Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye

M. Baxter, N. Joseph, V. R. Osborne, and G. Y. Bédécarrats¹

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G2W1, Canada

ABSTRACT Photoperiod is essential in manipulating sexual maturity and reproductive performance in avian species. Light can be perceived by photoreceptors in the retina of the eye, pineal gland, and hypothalamus. However, the relative sensitivity and specificity of each organ to wavelength, and consequently the physiological effects, may differ. The purpose of this experiment was to test the impacts of light wavelengths on reproduction, growth, and stress in laying hens maintained in cages and to determine whether the retina of the eye is necessary. Individual cages in 3 optically isolated sections of a single room were equipped with LED strips providing either pure green, pure red or white light (red, green, and blue) set to 10 lx (hens levels). The involvement of the retina on mediating the effects of light wavelength was assessed by using a naturally blind line (Smoky Joe) of chickens. Red and white lights resulted in higher estradiol concentrations after photostimulation, indicating stronger ovarian activation, which translated into a significantly lower age at first egg when compared with the green light. Similarly, hens maintained under red and white lights had a longer and higher peak production and higher cumulative egg number than hens under green light. No significant difference in BW gain was observed until sexual maturation. However, from 23 wk of age onward, birds exposed to green light showed higher body growth, which may be the result of their lower egg production. Although corticosterone levels were higher at 20 wk of age in hens under red light, concentrations were below levels that can be considered indicative of stress. Because no significant differences were observed between blind and sighted birds maintained under red and white light, the retina of the eye did not participate in the activation of reproduction. In summary, red light was required to stimulate the reproductive axis whereas green light was ineffective, and the effects of stimulatory wavelengths do not appear to require a functional retina of the eye.

Key words: reproduction, photoperiod, lighting, laying hen, light wavelength

INTRODUCTION

Avian species detect light through retinal and extra-retinal photoreceptors (Siopes and Wilson, 1980; Sharp, 1993; Saldanha et al., 1994; Dawson et al., 2001). The retina allows birds to see and respond to their environment and mediates the effect of light on growth and behavior (Wilson and Lindstrom, 2011). The retina contains 2 types of photoreceptors, cones and rods, with cones having a peak sensitivity to blue (450 nm), green (550 nm), red (700 nm), or violet (415 nm) light, allowing chickens to see some UV light (Lewis et al., 2007). Consequently, poultry perceive light differently than humans, resulting in some light sources appearing brighter (Pyrzak et al., 1987). Extra-retinal photoreceptors are located in the pineal gland and the hypothalamus (Siopes and Wilson, 1980; Saldanha et al., 1994; Dawson et al., 2001). The pineal gland is responsible for controlling bird’s circadian rhythm via the synthesis and release of melatonin (Pelham et al., 1972; Pang et al., 1974; Nir et al., 1987; Kumar and Rani, 1999). The hypothalamus, located deep within the brain tissue, directly controls or is involved in the control of most homeostatic and physiological processes, including reproduction.

In birds, reproduction is tightly regulated by stimulatory (gonadotropin releasing hormones; GnRH) and inhibitory (gonadotropin inhibitory hormone; GnIH) hypothalamic neuropeptides. Upon photostimulation, GnRH stimulates the release of gonadotropins (follicle stimulating hormone, FSH, and luteinizing hormone, LH) from the anterior pituitary gland, which in turn triggers gonadal development and the synthesis of steroid hormones (progesterone from the granulosa cells of the large follicles and estradiol from the small follicles) (Robinson and Etches, 1986; Ottinger and Bakst, 1995; Bédécarrats et al., 2009; Dunn et al., 2009; Tsutsui et
al., 2010). Although hypothalamic photoreceptors remain elusive, GnRH neurons have been shown to be directly innervated by opsin containing photoreceptor cells (Sharp, 1993; Saldanha et al., 2001) and exposure to long day photoperiod increases GnRH mRNA (Dunn and Sharp, 1999). On the other hand, GnIH acts on both the hypothalamus and the anterior pituitary to prevent the release of GnRH and gonadotropins, respectively (Tsutsui et al., 2010), and its release is stimulated by melatonin produced by both the retina and pineal gland during dark phases (Chowdhury et al., 2010). These findings have led us to propose a model in which increasing photoperiod reduces melatonin production, which decreases GnIH and indirectly stimulates the release of GnRH. Simultaneously, light stimulation of the hypothalamus also triggers the release of GnRH and as a result, under a short photoperiod sexually immature birds have high levels of GnIH resulting in the tonic inhibition of the reproductive axis, whereas exposure to a long photoperiod induces sexual maturity by reducing GnIH and increasing GnRH (Bédécarrats et al., 2009; Tsutsui et al., 2010).

Although the relative contribution of the retina of the eye and hypothalamic photoreceptors on reproduction is still controversial, experimental evidence showed that hypothalamic photostimulation activates the reproductive axis (Dawson et al., 2001; Saldanha et al., 2001), whereas retinal stimulation may decrease reproductive performance in chickens (Siopes and Wilson, 1980; Mobarkey et al., 2010; Gongruttanamun, 2011; Mobarkey et al., 2013). However, it is still unclear whether light wavelength is a deciding factor. For example, the retina can be stimulated with light at low intensities, whereas the hypothalamus requires higher levels (Harrison et al., 1970). As well, higher wavelengths contain more energy and are able to penetrate through the skull and brain tissue to easily stimulate the hypothalamus (Pang et al., 1974; Foster et al., 1985; Oishi and Ohashi, 1993; Mobarkey et al., 2010). Therefore, it was suggested that lower wavelengths (blue/green light) require higher intensities to stimulate hypothalamic photoreceptors (Pang et al., 1974). In addition to reproduction, light spectrum also plays a role in growth and behavior. Exposing broilers chicks to green light during initial production and blue light near the end of production caused satellite muscle cells proliferation and a subsequent increase in muscle mass (Rozenboim et al., 2004). Furthermore, behavioral studies performed on broilers found birds reared under green light are less active and spent more time sleeping and relaxing (Prayitno et al., 1997a), whereas birds reared under red and white light had increased walking activity, floor pecking, wing stretching, and aggression (Prayitno et al., 1997b).

Because photoperiod is essential to control sexual maturation and reproductive performance in avian species, artificial lighting is commonly used in commercial poultry production. Incandescent lights have been the primary lighting source used by the North American poultry industry; however, they are energy inefficient and the global push to reduce greenhouse gases has forced producers to find alternative sources. Light-emitting diodes (LED) are among the most efficient light sources and can be manufactured to deliver a defined and stable spectral output (Steranka et al., 2002). As the spectral characteristics of the light used may impact the reproductive performances, growth, behavior and health of the birds, it is essential to fully test and validate any new light source before it can be recommended for use by the industry. This study aims at establishing if light spectrum influences egg-production, body growth and stress in laying hens maintained in individual cages, and if these effects are mediated in part by the retina of the eye.

**MATERIALS AND METHODS**

**Experimental Birds**

To investigate whether the impact of light wavelength is mediated via retinal or extra-retinal photoreceptors, or both, this study was performed using Smoky Joe hens. This strain of Leghorn chickens harbors an autosomal recessive mutation that causes retinal degeneration (Salter et al., 1997; Tran et al., 2013). To obtain both blind and sighted birds, males and females from the parent colony were bred by artificial insemination following a strict pedigree protocol (Perttula and Bédécarrats, 2012). Photoperiod was kept at 24 h for the first 2 wk of age (woa) then decreased to 8 h thereafter. From hatch to 14 woa, light was provided by incandescent bulbs set to 10 lx intensity at birds’ level. Sight status (blind or sighted) was determined at 14 woa based on their reaction to visual stimulation (pupillary reflex), eye phenotypes (bulging, atrophied eyes), and pedigree. Throughout the trial, birds were fed ad libitum commercial diets (starter and layers) meeting or exceeding NRC (1994) requirements and had free access to water. All procedures were approved by the University of Guelph Animal Care Committee.

**Lighting Paradigm**

A single room at the University of Guelph research station was partitioned into 3 optically isolated sections each containing identical individual cages. The LED light strips (STR2 RGB) were purchased from GVA Lighting Inc. (Mississauga, Ontario, Canada) and mounted on top of cages. Each light strip section (1.2 m long) was assigned an individual Internet protocol (IP) address, and the intensity of individual diodes (red, green, and blue) within each section was remotely adjusted. Light fixtures were then connected to an E:cue Butler interface and light spectrum and intensity was controlled using the Terminal Emulator V5.2 computer software (e:cue Lighting Control, Paderborn, Germany). Each partition of the experimental room was illuminated with pure green (526 nm; G), pure red (632 nm; R), or white (combination of red, green, and
blue; W). Light intensities were adjusted to 10 lx at the hens’ level for all treatments and spectral output was verified using a portable spectroradiometer (LI-1800 Portable Spectroradiometer; Li-Cor Inc., Lincoln, NE). At 14 woa, 26 sighted and 34 blind hens were randomly assigned to the green (9 sighted and 11 blind birds), white (8 sighted and 12 blind birds), and red (9 sighted and 11 blind birds) treatments. To reduce placement variables, blind and sighted birds were staggered. Before LED were turned on, birds were given 1 wk to adjust to their new environment and were exposed to an 8 h photoperiod under incandescent light (10 lx). At 15 woa, LED lights were turned on with an 8 h photoperiod, and at 20 woa birds were photostimulated by an abrupt transfer to a 14 h photoperiod.

**Measurement of Growth, Stress, and Reproduction**

Body weights were recorded at placement (14 woa) and once every 2 wk from 14 to 23 woa and monthly from 37 to 52 woa. Sexual maturity and ovarian activation were determined by recording the age at first egg and measuring plasma levels of estradiol, respectively. Individual egg production was recorded daily throughout the trial (until 67 woa) and reproductive performances were expressed as weekly percent production (egg/hen per d). The effect of light treatment on stress was estimated by measuring plasma corticosterone concentrations.

**Hormone Analysis and Enzyme Immunoassays**

Blood samples were taken from each bird by venipuncture of the brachial vein at 14, 15, 20, and 23 woa, 2 to 4 h after lights were turned on. Approximately 2 mL of blood was collected and placed in a sodium heparin blood vacutainer. Immediately after collection, blood plasma was recovered by centrifugation at 900 × g for 15 min at 4°C and stored at −20°C until hormone extraction and assay. Prior to immuno-assays, corticosterone and estradiol were extracted from plasma using ethanol. In brief, thawed samples were diluted with cold ethanol at a 5:1 (ethanol:plasma) ratio. Samples were then vortexed, centrifuged for 5 min at 20°C at 1,800 × g, and frozen in a −80°C freezer. The organic (ethanol) phase was recovered, decanted into new tubes, and dried under nitrogen flow. Samples were reconstituted in half the original volume with Trizma assay buffer (20 mM Trizma, 0.3 M NaCl, 0.1% BSA; pH 7.5) and stored at −20°C until assayed. Plasma corticosterone was quantified using a corticosterone enzyme immunoassay developed by C. J. Munro (University of California, Davis) and modified by Graham et al. (2001). Briefly, microtiter plates were coated with affinity purified goat anti-rabbit gamma globulin (25 µg/plate; Sigma Chemicals, St. Louis, MO) dissolved in coating buffer (15 mM Na₂CO₃; 35 mM NaHCO₃; pH 9.6) and incubated overnight at room temperature. Coating buffer was removed, wells were refilled with Trizma assay buffer, and assay plates were stored at room temperature for at least 30 min. Coated plates were washed with 0.04% Tween 20 and samples (between 80–300 µL) or standards (3.9–500 pg/well) were added into the appropriate wells. Horseradish peroxidase-labeled corticosterone was then added to wells followed by anti-corticosterone antibody (reference number: CJM006) provided by C. J. Munro (University of California, Davis). Following incubation overnight at room temperature, plates were washed with 0.02% Tween 20 and incubated with substrate solution (0.5 mL of 16 mM tetramethylbenzidine and 0.1 mL of 0.175 M H₂O₂ diluted in 22 mL of 0.01 M C₂H₃O₂ Na; pH 5.0). After incubation (45 min, room temperature) the reaction was terminated with 50 µL of 3 M H₂SO₄ and the optical density was measured with a Microplate reader (model 550, Bio-Rad, Hercules, CA) at 450 nm (reference 595 nm). The standard curve and samples were then plotted and analyzed using Microsoft Excel (Microsoft Corp., Redmond, WA). The intra- and interassay CV were <15%.

Plasma estradiol was quantified using the same general procedure as described above for corticosterone except that an anti-estradiol antibody raised in rabbits and estradiol conjugated to horseradish peroxidase provided by C. J. Munro (University of California, Davis) were used. The standard curve for estradiol ranged from 3.9 to 500 pg/well, and the intra- and interassay CV were also <15%.

**Statistical Analysis**

All statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA). For each parameter, a 2-way ANOVA was used to determine the overall effect and possible interaction between light treatment and sight status (blind or sighted), and between light treatment and age/time. When significant differences were detected, further analyses were performed using Bonferroni post hoc tests. Significance was based on $P < 0.05$. To normalize for differences in initial BW between birds, cumulative body growth was measured by subtracting individual initial BW at each time point.

**RESULTS**

**Sexual Maturation and Egg Production**

The onset of sexual maturity, calculated based on the age at first egg (Table 1), was significantly delayed ($P < 0.0001$) for hens maintained under green light (189.6 ± 2.4 d) compared with hens under white (166.8 ± 1.1 d) and red (165.9 ± 1.3 d) lights. No significant difference was observed between blind and sighted hens for each light treatment and no significant interaction was detected between light and sight status (Supplemental Table S1A; http://dx.doi.org/10.3382/ps.2013-03799).
Estradiol concentrations in plasma were measured at different time points around sexual maturation to reflect activation of small follicles (Figure 1). At 15 woa, estradiol levels were not significantly different between light treatments (G: 0.84 ± 0.10 ng/mL; W: 0.82 ± 0.09 ng/mL; R: 0.87 ± 0.08 ng/mL), nor were there any significant differences between blind and sighted hens or interaction between sight status and light treatment (Supplemental Table S1; http://dx.doi.org/10.3382/ps.2013-03799). At 20 woa, levels of estradiol were significantly higher in birds maintained under red light compared with green light, regardless of sight status (G: 1.67 ± 0.23 ng/mL; W: 3.33 ± 0.39 ng/mL; R: 5.04 ± 0.44 ng/mL). At 23 woa, no significant difference was observed between light treatments or sight status, nor were there any interactions (G: 1.19 ± 0.22 ng/mL; W: 1.07 ± 0.094 ng/mL; R: 1.30 ± 0.13 ng/mL). Because no significant differences were observed between blind and sighted animals for each light treatment, blind and sighted data were combined and changes in estradiol concentration were analyzed over time (Figure 1D). Both age (P < 0.0001, F = 92.18, df = 2) and light treatment (P < 0.0001, F = 16.81, df = 2) had a significant effect with a significant interaction (P < 0.0001, F = 15.61, df = 2; Supplemental Table S1A; http://dx.doi.org/10.3382/ps.2013-03799). The red and white treatments resulted in significantly higher levels of estradiol at 20 woa compared with 15 and 23 woa. Conversely, no significant change in estradiol levels was observed in hens maintained under green light between 15 and 23 woa.

The effect of light treatment on egg production over the course of the trial is presented in Figure 2. There was a significant interaction between sight status and the light treatment on wk 25, 52, and 58–61 (Supplemental Table S1B; http://dx.doi.org/10.3382/ps.2013-03799). When the effects of sight and light treatment on egg production were analyzed on a weekly basis (Supplemental Table S1B; http://dx.doi.org/10.3382/ps.2013-03799), the effect of sight was significant for only 5 separate wk (29, 36, 39, 45, and 64 woa), whereas the effect of light treatment was significant for 16 out of 45 wk (from 23 to 28 woa and at 30, 33, 36, 40, 44, 45, 47, 50, 52, and 58 woa) with hens under red or red and white lights producing significantly more eggs than hens under green light. When egg production from blind and sighted hens was combined for each light treatment, it appeared that overall, light treatment had a significant effect on egg production throughout the course of the trial with hens under green light having the lowest production (Figure 2D). Interestingly, when analyzing the effect of sight for each light treatment individually, overall, sighted hens under green light had significantly lower production than blind animals (P < 0.0001; Figure 2A). However, Bonferroni post hoc test shows that the difference between sighted and blind hens was significant only at 61 and 64 woa. Under white light, age had a significant effect on egg production, yet there was no difference between blind and sighted birds (P < 0.5807), nor was there any interaction (Figure 2B). Under red light, sighted birds had a slightly higher level of production than blind birds (P < 0.0455; Figure 2C). However, when a Bonferroni post hoc was performed for each week no significant difference between blind and sighted birds was observed. Similar to the white treatment, age had a significant effect on production with no interaction between sight status and age.

The average cumulative number of eggs produced is displayed in Table 2. Hens from the red and white treatments had significantly higher cumulative egg production than those in the green treatment. There was no significant difference between blind and sighted hens for each treatment nor was there any significant interaction between light treatment and sight status (Supplemental Table S1A; http://dx.doi.org/10.3382/ps.2013-03799).

**Body Growth**

Following statistical analysis, sight status had a significant effect on BW at 14 and 15 woa (P = 0.003; Supplemental Table S1C; http://dx.doi.org/10.3382/ps.2013-03799) with blind birds lighter than sighted, regardless of light treatment. However, body growth was not significantly different between blind and sighted birds for each individual treatment (Figure 3). Until 23
Figure 1. Effect of light treatment on plasma estradiol concentrations. Estradiol concentrations in blind and sighted birds for the green, white, and red treatments are displayed in panels A, B, and C, respectively. Panel D corresponds to the combined values from blind and sighted hens for each light treatment at each time point. Different letters (a–c) indicate significant differences at \( P < 0.0001 \).

Figure 2. Effect of light treatment on weekly egg production. Egg production of blind and sighted birds for the green, white, and red treatments are displayed in panels A, B, and C, respectively. Panel D corresponds to the combined values from blind and sighted hens for each light treatment at each time point. *An asterisk indicates significant differences (\( P < 0.001 \)) between blind and sighted in panel A, and significant differences (\( P < 0.05 \)) between light treatments in panel D.
woa, there was no significant difference in BW change between light treatments, but after 23 woa, with the exception of 41 woa, the hens from the green treatment had significantly higher BW gain than those from the red and white treatments (Figure 3D). Overall, there was a significant effect of light treatment on body growth, regardless of age ($P = 0.0026$; Supplemental Table S1C; http://dx.doi.org/10.3382/ps.2013-03799).

### Stress

Stress was evaluated as per corticosterone levels in plasma (Figure 4; Supplemental Table S1D; http://dx.doi.org/10.3382/ps.2013-03799). At 14 woa, before birds were exposed to the various lights, no significant difference between light treatments (G: 0.35 ± 0.08 ng/mL; W: 0.29 ± 0.05 ng/mL; R: 0.30 ± 0.09 ng/mL) or between sight status was observed. Similarly, after 1 wk of exposure to the light treatment (15 woa) no difference in corticosterone levels was observed (G: 0.30 ± 0.04 ng/mL; W: 0.27 ± 0.04 ng/mL; R: 0.24 ± 0.03 ng/mL). Yet at that age, there was a significant difference between blind and sighted hens with sighted birds having significantly higher levels of corticosterone than blind birds, regardless of light treatment. At 20 woa (after 5 wk of exposure to light treatment), birds under red light had significantly higher levels of corticosterone (0.72 ± 0.12 ng/mL) than hens under green (0.30 ± 0.02 ng/mL) or white (0.37 ± 0.03 ng/mL) lights. As there was only a significant difference in corticosterone levels between the blind and sighted birds at 15 woa, data were combined and analyzed over time (Figure 4D; Supplemental Table S1D; http://dx.doi.org/10.3382/ps.2013-03799). A 2-way ANOVA revealed that the age had a significant effect on corticosterone levels, whereas light treatment did not have a significant effect on its own. However, the interaction between the age of hens and light treatment was significant (Supplemental Table S1D; http://dx.doi.org/10.3382/ps.2013-03799).

### DISCUSSION

#### Effect on Reproductive Performance

Based on our results, it is evident that wavelengths from the red spectrum are the most potent stimulator of sexual maturation and egg-laying in both blind and sighted Smoky Joe hens. This may be explained by the fact that wavelengths of lower frequencies possess

<table>
<thead>
<tr>
<th>Item</th>
<th>Cumulative egg number/bird (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blind green</td>
<td>151.4 ± 0.4a</td>
</tr>
<tr>
<td>Sighted green</td>
<td>135.0 ± 0.5a</td>
</tr>
<tr>
<td>Blind white</td>
<td>178.6 ± 0.3ab</td>
</tr>
<tr>
<td>Sighted white</td>
<td>182.3 ± 0.4B</td>
</tr>
<tr>
<td>Blind red</td>
<td>185.4 ± 0.4a</td>
</tr>
<tr>
<td>Sighted red</td>
<td>196.1 ± 0.4B</td>
</tr>
<tr>
<td>Green</td>
<td>140.5 ± 0.3A</td>
</tr>
<tr>
<td>White</td>
<td>180.2 ± 0.3B</td>
</tr>
<tr>
<td>Red</td>
<td>199.3 ± 0.3B</td>
</tr>
</tbody>
</table>

$^a,b P < 0.05$, $^A,B P < 0.001$. Different superscript letters indicate significantly different values.

Figure 3. Effect of light treatment on percent body growth. Body growth between blind and sighted birds for the green, white, and red treatments is displayed in panels A, B, and C, respectively. Panel D corresponds to the combined values from blind and sighted hens for each light treatment at each time point. Letters (a,b) correspond to significant differences ($P < 0.001$) between light treatments in panel D, whereas c indicates significant differences at $P < 0.01$. 

Table 2. Effect of light wavelength on cumulative egg production
higher power and are thus able to penetrate the skull and brain tissue more easily, as demonstrated by Oishi and Ohashi (1993). For example, in our experiment, adjusting light intensity to 10 lx resulted in 0.0871, 0.455, and 0.223 W/m² for the red, white, and green treatments, respectively.

Hens maintained under red and white light reached sexual maturity within 3 wk after photostimulation (23 woa), whereas birds exposed to green light displayed a 23.7 d (over 3 wk) delay. Interestingly, 27 wk is the age at which Smoky Joe hens mature spontaneously in our breeding colony when left under an 8 h photoperiod (unpublished data). This observed delay in egg production under green light is in agreement with previous studies (Harrison et al., 1970; Kumar and Rani, 1996; Mobarkey et al., 2010; Gongruttananun, 2011) and suggests that it is ineffective in properly mediating a stimulatory photoperiod. This is further strengthened by the fact that under our experimental conditions, green light failed to significantly elevate circulating estradiol levels, whereas red light was the most effective. Higher levels of estradiol during the initiation of egg laying have been correlated with the activity of small follicles (Robinson and Etches, 1986). This effect of light wavelength on ovarian activity was independent of a fully functional retina of the eye and is thus most likely mediated via extra-retinal photoreceptors, most likely hypothalamic.

As we and others previously proposed (Bédécarrats et al., 2009; Tsutsui et al., 2010), sexual maturation in chickens is tightly regulated by a balance of stimulatory (GnRH) and inhibitory (GnIH) inputs from the hypothalamus, coupled with a switch in sensitivity to these peptides at the level of the anterior pituitary. In that model, we postulated that the increase in GnRH observed upon photostimulation is under the direct influence of hypothalamic photoreceptors, whereas the synthesis and release of GnIH is under the control of melatonin produced during the dark phases. This is supported by evidence that melatonin from both the pineal gland and the retina of the eye contribute to the production of GnIH and is in part responsible for inhibiting LH release in both quails and chickens (Chowdhury et al., 2013). Moreover, stimulation of the retina of the eye with green light in broiler breeders results in decreased reproductive performances (Mobarkey et al., 2010), and supplementation with the serotonin inhibitor parachlorophenylalanine partially restored egg production (Mobarkey et al., 2013). It is possible that stimulation of the retina with green light promotes the synthesis of serotonin, which is further converted into melatonin during the dark phases, inhibiting the reproductive axis via GnIH. Interestingly, in our study no significant difference was observed between blind and sighted birds under red and white lights, suggesting that the inhibition from the retina of the eye may not be prevalent when sufficient stimulation of the hypothalamus is provided. On the other hand, when the stimulatory effect of red light is removed and hens are maintained under pure green light, sighted hens dropped out of production significantly earlier than blind birds, suggesting that without proper extra-retinal stimulation, an inhibitory effect via the retina can be observed. However, whether the degenerative retina in our blind
Smoky Joe line is associated with a decreased melatonin synthesis remains to be determined. Interestingly, in a previous study we performed on Smoky Joe roosters (Perttula and Bédécarrats, 2012), blindness resulted in advanced sexual maturity in both unphotostimulated and photostimulated birds. In this study, we used incandescent light bulbs that emit a broader spectrum, and it is possible that the ratio of green versus red is the determining factor to observe any antagonistic effect of stimulatory extra-retinal and inhibitory retinal photoreceptors on reproduction. However, although sighted birds began dropping out of production sooner under green light, no significant difference in cumulative egg number between blind and sighted birds was observed. This is most likely due to the fact the significantly different weeks occurred when both blind and sighted were below 60% production. Nonetheless, significance may have been reached if the trial had continued for a full 52-wk production cycle.

Although red light was the most effective wavelength to stimulate the production of estradiol, no significant difference in age at first egg or overall production were observed between hens under red and white lights. Thus, a white light containing 33% red may be sufficient to adequately stimulate and maintain reproduction. However, although not statistically significant, hens under red light did have slightly better reproductive performances than hens under white light, as they began laying earlier, and had a slightly higher level of production. This may be of importance for large commercial operations where slight increases in production translate into higher profit, and more than 33% of red spectrum may be beneficial to increase reproductive performances.

**Effect on BW**

The initial differences in body growth observed between blind and sighted hens could be explained by the fact that at placement, blind hens had to first “map” their new environment to locate feeders and water dishes. In a previous study, when Smoky Joe birds were monitored from an younger age and were kept in the same environment, no such difference between blind and sighted was observed (Perttula and Bédécarrats, 2012). However in the present study, the initial difference was no longer visible after 2 wk.

Prayitno et al. (1997b) previously reported that birds exposed to red light had higher feed intake and increased initial body growth, although higher activity levels also resulted in decreased final BW. Rozenboim et al. (1998) suggested that feed intake of caged laying hens was mainly affected by light intensity and not wavelength. On the other hand in broiler chicks, initial exposure to green and blue lights does increase growth later in development, and this effect is mainly due to proliferation of satellite muscle cells rather than changes in feed consumption (Rozenboim et al. 2004). In our experiment, light intensity was the same across all treatments and all birds were housed in individual cages, thus limiting physical activity. Although we did not monitor feed consumption, hens under green light grew at a significantly faster pace than those under white and red light from 23 to 52 woa. Because birds were exposed to their respective light treatment at 15 woa, this difference cannot be attributed to an effect of green light on satellite cells proliferation, nor can it be attributed to differences in physical activity. Instead, this may have resulted from changes in energy expenditure associated with the delayed entry in lay and lower overall egg production observed in the green treatment.

**Effect on Stress**

Corticosterone produced by the adrenal glands is the main mediator of the stress axis in birds and daily fluctuation in laying hens ranges from 7 to 11 ng/mL (Beuving and Vonder, 1978). Corticosterone levels in our study were lower than these values, suggesting that birds under the 3 light treatments were not physiologically stressed. However, we did observe significantly higher levels of corticosterone in the red treatment at 20 woa. At a behavioral level, prior studies have shown that birds reared under red and white light display increased walking activity, floor pecking, wing stretching, and aggression (Prayitno et al., 1997b), whereas birds reared under green light are less active with increased sleeping patterns, which may decrease the hens’ corticosterone levels (Prayitno et al., 1997a). Under our experimental conditions, birds were confined to individual cages and these behaviors could not be assessed. Thus, we cannot determine whether the observed increase was linked to behavioral changes and additional research should be conducted to investigate the effect of light spectrum. Nonetheless, the higher levels observed under red light at 20 woa were below what is deemed physiologically stressed (Beuving and Vonder, 1978) and had no negative impact on reproduction.

**Conclusion**

In conclusion, our study shows that red light is necessary to adequately initiate the activation of the reproductive axis, increase ovarian activity, maintain high levels of production, and increase total number of eggs. Although birds in the red treatment had higher levels of corticosterone, these levels were below what can be considered a physiological stress and reproduction was not compromised. Because we did not observe any significant difference between blind and sighted birds, it appears that under our experimental conditions, retinal stimulation or lack thereof does not affect initiation of reproduction.

**ACKNOWLEDGMENTS**

The authors acknowledge the Ontario Ministry of Agriculture Food and Rural Affairs and the Poultry
REFERENCES


