Continuous exposure to red light induces photorefractoriness in broiler breeder pullets

Charlene Hanlon,[*,†,1] Martin J. Zuidhof,[‡] Adriana Rodriguez,[†] Kayo Takeshima,[†] and Grégoy Y. Bédécarrats[†]

[*]*Department of Poultry Science, College of Agriculture, Auburn University, Auburn, AL 38649, USA;* [†]*Department of Animal Biosciences, Ontario Agricultural College, University of Guelph, Guelph, ON, Canada; and* [‡]*Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada*

ABSTRACT The management of body weight (**BW**) in broiler breeder pullets is critical to offset the negative correlation between their growth potential and reproductive success. Therefore, a precision feeding system was developed to allocate feed individually based on real-time BW in more frequent, smaller portions. However, this system requires access beyond the 8 h daylength of the rearing period. Since green and red spectra have been shown to stimulate growth and sexual maturation, respectively, this study aimed to evaluate the impact of continuous supplemental illumination of feeders with monochromatic wavelengths on sexual maturation. Furthermore, the best combination of supplemental and daytime lighting for optimizing the pullet-to-hen transition period was investigated. This study contained a 2 × 4 × 2 factorial arrangement, with 2 daytime lights (dtRED and dtGREEN; n = 2 rooms), 4 supplemental lights (sBLUE, sGREEN, sRED, and sCON; n = 12 pens), and 2 supplemental intensities (High and Low). At 3 wk of age (**woa**), 480 female Ross 708 chicks were randomly distributed across treatments (n = 10/pen). All birds were feed restricted per management guidelines and maintained under 8 h of dtRED or dtGREEN. Birds were photostimulated at 20 woa with 14L:10D. All birds were weighed weekly, with age at first egg (**AFE**) and production rate calculated weekly per pen. Birds under sRED were heavier than all other treatments from 27 woa to the end of the study ($P < 0.001$; 30 woa), resulting in hens that were over 400-g heavier. This resulted from a delayed AFE and lower production rate under sRED, with higher intensity further hindering reproductive performance ($P < 0.001$). Interestingly, despite the inhibitory effect of continuous red lighting (**sRED**) on reproduction, dtRED resulted in a 3.15% higher rate of lay than dtGREEN. Therefore, this study suggests that while red light remains superior at stimulating reproduction, continuous red supplemental lighting results in photorefractoriness. Thus, we recommend green light in PF systems.

Key words: broiler breeder, spectrum lighting, reproduction, production

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INTRODUCTION

Enhanced growth rate and improved feed efficiency in meat-type (broiler) chickens have been achieved through commercial genetic selection programs (Zuidhof et al., 2014). Unfortunately, the negative correlation between growth and reproductive traits (Decuypere et al., 2010) has resulted in negative consequences for the parent stock. While one of the primary breeding objectives of broiler breeder hens includes the production of high-quality fertile eggs, these hens still carry the growth potential obtained from genetic selection to transfer to their offspring. Therefore, when fed ad libitum, broiler breeders can easily become overweight, leading to reproductive issues such as multiple or internal ovulations, unfit body condition to mate, and a decline in fertility, shell quality, and hatchability (Leclercq et al., 1985; Cahaner et al., 1986; Decuypere et al., 2010). Consequently, broiler breeder hens are commonly placed on feed restriction programs, with particular emphasis on controlling their weight during the rearing phase and sexual maturation (Bruggeman et al., 1999; de Beer and Coon, 2007).

In avian species, sexual maturation is tightly regulated by the coordination of the hypothalamic-pituitary-gonadal (**HPG**) axis through the perception of light via

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[1]Corresponding author: chanlon@uoguelph.ca, clh0149@auburn.edu (CH)

the retina, pineal gland, and deep brain photoreceptors (Kumar et al., 2004). Thus, pullets are reared under short-day (**SD**) photoperiods to remain in an immature state through the pullet stage. Longer scotophases of up to 16 hours (**h**) result in the production of melatonin (**MEL**) from the pineal gland (Juss et al., 1993) and the retina of the eye (Underwood et al., 1984), which stimulates the release of gonadotropin-inhibitory hormone (**GnIH**) from the hypothalamus. Upon binding to its receptor (**GnIH-R**) in the hypothalamus and the anterior pituitary, GnIH maintains the inhibition of the HPG axis. A sudden increase in daylength above 12 h, referred to as photostimulation (**PS**), decreases the production of MEL and therefore causes a decline in GnIH (Ubuka et al., 2005) while activating deep brain photoreceptors. This allows an increase in the synthesis and release of gonadotropin-releasing hormone I (**GnRH-I**) (Tsutsui et al., 2000; Bentley et al., 2003), which binds to its receptor on the pituitary gland (**cGnRH-RIII**) and stimulates the release of the gonadotropins (Bédécarrats et al., 2006; Joseph et al., 2009). Activation of the HPG axis results in the development of small white follicles (**SWF**) and is associated with a rise in plasma estradiol ($\mathbf{E_2}$) concentration (Robinson and Etches, 1986; Johnson and Woods, 2009). Thus, a successful reproductive cycle in broiler breeder hens depends on lighting and feed restriction programs.

In an effort to manage individual body weight (**BW**) for optimal reproductive success and thus improve flock uniformity, daily or alternative feed restriction programs have been implemented in commercial settings (de Beer and Coon, 2007). Although successful at controlling BW, restricting diets up to 70% daily resulted in welfare concerns and poor BW uniformity (Savory and Maros, 1993; Savory et al., 1993; de Jong et al., 2002,2003), while alternative non-daily programs led to inefficient metabolic storage (de Beer and Coon, 2007). Thus, a precision feeding (**PF**) system was developed at the University of Alberta (Zuidhof et al., 2017). Using real-time BW, this system is able to allocate feed in restricted portions and durations, allowing the birds to eat several smaller meals per day, improving the BW coefficient of variation (**CV**) to less than 2% (Zuidhof et al., 2017; Zuidhof, 2018). This improvement in BW uniformity during pullet rearing results in a synchronization of the age at sexual maturation, culminating in the production of the maximum number of eggs overall (Hudson et al., 2001; Abbas et al., 2010). However, the economic viability of the PF system requires access beyond the 8 h of daylight traditionally provided during the pullet phase to ensure all birds have the opportunity to consume their daily allocation. Continuous exposure to supplemental lighting could cause photorefractoriness, which is a desensitization to a normally stimulatory light signal. Thus, PF systems would benefit from a 24 h supplemental illumination program that does not interfere with the process of photostimulation.

Due to differences in tissue penetration ability of the various wavelengths, spectrum lighting has been observed to impact physiological processes (Bédécarrats and Hanlon, 2017). In particular, red light has been shown to trigger sexual maturation and increase egg production in laying hens and broiler breeders (Mobarkey et al., 2010) as these longer wavelengths are better able to reach deep brain photoreceptors. Conversely, evidence suggests that wavelengths from the green spectrum are not only ineffective at triggering maturation but may potentially play an inhibitory role (Mobarkey et al., 2010; Baxter et al., 2014). On the contrary, other studies have reported that green light is able to elevate E_2 in chickens (Liu et al., 2015), as well as lead to an increase in egg production in pigeons Wang et al., 2019). Alternatively, green light has been primarily attributed to the enhancement of early growth in male broiler chicks and the stimulation of skeletal muscle cell proliferation (Halevy et al., 1998; Rozenboim et al., 1999). However, in this case perceived light intensity of the bird may have a larger role in providing these beneficial growth effects, rather than spectrum itself (Remonato Franco et al., 2022). On the other end, while monochromatic blue light can result in heavier birds during the late growth stage in broilers (Rozenboim et al., 1999; Li et al., 2014), in layers it was shown to cause a decline in egg production rate despite elevated gonadotropin serum levels similar to that of monochromatic red light (Li et al., 2014). While the impact of spectrum lighting on egg laying using standard photoperiod has been relatively well studied, little is known about the impact of continuous (24 h) illumination using monochromatic light sources on growth and reproduction.

Thus, the aim of this study was to evaluate the impact of continuous supplemental illumination of feeders with pure green, red, and blue light on the growth and sexual maturation of broiler breeder hens. Furthermore, the impact of daytime (main barn light) light spectrum was also investigated to determine any potential interaction between daytime and supplemental light systems. Ultimately, this study aimed to determine the best combination of daytime and supplemental spectrum lighting that could be used in PF stations to optimize the pullet-to-hen transition in broiler breeder hens.

MATERIALS AND METHODS

This experiment was approved by the Animal Care Committee of the University of Guelph, and all procedures were performed in accordance with recommendations of the Canadian Council on Animal Care guidelines (CCAC, 2009).

Birds and Housing

Four hundred and eighty Ross 708 female broiler breeder chicks (Aviagen, Elkmont, AL) were housed at the Arkell Poultry Research Station of the University of Guelph (Guelph, ON, Canada). From 1 day of age (**doa**) to 15 doa, chicks were randomly placed in 12 floor pens (2.4 × 1.8 m) in a single room, with 40 chicks per

pen. During this time, feed and water were provided ad libitum. At 2 weeks of age (**woa**), chicks were individually wing tagged and randomly distributed throughout 4 identical rooms, each containing 12 visually and optically isolated pens (n = 48 pens), with 10 chicks placed in each pen. Following this transfer, at 2 woa, birds were placed on a daily restricted feeding program in accordance with the recommended BW provided in the breeder's guidelines (Aviagen, 2016). Feed allocation was determined on a flock basis, calculated from the weekly average flock BW for the remainder of the study. The same per-bird feed allocation was provided to all pens. Water was provided ad libitum throughout the trial. At 160 doa, three-hole roll-out nesting boxes were installed in each pen.

Experimental Design

This experiment was comprised of a 2 × 4 × 2 factorial arrangement of treatments, with 2 types of main house daytime lights, 4 supplemental light colors, and 2 intensities. Supplemental lighting treatments were applied from 3 to 30 woa. At placement, chicks were raised under white light-emitting diode (**LED**) luminaires (Think-A19 LED lighting eclairageDEL, Reonac Energy Systems, Canada) for 23 hours (**h**) per day at an intensity of 30 lux, with the photoperiod decreased to 12 h of light at 4 doa. At 2 woa, once the birds were randomly placed within the 4 rooms, the photoperiod was further reduced to 8 h of light at 10 lux.

The experimental lighting regime was applied at 3 woa to provide chicks with an adjustment period to their feed restriction and new environment. To investigate the effect of daytime light spectrum on rearing and sexual maturation, birds were housed in rooms with either 60% red LED daytime light (**dtRED;** 60% red, 20% green, 20% blue spectrum LED light; AgriLux PLR, Thies Electrical Distributing Inc., Canada; n = 2 rooms) or 60% green LED daytime light (**dtGREEN**; 60% green, 20% red, 20% blue spectrum LED light; AgriLux PGR, Thies Electrical Distributing Inc., Canada; n = 2 rooms). In addition to these daytime spectrum light treatments, a supplemental LED strip lighting (RGBW 5050 LED Strip lighting: ALED-CN Lighting Co. Ltd., China) was placed around the hanging feeder with shades directing the light into the pan. These supplemental lights provided 24 h illumination of feeders to mimic the visibility and accessibility of feed in a PF system. Each pen was randomly assigned within each daytime spectrum light block to one of 4 supplemental LED strip feeder light treatments: monochromatic red (**sRED**; 630 nm), monochromatic green (**sGREEN**; 508 nm), monochromatic blue (**sBLUE**; 450 nm), or no illumination (**sCON**). Finally, each supplemental feeder light treatment was assigned to either high intensity (**INT**) (10 lux for sRED-H, sGREEN-H; 20 lux for sBLUE-H) or low INT (1 lux for sRED-L, sGREEN-L; 2 lux for sBLUE-L), resulting in 3 replicates per 2 × 4 × 2 treatment. At 20 woa, all birds were photostimulated with an abrupt increase of the daytime lighting photoperiod to 14 h at 30 lux, which was maintained for the remainder of the study period. All lighting spectral output was measured using the QStick USB subminiature Spectrometer and analyzed via the Waves spectroscopy software (RBG Lasersystems Leading photonics, Germany). The intensity of the daytime and supplemental lights was set using an LED light meter (LT40, Extech, Nashua, NH).

Measurements of Growth and Reproduction

Each bird was individually weighed weekly from 2 to 30 woa. Flock uniformity was calculated as a CV using the pen BW mean and standard deviation. From the onset of lay, eggs were collected and recorded daily, with weekly egg production rates determined per hen housed for each pen. To determine the age at sexual maturation, age at first egg (**AFE**) was recorded for each pen. Final cumulative egg number per hen housed throughout the experiment was analyzed per pen.

Blood Sampling and Estradiol Analysis

Repeated blood samples were collected from the brachial vein of the same 3 hens from each pen (n = 144 total) biweekly from 3 to 30 woa. At this time, 2 mL of blood was placed in a 4-mL sodium heparin vacutainer and placed on ice. Plasma was recovered after centrifugation at 900 × g at 4°C for 15 minutes and stored at −20°C until extracted. To determine plasma E_2 levels, plasma samples were extracted using the cold ethanol extraction protocol outlined by Baxter et al. (2014). Extracted plasma samples were processed according to the manufacturer's protocol outlined in the DetectX commercial estradiol ELISA kit (DetectX 17β-estradiol enzyme immunoassay #K030-H5, Arbor Assays, Ann Arbor, MI). The standard curve and samples were plotted and analyzed using the MyAssays software with the built-in 4 parameter logistic curve (www.myassays.com/arbor-assays-estradiol-eia-kit.assay). The intra and inter-assay CV were determined to be < 5% and 12.6%, respectively.

Statistical Analysis

All statistical analyses were performed using the HPMIXED and MIXED procedures of SAS version 9.4 (SAS Institute, Cary, NC). Normality was confirmed for all datasets with the Shapiro-Wilks test. For BW, CV of BW, egg production rate, and E_2 concentration, fixed effects included daytime light (dtRED and dtGREEN), supplemental feeder light (sRED, sGREEN, sBLUE, and sCON), supplemental light INT (L and H), age, and their interactions. Random effects included room and pen. For parameters collected as repeated measures from individual birds, bird ID was used to identify the subject in the repeated statement. An F-test was performed to determine the significance of the fixed effects, and Tukey's multiple range test was used to test the

significance of least squares treatment means. Differences were reported where $P \leq 0.05$.

RESULTS

Body Weight

Throughout the study, BW was recorded weekly and feed allocation adjusted to closely follow the target weight recommended by the Ross 708 Parent Stock management guidelines (Figure 1; Aviagen, 2016). There was no significant effect of DTL or INT on BW. However, BW was dependent on age ($P < 0.001$) and SFL ($P < 0.001$), along with an interaction observed between age and SFL ($P < 0.001$). Pairwise differences indicated that hens under sRED, regardless of DTL treatment, were 145-g and 198-g heavier than sBLUE and sGREEN respectively at 26 woa but did not differ from sCON. From 27 woa through to the end of the study (30 woa), hens under sRED were significantly heavier than all other treatments ($P < 0.001$). This led to hens under sRED reaching a BW that was over 400 g heavier than hens under any other light.

Coefficient of Variation

The CV, presented in Figure 2, was used to determine flock uniformity. An age effect was observed ($P < 0.001$), with the CV reaching 10 to 11% from 26 to 30 woa. There was an effect of INT ($P = 0.0348$), with hens under high INT demonstrating less uniformity than those under low INT treatment. While there was an interaction between age and INT ($P < 0.001$), the only pairwise difference observed was at the initiation of the treatments (3 woa), with high INT hens displaying a higher CV than low INT hens ($P = 0.021$). Thus, this is likely a carryover effect of the rearing environment, rather than a direct consequence of light intensity at this time, as the trend did not persist.

Sexual Maturation

Age at first egg (**AFE**) was determined per pen and displayed in Table 1. There was an effect of SFL ($P < 0.001$) and a SFL × INT interaction ($P = 0.041$). Overall, AFE was found to be delayed by approximately 9 d in hens under sRED light compared to all other SFL, regardless of INT. When considering the interaction, there were no differences observed between sRED-H and sRED-L (181 and 175 d, respectively), yet sRED-L also did not differ from sCON-L (169.2 doa) and sBLUE-H (170 doa). Overall, the earliest entry into lay was observed at 167 doa under sBLUE-L, sCON-H, sGREEN-H, and 168.2 doa under sGREEN-L.

E_2 concentrations were determined bi-weekly between 15 and 29 woa and displayed in Figure 3. While there was an effect of age ($P < 0.001$), SFL ($P = 0.022$), and an interaction between age and DTL ($P < 0.001$), no further pairwise differences were identified. Interestingly, there was a tendency for sRED to have a lower E_2 concentration than sGREEN at 23 woa ($P = 0.058$). Additionally, all treatments were elevated at 29 woa, yet the peak in E_2 traditionally associated with sexual maturation (Renema et al., 1999; Onagbesan et al., 2006; van der Klein et al., 2019) was not identified in these hens.

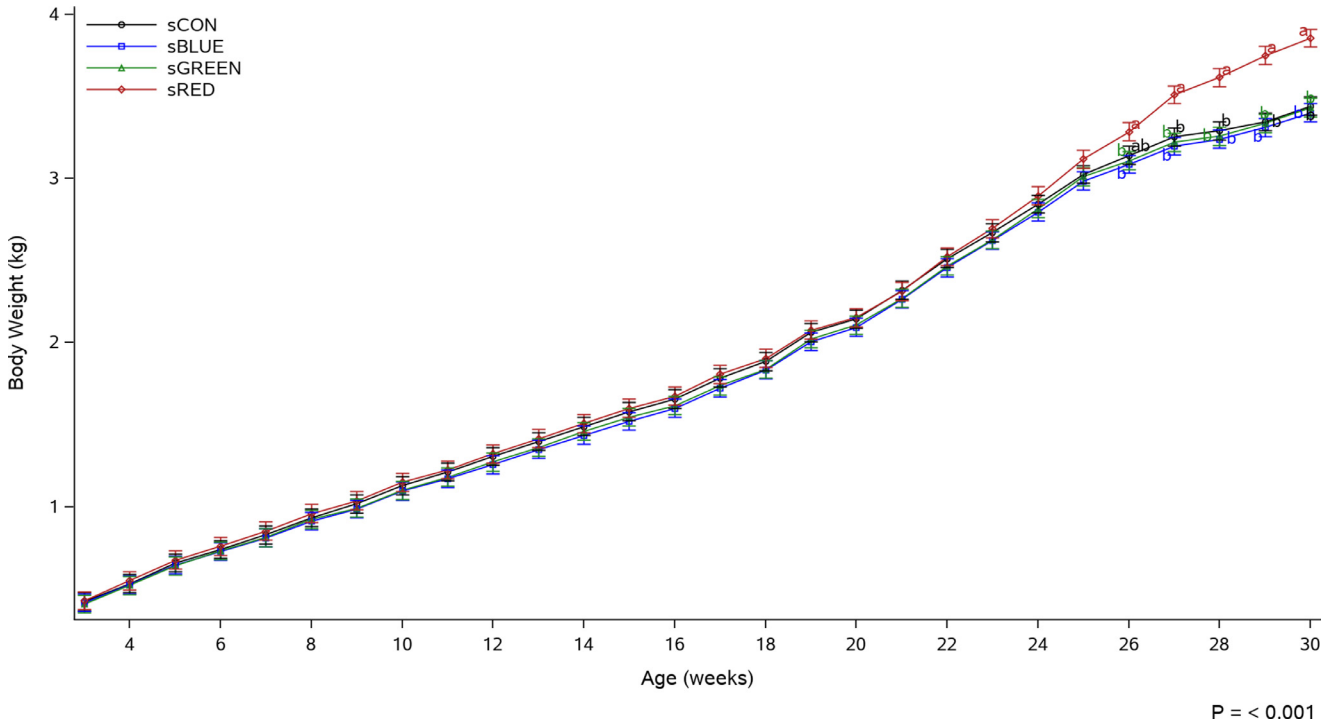

Figure 1. Body weight (BW) of broiler breeder hens maintained under blue (sBLUE), green (sGREEN), red (sRED) and control (sCON) supplemental feeder lights (SFL) from 3 to 30 woa. [a-b] Data points lacking a common superscript differ significantly at specific ages ($P < 0.05$).

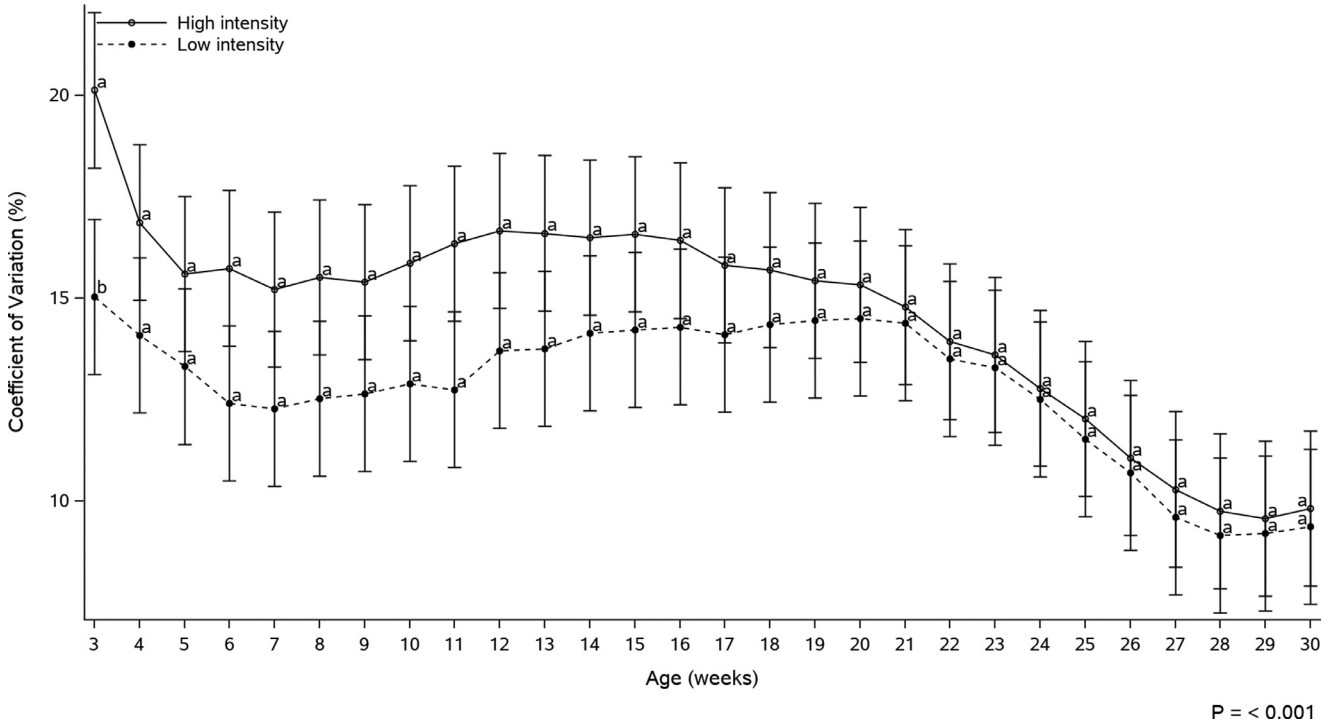

Figure 2. Coefficient of variation (CV) for BW from 3 to 30 wk of age (woa) under High or Low supplemental feeder light (SFL) intensity (INT). [a-b] Data points lacking a common superscript differ significantly at specific ages ($P < 0.05$).

Table 1. Age at first egg (AFE) and cumulative egg number of female broiler breeder hens to 30 wk of age housed under 60% red (dtRED) or 60% green (dtGREEN) daytime light (DTL), monochromatic blue (sBLUE), control dark (sCON), green (sGREEN), or red (sRED) supplemental feeder light (SFL), and high (H) or low (L) SFL intensities (INT).

Effect	DTL[1]	SFL[2]	INT[3]	AFE[4] (days)	Cumulative egg (n)
DTL	dtRED			171.5	21.6
	dtGREEN			170.1	20.0
SEM				0.74	0.69
SFL		sBLUE		168.9[b]	24.8[a]
		sCON		168.4[b]	24.2[a]
		sGREEN		167.8[b]	24.2[a]
		sRED		178.2[a]	10.1[b]
SEM				1.04	0.98
INT			H	171.7	19.9
			L	169.9	21.7
SEM				0.74	0.69
SFL		sBLUE	H	170.5[bc]	23.8
x			L	167.3[c]	25.7
INT		sCON	H	167.7[c]	24.3
			L	169.2[bc]	24.1
		sGREEN	H	167.3[c]	24.6
			L	168.2[c]	23.8
		sRED	H	181.3[a]	6.9
			L	175.0[ab]	13.3
SEM				1.47	1.38
Source of variation				——— *P*-value ———	
DTL				0.173	0.123
SFL				<0.001	<0.001
INT				0.095	0.072
DTL x SFL				0.997	0.561
DTL x INT				0.968	0.110
SFL x INT				0.041	0.058
DTL x SFL x INT				0.974	0.959

[1]DTL, Daytime light.
[2]SFL, Supplemental feeder light.
[3]INT, Intensity.
[4]Age at first egg found in each pen, which housed 10 birds.
[a-c]Means within a column and effect treatment group lacking a common superscript differ significantly ($P < 0.05$).

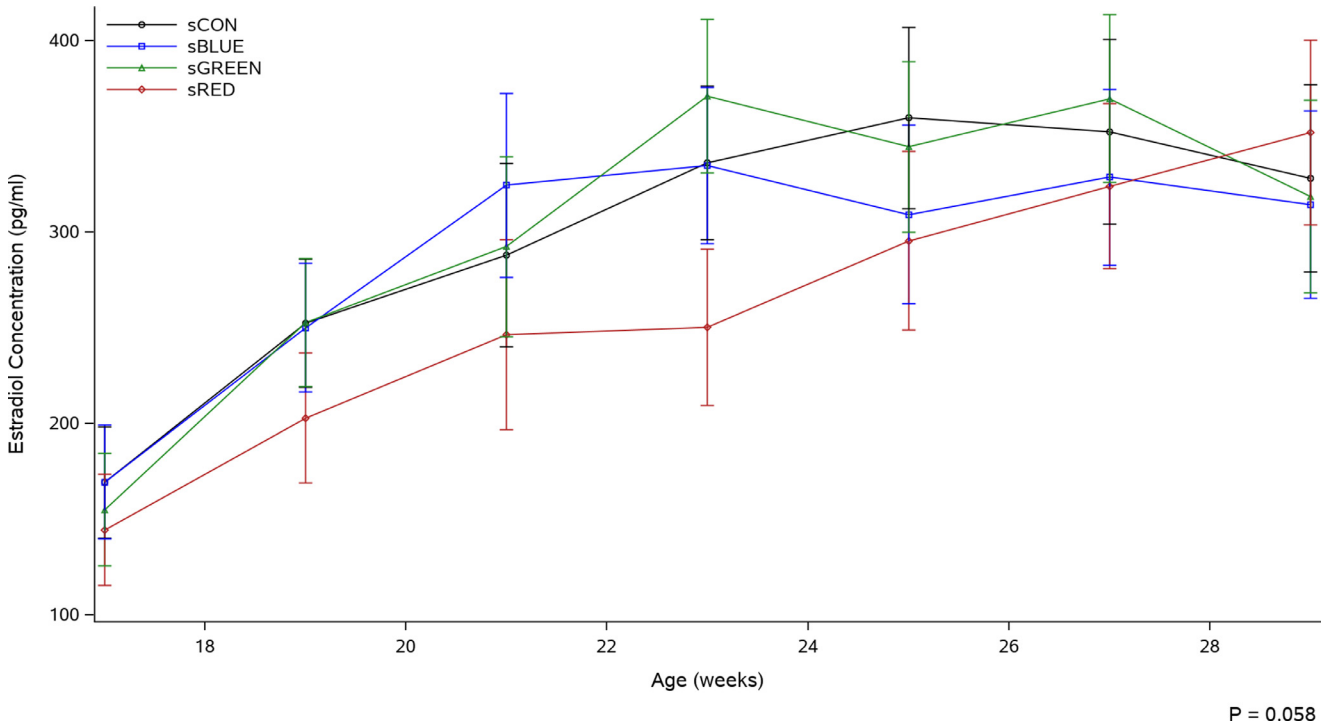

Figure 3. Plasma estradiol (E_2) concentration of broiler breeder hens maintained under blue (sBLUE), green (sGREEN), red (sRED) and control (sCON) supplemental feeder lights (SFL) measured biweekly from 17 to 29 wk of age (woa).

Egg Production

Egg production was recorded per pen daily and displayed in Figure 4 as a percentage of hens housed weekly. DTL had a significant effect on egg production ($P = 0.008$) with the rate of lay 3.15% higher throughout the study in hens under dtRED. There was also an interaction between age and DTL ($P = 0.001$), but pairwise differences were not identified. Concurrent with the delayed entry into lay, sRED had the lowest production rate overall (20.55%; $P < 0.001$) compared to sBLUE (50.51%), sGREEN (49.37%), and sCON (49.32%). This led to an interaction between SFL and age ($P < 0.001$), with sRED displaying a lower weekly production

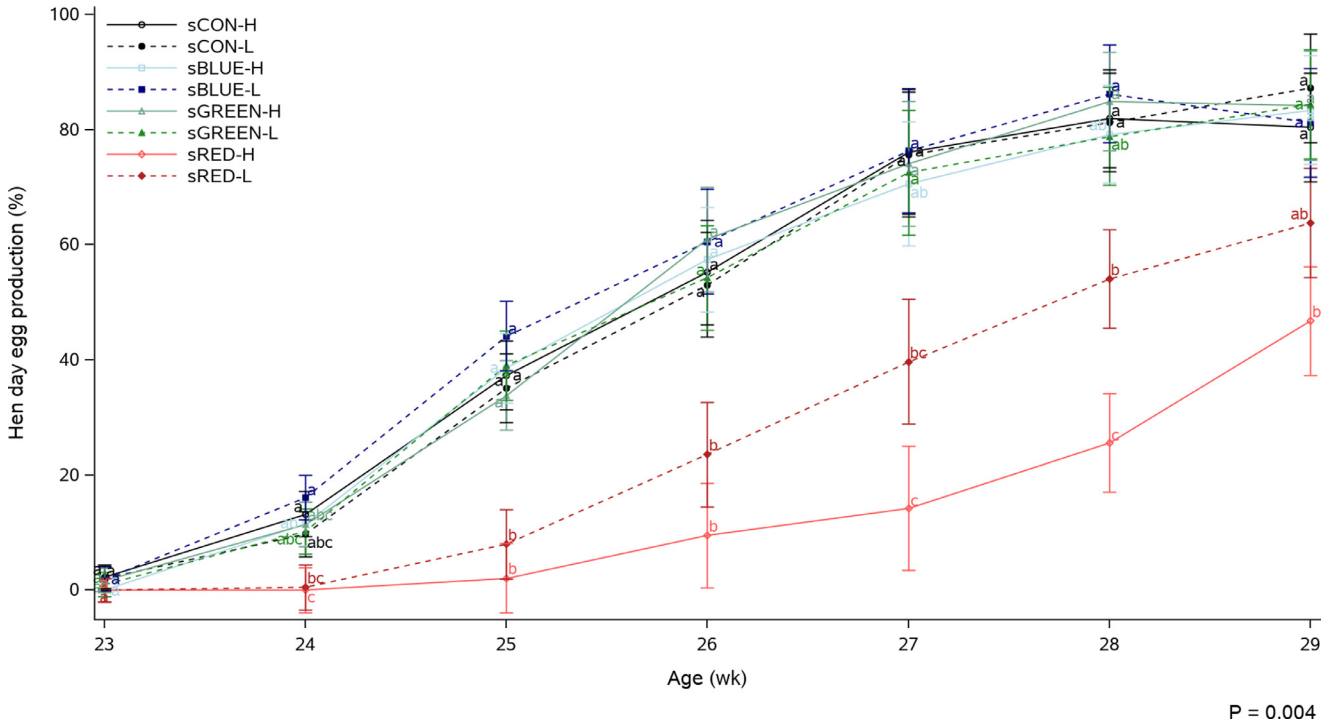

Figure 4. Weekly egg production rate (%) of broiler breeder hens maintained under high (H) and low (L) intensity supplemental feeder lights (SFLs), including control (sCON-H and sCON-L), blue (sBLUE-H and sBLUE-L), green (sGREEN-H and sGREEN-L), and red (sRED-H and sRED-L) from 23 to 29 wk of age (woa). [a-c] Data points lacking a common superscript differ significantly at specific ages ($P < 0.05$).

from 24 to 29 woa. However, this impact was further exacerbated by the effect of INT ($P = 0.002$) and its interaction with SFL and age ($P = 0.004$). No differences were present at 23 woa, yet by 24 woa sBLUE-L and sCON-H displayed the highest weekly laying rate, while sRED-H had the lowest. From 25 to 27 woa, sRED-H and sRED-L had the lowest production rate compared to all other treatments. While sRED-H was lowest at 28 woa, sRED-L did not differ from sBLUE-H and sGREEN-L. By 29 woa, while sRED-L no longer differed from any other treatment, sRED-H remained at the lowest production rate. Interestingly, the interaction between INT and DTL demonstrated that hens under dtRED-L reached an average production rate that was ~6% higher (47.50%) compared to those under dtGREEN-L (41.08%) or any hens under high INT (dtRED-H:40.52%; dtGREEN-H:40.64%). Overall, this resulted in a SFL effect on cumulative egg production ($P < 0.001$), with 14 fewer eggs produced per hen-housed under sRED compared to the other treatments, as shown in Table 1.

DISCUSSION

While strict feed restriction programs control BW during growth and maturation in broiler breeders, concerns regarding flock uniformity and welfare persist within the industry. The introduction of the PF system (Zuidhof et al., 2017) demonstrated a novel approach to feeding programs, providing consistent access to nutrients during the energy-demanding processes of growth and reproduction and subsequently maintaining a more stable energy balance. However, these feeding systems require 24 h access to be used commercially, hence requiring constant illumination. Thus, this study aimed to determine the ideal combination of daytime and feeder supplemental spectrum lighting to ensure optimum reproductive performance.

Body weight remained unaffected during pullet growth regardless of light treatment. Previous studies have reported changes in rearing BW in broiler breeders housed under varying photoperiods due to altered energy partitioning (van der Klein et al., 2020). However, there is no consistent evidence of wavelengths' ability to impact energy partitioning during rearing, as no differences in feed conversion ratio were observed in broilers raised under 23 h photoperiods of red, blue, or green monochromatic LED lighting (Kim et al., 2013). Similar results have also been shown in layer pullets, with no influence of spectrum lighting on BW during rearing (Lewis et al., 2007; Takeshima et al., 2019). Thus, the absence of an effect of lighting on pullet growth was anticipated, mainly due to the strict feeding program of these broiler breeders and the consistent photoperiods between treatments.

The relationship between spectrum lighting and body weight during the laying cycle outlined in the literature remains complex and inconsistent. However, much of the conflicting evidence outlined in these studies results from the management style, feeding level, or lighting source. While studies in laying hens and quail had demonstrated altered growth under red and green lighting (Woodard et al., 1969; Reddy et al., 2012; Baxter et al., 2014; Li et al., 2014), no BW differences were anticipated in the present study due to equal per-bird feed allocations in all pens. Interestingly, BW differences were observed following maturation under the sRED treatment regardless of daytime light, with these hens displaying an elevated weight beginning at 25 woa. In fact, by 30 woa, hens under sRED weighed over 400 g more than any other lighting treatment, despite all hens being provided the same feed allocation. While this appears consistent with the studies in quail and layers demonstrating a heavier BW under red lighting during early lay (Reddy et al., 2012; Li et al., 2014), these previous studies were conducted in birds fed ad libitum, and the additional BW was attributed to increasing reproductive organ weight and ovarian follicles. This was not the case in our study. In fact, maturation was instead delayed under sRED treatment, likely resulting in nutrients being diverted toward growth instead of reproduction, hence the heavier BW observed in this group. This is contrary to many studies reporting that AFE can be reduced under exposure to monochromatic red-light sources (Khosravinia, 2007; Mobarkey et al., 2010; Baxter et al., 2014; Yang et al., 2016; Elkomy et al., 2019). However, it is important to note that the aforementioned studies used photoperiods of 16 h of light or less while our supplemental light treatment was continuous. To our knowledge, the present study is the first to place hens under constant (24 h) monochromatic red LED light sources at the level of the bird during the laying cycle.

As van der Klein et al. (2018) demonstrated, broiler breeder hens must reach a body fat threshold to initiate lay. In the present study, as there were no differences in BW between treatments at 24 woa when hens under sBLUE, sGREEN, and sCON entered lay, this would suggest that the metabolic threshold is not the cause of the delayed AFE under sRED. Interestingly, laying hens and broiler breeders placed under non-stimulatory photoperiods have been shown to enter lay once they are metabolically fit (Ciacciariello and Gous, 2005; Baxter and Bédécarrats, 2019; Ferreira et al., 2019; Bahry et al., 2021; Hanlon et al., 2021). Thus, we hypothesize that the constant exposure to a long (red) wavelength, which is known to penetrate the skull more easily to stimulate the HPG axis (Benoit, 1964; Menaker and Underwood, 1976; Mobarkey et al., 2010), resulted in the desensitization of deep brain photoreceptors. This would effectively impair the activation of the HPG axis, rendering the photoreceptors unable to respond to stimulatory photoperiods or wavelengths, despite the removal of inhibition via GnIH that has been linked to metabolic thresholds (Bédécarrats et al., 2022). Alternatively, it is possible that red light results in an overproduction of GnRH, thus desensitizing its receptor on the anterior pituitary gland. This is supported by Haas et al. (2017), who showed that an

elevation in GnRH mRNA was observed under exposure to LD (18L:6D) white and red fluorescent light, while expression was downregulated under LD blue and SD (6L:18D) white light in Pekin drakes. However, in the case of sRED in our study, the tendency for a lower E_2 concentration at 23 woa compared to the other treatments suggests that this is not the case, with the HPG axis likely downregulated due to the 24-h red exposure. The further delay present in the sRED-H treatment compared to all treatments suggests that intensity can further amplify the desensitization of these photoreceptors. This is particularly interesting, as sBLUE and sGREEN did not observe this same response to intensity. However, no prior studies have investigated the impact of light intensity on the reproductive performance of layers or broiler breeders.

Since the photoperiod was not altered to allow for resensitization of these photoreceptors in this study, it is likely that hens under sRED displayed relative photorefractoriness, as demonstrated in turkey hens returning to lay spontaneously (Siopes, 2005). This may have only been present in the sRED hens due to the ability of the longer wavelengths to penetrate the skull (Foster and Follett, 1985). Conversely, the hens maintained under the shorter blue and green wavelengths likely did not experience this same phenomenon and were able to naturally undergo the dissipation of juvenile photorefractoriness during the maturation process (Benoit, 1964). Additionally, broiler breeder hens exposed to LD as pullets experienced a delayed AFE yet were still able to mature, thus overcoming juvenile photorefractoriness (Lewis et al., 2004). However, it has been shown that broiler breeder hens will spontaneously mature under constant 8 h photoperiods, albeit delayed and at a significantly heavier BW than those reared under photostimulatory periods (Ciacciariello and Gous, 2005). In fact, Ferreira et al. (2019) demonstrated that broiler breeders remaining under non-stimulatory photoperiods of 8 or 10 h of light will still enter lay at ~27 woa. This is likely due to the influence of body weight and metabolic factors, which also control the expression of GnIH (Bédécarrats et al., 2022). Thus, it is possible that hens under sRED lighting overcame the desensitization of the photoreceptors. Alternatively, a previous study by van der Klein et al. (2019) demonstrated that hens can overcome exposure to photostimulatory rearing daylengths (12L:12D) to enter lay, albeit at a delayed age and a higher BW. This provides supporting evidence that elevated BW may lead to an eventual sexual maturation in broiler breeder hens. In fact, there is potentially a BW threshold in which this maturation will occur. In the aforementioned study, hens under SD and LD rearing photoperiods entered lay at ~2.7 and 3.4-kg, respectively. This is consistent with the results of the present study, with hens under sGREEN, sBLUE, and sCON identified to enter lay at ~2.8-kg and those under sRED at ~3.3-kg. Altogether, these results suggest that impeding the impact of photostimulatory cues can be overcome via metabolic status (Zhang et al., 2017). However, the specific mechanism controlling this interaction between photoperiod and metabolic cues remains unknown.

In agreement with the many studies that have suggested improvements in early production rates under red light (Mobarkey et al., 2010; Min et al., 2012; Hassan et al., 2013; Baxter et al., 2014), the present study demonstrated a 3.15% higher rate of lay under dtRED than dtGREEN throughout the study. In an effort to elucidate the mechanisms driving these production differences, Reddy et al. (2012) demonstrated that red light stimulated higher GnRH mRNA expression compared to exposure to incandescent light. A similar result was observed in broiler breeders under 14 h of red combined with 6 h of supplemental blue or green monochromatic LED lights, with GnRH mRNA significantly upregulated during periods of persistently higher production (Zaguri et al., 2020). Thus, this study continues to add to the growing body of literature supporting red spectrum lighting as a daytime light source during the laying cycle. Previous studies have also reported that green wavelengths inhibit GnRH via an elevation in melatonin production in the chick brain of male broilers (Zhang et al., 2017). However, this spectral output did not appear to impact sexual maturation of breeder hens in our study.

In the present study, delayed sexual maturation of the hens under 24 h sRED resulted in a lower production rate throughout the study period. The timing of this delayed AFE aligns with the elevated BW observed in sRED hens, suggesting that while all hens were provided with the same feed allocation, the nutrient partitioning was altered between treatments. Typically, a higher growth curve is associated with earlier photosensitivity (Dunn and Sharp, 1990; van der Klein et al., 2018; Hadinia et al., 2020) and, thus, earlier production. In the case of sRED, these hens continued to store nutrients in the absence of sexual maturation. Conversely, hens under sGREEN, sBLUE, and sCON utilized available energy for the maturation of the reproductive axis and the initiation of the egg formation process, thus altering nutrient partitioning to divert energy away from growth (Leeson and Summers, 2005). This is highlighted by the slower growth curves observed within these treatments, along with a gradual elevation in E_2 concentration by 23 woa, prior to first egg. Interestingly, while previous broiler breeder studies had reported an initial peak at or 1-wk post-AFE (Onagbesan et al., 2006; Hadinia et al., 2020), this present study did not determine a subsequent decline. This may be due to the biweekly sampling paradigm or a consequence of the 24-h exposure leading to a flock desynchronization and larger variation. This was highlighted by a previous study in our laboratory, in which significant individual variation in the timing of E_2 peaks of laying hens was observed, despite similar production rates (Hanlon et al., 2021). Alternatively, E_2 levels in hens under sRED continued to rise up until the end of the study at 29 woa, subsequent to the AFE. This may be attributed to the slowed production rates and the number of hens still undergoing sexual maturation. Regardless, this is one of the first studies to report a disconnect

between the timing of AFE and the E_2 peak. As E_2 is well established to be linked to the processes associated with initiating the production of all egg components (Hanlon et al., 2022), this disconnect requires further investigation. Overall, this provides further evidence that the activation of the HPG axis has been impeded under continuous exposure to the supplemental red-light source.

CONCLUSIONS

In the present study, the results indicate that the implementation of 24 h red LED supplemental lighting is detrimental to sexual maturation and early reproductive performance of broiler breeder hens. Conversely, these effects of supplemental lighting on growth and reproduction were not observed under green and blue lights. Thus, these shorter wavelengths should be considered for the continuous illumination of PF stations. Meanwhile, dtRED demonstrated a 3.15% improvement in production rate compared to dtGREEN, despite no differences in BW. This suggests that while red light is better at stimulating deep brain photoreceptors, continuous exposure desensitizes the HPG axis. Further studies are required to identify the mechanisms driving these differential responses to red wavelengths based on the duration of exposure.

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DISCLOSURES

The authors affirm that there is no conflict of interest.

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