assigned to one of six specific lighting treatments - one of 5 different PAWS lighting treatments: P1, P2, P3, P4 or P5; or a white, incandescent light (CON) to serve as the control. While under these lighting conditions, a novel object test was performed in each housing enclosure by placing two novel objects, a black wiffle ball and a white wiffle ball, into the enclosure for 30 minutes (min). Continuous video recordings were collected and evaluated at 15 second (s) scan intervals for specific behaviors, including approaching either of the objects, moving away from the objects, standing still, and eating and drinking. The number of pecks at each colored object, number of flight events and latency to the first and second peck were also recorded. Linear mixed models were used to evaluate the effect of lighting treatment and time interval on pullet behavior. All observed behaviors, with the exception of approaching the black wiffle ball, were impacted by the duration of exposure to the novel wiffle balls ($P < 0.01$). The proportion of birds eating and drinking was impacted by the lighting treatment and the duration of exposure to the balls ($P < 0.01$) with greater proportions observed in the CON lighting. Birds housed in PAWS lighting pecked the novel wiffle balls more than birds housed in CON lighting ($P < 0.001$). Although this is a preliminary study, results suggest that PAWS lighting environments may have a positive impact on pullet behavior as shown by increased novel object interaction and exploration. Further research should be performed using this PAWS technology to determine the impact on other behavioral and well-being related responses, as well as, production parameters in pullets and laying hens.

Introduction

While many management factors can influence performance and well-being of poultry, lighting conditions are one of the most influential components of their indoor housing environment (Mohammed et al., 2010). Substantial research implementing different lighting types, intensities, and colors has demonstrated that the behavior and welfare of laying hens can be drastically affected by the environmental lighting conditions (Manser, 1996; Morris, 1967). Lighting, as explained by Manser (1996), is defined by its source, intensity, wavelength spectrum, and its photoperiod duration. Understanding the lighting requirements of birds and the effects of different lighting strategies is crucial in choosing the optimal lighting sources for enhanced hen welfare.

Some commonly researched types of lighting applications utilized in hen housing systems are incandescent, fluorescent, high intensity discharge lighting, UV radiation, light emitting diode (LED), general lighting with tungsten filaments and high-pressure sodium vapor discharge lamps (Lewis and Morris, 1998; Parvin et al., 2014). Studies have assessed the impact of different lighting strategies on a multitude of outcomes including sexual maturity, egg production and quality, growth, facilitation of feeding and digestion, and mortality (Patel et al., 2016; Jácome et al., 2014). These lighting environments, differing in intensity, wavelength and duration, have been shown to have both negative and positive effects on hen production and behavior as discussed in reviews by Patel et al. (2016) and Manser et al. (1996).

It has been suggested that the stimulation of activity in birds under fluorescent lighting compared to incandescent lighting could be due to the differences in spectral characteristics of the light sources or by the perceived flickering of the light (Boshouwers and Nicaise, 1993). LED lighting has also grown very popular due to its lower energy costs over conventional light sources. Additionally, the shorter wavelengths of white, yellow, blue, green or a combination LED have shown improved muscle structure and immune responses in broilers (Parvin et al., 2014; Li et al., 2019). Birds have an increased sensitivity to wavelength as compared to humans (Mohammed 2010). For example, long wavelength radiation from red light can pass through the hypothalamic extra-retinal photoreceptors to stimulate the reproductive axis, thus accelerating sexual development and maturity, and may increase aggressiveness (Lewis and Morris, 2000; Baxter et al., 2014; Li et al., 2014) and stimulates more activity such as walking and pecking behaviors in birds (Sultana et al., 2013).

Many of the aforementioned studies focus on physiological, health, and performance outcomes in response to lighting strategies. Behavioral tests aimed to measure fear and anxiety can also be used to quantify the impact of environmental factors such as lighting on poultry well-being. In general, fear inhibits other motivational systems and may impair the ability of the animals to adapt to environmental changes, interact successfully with cohorts or with humans, and utilize new resources (Jones, 1996). Therefore, emotional and mental states of high fear are unwanted and the reduction of fear is of major importance (Jones, 1996). The novel object test is a commonly used assessment involving the introduction of a novel object to the animal's environment, during which behavior observation and other parameters may be recorded. Due to the fact that fear tends to promote inactivity and silence, the bird's activity and willingness to approach and/or avoid a novel object are often assessed (Jones, 1987). Various objects, such as novel food, colored fishing floats, wooden rods, and light bulbs, have been used to perform these tests (Forkman et al., 2007). Incorporating this type of assessment into trials evaluating new lighting technologies could be useful in providing insight on impacts that light strategies have on bird affective state.

A new lighting technology for application in pullet and hen housing systems was developed in 2017 by Xiant Technologies (Greeley, CO) called PAWS (Pulsed Alternating Wavelength System) as a method of boosting endogenous hormones in order to maximize efficiency and production in animals (Xiant Technologies, 2017). PAWS lighting is a patented technology that implements a modified LED apparatus that creates electro-magnetic wave emission pulse trains (photons) of individual color spectrums (Suntych, 2017). These photons are released at a sufficient intensity to initiate a photochemical response in a bird and stimulate a desired response, such as egg production, fertility and ovulation. The PAWS system consists of different photon modulation of multiple colors (i.e. different combinations of wavelengths pulsing between "on" and "off" at different intervals; personal communication, M. Reinhardt).

The hypothesis is that PAWS maximizes an organism's growth and production capacity by providing light only when and how the organism is capable of utilizing it. Unpublished data from a poultry trial with this lighting resulted in changes in production performance of eggs/bird/day compared to the Leghorn breed standard, and exponentially more than commercial producers (Xiant Technologies, 2017). However, there is no available literature exploring the impacts of PAWS lighting on poultry behavior and affective state.

The main objective of this preliminary pilot study was to determine the impact of PAWS lighting environments on the behavioral reaction of young pullets to a novel object. In addition, due to the potential differences in visual perception of PAWS, the researchers wanted to evaluate the impact of the color of the novel object on the behavioral reaction of the pullets in a PAWS environment. The hypothesis was that young pullets exposed to PAWS lighting environment would respond to novel object tests with increased investigatory behaviors as compared to those birds housed in a conventional, control type lighting.

Materials and Methods

IACUC Protocol

Prior to the initiation of this study, animal use and associated procedures were reviewed and deemed exempt by the Colorado State University (CSU) Institutional Animal Care and Use Committee (IACUC Exemption #2018-067-ANSCI) due to the non-invasive and observational data collection techniques.

Birds, Housing and Management

The study was conducted using 299 Bovan pullets (approx. 10 weeks of age) over a period of two days in July of 2018. The pullets were housed in hand-made wooden square enclosures (1.02 m by 1.02 m by 1.22 m; L x W x H) set on wooden pallets to elevate them above the cement floor of the indoor warehouse environment. Each side of the plywood tent enclosure was wrapped with thick, opaque, black plastic (6 mil plastic sheeting, HDX, Atlanta, GA) and fitted with an air exchange system for ventilation (Figure 1). Each tent housed 16-18 birds and consisted of shavings (softwood shavings, Bitterroot Valley Forest Products, Missoula, MT) as bedding, one 4.5 kg plastic, poultry feeder (free range feeder, Harris Farms LLC, Nolensville, TN), one 0.95 L poultry waterer (free range waterer, Harris Farms LLC) and one cement cinder block to be used as a small perch (Figure 2). A door on the side of the tent was used to facilitate moving birds in and out when needed for cleaning, observation and general care. The door was a cut-out portion of the wooden wall itself fitted with additional hardware to allow a mode of entry into the enclosure. The internal tent environment was closely monitored twice daily and maintained at approximately 23.8 ± 0.8 °C and 53.5 ± 7.1 % relative humidity via individual, digital thermometers (HC520 Digital Thermo Hygrometer, Samshow, Shenzhen Guangdong) that were also present in each tent.

Animals were fed a diet (Homestead® Poultry Developer, Hubbard Feeds, Mankato, MN) that met all of their nutrient requirements once daily in the morning. Feeders were cleaned weekly. Waterers were filled twice daily and cleaned daily. All birds remained inside their enclosures throughout the experiment, with the exception of weekly cleaning procedures. During cleaning, all birds were gently transferred to a dark, opaque plastic tote for minimal time until cleaning was concluded. A routine cleaning consisted of the removal of soiled bedding, vacuuming to reduce the dust collection, sanitization with cleaning wipes (Lysol disinfecting wipes, Parsippany, NJ), the addition of new shavings, and cleaning of the feeder and waterer.

Lighting Treatment

The 299 pullets were randomly assigned to 17 different individual enclosures. Each enclosure was randomly assigned to one of six specific lighting treatments that included the following: PAWS lighting recipe 1 (P1), PAWS lighting recipe 2 (P2), PAWS lighting recipe 3 (P3), PAWS lighting recipe 4 (P4), PAWS lighting recipe 5 (P5) and white, incandescent light (CON) to serve as a control. All lighting 'recipes' were designed to enhance the growing performance of young pullets before transitioning into a commercial egg laying environment. The PAWS system uses very rapid (microsecond) pulses of alternating wavelengths of light in specially designed patterns to target specific photoreceptors. By managing the naturally existing ability of photo-chemicals to perform latching and resetting functions, the electron supply chains for biological activity are controlled. The exact 'recipes' represent proprietary information of the company. Each lighting treatment occurred in three replicates, with the exception of the control lighting treatment only including two replicates (Table 1).

Novel Object Test

A novel object test was performed in each tent in multiple replicates across lighting treatments. Two plastic wiffle balls (Truscope Sports, Truscope Holdings, LLC, Kearny, NJ) were used as the novel objects, one white colored and the other black colored. The colors of the balls were chosen because the impact of the PAWS lighting on the pullets' perceptions of color is still unknown; black and white were selected because they do not have specific wavelengths related to the lights and are opposite in light reflection and absorption properties. The wiffle balls were fastened around the enclosure wire and/or supporting wood structures with transparent fishing wire (Berkley 7Strand uncoated, Columbia, SC) at a length that was relatively below eye level for the birds (approx. 0.18 m above the floor of the enclosure). One white and one black ball was placed in each tent for the test and hung from either the left or right side of the inside of the tent. The placement of the colored wiffle balls was alternated between the left and right side for each replicate of lighting treatment. For those treatments with three replicates, the wiffle ball placement for the third replicate was based on randomly assigning each ball color to a specific side.

A video camera (Hero Black 4 and 5, GoPro, San Mateo, CA) was mounted on the ceiling of each tent that continuously recorded the novel object test. The wiffle balls were held out of sight of the birds until both novel objects were secured. Once set up was complete, the wiffle balls were gently allowed to hang on their appropriate sides of the tent. Before initiating the start of the test, both wiffle balls were ensured to be motionless so that any unintended movement of the novel object would not affect the birds' attention or behavioral responses. Each tent was recorded for 30 minutes after the initial exposure to the wiffle balls. The observation period began once the set up was complete and the wooden door was securely closed. Due to the limited number of video cameras, 9 tents were recorded on the first day and the remaining 8 tents were recorded on the second day of experimentation.

Behavioral Observation

After performing the novel object tests, the video recordings were observed and evaluated by two trained researchers. One researcher performed scan sampling with 15 s intervals for the following behaviors: approach black, approach white, eat/drink, flight, move away, peck, stand still, other (Table 2). The second researcher performed continuous behavior sampling to record all instances of pecking at the novel object and flight events throughout the entire 30 min observation period. The latency to interact with the ball, defined as the time elapsed from the initial exposure to the balls (i.e. the start of the test) to the first and second peck of both colored novel objects, was also calculated. It was also noted if the first two initial contact events with each of the wiffle balls was performed by the same bird or two different birds. One of the tent recordings for the P2 lighting treatment did not record for a full 30 min; therefore, this data was not included for the scan sampling or continuous sampling behavioral observations. This tent was included in the latency to peck each of the colored wiffle balls.

Statistical Analysis

Data were analyzed using the software R version 3.4.1 (R Core Team, Vienna, Austria) in RStudio using the lme4 (Bates et al., 2015), emmeans (Lenth, 2019), and dunn.test (Dinno, 2017) packages. The 30 minute observation period was divided into six 5 minute intervals for analysis. The data were converted from the number of birds performing each behavior at 15 s intervals to the proportion of birds performing each behavior at each scan sample. These proportions were averaged resulting in the mean percentage of birds performing each behavior during each 5 min interval. Each 5 min interval contained approximately twenty 15 s scan samples (interval 1: scans within 0-300 s, interval 2: scans within 315- 600 s, interval 3: scans within 615-900 s, interval 4: scans within 915-1200 s, interval 5: scans within 1215-1500, interval 6: scans within 1515-1800 s). Summary statistics and data graphing were performed to check normality. The percentage of birds recorded for the move away behavior and approach white behavior was transformed (cube root and square root, respectively) to achieve normality and satisfy all model assumptions. Linear mixed models were created for each response variable (approach black, approach white, eat/drink, move away, peck, stand still) with the lighting treatments and time interval as fixed effects, tent as random effect and an interaction between treatment and interval to account for repeated measures. Tent was considered to be the experimental unit. A Mann-Whitney test was used for the response variables containing counts of behavior or time (total number of pecks for each colored object and time to initial peck) to evaluate any differences in lighting treatment. Flight events were considered extremely rare and therefore not analyzed. Significant differences were recognized at $\alpha \leq 0.05$.

Results

Effects of Treatment and Interval on Behavior

Table 3 shows the mean percentage of birds exhibiting recorded behaviors by treatment via linear mixed model approach. In general, all birds spent more time moving away from the novel objects (white or black wiffle balls) within the first 5-minute interval of observations ($P < 0.02$). More specifically, a greater percentage of control (CON) birds were observed moving away from the wiffle balls during the first 5-minute interval compared to the rest of the observation period ($P = 0.04$), and a greater percentage of birds in the P5 lighting treatment moved away from the wiffle balls in the first 5-minute interval compared to intervals 3 and 5 later on ($P < 0.01$; Figure 5c). Generally, this model showed that the proportion of birds that moved away from the wiffle balls was significantly impacted by the duration of exposure to the novel objects ($P < 0.001$).

The proportion of birds approaching the white wiffle balls was also impacted by the duration of exposure to the object ($P < 0.01$), and it is important to note that the lighting treatments yielded a tendency for this behavior ($P < 0.07$). However, the proportion of birds approaching the black wiffle balls was not impacted by lighting treatment nor the duration of exposure to the object ($P = 0.14$ and 0.47, respectively). Within the first 5-minute interval of observations, a greater percentage of CON birds approached the white wiffle ball than PAWS treatments P3 and P5 ($P = 0.01$, $P < 0.01$, respectively; Figure 5b). Furthermore, a greater percentage of P5 birds approached the white wiffle ball in the first half of the observation period compared to the last 5-minute interval ($P < 0.05$).

The proportion of birds eating and drinking was impacted by both the lighting treatment and the duration of exposure to the balls ($P < 0.01$, $P < 0.001$, respectively). The proportion of birds standing still was also impacted by the duration of exposure to the balls ($P < 0.001$). Birds in the CON lighting group spent more time consuming feed and drinking water compared to all ($P \leq 0.04$). Fewer birds were observed eating and drinking in the first 5-minute interval compared to the last half of the 30-minute observation period across all treatment groups ($P \leq 0.04$; Figure 5d). More specifically, a greater percentage of CON birds were observed eating and drinking in the third 5-minute interval compared to the PAWS treatment groups ($P < 0.001$). The birds exhibited less time standing still in the first 5-minute interval compared to the rest of the observation period, except interval 3, when averaged across the treatments ($P \leq 0.04$; Figure 5e). Fewer birds in the P5 treatment group were observed standing still in the first 5-minute interval compared to intervals 3, 4 and 6 ($P \leq 0.03$).

Impact of Novel Object Color on Behavior

Across all treatment groups, the birds interacted with black wiffle balls more than the white wiffle balls ($P = 0.002$). Birds in the P3 and P4 treatments pecked the black wiffle ball significantly more than the white wiffle ball ($P = 0.002$ and $= 0.008$ for P3 and P4, respectively; Figure 3B). In general, the behavior of pecking the novel object occurred at a higher incidence by the birds housed in PAWS treatment lightings versus the CON lighting ($P < 0.001$). There was no effect of lighting treatment on the latency to peck either of the colored wiffle balls ($P = 0.8$; Figure 4).

Discussion

Novel object tests aim to gauge the response of an animal to a new item placed in or near its environment and have been used to assess neophobia and fear in a variety of animals (cattle, Van Reenen et al., 2004; horses, Visser et al., 2002; pigs, van Kooij et al., 2002; parrots, Meehan and Mench, 2002; songbirds, Baugh et al., 2017; chickens, Uitdehaag et al., 2008; and rainbow trout, Sneddon et al., 2003). Physiological measures, such as heart rate, neurological functioning, nociception, and behavioral measures, such as avoidance and exploration, are often observed and recorded as outcomes in these tests (Visser et al., 2002; Sneddon et al., 2003; Dalmau et al., 2009). The general interpretation of observed outcomes in novel object tests is that a fearful animal would be less active with less engagement with the object or avoiding it all together; a non-fearful animal would be more likely to explore the environment, be active and interact with the object (Hogan, 1965; Faure, 1975; Jones, 1987b). Commonly, fear is observed as a reaction to a novel and/or potentially dangerous environment (Boissy et al., 1998) and states of high fear are considered a negative state for a domesticated animal that should be reduced (Jones, 1996). Therefore, tests, such as a novel object test assist with the evaluation of the amount of fear a bird may experience when introduced to a novel environment. Furthermore, understanding behavioral changes can provide us with an understanding of an animal’s affective state and help determine the bird’s optimal conditions towards promoting a positive welfare state. In fact, fearful laying hens (i.e. displayed no walking or vocalizing during an open field test) may be more impacted by changes in environmental cues that could impact learning and judgement (Haas et al., 2017).

Factors, such as housing environment, have been shown to impact birds’ responses to novel object tests. For example, increased environmental complexity as indicated by group housing environments with novel objects in the enclosure, such as a golf ball, string, table tennis balls, and colored leg bands, has resulted in chicks with reduced fear (i.e. increased activity, increased pecking, lower duration of freezing behavior) when placed in a novel environment during an open-field or novel environment test (Jones, 1982). Additionally, an increased approach response toward a novel object paired with more time spent near the novel object was observed in birds housed in an enriched environment (Jones and Waddington, 1992). In contrast, another study providing environmental enrichments to chickens reported that chickens did not exhibit any behaviors related to a reduction in fear as a result of receiving enrichment (Pichova et al., 2016). In the current study, the lighting treatment had limited impact on the birds' behaviors in response to the novel object. There was no difference in avoidance behavior (e.g. moving away) between treatments across the entire observation period and actually the percentage of birds avoiding the objects was relatively low. Although there was no difference between treatments in the percentage of birds approaching the black wiffle ball, there was a tendency for birds in PAWS treatments to approach the white wiffle ball. This tendency and the high variation in treatments means highlight the need to further explore the mechanism behind the behavioral impact PAWS lighting on bird behavior. While future work is needed, these preliminary results suggest PAWS lighting does not negatively impact bird affective state and may actually create opportunities for increased expression of goal-directed and highly motivated behaviors.

The relationship between behavior and the environment is complex as there are many impacting factors that need to be considered when interpreting study outcomes. In the current study, there was a clear effect of time (i.e. interval) on many of the recorded behaviors. After the initial five minutes, fewer birds approached the black novel object as compared with the first five minutes of interaction. Additionally, the percentage of birds eating and drinking generally increased after the first five minutes, perhaps indicating the redirection of birds' attention from the new objects in their environment to other behaviors. Providing animals with substrates or other items in their environment that promote the expression of positive behavior, such as exploratory behaviors, can improve welfare but the objects must remain novel. The results of this study along with previously published work (Apple and Craig, 1992; Gifford et al., 2007; Trickett et al., 2009) indicate the importance of object exposure time on maintaining object novelty and thus the amount animals engage with an enrichment object. Over time animals may habituate to an enrichment item in their environment and therefore it is necessary to limit exposure time and potentially increase time between re-exposure of the particular object to maintain the effectiveness of the enrichment item (Gifford et al., 2007).

Birds are highly visual animals with complex visual systems. The different parts of the photo-stimulation of the bird's phytochromes located in the hypothalamus and the retina (i.e. red opsins and green opsins) photoreceptors are maximized allowing for optimal influence on the desired response (such as egg laying) while also allowing for control of a bird’s response (Xiant Technologies, 2017). Opsins are a type of membrane bound phytochrome receptors found in the retina and the hypothalamus region of the brain of birds and mammals. Opsins mediate a variety of functions in birds and mammals, including ovulation, egg laying and behavior, through the conversion of photons of light into an electrochemical signal. Thus, the different types of lighting and colors that birds are housed in relay into behavioral outputs.

Although human color perception is different from chicken visual perception, it is worth noting that to humans, the PAWS lighting treatments used in this study appeared blue. In previous literature, despite the fact that poultry perception of color has been confounded with illuminance, wavelength has shown undeniable effects on poultry production and behavior, meaning changes in lighting conditions involving color(s) can positively affect important production parameters (Lewis and Morris, 2000). While red, green and white light environments have commonly been used to reduce cannibalism in laying hens (Schumaier et al., 1968), blue, green and white lighting environments were also found to improve growth, body development and carcass characteristics from increased feed consumption, calmer demeanors, improved well-being and enhanced immune responses (Prayitno et al. 1997; Lewis and Morris, 2000; Cao et al., 2008; Sultana et al., 2013; Riber 2015). Location preference was also altered by lighting color, where Li et al. (2018, 2019) discovered that pullets spent the majority of their time present and feeding under blue and white lights. Additionally, birds have been shown to spend more time sitting or standing under short wavelengths (blue/green) and exhibited more locomotion under longer (red/yellow) wavelengths (Sultana et al., 2013). In the present study, the birds in all PAWS treatment group environments spent less time drinking and eating compared to the control possibly due to the white lighting environments. However, this still contradicts the previous literature by Li et al., (2018) indicating blue lights, as were the PAWS lighting, promote feeding behaviors. This may indicate that the PAWS lighting environments, appearing blue to the human eye, could be perceived differently by laying hens. Standing still was not impacted by treatment, but was, however, impacted by duration of exposure to the novel object balls. This further indicates the birds' curiosity to investigate it, or move away from it as shown in our results, until they were more used to it being there. These results contradict previous literature showing less activity under perceived blue lights considering there was no effect of lighting treatment on standing still in the present study.

As discussed earlier, the colored lighting exhibited in PAWS lighting systems has shown beneficial effects on increased interactions with novel objects in hen production as seen with the wiffle balls in PAWS lighting compared to the control lighting environments. Additionally, the interactions with the black wiffle balls more than the white wiffle balls in all treatments could be due in part to the bird's perception of color as the reflective and absorptive properties of the colors of the wiffle balls compounded with the lighting recipes varied. Ham and Osorio (2007) discussed that behavioral preferences can be related to multiple models of perceiving and processing color signals, such as chromatic contrast against the background, distinct ranges of colors leading to color categorization or an innate or learned internal standard. Innate or learned behaviors bias the bird's decisions and therefore account for a large portion of their color preferences. One example of innate behaviors is that related to feeding; animals such as chickens are attracted to food objects, or objects that resemble food, through several means of stimulation, however, minimum stimulation is needed to excite a reaction (Hess, 1956). One of these sources of stimulation is color and color of food items, and color preferences of chickens have been well studied and explored (Hess, 1956; Fischer et al., 1975; Ham and Osorio, 2007). Well-defined chromatic stimuli can help begin investigating what aspects of color stimuli control simple visual tasks such as pecking at possible food items (Ham and Osorio, 2007). On the other hand, chickens are notorious for clustering in groups around a particular stimulus that one bird may have been attracted to and therefore, through social facilitation, attracted other birds to the stimulus as well, and thus adding bias to the results (Hess, 1956). The PAWS lighting environments may have allowed the birds to perceive the black wiffle ball as a food source, or from social facilitation of exploring an object more novel than a white wiffle ball.

Other factors such as light intensity and flicker rate (pulsing) may also impact bird response to different lighting regimens. Illuminance levels, spectrum of light sources, photoperiod and flicker (or pulse) can influence birds directly through stimulation of photosensitive physiological processes, or indirectly through behavioral modification (Mihailov et al., 2014). Fluorescent lighting is generally regulated at low frequencies, for example 120Hz in the United States or 100Hz in Europe. The fluorescent illumination is a discontinuous type of lighting, but perceived as continuous to humans because the highest flicker rate detected by us is only between 50 to 60 Hz, whereas poultry and other fowl have a maximum value around 105Hz allowing them to see the flickering of the light (Lewis and Morris, 1998). The PAWS environments in the present study involved pulsing (or flickering) lights. A bird's CFF (critical flicker fusion frequency), which is the highest flicker fusion frequency at any given light intensity, is higher compared to humans (Lisney et al., 2011). This high temporal resolution (ability to recognize pictures per second) that birds have allows them to be more susceptible to lighting conditions leading to general stress, impaired welfare and difficulties in object recognition (Prescott et al., 2003; Greenwood et al., 2004; Mihailov et al., 2014). These lighting attributes will be valuable to consider in future PAWS studies.

Conclusion

This was a preliminary study designed to determine the impact of PAWS lighting on pullet behavior in response to a novel object. Different lighting strategies have been used in poultry production settings to improve performance parameters of hens. As new technologies are developed, such as PAWS, it is essential to assess both performance indicators and also behavioral outcomes that could help provide insight into the overall welfare state of the animals. This study provides initial evidence that PAWS lighting may make a positive impact on bird behavior as demonstrated by increased approach and engagement with a novel object during the test scenario. Further research should focus on how PAWS lighting impacts the activity budget of birds and their response to other fear tests, such as a tonic immobility test, as compared with other more traditional lighting systems to help inform overall impacts of this new lighting technology on bird physiology, performance, and behavior. Although novel object tests have been used in research, there is little consistency or guidance in what novel objects should be selected for use. Future work could explore characteristics of novel objects that may influence a bird's reaction such as color, shape, and other visual cues. Factors such as color, flicker rate and intensity are key components that can impact a bird's reaction to certain lighting strategies and further exploration of how PAWS technology varies in these factors would be valuable for consideration in future work.

Conflict of Interest

To be filled in.

Author Contribution

FB and LE-C have substantial contribution to the work through concept, design, data collection, and interpretation. PP assisted with project concept and design. JH and MG performed statistical analyses. FB, MD and LE-C composed the manuscript content. All authors revised and edited the draft and approved of publication.

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Figure Legend

A **B**

Figure 1 Figure 1. Individual "tent" housing structures constructed of wooden boxes, placed on pallets that were covered with black, opaque plastic to create an individually controlled lighting environment. (A) Three separate tent housing enclosures that included one treatment per tent (approximately 16-18 young pullets/tent). (B) A tent enclosure with the outside plastic opened to show the wooden door that was used for accessing the bird housing area.

Figure 2 A control tent housing set up, which included the white, control light hanging from the top of the enclosure, a feeder, a waterer, a cement cinder block perch, and two novel objects (black and white wiffle balls).

Top: A, Bottom: B

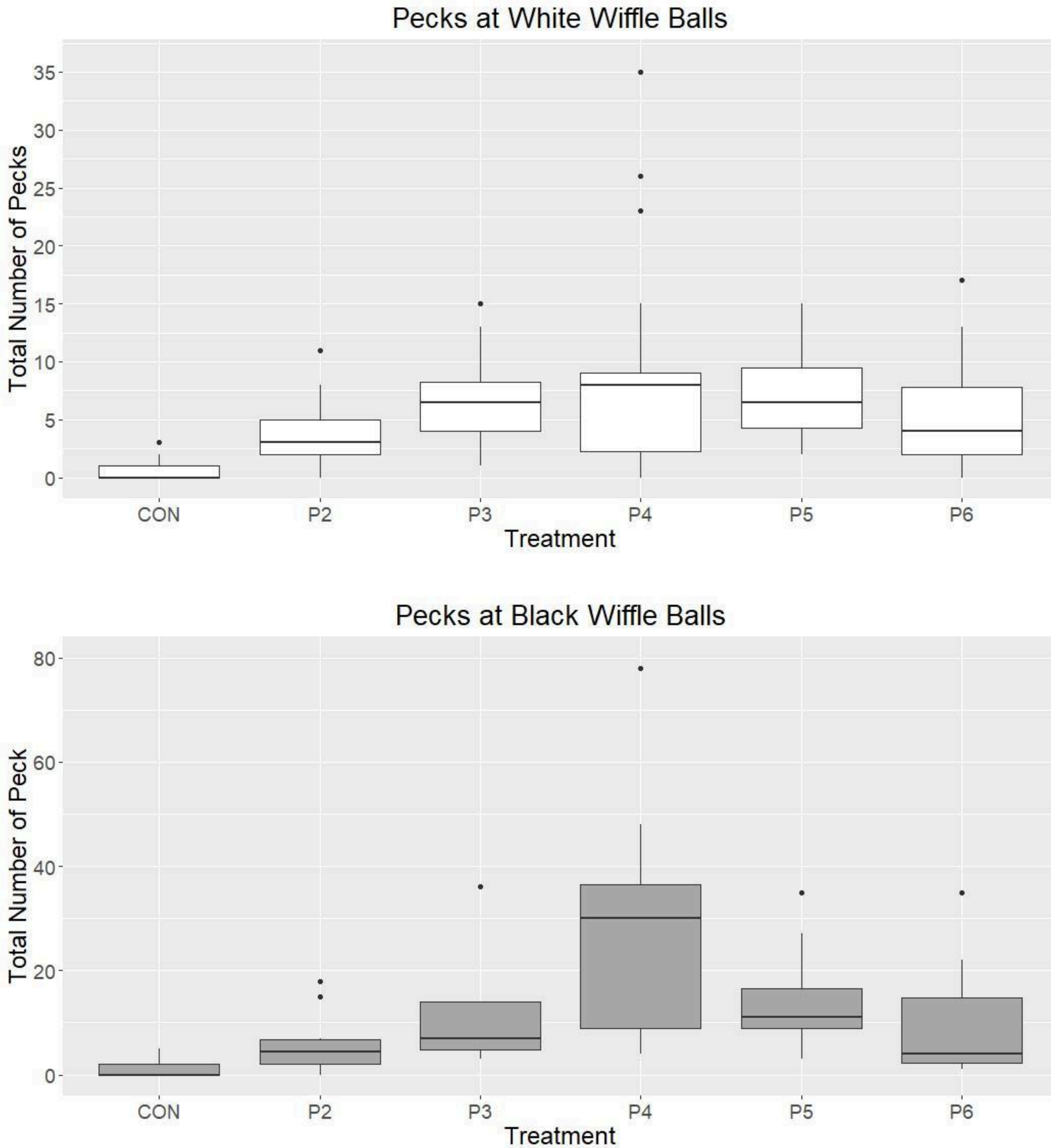


Figure 3 Summary boxplots of the total number of pecks for each novel object color – A) white and B) black – observed during the observation period for each treatment summarized at the tent enclosure level. Housing light treatments were defined as follows: P1, P2, P3, P4, P5 = specific recipe of pulsed alternating wavelength system (PAWS) lighting; CON = conventional, white lighting.

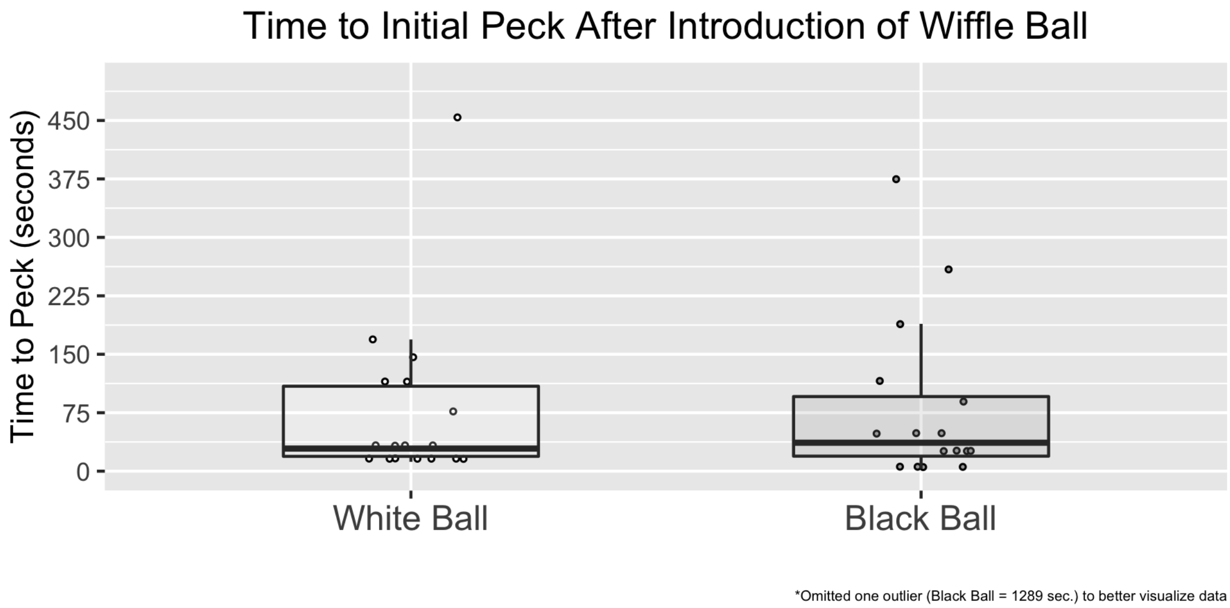
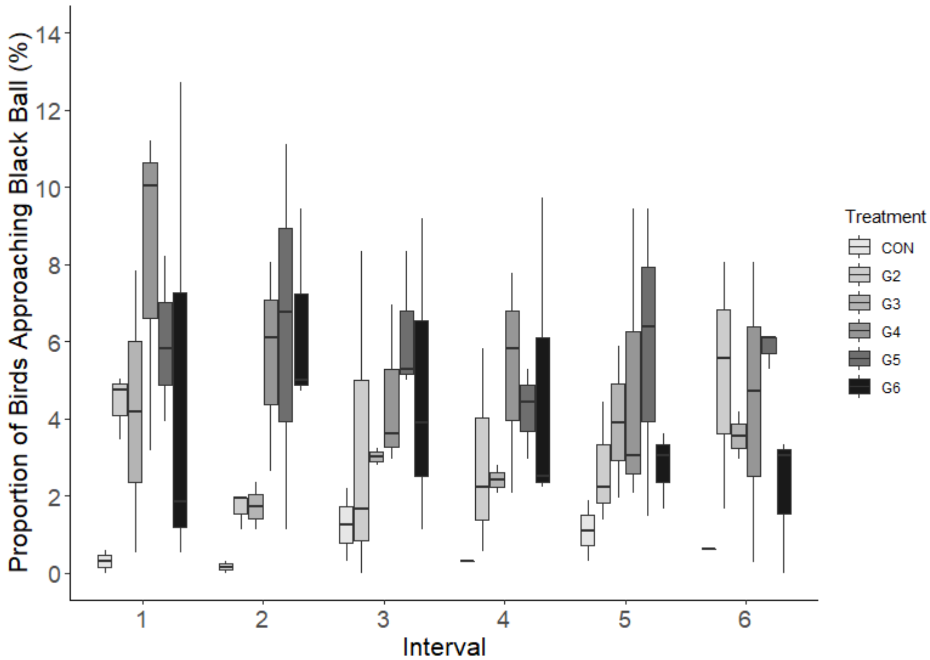


Figure 4 Boxplot of the latency of the first peck for each novel object color - white and black - observed during the observation period across all treatments.

Figure 5. A. and B.



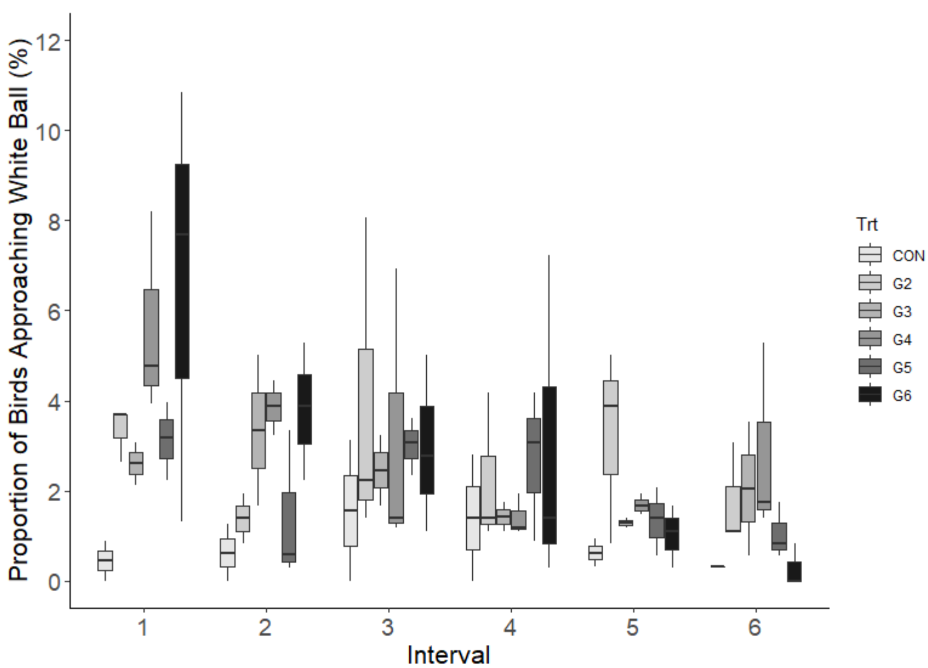
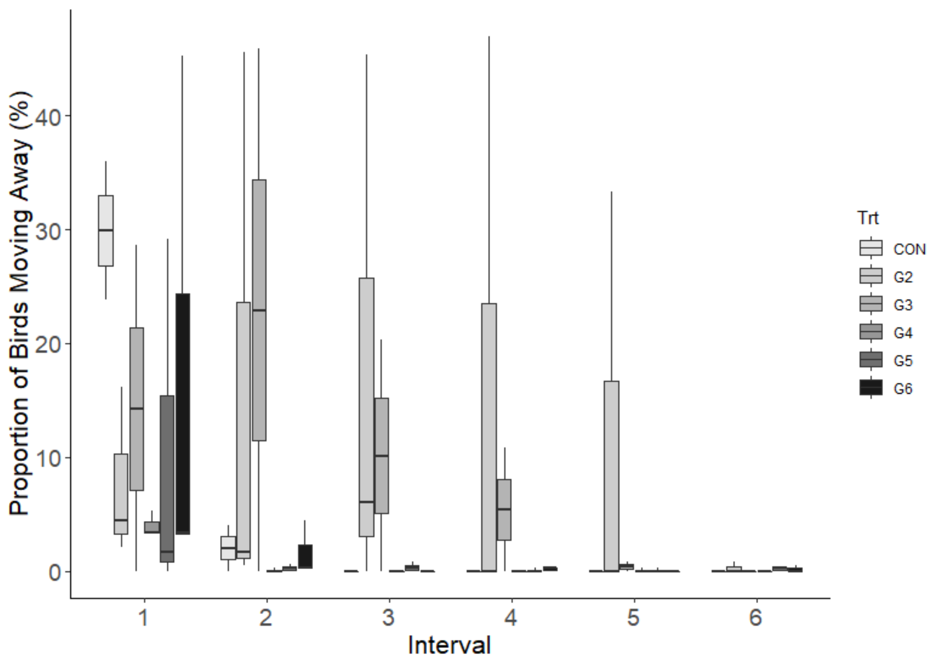


Figure 5. C. and D.



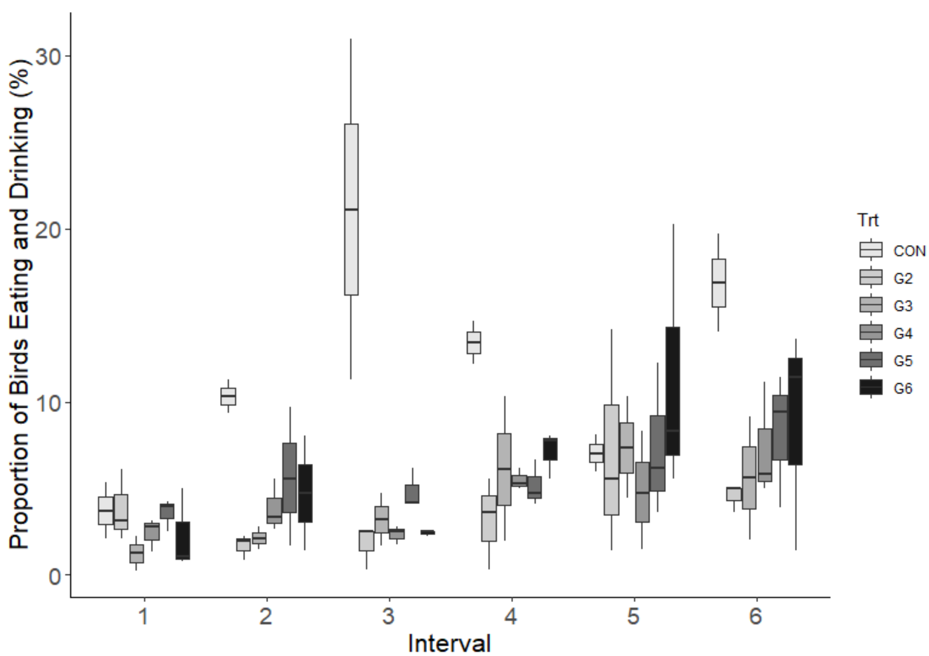


Figure 5. E.

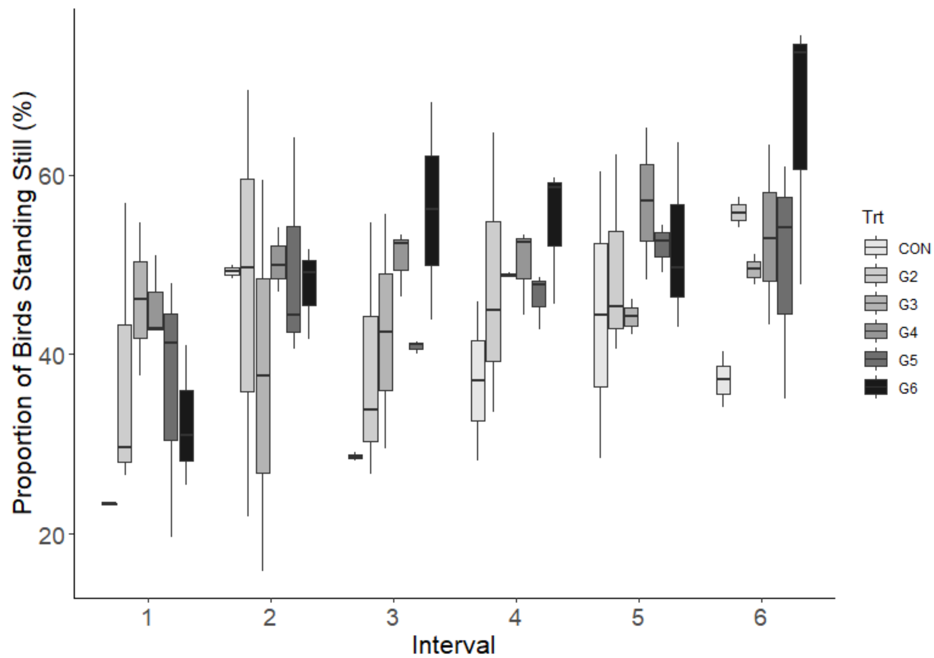


Figure 5. Summary boxplots of the proportion of birds displaying a specific behavior within each interval for each lighting treatment. A) Approach Black, B) Approach White, C) Move Away, D) Eat and Drink and E) Stand Still. The entire 30 min observation period was divided into six 5 min intervals to chronologically assess the impact of the duration of novelty exposure.Housing light treatments were defined as follows: P1, P2, P3, P4, P5 = specific recipe of pulsed alternating wavelength system (PAWS) lighting; CON = conventional, white lighting.

Table 1. Treatment assignments and replication as shown by the numbers of birds per treatment.

	Number of Birds/Treatment			
Lighting Treatment	Replicate 1	Replicate 2	Replicate 3	Total Birds/Treatment
PAWS[1]	18	18	17	53
P1	18	18	18	54
P2	18	18	17	53
P3	18	18	18	54
P4	18	18	17	53
P5	18	18	18	54
CON[2]	16	16	-	32

[1] Pulsed alternating wavelength system (PAWS)

[2] Conventional, white lighting serving as the control (CON)

Table 2. Ethogram of the recorded pullet behaviors when exposed to a novel object for 30 minutes.

Behavior	Description
Approach Black	Forward movement – walking or stepping toward the black wiffle ball or standing nearby and moving head toward black ball. This did not include when the bird was standing still near or touching the black ball. The movement had to be forward progression toward the black ball via whole body or head movement.
Approach White	Same definition as Approach Black but for the white wiffle ball.
Eat/Drink	Active consumption of feed at the feeder or consumption of water at the waterer. This did not include idle or still behavior near the feeder/waterer.
Move Away	Walking away from one of the novel objects (black or white).
Stand Still	No movement; standing motionless and/or resting.
Other	Any behavior that did not fit any other defined behavior.
Flight Event	The display of flapping wings and elevation of the entire body, including the feet, above the ground for a brief period of time.
Peck	The contact of the bird’s beak with one of the novel objects (black or white). This involved the wiffle ball specifically, not the string used to attach the novel object to the side of the enclosure.

Table 3. Effect of housing light treatment on the behaviors of young pullets when exposed to two novel objects.

Behavior	Treatment[1] (%)						SE[2]	*P-values*		
	P1	P2	P3	P4	P5	CON		Trt	Interval	Trt*Interval
Approach Black	3.34	3.14	5.45	5.72	4.31	0.623	1.21	0.14	0.74	0.95
Approach White[3]	2.37	2.03	2.79	1.88	2.12	0.46	0.18	0.07	< 0.01	0.38
Eat/ Drink	3.66[b]	4.28[b]	4.38[b]	5.80[b]	6.12[b]	12.08[a]	1.12	< 0.01	< 0.001	0.07
Move Away[4]	2.43	1.13	0.04	0.17	0.27	0.27	0.08	0.59	< 0.001	0.51
Stand Still	46.01	44.80	51.12	45.88	51.40	36.63	6.92	0.42	< 0.001	0.58

[1] Means of the percentage of behavior occurrences were observed throughout the 30 min period (averaged across all 5 min intervals) after the initial exposure to two novel objects (one black and one white wiffle ball). Housing light treatments were defined as follows: P1, P2, P3, P4, P5 = specific recipe of pulsed alternating wavelength system (PAWS) lighting; CON = conventional, white lighting.
[2] Pooled standard error across all treatments.
[3] Means and pooled standard errors are reported on the original scale, while the *P*-value is reported based on the square root transformed values used to achieve a normal distribution of data.
[4] Means and pooled standard error are reported on the original scale, while the *P*-value is reported based on the cube root transformed values used to achieve a normal distribution of data.
[a,b] Means with different superscripts within the same row are significantly ($P < 0.05$) different.