Short-wavelength light induces broiler's behavioral and physiological syndrome through a misaligned eating rhythm

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Abstract: Previous work shows that long-wavelength light has a robust circadian rhythmic pattern in the expression of clock genes of chickens, whereas short-wavelength light leads to an arrhythmic oscillation of some clock genes (e.g., *cClock*, *cCry1*, *cCry2*, *cPer2*, and *cPer3*). However, knowledge about the consequences of LED lights on the physiological and behavioral phenotype was still not clear. This experiment hypothesizes that short-wavelength light disturbs chickens' eating rhythm and leads to the wrong time to eat, resulting in metabolic syndrome. "Meihuang" broilers were housed in monochromatic LED blue light, green light, yellow light, red light, or white light with a very low dose (15 lx). Multiply physiological parameters were measured and the 24-h eating behavior was determined. The effects of LED light on physiological status and behavioral phenotype showed a wavelength-dependent manner. Short-wavelength light significantly decreased the level of total triglycerides and total cholesterol but increased triiodothyronine concentration. Inversely, long-wavelength light increased the triglycerides and total cholesterol and reduced the level of triiodothyronine. Further, it was found that short-wavelength light significantly boosted body weight compared with long-wavelength light, despite equivalent levels of food intake. Short-wavelength light-induced 23.4% and 14.1% of food consumption during subjective nights, but long-wavelength light did not. These results imply that when chickens eat matters, not just what they eat. Thus, low as 15 lx of blue light exposure during the typical dark period is sufficient to lead an individual to eat at the "wrong" time, causing metabolic dysfunction. Blue light should be cautiously considered to be used in the poultry breeding process.

Keywords: short-wavelength light, broiler, behavior, physiology, environmental control, circadian rhythm, intermittent eating
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1 Introduction

The use of artificial light at night has rapidly increased in recent decades, causing negative impacts on sleep, health, and metabolism[1,2]. The current trend in global lighting is shifting from "yellow" sodium lamps toward a new generation of broad-spectrum, energy-efficient, "white" light-emitting diodes (LEDs)[3]. Replacement of traditional lighting technologies with energy-efficient LEDs is being implemented worldwide to decrease CO_2 emissions, environmental impacts, energy consumption, and lighting costs[4]. However, the metabolic and physiological consequences of LED lighting remain poorly understood. Recent studies have raised awareness that artificial night lighting can have other, more subtle effects on individuals, in particular, effects related to the modification of circadian rhythms. Especially, white LEDs emit a high content of blue spectra, which may disrupt circadian regulation.

Biological rhythms are fundamental to the behavior and physiology of organisms from *Drosophila* to humans, with 24 h oscillations that are driven by a circadian pacemaker[5,6]. In mammals, the circadian pacemaker is located in the hypothalamic suprachiasmatic nucleus (SCN)[7]. SCN serves as the master circadian clock controlling behavioral and physiological rhythms, which is entrained by environmental light, while the peripheral clocks in tissues such as the liver are entrained by food intake[8]. Daily couplings between the SCN and peripheral clocks regulate behavior, physiology, and metabolism to set temporal oscillators in homeostatic regulation. However, because of the sophisticated composition, the circadian system is more complex in non-mammalian vertebrates than in mammals, including not only SCN, but also the retina and the pineal gland[9-11]. In the case of avian species, the chick pineal gland as a pacemaker is suggested to function similarly to the mammalian SCN[12]. The pineal gland has the primary role of synthesizing and releasing the hormone melatonin[13]. Phase coherence of circadian oscillators is achieved by entraining the pacemaker to the environmental light. The pacemaker coordinates activities and eating rhythms, thus setting the timing of food intake, energy expenditure, rest, and basal

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metabolism. Besides, with the opsin-based photopigments and cryptochromes, the pineal gland directly receives external photic information and responds to light entrainment in broilers[14,15].

Multiple studies have linked disrupted circadian clock and metabolic disorder[16,17]. Clock mutants show profound changes in circadian rhythmicity as well as body weight gain[16,18]. Further, circadian rhythms are wavelength-dependent, which show the highest sensitivity to the short-wavelength light in mammals[19-21]. Reports showed that the short-wavelength-enriched light delayed circadian rhythm[22] and suppressed melatonin secretion[23,24]. In consistency with mammals, the spectral composition of light plays a vital role in the avian circadian system. A previous study suggested that the wavelength-dependent responses of the photoperiodic clock could be part of an adaptive strategy in the evolution of seasonality in reproduction[25]. The spectral composition of light also synchronized the circadian physiology in blackheaded buntings[26] and affected the melatonin rhythm in chicks[27,28]. Blue light and red light showed different roles in regulating circadian behavior and plasma melatonin level in *E. melanocephala*[26]. Cryptochrome (CRY) is a short-wavelength light-sensitive photo-pigment in the chick pineal gland. Blue light advanced the acrophases of the *cCry1*, *cCry2*[29].

The chicken acts as an excellent model for assessing the photobiology responses of artificial light. The previous studies showed the authors that the expression of genes (both positive and negative core clock genes) were wavelength-dependent in chicken. Long-wavelength LED light maintains a robust circadian rhythmic pattern in clock genes expression of chickens, whereas short-wavelength LED light results in arrhythmic oscillation of some clock genes (e.g., *cClock*, *cCry1*, *cCry2*, *cPer2*, and *cPer3*)[30]. However, there is little information about the consequences of LED lights on the physiological and behavioral phenotype. Here, it can be hypothesized that short-wavelength light disturbs chicken's eating rhythms and leads to the wrong time to eat, resulting in metabolic syndrome. Two empirical experiments were used to test this hypothesis by subjecting chickens to various spectral components of LED light and measuring multiple physiological traits and the circadian rhythm of the behavioral phenotype.

2 Materials and methods

2.1 Experimental environment

Animal. Upon arrival, all broilers ("Meihuang"; age 1 d; mean body weight 30.5 g) were raised at an ambient temperature of (33±2)°C in the first two weeks and (22±1)°C in the following period. Birds were provided with a regular diet (13.4 MJ Metabolizable energy (ME)/kg; 220 g/kg crude protein in the first two weeks, followed by a 13.6 MJ ME/kg, and 200 g/kg crude protein for the remainder of the experiment) and filtered tap water *ad libitum*. All broilers were randomly housed in four light-controlled rooms and each light-controlled room was divided into five equal-sized pens (1 m×1 m×1 m). Each pen had its own independent light system and was covered with fluorescent fabrics to avoid light pollution from other sources. Each pen of treated birds (n=15, n is the number of birds in the group) was exposed to either red light (λ=620 nm, RL group), yellow light (λ=580 nm, YL group), green light (λ=514 nm, GL group), blue light (λ=455 nm, BL group) or white light (λ=380-780 nm, WL group). Totally 300 birds were used. Each light treatment contains four replicates, and each replicate contains 15 birds.

The light intensity was adjusted to the same level according to the spectral sensitivity[31]. All birds were maintained under a 16:8 light/dark cycle (lights on at 08:00, off at 24:00) with 15 lx. All the illumination is provided by LED lamps (Langtuo Biological Technology Co. Ltd., China). The detailed parameters of those LED lamps can be found in Figure S1. Animal care and experimental procedures were under the Animal Research Committee guidelines of Zhejiang University.

2.2 Physiological measurement

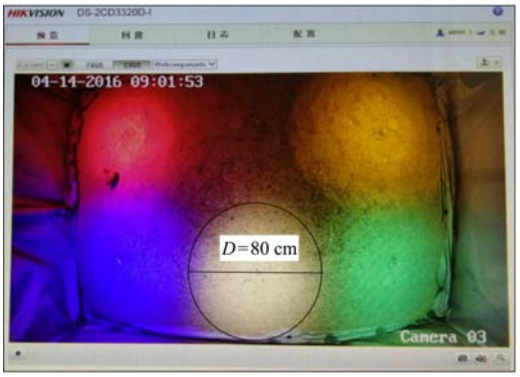
The food consumption was measured by recording the supplied and remaining amounts daily. Body weight was individually measured using an electronic scale with an accuracy of 0.1 g (CW Electronic Scale, Lisite, Inc., Zhejiang, China) weekly. A solid board covered with the plastic film was placed 0.35 m below the pens to collect manure. Detailed methods for broiler manure collection were described in our previous study[32]. The fecal organic substance (FOS) of the thawed manure samples was measured at 25°C according to the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (NREL, 2008). At the end of the experiment (42 d of age), three birds were randomly selected from each replicate pen so that each replicate pen was represented equally. The selected birds were killed by cervical dislocation to collect 5-mL blood samples. The blood samples were centrifuged at 4°C for 30 min at 3000 r/min to separate the serum. The serum was transferred into polypropylene microcentrifuge tubes and stored at −70°C for subsequent use. Thyroid hormone secretion (triiodothyronine: T3, and thyroxine: T4), total triglycerides concentration (TG), total cholesterol concentration (TC), and glucose concentration (GLU) were determined by an ELISA kit with intra- and inter-assay coefficients of variation (% CV) of (1.5±0.4)% and (3.2±1.3)% (Jiancheng Bioengineering, Nanjing, China) respectively. The limit of sensitivity of those parameters assay was 0.3-0.6 ng/mL. Nutritional ion concentration (Ca and P), and electrolytes concentration (K^+ and Na^+) were determined using an Automatic Biochemistry Analyzer (AU5400, Olympus Co. Ltd., Japan).

2.3 Behavioral measurement

A tailored behavioral assay system was used to test the behavior patterns of chickens exposed to different light environments (Figure 1). We installed the surveillance video (HIKVISION NO. DS-2CD3320(D)-I) on the top of the behavioral test system. The size is length × width × height (240 cm×160 cm×200 cm). The top of the system was divided into six parts, of which one part was not equipped with lamps as a control (Black), and the other five parts were respectively installed with RL-, YL-, GL-, BL-, and WL-LEDs. Each LED lamp circumscribed a light channel device with a radius of 5 cm, so that the projection area of each lamp projected to the bottom of the device was the same size, and the projection area was a circle with a diameter of 80 cm. The illuminance of each lamp in the behavioral test system was set at 15 lx, and the illuminance under each lamp was guaranteed to be consistent through a stepless dimmer (Philips NO. SED-200A). During the test, the lighting area changes randomly to prevent the chickens from generating environmental inertia.

The behavior of the birds was video recorded for 48 continuous hours per week for each replicated pen at 35 d of age. Instantaneous scan sampling was used to decode the behavioral expression from the electronic media at an interval of 1 min for an observation period of 24 h. The recorded behaviors include eating, walking, standing, sleeping, wing flapping, and looking around. It is very difficult to differentiate between resting and sleeping, thus they were jointly categorized as sleeping. For calculating the percentage of time budget for a particular behavior, occurrence

over the 1 min intervals was calculated and then averaged over the 24 h period. Further, diurnal rhythms of eating behavior of each group were recorded at four zeitgeber time phases (ZT), including ZT 2-4 (Morning phase), ZT 8-10 (Afternoon phase), ZT 14-16 (Evening phase), and ZT 20-22 (Night phase).



Note: *D* is the diameter of the projection area, cm.

Figure 1 Screenshot of behavioral measurement system

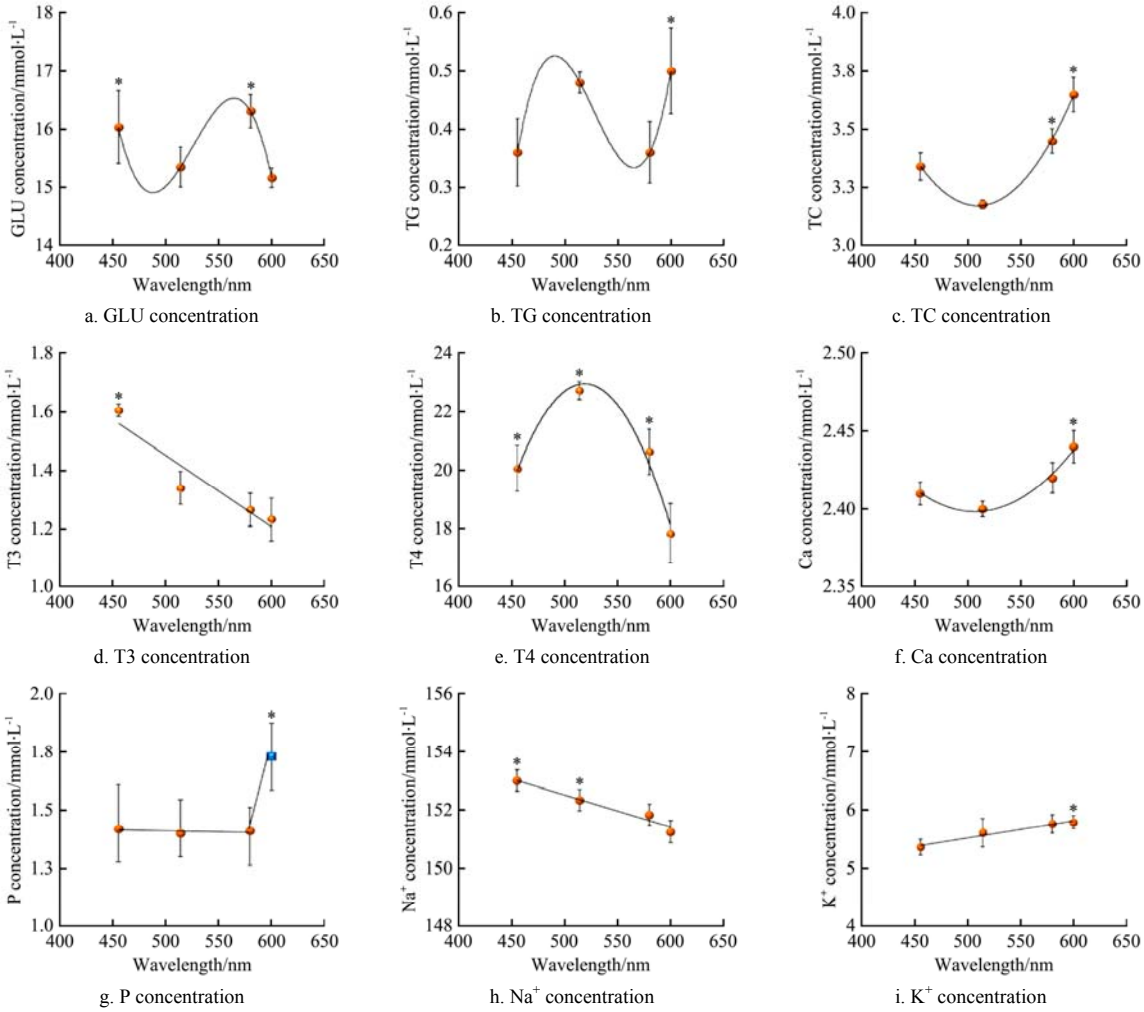
2.4 Statistical analysis

Data are presented as means±SD. Grubbs' test was used to detect outliers, which were discarded before subsequent analyses. Statistical analyses were performed using SPSS Statistical Software (V. 20). Data were processed with analyses of variance (ANOVA) with repeated measures. If significant main effects were detected (p<0.05), post hoc comparisons were performed with Duncan's test. The student's t-test was used for single statistical comparisons. The level of statistical significance was set at bilateral 5%. Correlations between body weight and eating frequency were made using Pearson's correlation. Associations of metabolic variables with wavelength were assessed by linear, quadratic, and polynomial regression analysis. Principal component analysis (PCA) was performed after normalization of physiological data using Matlab 2017b.

3 Results

3.1 Physiological states

An ANOVA indicated that the birds exposed to blue light (BL) had greater GLU concentrations in the blood in contrast to birds exposed to red light (RL) (p=0.012) (Figure 2a), whereas no significant difference was observed in birds exposed to green light and red light (p=0.280). A polynomial regression model was applied to depict the relationship between light wavelength and GLU levels: GLU=y=$-7\times10^{-6}\times$wavelength^3+0.0114×wavelength^2−5.9624 × wavelength+1050.3, $R^2=1$, p<0.01. Reverse to GLU levels, TG concentrations were significantly greater on birds raised with red light compared with blue light (p=0.036) (Figure 2b). No significant differences were observed between birds raised with red light (RL) and green light (GL) (p=0.98). The phase of the regression model of TG is just opposite to that of GLU: TG=$9\times10^{-7}\times$ wavelength^3 − 0.0015 × wavelength^2 + 0.7667 × wavelength − 33.06, R^2=1, p<0.001.



a. GLU concentration b. TG concentration c. TC concentration

d. T3 concentration e. T4 concentration f. Ca concentration

g. P concentration h. Na^+ concentration i. K^+ concentration

Note: Thyroid hormone secretion (triiodothyronine: T3, and thyroxine: T4), nutritional ion concentration (Ca and P), total triglycerides concentration (TG), total cholesterol concentration (TC), glucose concentration (GLU), and electrolytes concentration (Na^+ and K^+) were altered in birds exposed to short-wavelength light.. p <0.05.

Figure 2 The metabolic disorder induced by chronic artificial light exposure as a function of light wavelength in chicks

In contrast to blue light-treated birds, yellow (YL) and red light-treated birds obtained significantly greater TC concentrations (p=0.02) (Figure 2c). However, no differences were observed among those treated by green, yellow, and red light (p = 0.32). Further, a quadratic regression was fitted to describe the relationship between the TC concentrations and the light wavelength: TC=$6\times10^{-5}\times$wavelength$^2-0.0586\times$wavelength+18.112, R^2=0.9989, p<0.001.

For T3 concentrations, the birds raised with blue light were significantly greater than the birds raised with longer wavelength light (YL: p<0.01; RL: p<0.01) (Figure 2d). No significant differences were observed among the longer wavelength light (p=0.91) (T3=−0.0024×wavelength+2.6677, R^2=0.899, p<0.001). A quadratic model was established between the light period and T4 concentrations in blood (T4 = $-7\times10^{-4}\times$wavelength^2 + 0.7632 × wavelength − 175.02, R^2 = 0.9747, p<0.001) (Figure 2e), which showed that the maximum T4 concentration in blood occurred when green light was used. Moreover, an ANOVA indicated that birds raised with green and blue light reached greater T4 concentrations in blood compared with the birds raised with red light (BL: p = 0.02; GL: p = 0.003), whereas no significant differences were found among birds raised with yellow, green, and blue light (p=0.13).

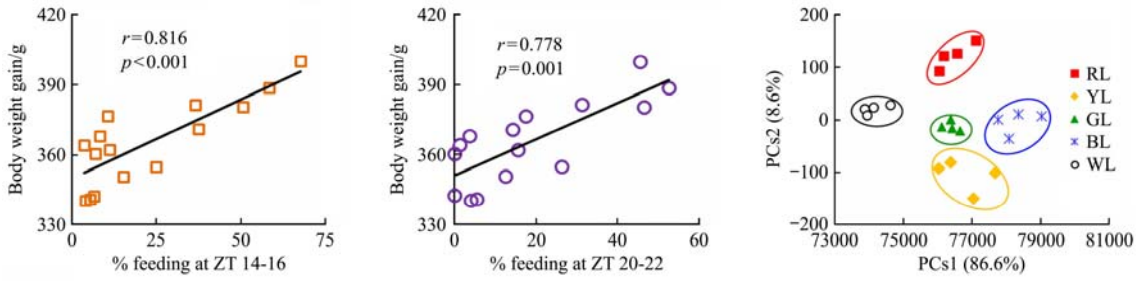
Ca is a ubiquitous second messenger in almost all cells that regulates various cell functions including gene expression, cell migration, neural activity, and muscle contraction[33]. There is a positive correlation between shank weight and Ca levels as well as body weight. Nutritional ion absorption (Ca and P) was reported vital to affect skeletal growth[34]. For nutritional ion status, Ca level in birds exposed to red light reached their greatest compared with other light wavelengths (p=0.047) (Figure 2f). Ca levels in birds exposed to blue, green, and yellow light did not significantly differ from each other (p=0.91). Ca levels exhibited a quadratic response to light wavelength (Ca=$5\times10^{-6}\times$wavelength$^2-$0.0046×wavelength+3.5726; R^2=0.9839; p<0.01). A greatest P concentration was found in birds raised with long wavelength light (RL: p<0.01) (Figure 2g). A broken-stick analysis suggested that T4 concentration would be similar for birds exposed to blue, green, and yellow light (p=0.99).

The monovalent ions (Na^+ and K^+) are the critical minerals for participating in the acid-base balance of the body fluids[35]. Changes of K^+ level were requisite for altering metabolism, because it has been proved that potassium ions are more involved in metabolic processes including amino acid absorption and transport, protein synthesis and acid-base balance[36]. Also, some clock neurons were reported to drive their rhythmic activity and daily behavior by employing daily antiphase K^+ and Na^+ conductances[37]. Na^+ levels differed significantly with light wavelength (p=0.03) (Figure 2h). Regression analysis indicated that Na^+ levels were negatively correlated with light wavelength (Na = −0.0111 × wavelength + 158.04, R^2 = 0.9601, p < 0.001), demonstrating the greatest Na^+ level was researched by the birds raised with blue light. In contrast to Na^+, the greatest K^+ levels were observed in birds raised with red light, which was significantly greater than in birds raised with blue light (p=0.045) (Figure 2i). Naturally, K^+ levels positively correlated with light wavelength (K=0.0029×wavelength+4.0541, R^2=0.9672, p<0.001).

To reveal the physiological status of the light-treated birds, PCA was adopted on all the sixty physiological parameters (three technical replicates for each biological replicate and four biological replicates from each group), then twenty principal components (PCs) were generated. The variances explained by the first two PCs are shown in the labels of Figure 3c. It can be found that the PC1 could explain 86.6% variance of all physiological parameters, and variances explained by the second PC decreased to 8.6%. In total, the accumulative variance of the first two PCs was up to 95.2%. So, it could be concluded that the first two PCs could explain most of the variance of the sixty physiological parameters. After PCA, a series of new PCs were generated with the reconstruction of the sixty physiological parameters, so every sample could be denoted with the PCs, and the score plot is a description of samples in the new PCs space. As the PCA had compressed most variance of the physiological parameters into the first two PCs, the score plot of the first two PCs may reveal important information of recognition. Figure 3c is the score plot of the first two PCs. It could be seen that all the samples of each light-treated bird were clustered together, and the boundaries of different light groups were absolutely clear.

3.2 Body weight and energy intake

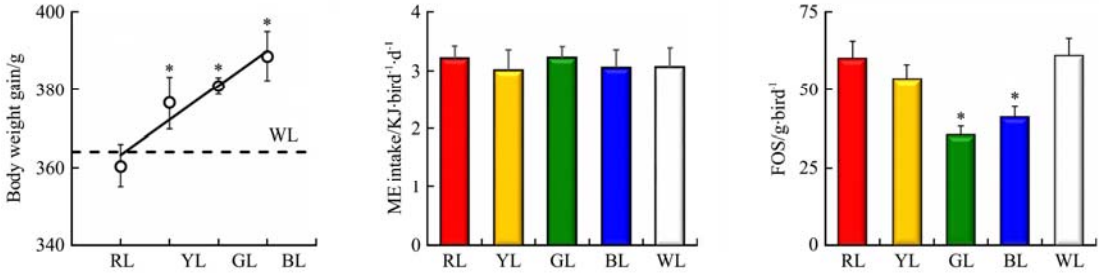
An ANOVA indicated that the body weight gain was significantly affected by the light wavelength (p=0.031) (Figure 4a). The birds raised with blue light were significantly heavier than the birds raised with long-wavelength light (RL: p<0.01; YL: p=0.025). Regression analysis indicated that the body weight gain responded to light wavelength in a negative linear fashion (body weight = −0.1536×wavelength+459.97, R^2=0.8752, p<0.001) (Figure 4a), which suggested that longer-wavelength light exposure inhibited body weight.



a. ZT 14-16 b. ZT 20-22 c. Scores of the first two principal components (PCs) of metabolic parameters

Note: a. Percentage of eating behavior during ZT 14-16 correlated positively with body weight gain (r=0.816, p<0.01). b. Percentage of eating behavior during ZT 20-22 correlated positively with body weight gain (r=0.778, p=0.01). c. Scores of the first two principal components (PCs) of metabolic parameters from RL, YL, GL, and BL. PCs plots explained 86.6% and 8.6% of the variance of metabolic parameters. Samples of each group were clustered together, and the boundaries of different groups were absolutely clear, suggesting artificial light effects on physiological homeostasis in a wavelength-dependent manner.

Figure 3 Body weight gain is associated with eating behavior percentage during subjective nights (ZT 14-16 and ZT 20-22)



a. Body weight gain b. The total metabolizable energy (ME) c. Fecal organic substance (FOS)

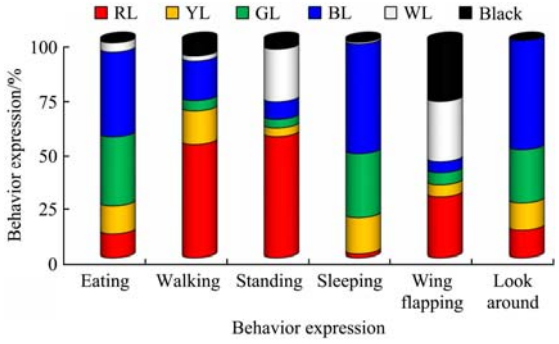
Note: a. Increased body weight of 42-day-old-birds compared to the end of the brooding period exposed to red light (RL), yellow light (YL), green light (GL), blue light (BL), and white light (WL). Chicks exposed to short-wavelength light (BL and GL) had elevated body weight. Data are expressed as the mean value±SD, $p<0.05$ using ANOVA. b. The total metabolizable energy (ME) for RL, YL, GL, BL, and WL groups. There were no differences in the total ME among groups. c. Fecal organic substance (FOS) differed among groups. The FOS residual was less in the short-wavelength light treatment (BL and GL). Data are expressed as the mean value ± SD, $p<0.05$ using ANOVA.

Figure 4 Contribution of light wavelength to body weight gain

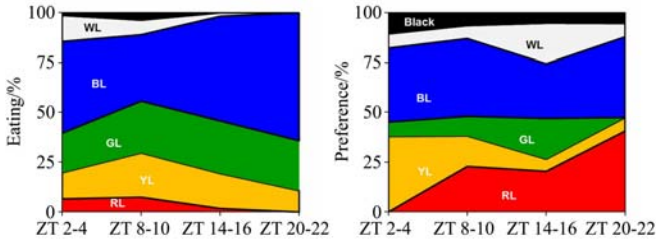
Total 24-h metabolizable energy (ME) intake did not differ among groups (p=0.24) (Figure 4b). Although no differences in total energy intake, the residual energy was measured among groups, finding that fecal organic substance (FOS) was less in the blue light treated birds (Figure 4c).

3.3 Daily behavior phenotype

Significant differences were observed in various behavior expressions among groups. Birds raised with blue and green light expressed more eating behavior than did birds raised with red light (BL: p=0.031; GL: p=0.024) (Figure 5a). However, walking behavior in red light-treated birds was significantly greater than those in blue ($p<0.01$) and green light ($p<0.01$). Standing was similar to walking. Birds raised with blue light had more sleeping ($p<0.01$) and preference ($p<0.01$) than birds raised with red light.



a. The diurnal distribution of behavior



b. The diurnal distribution of the eating behavior c. The diurnal distribution of preference expression

Note: a. BL and GL significantly increased the eating behavior percentage, whereas RL increased the walking and standing behavior percentage. The diurnal distribution of the eating behavior b. and preference expression c. from each group. Chicks exposed to BL and GL ate more food than RL and WL during subjective nights (ZT 14-16 and ZT 20-22).

Figure 5 Averaged behavior expression percentage of 42-day-old-birds exposed to various light treatments

Further, the diurnal rhythm of eating and preference behavior was examined (Figures 4b and 4c). Eating frequency in ZT 2-4 and ZT 8-10 were similar for all light groups. However, birds raised with blue light consume 23.4% of food during ZT 14-16, as compared with 6.2% food in birds raised with red light and 9.1% in white light (Figure 5b).

During ZT 20-22, birds raised with blue light consume 14.1% of food, whereas birds raised with red and white light did not intake any food (Figure 5c). Moreover, correlation analyses confirmed that the percentage of eating frequency in ZT 14-16 and ZT 20-22 were positively related to body weight gain (r=0.816, $p<0.001$; r=0.778, p=0.001) (Figures 3b and 3c).

4 Discussion

In this study, it was found a significant increase in body weight gain among 42-day-old-birds in the blue light and green light groups, relative to the red light group. In contrast, red light and white light showed non-significant differences in body weight gain. Because body weight gain normally coincides with excess calorie intake[38,39], whether the greater body weight in blue light and green light was due to the extra energy intake was considered. No differences in metabolizable energy intake were observed among all the light treatments, indicating that body weight gain in blue light and green light was not due to the excess energy intake. However, by measuring the FOS residual from each group, less FOS in blue light and green light was found significantly, relative to white light (48.5% and 72.2%). Therefore, although the food intakes of broilers were not altered by the short-wavelength light, the substrates' emissions were decreased by the short-wavelength light. Green and blue light could increase the villus height of the small intestine[40], suggesting a better mucosal structure of the small intestine, resulting in superior intestinal absorption[41]. In the white light group, the substrate intake and emission were balanced, leading to a regular body weight gain. The substrate intake from short-wavelength light treatments was similar to that in the white treatment, whereas the absorption of the nutrients was enhanced by short-wavelength light, which disturbed the normal energy homeostasis.

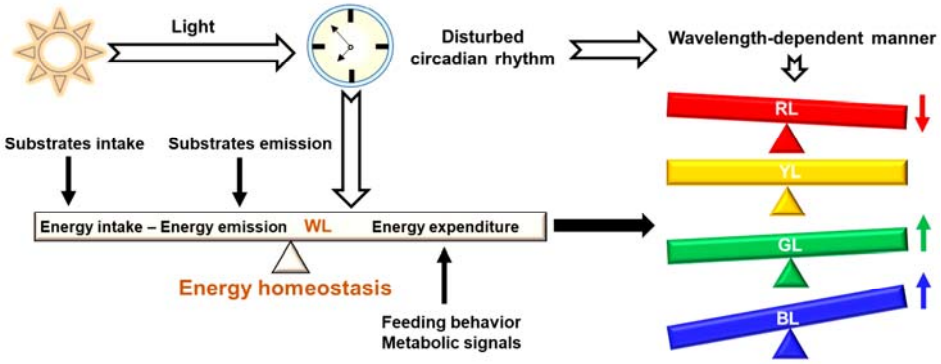
An important component of nutrient homeostasis in many terrestrial vertebrates is the coordination of daily rhythms in rest and activity, eating behavior, energy utilization, and energy storage across the daily light cycle. Thereby, whether the behavior was altered by the short-wavelength light was examined. The total eating behavior frequency in blue light and green light was greater than those in white light, indicating that short-wavelength light increased eating behavior expression. However, the expression of the energy expenditure behavior such as walking and standing were greater in red light than in blue

light (walking: 193%; standing: 601%) and GL (walking: 1000%; standing: 1342%). Further, the different total eating behavior frequencies might be accomplished with different body weights (Table S1). The daily rhythms in eating behavior and preference expression significantly differentiated were observed between blue light and white light, suggesting that although no differences in total food intake were observed among groups, eating behavior was altered in the blue light and green light.

Further, the daily food intake frequency was measured at four zeitgeber time phases (ZT 2-4, ZT 8-10, ZT 14-16, and ZT 20-22). Food intake frequency in ZT 2-4 and ZT 8-10, as measured in the pen via intersecting Charge Coupled Device (CCD) camera, were similar for all light treatments. However, blue light consumes 23.4% of food during ZT 14-16, as compared with 6.2% food in red light and 9.1% in WL. During ZT 20-22, BL consumes 14.1% of food, whereas red light and white light did not intake any food. Moreover, correlation analyses confirmed that the percentage of food intake in ZT 14-16 and ZT 20-22 were positively related to body weight gain. Blue light displays asynchrony between internal metabolic activity and food intake, as suggested by the disturbed rhythm of food consumption. The desynchronization may be the primary factor causing abnormal body weight gain. Short-wavelength light may have disrupted melatonin signaling leading to a misalignment of food intake and resulting in altered fuel metabolism. Disturbed timing of food intake coincides with metabolic syndrome in other animal models[16,42]. Blue light and red light differentiated circadian behavior and plasma melatonin level in blackheaded buntings[43]. In total, the present study found that nine types of metabolic signal variations were induced by chronic artificial light exposure as a function of light wavelength. Short-wavelength light significantly decreased the level of total triglycerides and total cholesterol, whereas increasing triiodothyronine concentration. Inversely, the long-wavelength light increased the triglycerides and total cholesterol and reduced the level of triiodothyronine. Furthermore, the PCA combined with sixty metabolic parameters demonstrated that each light group was clustered together, and the boundaries of different light groups were absolutely clear, suggesting artificial light has effects on physiological homeostasis in a wavelength-dependent manner.

Metabolism and the circadian clock are intrinsically related[44], with desynchronized eating and behavior expression inducing metabolic alterations[45]. A misaligned circadian rhythm leads to adverse metabolic and cardiovascular consequences[46]. Short-wavelength light increased the *cClock* and *cBmal1* gene expression, followed by a corresponding great expression of the mRNA level. However, the long-wavelength light decreased the expression of these core clock genes[47]. These results indicate that apart from a light cycle, the spectral composition of light also programs circadian time generation and changes the circadian cycle process. The seemingly innocuous manipulation of environmental light used in this study may have important implications for humans. Night-eating syndromes in patients have coincided with increased body mass index[48]. Moreover, the use of light-emitting electronic devices for reading, communication, and entertainment has greatly increased recently. Light from the electronic devices is short-wavelength-enriched (blue component). The use of these devices delays the circadian clock and suppresses melatonin, compared with white light[22].

Improvement in the eating-fasting cycle that sustains a robust circadian oscillation in peripheral organs imparts health benefits. For example, wild-type mice fed a high-fat diet only during normal waking hours by restricting the time of eating showed a larger amplitude of expression of circadian clock genes and staved off obesity, metabolic dysfunction, and liver damage compared to mice fed ad libitum[49,50]. The present study implied that when animals eat matters, not just what they eat. Thus, low as 15 lx of blue light exposure during the typical dark period is sufficient to lead an individual to eat at the "wrong" time, causing metabolic dysfunction (Figure 6).



Note: Short-wavelength light disturbs the energy homeostasis through the disorder of eating behavior and metabolic signals. Environmental light is perceived by the pineal gland and suprachiasmatic nucleus, which modulates the circadian regulation of energy homeostasis. Short-wavelength light delays the circadian rhythm, resulting in prolonged eating behavior. During subjective nights (ZT 14-16 and ZT 20-22), short-wavelength light still induced a great amount of eating

Figure 6 Proposed model on the spectral composition of light programing body weight gain

5 Conclusions

The following conclusions can be drawn from this study.

1) Short-wavelength light significantly decreased the level of total triglycerides and total cholesterol but increased triiodothyronine concentration. Inversely, long-wavelength light increased the triglycerides and total cholesterol and reduced the level of triiodothyronine.

2) Short-wavelength light significantly boosted body weight compared with long-wavelength light, despite equivalent levels of food intake. Short-wavelength light-induced 23.4% and 14.1% of food consumption during subjective nights, but long-wavelength light did not.

3) Short-wavelength light disturbs the energy homeostasis through the disorder of eating behavior and metabolic signals. Environmental light is perceived by the pineal gland and suprachiasmatic nucleus, which modulates the circadian regulation of energy homeostasis. Short-wavelength light delays the circadian rhythm, resulting in prolonged eating behavior.

4) Low as 15 lx of blue light exposure during the typical dark

period is sufficient to leads an individual to eat at “wrong” time, causing metabolic dysfunction. Blue light should be cautiously considered to be used in poultry breeding process.

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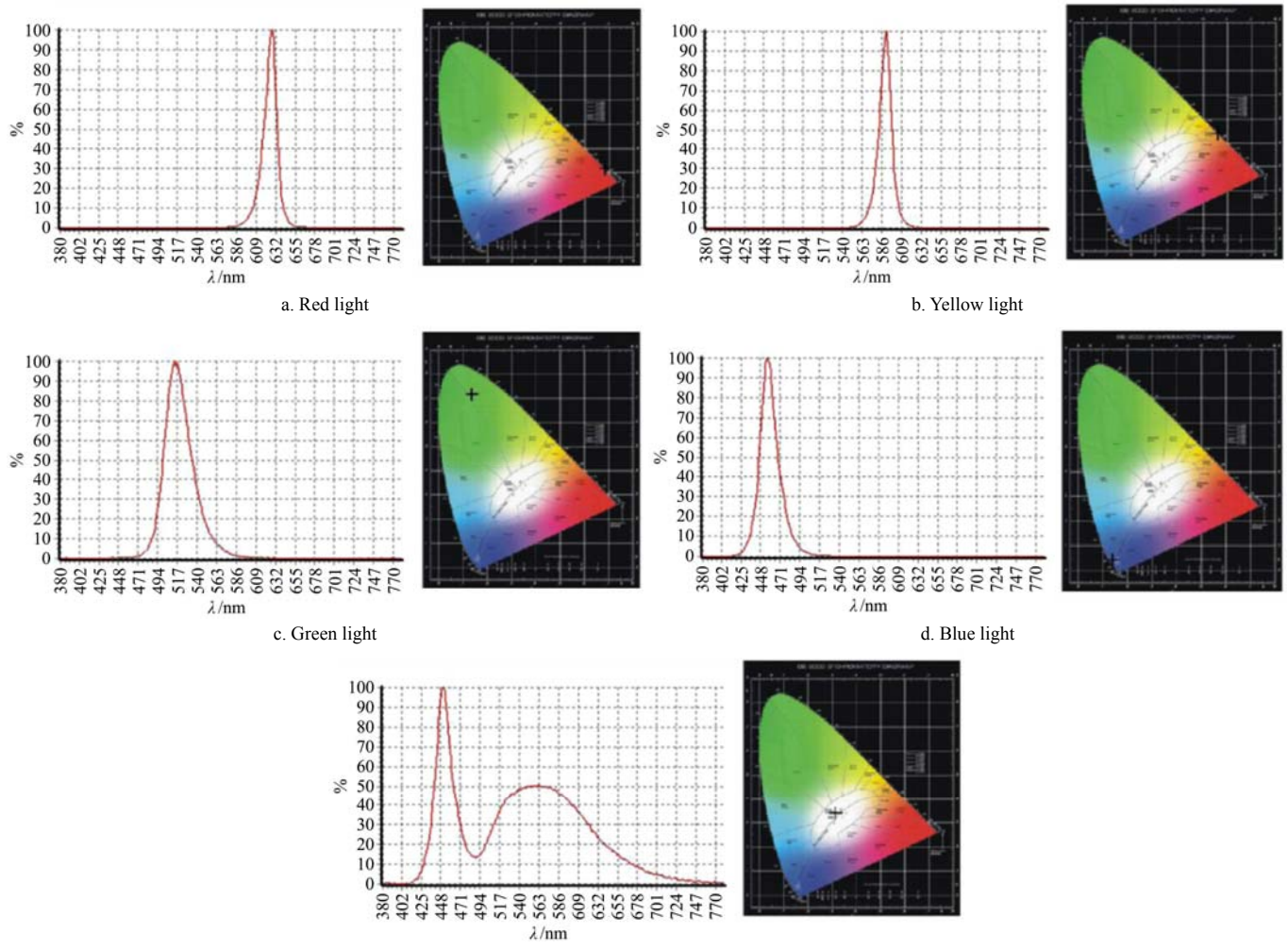
Appendix



a. Red light

b. Yellow light

c. Green light

d. Blue light

e. White light

Note: Red light (620 nm, RL group), yellow light (580 nm, YL group), green light (514 nm, GL group), blue light (455 nm, BL group) and white light (WL group) used in this study. Horizontal axis is light spectrum in nm and vertical axis is relative spectral power.

Figure S1 Comparison of spectrum readings of the three different lighting sources

Table S1 Body mass, growth rate, food intake, ME intake, FOS, and FOS/I of chick from each group

Item	RL	YL	GL	BL	WL
Body mass/g	360.4±5.6^{c}	376.6±6.7^{bc}	381.0±2.2^{ab}	388.7±6.2^{a}	364.1±2.4^{c}
Growth rate/%	98.9±2.3^{c}	103.4±2.8^{abc}	104.6±0.9^{ab}	106.8±2.5^{a}	100±0.9^{c}
Food intake/g·(bird·d)$^{-1}$	236.2±15.7	221.5±25.3	236.7±14.1	224.7±22.1	225.0±24.1
ME1 intake/kJ·(bird·d)$^{-1}$	3.21±0.21	3.01±0.34	3.21±0.19	3.05±0.30	3.06±0.33
FOS2/g	60.3±5.2^{b}	53.7±4.6^{b}	35.5±2.8^{a}	31.1±3.3^{a}	61.2±5.3^{b}
FOS/I3/g·g^{-1}	25.5±3.0^{b}	24.2±2.9^{b}	15.0±2.0^{a}	18.3±2.3^{a}	27.2±3.2^{b}

Note: a, b, and c mean $p<0.05$ indicates significant difference (ANOVA). ME: Metabolizable energy; FOS: Fecal organic substance; FOS/I means Fecal organic substance/food intake; RL: Red light; YL: Yellow light; GL: Green light; BL: Blue light; WL: White light.