

**COMPARISON OF RED AND WHITE LED LIGHT BULBS AND THEIR
EFFECTS ON LAYING HEN PERFORMANCE, STRESS, AND
BEHAVIOR**

An Undergraduate Research Scholars Thesis

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EMILY C. BERGER

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Dr. Gregory S. Archer

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ABSTRACT

Comparison Of Red And White LED Light Bulbs And Their Effects On Laying Hen Performance, Stress, And Behavior

Emily C. Berger
Department of Poultry Science
Texas A&M University

Research Advisor: Dr. Gregory S. Archer
Department of Poultry Science

Lighting is a vital aspect of every commercial poultry operation. Since chickens are a photorefractory species, an inadequate lighting program could be detrimental to their growth and productivity. Few studies have looked at the effects of lighting on laying hens, but it has been shown that red light can potentially stimulate egg production.

The company Once Innovations has produced a red LED light bulb marketed to poultry producers. However, this new light bulb costs \$23 to Overdrive's \$10 white LED. The effects of the two different light bulbs on bird performance, stress, and behavior were compared to determine which type is more beneficial to the birds and more cost-effective for poultry producers.

Three replicates were run for a total of six treatments, with 36 birds per treatment. Initially there were no differences between the two treatments. At the end of the study, after exposing the birds to the two separate treatments, no difference ($P < 0.05$) was found in Haugh units (Red, 105.81 ± 0.54 ; White, 105.75 ± 0.63), but eggs laid under white light had a higher breaking strength (Red, 3195.56 ± 29.28 g; White, 3251.07 ± 24.32 g). The feed conversion ratio was better for hens

housed under red light (Red, 2.04; White, 2.06). Birds housed under white light were more stressed, with higher blood corticosterone levels (Red, 550 ± 84 ng/dl; White, 1884 ± 195 ng/dl), a higher blood heterophil/lymphocyte ratio (Red, 0.57 ± 0.08 ; White, 1.14 ± 0.11), and a greater increase in physical asymmetry (Red Start, 1.52 ± 0.09 & Red End, 1.15 ± 0.06 ; White Start, 1.47 ± 0.11 & White End, 1.59 ± 0.10). No differences in Tonic Immobility were observed ($P > 0.05$). All data was analyzed using PROC GLM and significant differences were considered at $P < 0.05$.

Results indicated that birds housed under red light were less stressed and more productive; therefore, red lighting may be a beneficial means to increase production while also decreasing stress in the birds. However, more research is needed to determine the effects of red lighting through the second half of the lay cycle.

CHAPTER I

INTRODUCTION

Background on lighting choices

The majority of commercial layer operations require artificial lighting to photo-stimulate the birds and regulate egg production. When implementing lighting programs for their flocks, poultry producers must make decisions based on a number of different factors, namely the type of light bulb, the color of light that will be used, the intensity of the lighting, and the lighting schedule of light and dark periods. This study chose a specific light bulb type, then focused on the effects of light color, seeking to determine the benefits of an LED light bulb that produced light in the red spectrum.

Choosing a lightbulb type

The decreasing price of light-emitting diode (LED) lights has brought about a much more cost-effective lighting option for poultry producers. LED light bulbs are 80-85% more energy efficient than incandescent light bulbs (Watkins, 2014) and also have greater longevity, lasting for approximately 50,000 hours (Burrow, 2008). Besides preventing excess energy usage, LEDs can also prevent excess heat generation and rarely exhibit early failures (Benson, et al., 2013).

In addition, LED light bulbs can be dimmed to the appropriate lighting levels for poultry and allow for a variety of color options. Poultry producers that already use red or blue lights to increase production or calm birds can switch to LEDs to reduce energy costs (Burrow, 2008).

Alternatively, the spectrum available using LED lights can be set to more closely match natural

daylight, as opposed to the artificial appearance of compact fluorescent lamp (CFL) lights (Morrison, 2013).

A study conducted by Huth and Archer (2015) at Texas A&M University compared the effects of LEDs and CFLs on broiler performance, behavior, stress, and overall well-being. The study showed that LEDs improved both broiler growth and the overall well-being of the birds. A subsequent study by Archer (2015), which compared the effects of CFLs, LEDs, and incandescent light bulbs on broiler production, behavior, and stress, indicated that LED light caused the greatest increases in growth and feed conversion, and also allowed birds to be the least fearful and stress susceptible. The same benefits seen in these studies would likely hold true in laying hens as well, though few studies have been conducted to determine if that is the case.

Overall, it has been proven that LEDs are currently the best lighting type to use in poultry housing from both a production and a welfare standpoint, but to maximize lighting benefits, light bulb color must also be considered.

Choosing a light color

In addition to lightbulb type, light color can also have an effect on the productivity of laying hens. Chickens have highly specialized visual systems, and much of their behavior, including feeding and social interaction, is mediated by vision (Mendes et al., 2013). Poultry lighting systems that are currently in place are influenced by human vision, failing to account for the requirements of poultry vision. This shortcoming can negatively impact various visually-

mediated behaviors of poultry, ultimately causing distress and poor welfare (Prescott et al., 2003).

Poultry have 4 types of single-cone receptors in their eyes, which are sensitive to ultraviolet, short-wavelength, medium-wavelength, and long-wavelength light (Osorio et al., 1999). These color receptors are different than those found in humans; therefore, further analysis must be completed to determine the way that chickens perceive color intensity (Bennett et al., 1994). It has been shown that chickens have a higher sensitivity to light at 620–750 nm than humans, meaning that they can see red light much more clearly (Prescott and Wathes, 1999). Red light is also more sexually stimulatory to the birds than other colors, which means that red lighting can cause birds to come into lay significantly earlier than white lighting (Gongruttananun, 2011) and that red lighting could potentially help improve egg production (Lewis and Morris, 2000).

In a study conducted by Borille et al. (2015) observing the effects of monochromatic light on laying hens, the best production results were obtained using red (600–630 nm) and white (2,800–3,200 K) LED lights as opposed to green (510–530 nm), yellow (580–590 nm), or blue (450–460 nm) lights. Therefore, white and red lights are the best colors to continue comparing for poultry production.

Purpose of lightbulb comparison

In order to meet the growing demand for commercial poultry lighting, some lighting companies have designed specialized products specifically to meet the needs of poultry producers. One such

company is Once Innovations, which produces a variety of different dimmers and lightbulbs for layer, broiler, and turkey operations within the poultry industry.

Marketing a new product

Based on research revealing the potential benefits of using red lighting for poultry, Once Innovations has designed a red lightbulb to be marketed to commercial layer operations. The company's goal is to produce a lightbulb that will improve the productivity of laying hens by decreasing the amount of time that it takes for the birds to reach peak production. In addition, the lightbulbs are designed to help decrease the birds' stress levels and thereby improve the birds' welfare.

As this new product is placed on the market, it is important to field-test it to determine its effectiveness. Commercial layer companies need lightbulbs that are economical, and will only purchase this new product if its benefits are substantial enough to justify spending more at first.

Testing the new lightbulb

In order to determine whether the lightbulb's design is effective, it was tested against a traditional white LED lightbulb produced by Overdrive. Bird performance was evaluated through measurements of egg production, egg quality, and feed efficiency; general bird behavior was assessed through fear tests; and bird stress levels were determined through measurements of physical asymmetry as well as stress hormone levels and immune cell populations in the blood.

All of these measurements were compared between the two lighting treatments to determine any differences in their effects. These differences were then analyzed to determine whether or not the new Once Innovations lightbulb is beneficial to bird performance and welfare, and still cost-effective for poultry producers.

CHAPTER II

METHODOLOGY

Setup of housing

The test flock consisted of 216 white Leghorn hens, which were caged in trios in three different rooms at the TAMU Poultry Science Extension Center. The rooms were kept at a consistent temperature of 70°F, and the light schedule had 16 hours of light to 8 hours of darkness with an average illumination of 4 foot candles.

Half of the birds were housed under the red Once Innovations light bulb (Once, AgriShift® MLM-Red; Red) while the other half were housed under the white Overdrive light bulb (Overdrive, L10NA19DIM 3000K; White). Each of the three rooms was split down the middle with a light-proof barrier. Both treatments took place in every room for a total of three replicates and six separate treatments overall.

Measurements of performance

Bird performance was evaluated through daily measurements of egg production and egg weight. The former was used to calculate flock production by determining the percentage of the flock that was in lay from day to day; the latter allowed for calculation of the flock's feed conversion rate. Measurements of feed consumption and feed conversion were calculated monthly based on the weight of the eggs produced and the weight of the feed given to the birds throughout each month.

On a biweekly basis, each egg's Haugh unit, which is an egg quality measurement based on the correlation between egg weight and albumen height, was measured. To calculate Haugh unit, the egg is first weighed and then cracked open so that the albumen height can be measured. A taller albumen indicates a higher quality egg, but since bigger eggs will naturally have taller albumens than smaller ones, the disparity in egg sizes must be taken into account via the Haugh unit formula, which is $HU=100*\log(h-1.7w^{0.37}+7.6)$ where HU=Haugh unit, h =observed height of the albumen in millimeters, and w =weight of egg in grams. The higher the Haugh unit, the better the quality of the egg.

Breaking strength was also measured biweekly using the QC-SPA System (Technical Services and Supplies, York, England). To use the QC-SPA machine, an egg was placed on the test bed, and then a lever automatically lowered to crush in the top of the egg shell, determining the weight in grams that the shell could withstand before cracking. Shell breaking strength is an important production factor from a transportation standpoint — stronger shells are necessary so that eggs don't break while they are shipped from the producer to the consumer.

Measurements of stress

Stress susceptibility of the birds was evaluated by measuring physical asymmetry, which is a combined assessment of the metatarsal length, metatarsal width, and middle toe length on both legs of each bird. These measurements were taken using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL), and were all taken by the same person to avoid comparison errors.

A composite score of the three asymmetry measurements indicates the long-term stress levels of the birds via comparison of the symmetry between the left and right legs, specifically by calculating the average of the signed difference of the traits measured (Campo, et al., 2008). This score was achieved by adding the absolute values of left minus right of each trait, then dividing by the total number of traits; therefore, the formula for this study would be $(|L-R|_{MTL} + |L-R|_{ML} + |L-R|_{MW}) / 3 = \text{composite asymmetry score}$. If birds are more stressed, they will become more asymmetrical through the production cycle; if they are less stressed, they will stay roughly symmetrical over time.

Blood samples were taken twice during the lay cycle of the birds — at start of lay and mid-lay— and the plasma was analyzed for stress hormone levels, specifically corticosterone, and immune cell populations, namely the heterophil/lymphocyte ratio. To minimize the number of birds that were stressed by having their blood drawn, one bird from each cage was tested rather than every single bird. This added up to 12 birds per replicate for a total of 36 birds per treatment that were sampled.

Consistent testing and handling for blood sampling was vital since corticosterone levels can easily fluctuate due to outside stressors to the birds. To make sure that the end data would be accurate, each bird was gently handled in the same manner. In addition, each sample, from the moment each bird was removed from its cage to the last of its blood being drawn, was taken within a minute. It takes approximately 1-2 minutes for corticosterone levels in the plasma to change, so conducting each sample quickly eliminated the risk of inaccuracy due to sudden

stressors. Corticosterone levels can also be affected by circadian rhythms; in order to account for this natural fluctuation, samples were taken at the same time of day throughout the flock.

Once blood samples were collected, corticosterone levels were analyzed via a commercially viable ELISA (Enzo Life Sciences, ADI-901-097, Farmingdale, NY). The inter- and intra-assay %CV were both under 5%, while the heterophil/lymphocyte ratio was determined using microscopy. For measuring the latter, 100 cells were counted per sample by making smears on slides, then staining them to increase visibility, and then observing them underneath a microscope. A simple ratio between the number of heterophils and lymphocytes present was then calculated (Campo, et al., 2000). This ratio is less variable than counting individual numbers of heterophils or lymphocytes and is a more reliable measurement than observing corticosterone levels alone (Gross & Siegel, 1983).

Measurements of behavior

The birds' behavior was appraised using tonic immobility, which is based on Ratner's (1967) model and is the most commonly used method of fear testing in the poultry industry. According to Ratner, tonic immobility is the final stage of the predator-prey interaction. Tonic immobility works by using physical restraint to cause a prolonged period of non-responsiveness (Maser et al 1973, Jones 1989). A bird will enter this state if it is unable to escape its captor, at which point it will become temporarily rigid, still, and slow to right itself (Jones 1996).

The first round of tonic immobility tests was conducted at start of lay and the second round took place at mid-lay, five months into the production cycle. For tonic immobility testing, each bird

was laid on its back in a cradle constructed of PVC pipe and covered with black fabric. For a duration of 15 seconds, one hand was placed on the bird's breast while the other covered the head. This simulated the process of being caught and restrained by a predator, inducing an immobile state in the birds. The person conducting the test then stepped back to observe the bird and record the amount of time it took for the bird to stand up; this time period is referred to as latency to right. Longer latency to right indicated a more fearful bird, whereas shorter latency to right indicated a less fearful bird. The time of each bird's first head movement before rising was also recorded as the bird's initial attempt to observe its surroundings for danger.

CHAPTER III

RESULTS

At the beginning of the study, all birds exhibited similar fear and stress responses, and the two treatments had minimal differences between numerical measurements of production. After being exposed to the different treatments, the birds' behavior was not affected, but they showed slight numerical differences in production and drastic statistical differences in stress measurements.

Effects on performance

When the flock first came into lay, some birds housed under white light started laying sooner, but birds housed under red light reached higher production levels more quickly. As is illustrated by Figure 1, red light also aided birds in holding those higher production levels throughout the first half of the lay cycle. On average, 95.6% of the birds under red lighting were laying at any given time, as opposed to 93.6% of the birds under white lighting.

A drop in production occurred at around 40 weeks of age following the collection of blood samples for stress analyses, but otherwise the birds were allowed to lay normally without the interference of outside stressors. In a normal production environment, the percentage of birds under white light that were laying was lower than that of birds under red light at any given time. The birds housed under red light also achieved 100% production more often than the birds housed under white light.

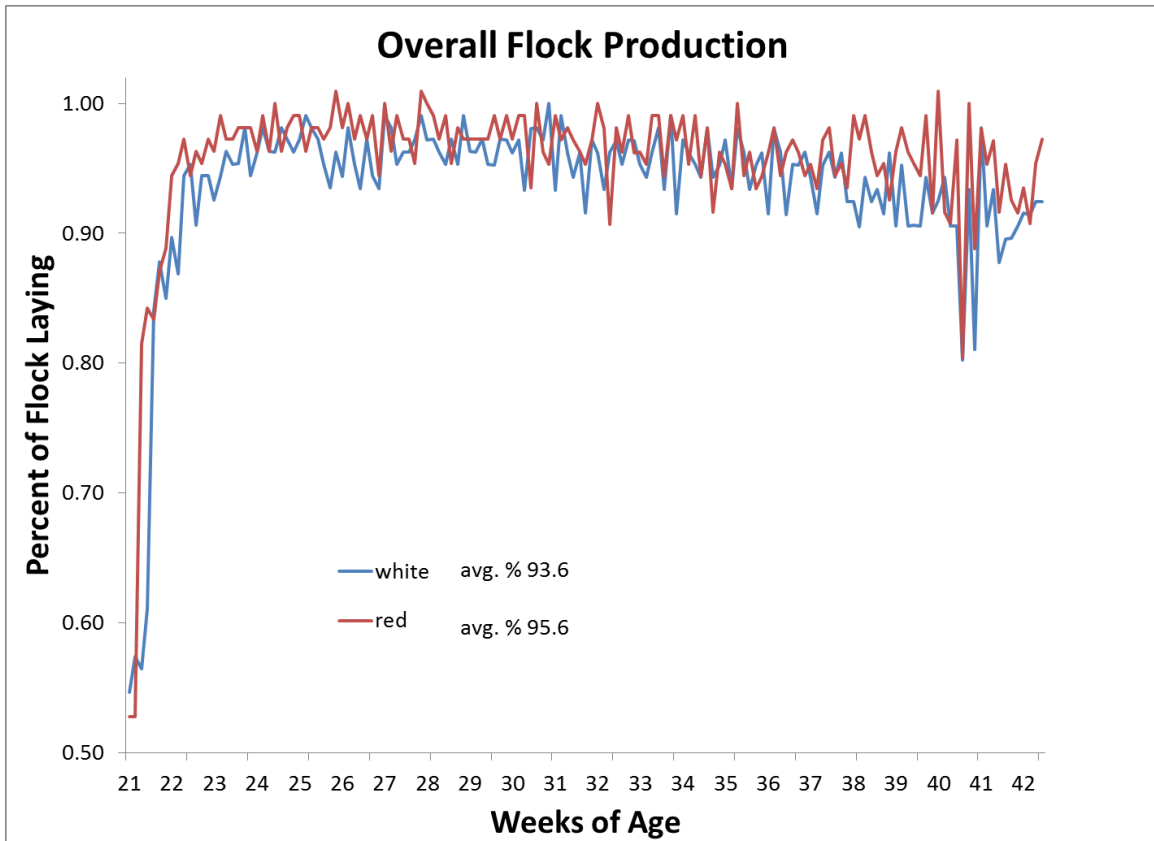


Figure 1. Flock production as a function of the percentage of the flock in lay. Red lighting caused the percent of flock laying to be consistently higher.

While there were no significant statistical differences in egg quality, numerically, white light caused stronger egg shells overall (Red, 3195.56 ± 29.28 g; White, 3251.07 ± 24.32 g), but red light caused greater Haugh unit overall (Red, 105.81 ± 0.54 ; White, 105.75 ± 0.63).

Table 1. Egg quality as measured by shell strength and Haugh unit

| | Egg Weight | Albumen Height | Shell Strength | Haugh Unit |
|-----------|-------------------|-----------------------|-----------------------|-------------------|
| | g | mm | g | |
| Treatment | Week 21 | | | |
| Overdrive | 49.33 | 11.70 | 3191.14 | 108.17 |
| Once | 49.27 | 11.48 | 2942.72 | 107.36 |

Table 1. Continued

| | Egg Weight | Albumen Height | Shell Strength | Haugh Unit |
|-----------|-------------------|-----------------------|-----------------------|-------------------|
| | g | mm | g | |
| Treatment | Week 23 | | | |
| Overdrive | 49.21 | 11.58 | 3241.04 | 107.70 |
| Once | 49.36 | 11.61 | 2880.62 | 107.82 |
| | Week 25 | | | |
| Overdrive | 49.04 | 11.67 | 3193.59 | 108.12 |
| Once | 49.50 | 11.52 | 2927.31 | 107.44 |
| | Week 28 | | | |
| Overdrive | 48.96 | 11.61 | 3156.88 | 107.89 |
| Once | 49.61 | 11.57 | 2952.91 | 107.60 |
| | Week 30 | | | |
| Overdrive | 49.13 | 11.68 | 3144.72 | 108.13 |
| Once | 49.57 | 11.54 | 2968.82 | 107.48 |
| | Week 32 | | | |
| Overdrive | 57.58 | 11.23 | 3341.61 | 104.15 |
| Once | 58.65 | 11.39 | 3204.61 | 105.28 |
| | Week 34 | | | |
| Overdrive | 58.28 | 11.65 | 3274.82 | 106.33 |
| Once | 58.69 | 11.91 | 3239.56 | 107.22 |
| | Week 36 | | | |
| Overdrive | 59.28 | 11.63 | 3261.47 | 106.09 |
| Once | 59.24 | 11.40 | 3241.34 | 105.08 |
| | Week 38 | | | |
| Overdrive | 59.52 | 11.81 | 3125.36 | 106.23 |
| Once | 59.69 | 11.62 | 3195.34 | 105.98 |
| | Week 40 | | | |
| Overdrive | 59.42 | 11.00 | 3328.63 | 103.55 |
| Once | 59.56 | 10.94 | 3238.42 | 103.31 |
| | Week 42 | | | |
| Overdrive | 60.11 | 10.57 | 3266.95 | 101.66 |
| Once | 60.38 | 10.93 | 3334.87 | 103.09 |

Numerically, birds housed under red lighting had a better feed conversion rate than birds housed under white lighting. Feed conversion of the flock from 20-42 weeks of age was 2.06 ± 0.02 under the white Overdrive light bulb and 2.04 ± 0.02 under the red Once Innovations light bulb.

Effects on stress

Over the life of the flock, birds housed under white light became more asymmetrical (White Start, 1.47 ± 0.11 & White End, 1.59 ± 0.10), while birds housed under red light actually became more symmetrical over time (Red Start, 1.52 ± 0.09 ; Red End, 1.15 ± 0.06).

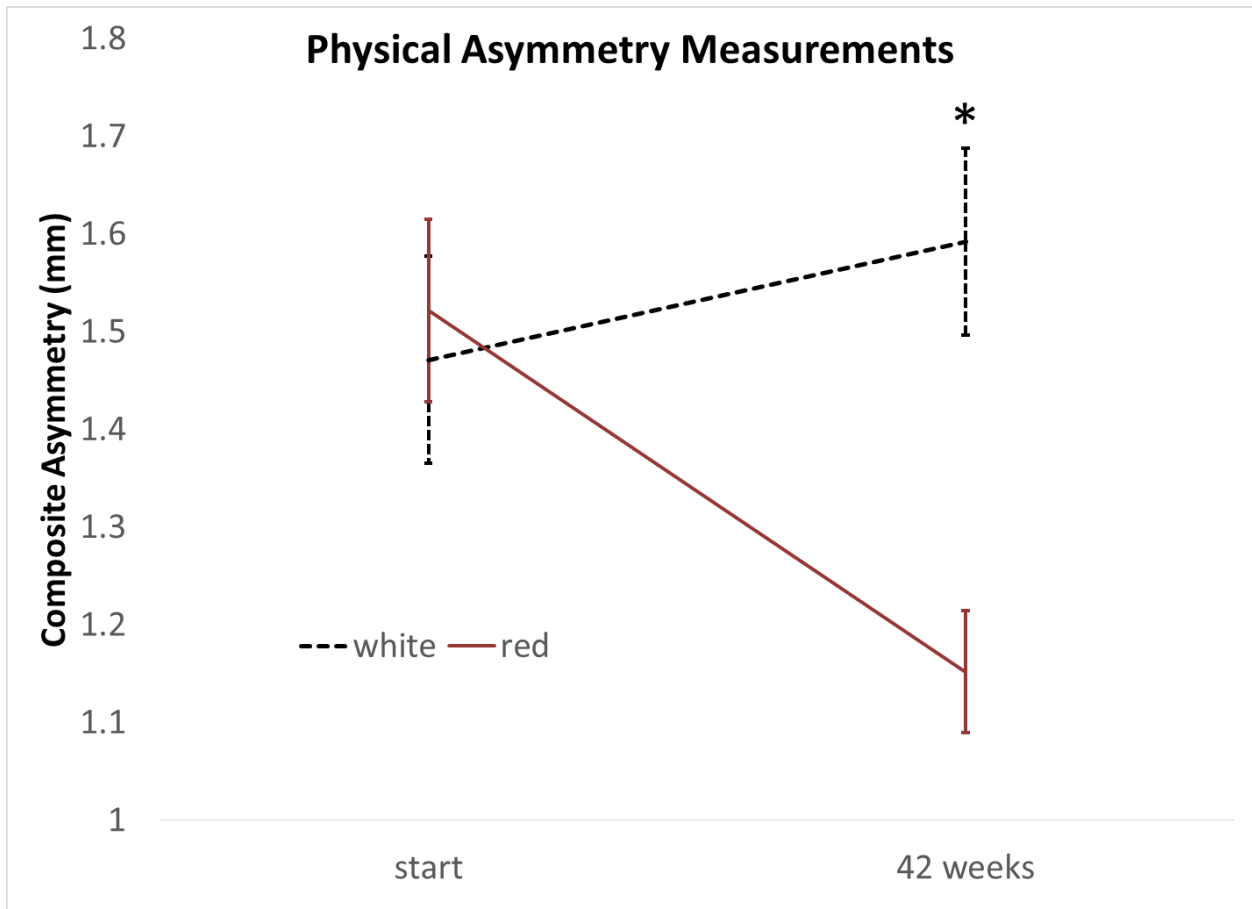


Figure 2. Degree of physical asymmetry changes during the production cycle. Birds housed under red light were more stress susceptible throughout the first half of the lay cycle, while birds housed under red light had their stress susceptibility decreased. * Indicates that red and white treatments are significantly different: $P < 0.05$.

Analysis of blood plasma via ELISA revealed that samples taken from birds housed under white lighting had greatly increased corticosterone levels by the mid-lay time point, reaching an average of 1884 ± 195 ng/dl. Samples taken from birds housed under red lighting only had a slight increase in corticosterone levels, reaching 550 ± 84 ng/dl, which was significantly lower than the increase seen under red lighting.

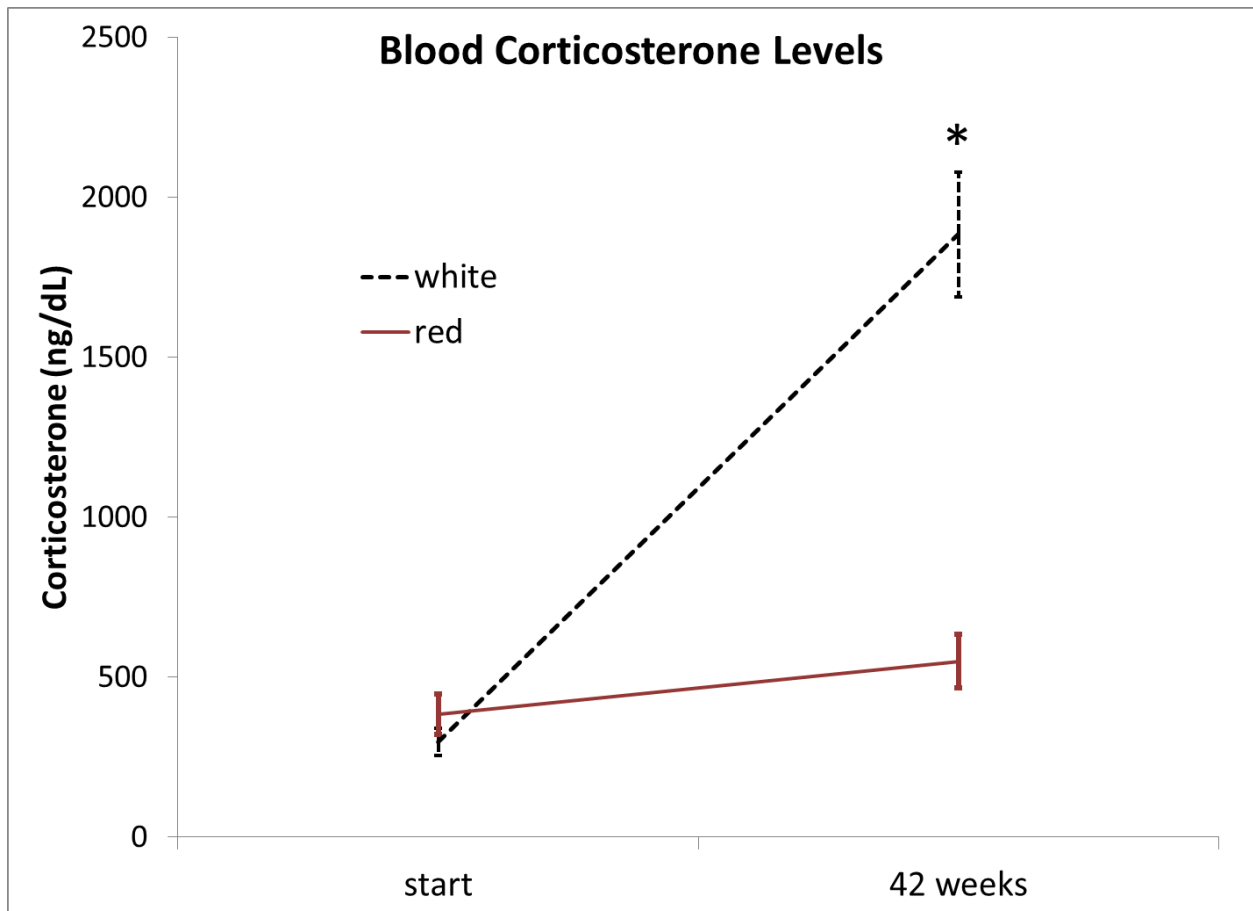


Figure 3. Changes in blood corticosterone levels due to the stress of production. Birds housed under white light saw significant increases in their blood corticosterone levels, but birds housed under red light only saw slight increases. * Indicates that red and white treatments are significantly different: $P < 0.05$.

Analyzing blood plasma via microscopy showed that samples taken from birds housed under white lighting had greatly increased heterophil/lymphocyte ratios by the end of the first half of the lay cycle, averaging at 1.14 ± 0.11 . Samples taken from birds housed under red lighting had much lower heterophil/lymphocyte ratios, only increasing to 0.57 ± 0.08 on average.

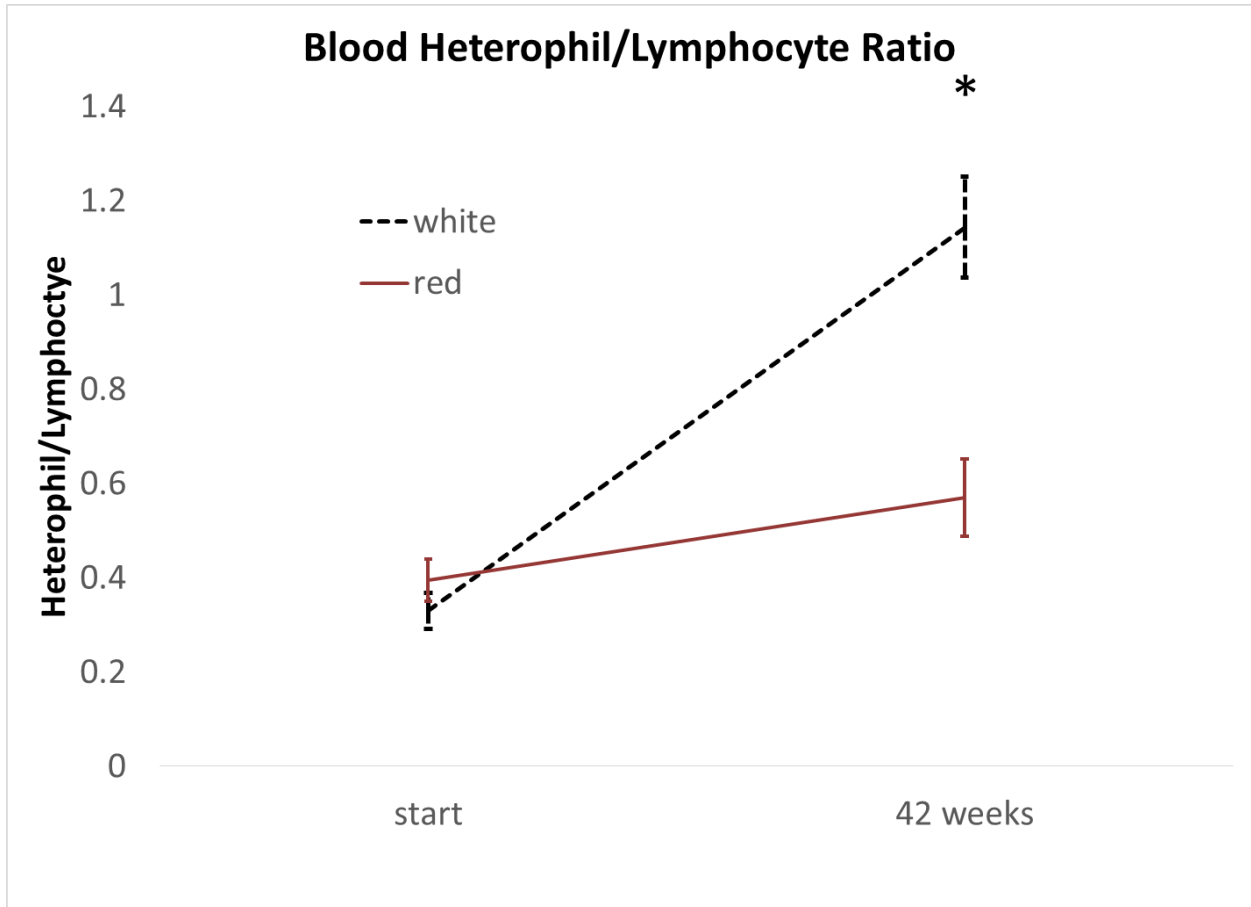


Figure 4. Changes in the heterophil/lymphocyte ratio due to the stress of production. The ratio increased to a greater degree for birds housed under white light than for birds housed under red light. * Indicates that red and white treatments are significantly different: $P < 0.05$.

Effects on behavior

Preliminary testing showed minimal behavioral differences between birds across the two treatments, and results at the end of the study were roughly the same as well. Statistically, there were no significant differences. Numerically, both birds under white light and birds under red light became less fearful over time, though the decrease in fearfulness was greater for birds housed under red light.

Table 2. Changes in fear response as measured by tonic immobility (means \pm SE)

| | Initial Head Movement | Latency to Right |
|-----------|------------------------------|-------------------------|
| | seconds | seconds |
| | Start of Lay | |
| Overdrive | 13.73 \pm 2.73 | 278.56 \pm 20.0 |
| Once | 11.82 \pm 2.00 | 310.44 \pm 21.2 |
| | Mid-Lay | |
| Overdrive | 11.40 \pm 2.73 | 274.11 \pm 18.8 |
| Once | 11.96 \pm 2.27 | 276.35 \pm 18.2 |

CHAPTER IV

CONCLUSION

At the beginning of the lay cycle, all of the birds were uniform in behavior and stress levels, but as time went on, the strain of production led to increased fear and stress susceptibility. When comparing the red and white lighting treatments, drastic differences arose between the two with regards to stress levels. Other studies have indicated production benefits from the use of red light (Gongruttananun, 2011; Lewis and Morris, 2000; Borille et al., 2015), but this study's results suggest that light color can also have a huge effect on the welfare of the birds.

Corticosterone levels and heterophil/lymphocyte ratios started out the same amongst all the birds, but differences between treatments occurred over the life of the flock, indicating that light color can affect stress levels in the birds. Standard white LED lighting caused large increases in stress hormone levels and immune cell populations following the typical pressures of production, while the new Once Innovations red LED only caused slight increases in stress levels. When measuring physical asymmetry, white lighting led to a large increase in asymmetry. It was expected that red lighting would cause asymmetry to hold constant or increase slightly, but it actually decreased over time, which means that red lighting helped the birds become less susceptible to the daily stresses of production.

The differences between the two treatments for each of the three stress measurements were statistically significant, strongly suggesting that red lighting can prevent bird stress levels from increasing as much as they do under white lighting. Therefore, from an animal welfare

standpoint, red lighting was shown to be better for the birds because it lead to decreased stress levels when compared with white lighting. Ultimately, this could also affect bird performance, since calmer birds can expend more of their energy towards increased productivity; however, significant differences in production have not yet been observed in this study. There were no significant differences in bird behavior between the two treatments either, though previous studies have shown that light color can exert behavioral effects (Mendes et al., 2013; Prescott et al., 2003).

Further observation is required to determine the degree to which performance and behavior parameters could be affected by light color as the birds continue to age. It is important to assess the effects of the two lighting treatments in both the short-term and the long-term, so more research is needed on how the treatments affect the birds during the second half of the lay cycle. This study will continue to take place until end of lay to determine whether the results stay consistent throughout the life of the birds.

In addition, more investigation is needed into the potential for red light to cause earlier start of lay. While this study indicates that red lighting can cause the flock to reach peak production sooner, further studies should be conducted to confirm that principle.

In the end, the results of this study are consistent with those found by Prescott and Wathes (1999) — chickens are more responsive to red light, and thus using it in a production setting can have positive effects on flock welfare.

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