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Sustainable poultry practices: integrating green light interventions to control pecking in chicken

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Abstract

Background The present study aimed to investigate the impact of the light-emitting diode (LED) green light alone or in combination with melatonin on pecking-related hormone regulation during incubation under normal and under hormonal stress conditions in breeder eggs. This study was divided into 2 experiments: In the first experiment effect of LED green light incubation on pecking-related hormones under normal conditions, on Hy-line brown (low pecking phenotype) and Roman pink (high pecking phenotype) eggs were tested. The 296 eggs of each strain were divided into two groups: LED green light incubation and dark incubation (control), each containing four replicates (37 eggs/replicate). The second experiment was conducted to evaluate the effect of LED green light incubation alone or in combination with melatonin under hormonal stress conditions on Roman pink eggs. A total of 704 Roman pink eggs were taken and divided into four groups, each consisting of 176 eggs. Each group was further divided into 2 subgroups, LED green light-regulated incubation and dark incubation with 88 eggs per subgroup, having 4 replicates of 22 eggs each. The groups were as follows: corticosterone solution injection (CI), corticosterone + melatonin mixed solution injection (CMI), Phosphate buffer solution injection (PI), and no injection (UI).

Results Results of the first experiment revealed a higher level of serotonin hormone and lower corticosterone hormone in Hy-Line brown embryos compared to Roman pink embryos during dark incubation. The LED green light incubation significantly ($P < 0.05$) increased the level of 5-HT while decreasing the CORT level in Roman pink embryos indicating its regulatory effect on pecking-related hormones. Results of the second experiment showed that LED green light incubation significantly ($P < 0.05$) alleviated the CORT-induced hyperactivity of plasma 5-HT in Roman pink embryos. Furthermore, Melatonin (MLT) injection and LED green light together significantly ($P < 0.05$) reduced the hormonal stress caused by corticosterone injection in the eggs.

Conclusions Overall, the LED green light regulatory incubation demonstrated a regulatory effect on hormones that influence pecking habits. Additionally, when coupled with MLT injection, it synergistically mitigated hormonal stress in the embryos. So, LED green light incubation emerged as a novel method to reduce the damaging pecking habits of poultry birds.

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Keywords Breeder, Poultry, Pecking, Hormones, LED, Green light, Incubation

Background

In pursuing sustainable livestock systems, novel techniques that improve animal welfare and production are paramount. Exploring environmental alterations, particularly through controlled lighting conditions, is one interesting line of inquiry. It has been demonstrated that different light regimens have a considerable impact on the physiological and behavioural outcomes of chickens [1, 2]. In the poultry business, pecking is a major welfare and financial problem. It has a substantial impact on disease prevention, management, and breeding efficiency, leading to large financial losses [3, 4]. Along with environmental and genetic factors behaviour management is also dependent on hormone regulations creating high-pecking and low-pecking phenotypes in the chicken by transferring hormones and genetic information from the mother into the eggs [5, 6]. The hormonal imbalance can create a range of physiological issues, for instance, an imbalance in serotonin will lead to damaging behaviour in chickens, such as cannibalism and pecking [7, 8]. The physiological response in chicken farms is further exacerbated by environmental stresses that raise corticosterone levels, which can amplify these responses by making serotonin receptors more susceptible [9, 10]. While, testosterone affects the subcortical region of the brain that promotes emotional aggressiveness [11], and dopamine stimulates the chicken brain, causing hunger, foraging, and aggression [12]. Conventional approaches to control damaging habits e.g. pecking, include trimming beaks, giving medication, lowering stocking density and feeding density, and dimming light, frequently fail to address the underlying hormonal and genetic reasons and have a negative impact on animal welfare [4]. The wavelength and duration of light have an important role in controlling a wide range of biological processes, including circadian cycles, hormone balance, and overall health in avian species [13]. Studies have demonstrated that some light wavelengths, especially those in the blue-green range, can significantly reduce pecking, most likely by influencing hormones [2]. This impact may be mediated by the action of light on the pineal gland, which plays an important role in hormone control by regulating melatonin release, therefore influencing circadian rhythms and hormonal balance [14, 15]. Advancements in lighting technology make it easier to employ particular wavelengths to trigger desired physiological reactions. Green light has drawn attention since it has an impact on melatonin release, which is essential for preserving circadian rhythms and controlling metabolic activities [16]. Melatonin (MLT) not only influences sleep-wake cycles but also has substantial consequences for immunological function and stress control, making it a key area for studies aimed at improving poultry management and welfare [17]. Furthermore, several key hormones related to physiological behaviour and stress reactions, including serotonin (5-HT), corticosterone (CORT), dopamine (DA), and testosterone (T), respond to variations in light exposure [18, 19]. Hens will transfer their physiological and genetic information, including hormones, to the yolk of their eggs, which impacts offspring behaviour. If a mother hen is stressed, her hormonal balance will be dysregulated, producing damaging behaviour in her offspring [6, 20]. Understanding how this works might give insights into regulating and perhaps alleviating problems like damaging pecking, common in commercial poultry production. Given these intricate relationships, our research seeks to elucidate the impact of LED green light on the hormonal pathways linked to physiological behavioural activities. Focusing on the regulation of behaviour-related hormones during the incubation aims to explain the complicated processes by which light conditions may contribute to reducing prenatal stress and lead to more successful and humane poultry husbandry techniques. This comprehensive approach intends to provide novel perspectives and theoretical directions to solve the widespread issue of damaging pecking in the poultry industry, advancing sustainable and genetically improved animal management techniques. Therefore, this study aimed to address the identified issues by exploring hormonal changes in Hy-line brown and Roman pink embryos under LED green light incubation, investigating the regulatory effects of LED green light on eggs predisposed to hormonal stress through corticosterone injections, and assessing the combined impact of LED green light incubation and melatonin hormone on circadian rhythms and physiological behavioural responses in birds.

Materials and methods

Experiment 1: effect of LED green light incubation on pecking hormones under normal conditions

Experimental design

The experiment was conducted at the Key Laboratory of Intelligent Equipment, College of Biosystems Engineering and Food Science, Zhejiang University Hangzhou, 310,058, China. A total of 296 eggs of each Roman pink and Hy-line brown breeder were taken and randomly divided into 2 groups; LED green light incubation (experimental group), and dark incubation

(control group). Each group was further divided into 4 replicates (37 eggs/replicate).

Incubation process

The experiment adopted two NK-525 microcomputer automatic incubation control boxes (Shandong Agricultural Science Incubation Equipment Co., Ltd., Shandong Dezhou) one for dark incubation and one for LED green light incubation, and their size specifications were 1100×1000×900 mm. In LED green light incubation two 60 cm long LED green light tubes were fixed in the egg incubator at 15 cm above the two sides of the egg tray. To measure the light intensity, 9 points were selected on each egg plate, and a lux meter was used to measure the light intensity of each point in the left, right and horizontal directions. An electromagnetic pulse controller was used to adjust the light intensity of each test point, and the light intensity was controlled at 20 to 50 lx. The light cycle was adjusted to 16-hour light: and 8-hour dark (16 L:8 D). The incubation temperature throughout the experiment was 38.0±0.1 °C (1 to 7 days), 37.8±0.1 °C (7 to 14 days), and 37.6±0.1 °C (14 to 21 days), and the humidity was 60±2%. The egg turning interval was 1.5 h throughout the experiment, and the egg pan was adjusted at the angle of 45° from 0 to 18 days of incubation. On days 9, 12, and 15, the eggs were examined using a Cool-lite tester to sort out dead embryos and azoospermia from the eggs. On day 18, all eggs were transferred from egg trays to the hatching baskets.

Tissue sample extraction

Embryonic sample extraction

On day 18, four eggs/replicate were randomly chosen from the same positions within egg trays of each experimental group at 6:00, 12:00, 18:00 and 24:00 O-clock to validate the survival rates. Eight eggs/group were randomly broken at the blunt end to take the samples of embryos. Liver tissue (150 to 300 mg weight) was extracted from each embryo, put into Eppendorf (EP) tubes and stored at -80 °C for further analysis.

Detection of hormones from liver samples

The concentrations of 5-HT, CORT, DA, and T were detected by ELISA as given below.

First of all, set up the standard curve and experimental sample wells in the microplate, adding 50 µL of different concentration standard samples to the standard curve wells and using one blank well as a control. In the sample detection wells, add 40 µL of sample dilution and 10 µL of the sample to be tested (5x dilution). For incubation, add 100 µL of microplate reagent to each well (excluding the blank wells), seal the plate, and incubate at 37 °C for 60 min. After incubation, prepare the washing solution by diluting the concentrated washing liquid 20 times with distilled water, then wash each well 5 times after discarding the liquid. For colour development, add 50 µL each of colour developers A and B to all wells, including the blank, and incubate the plate at 37 °C for 15 min, protected from light. To terminate the reaction, add 50 µL of stop solution to each well, including the blank wells. Finally, measure the OD value of each well at a 450 nm wavelength within 15 min using a microplate reader, with the blank well used to zero the measurement.

Experiment 2: effect of LED green light incubation on different hormones during hormonal stress

Experimental design

A total of 704, 52-week-old Raman pink eggs were selected and weighed 55.4±0.41 g (average±SEM).

The eggs were sterilized and randomly divided into four groups of 176 eggs each for the follow-up trials. The experimental groups included: 100 µL corticosterone solution injection (CI), 100 µL corticosterone and melatonin mixed solution injection (CMI), 100 µL PBS solution injection (PI), and a no injection group (UI). Each group was further subdivided into two subgroups of 88 eggs each, with one subgroup undergoing LED green light-regulated incubation (G) and the other dark incubation (D). This resulted in the following groups: G+CI, D+CI, G+CMI, D+CMI, G+PI, D+PI (experimental groups), and G+UI and D+UI (control groups). Each subgroup was further divided into four replicates with 22 eggs each. The blunt ends of all eggs were punched, and a Hamilton injection needle (Hangzhou Cyst Firefly Technology Co., Ltd., Hangzhou) was carefully inserted 20 mm into the central hole at the blunt end of each egg to ensure accurate injection into the yolk. Paraffin wax was used to seal the pores of eggs to prevent contamination during incubation. All injections in the experiment were performed by a single experimenter to eliminate non-systematic errors.

Corticosterone/melatonin solution preparation

To produce CORT and MLT solution, 100 mg of CORT/MLT dry powder was dissolved in 6 mL of absolute ethanol, and then 10 mL of sodium bicarbonate buffer was added. Distilled water was used to set the volume to 100 mL to prepare a stock solution having a concentration of 1 µg/µL for later use. One mL of the mother solution was mixed with distilled water to make a total volume of 100 mL, and a concentration of 1 µg/100 µL was obtained for later use.

Corticosterone + melatonin mixed solution preparation

A solution was prepared by combining 1 mL of CORT mother liquor with 3 mL of MLT mother liquor and adjusting the final volume to 100 mL using distilled water. The resulting solution contains 1 μg of CORT and 3 μg of MLT per 100 mL, ready for later use.

Sample preparation and detection of physiological hormone levels

On the first day of hatching (HD1), 2 chicks/replicate in each group were selected at 6:00, 12:00, 18:00, and 24:00. A blood sample of 1-1.5 mL was obtained from each chick, mixed with sodium citrate for anticoagulation, and agitated for 15 min. The mixture was then centrifuged at 2000–3000 rpm for 20 min, and the supernatant was collected and stored at -20 °C. The ELISA method was used to detect the concentrations of 5-HT and CORT in the blood serum.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 software. One-way ANOVA (with nonparametric or mixed methods) was used to compare hormone concentrations. All the data are expressed as the standard error mean ± (SEM±). A probability value of $P<0.05$ was considered to indicate statistical significance. The notable differences between groups were identified by Duncan's multiple comparisons test.

Results

Analysis of different hormones during the incubation period

Liver 5-HT content analysis

The 5-HT liver content of Hy-line brown embryos hatched in the dark group at 12:00 PM and 18:00 was significantly ($P<0.05$) higher than that of Roman pink, while non-significant at the other two time points. Green light incubation significantly increased the liver 5-HT content of Roman pink embryos at all four stages

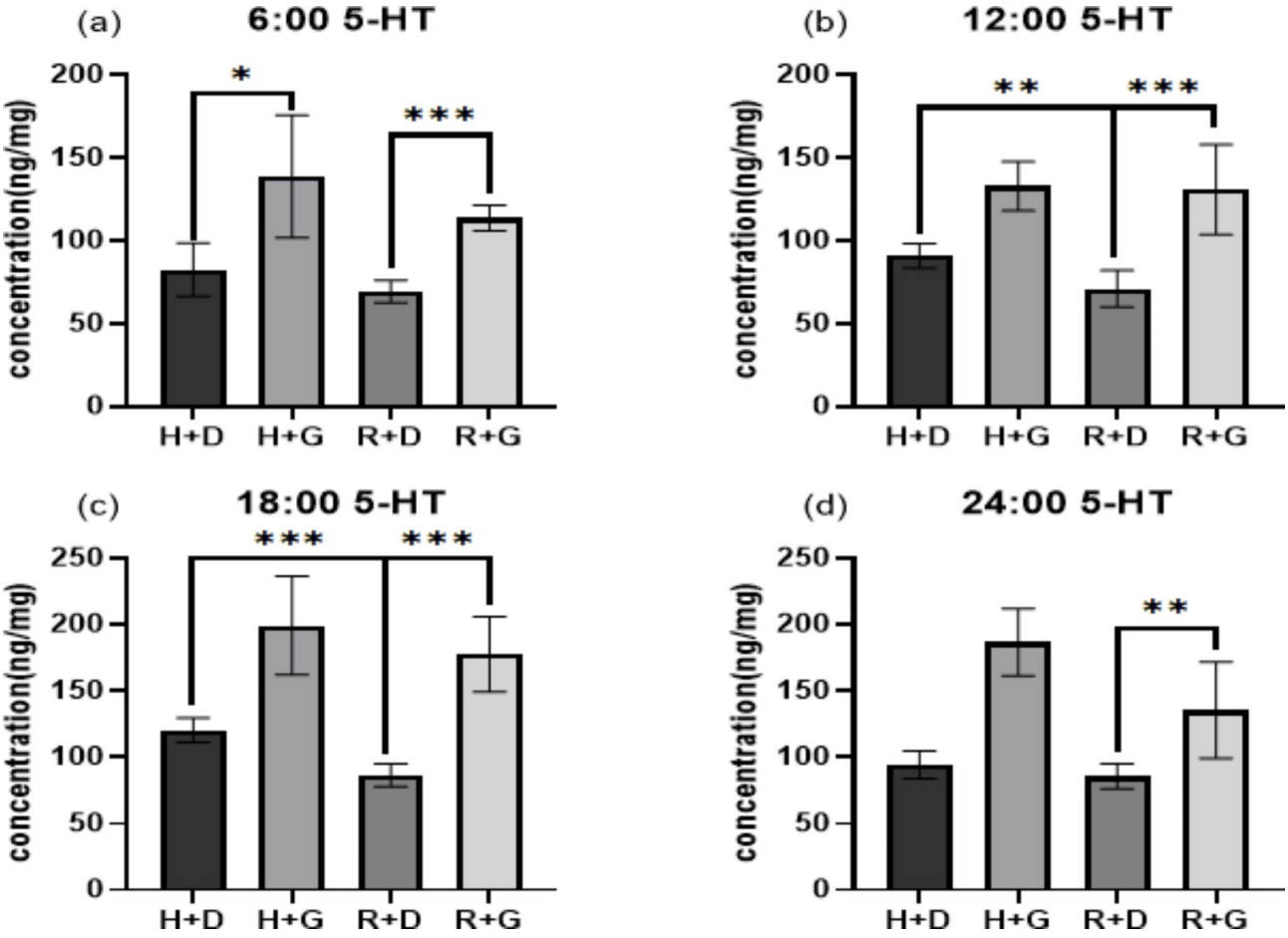

Fig. 1 The content of serotonin (5-HT) in the liver of Hy-line Brown/Roman Pink embryos at the 18th day of embryo hatching. H: Hy-line brown; R: Roman pink; G: Green light incubation; D: Dark incubation; H + D: Hy-line Brown hatched in the dark; H + G: Hy-line Brown hatched in green light; R + D: Roman pink hatched in the dark; R + G: Roman pink hatched in green light; 6:00, 12:00, 18:00, 24:00 are the time points of the day. Note: * Represent the significance difference; *$P<0.05$, ** $P<0.01$, *** $P<0.001$

(6:00, 12:00, 18:00, and 24:00 o clock). Green light incubation also significantly increased the liver 5-HT content of Hy-line brown in embryos at 6:00, 12:00, and 18:00 o clock ($P<0.05$) Fig. 1.

Analysis of liver CORT content

The liver CORT content of Hy-line brown embryos in the dark group was significantly ($P<0.05$) lower at four sampling points than that of Roman pink. The green light incubation significantly ($P<0.05$) reduced the CORT content of the Roman pink embryos at 6:00 and 24:00, while it was non-significant at the other two points (Fig. 2). However, increased CORT content was observed in Hy-line brown embryos under green light incubation at 12:00 and 18:00.

Liver DA content analysis

As shown in Fig. 3, at E18, the DA content in the liver of Hy-line brown and Roman pink embryos in the dark group did not differ significantly at all four-time points ($P<0.05$). However, LED green light regulatory incubation significantly ($P<0.05$) increased the DA contents of Hy-line brown and Roman pink embryo livers at all four-time points.

Liver TES content analysis

As shown in Fig. 4, the TES content in the liver of the Hy-line-brown and Roman pink in the dark group did not differ significantly ($P<0.05$) at any of the four-time points. For Hy-line brown embryos, compared with the control group, LED green light incubation significantly increased the liver TES content at all of the four-time points. However, for the Roman pink embryos, compared with the control group, LED green incubation significantly reduced their liver TES content at 24:00 AM. While showing non-significant effects at other time points ($P<0.05$).

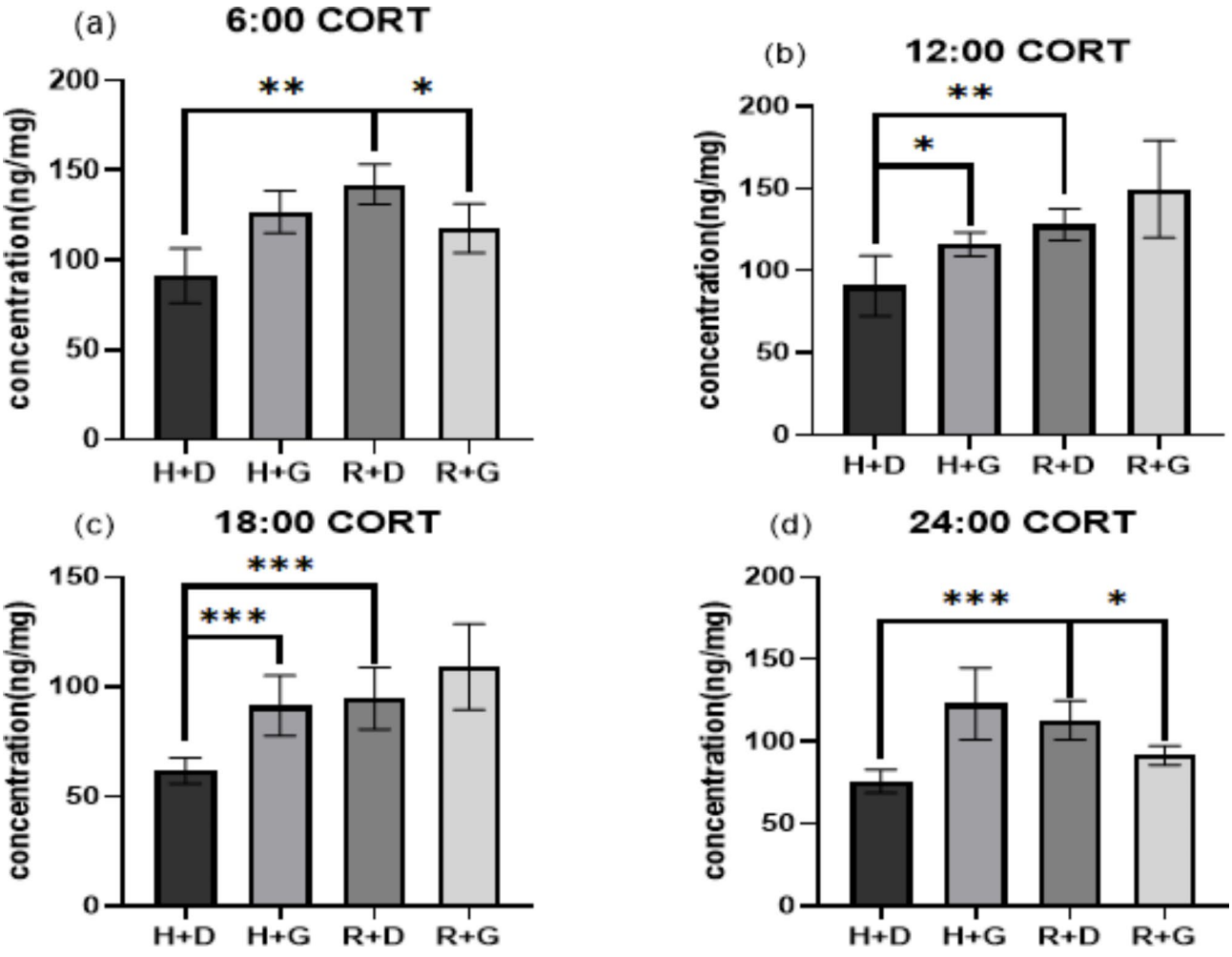

Fig. 2 The content of Corticosterone (CORT) in the liver of Hy-line Brown/Roman Pink embryos at the 18th day of embryo hatching. H + D: Hy-line Brown hatched in the dark; H + G: Hy-line Brown hatched in green light; R + D: Roman pink hatched in the dark; R + G: Roman pink hatched in green light; 6:00, 12:00, 18:00, 24:00 are the time points of the day. Note: * Represent the significance difference; *$P<0.05$, ** $P<0.01$, *** $P<0.001$

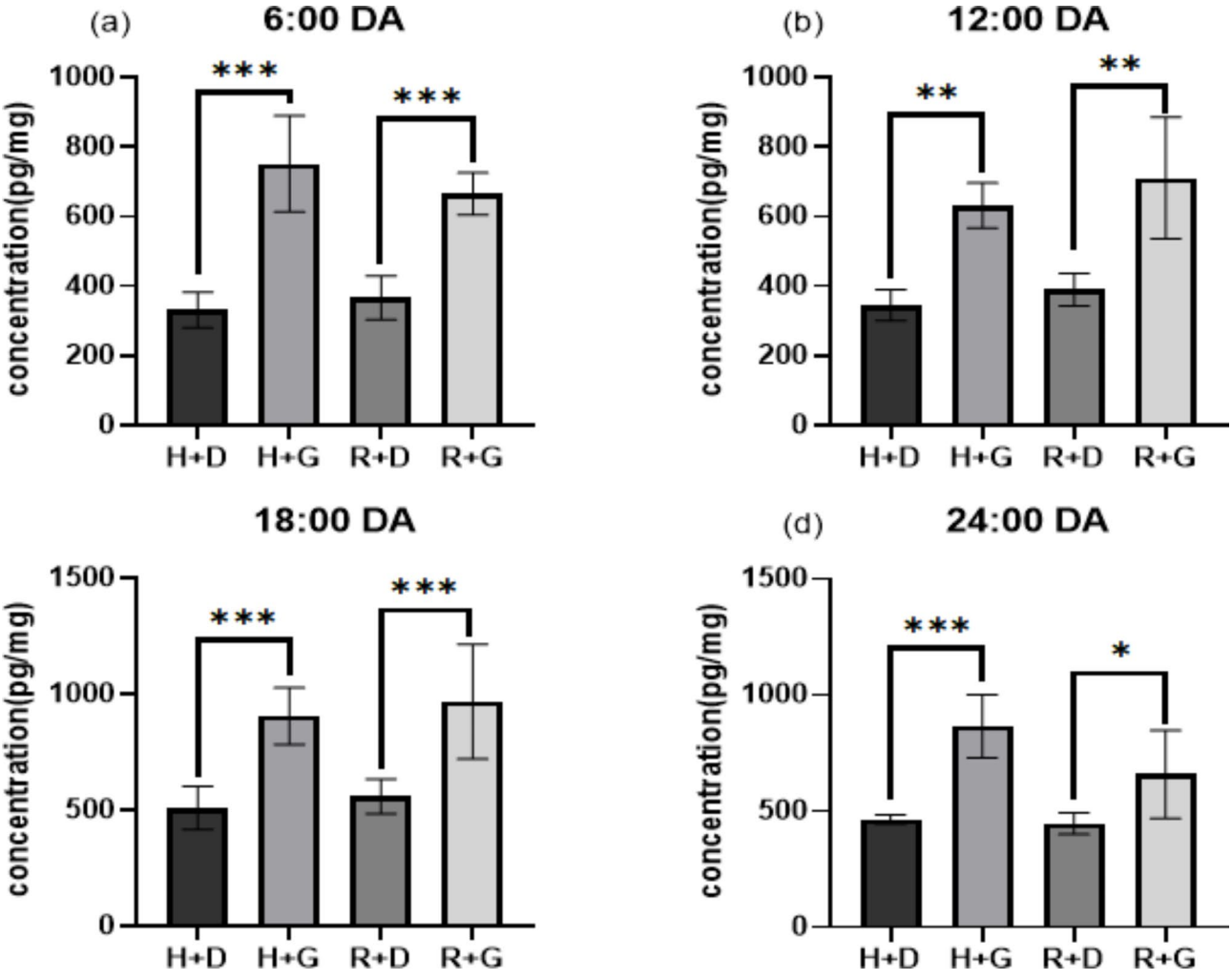

Fig. 3 The content of dopamine (DA) in the liver of Hy-line Brown/Roman Pink embryos at the 18th day of embryo hatching. H + D: Hy-line Brown hatched in the dark; H + G: Hy-line Brown hatched in green light; R + D: Roman pink hatched in the dark; R + G: Roman pink hatched in green light; 6:00, 12:00, 18:00, 24:00 are the time points of the day. Note: * Represent the significance difference; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Analysis of hormonal rhythms during the incubation period

Figure 5 illustrates the impact of LED green light on the daily rhythms of different hormones in Hy-line brown and Roman pink embryos under dark incubation and LED green light conditions. At day 18 of incubation LED green light significantly increased the liver's 5-HT content in both varieties, regulating the exogenous rhythm in Hy-line brown embryos while having no significant effect on the 5-HT rhythm in Roman pink embryos. The CORT rhythm was altered by LED green light, with Roman pink embryos exhibiting significantly higher changes than Hy-line brown ($P < 0.05$). Similarly, the DA content in the liver of both varieties increased significantly under LED green light. Additionally, LED green light significantly affects the liver TES rhythms in Hy-line brown embryos during the dark cycle ($P < 0.05$).

Effect of LED green light incubation under hormonal stress on breeder eggs

Plasma 5-HT content analysis

As shown in Fig. 6, at HD1, CORT injection abruptly increased the plasma 5-HT concentrations of CI chicks in the dark incubation group at 6:00, 12:00, and 18:00 as compared to LED green light incubation. LED green light treatment significantly ($P < 0.05$) reduced hyperactive plasma 5-HT concentration in the embryos and neutralized the effect of CORT injection. Furthermore, CORT + MLT injection under LED green light incubation reduced the hyperactive 5-HT at 6:00 and 12:00, while the level was increased at 24:00. Furthermore, the plasma 5-HT concentration of CMI chicks in the dark group was regulated by MLT mixed injection at 18:00 and 24:00 and was significantly lower than that of chicks in the CI group. While there was no

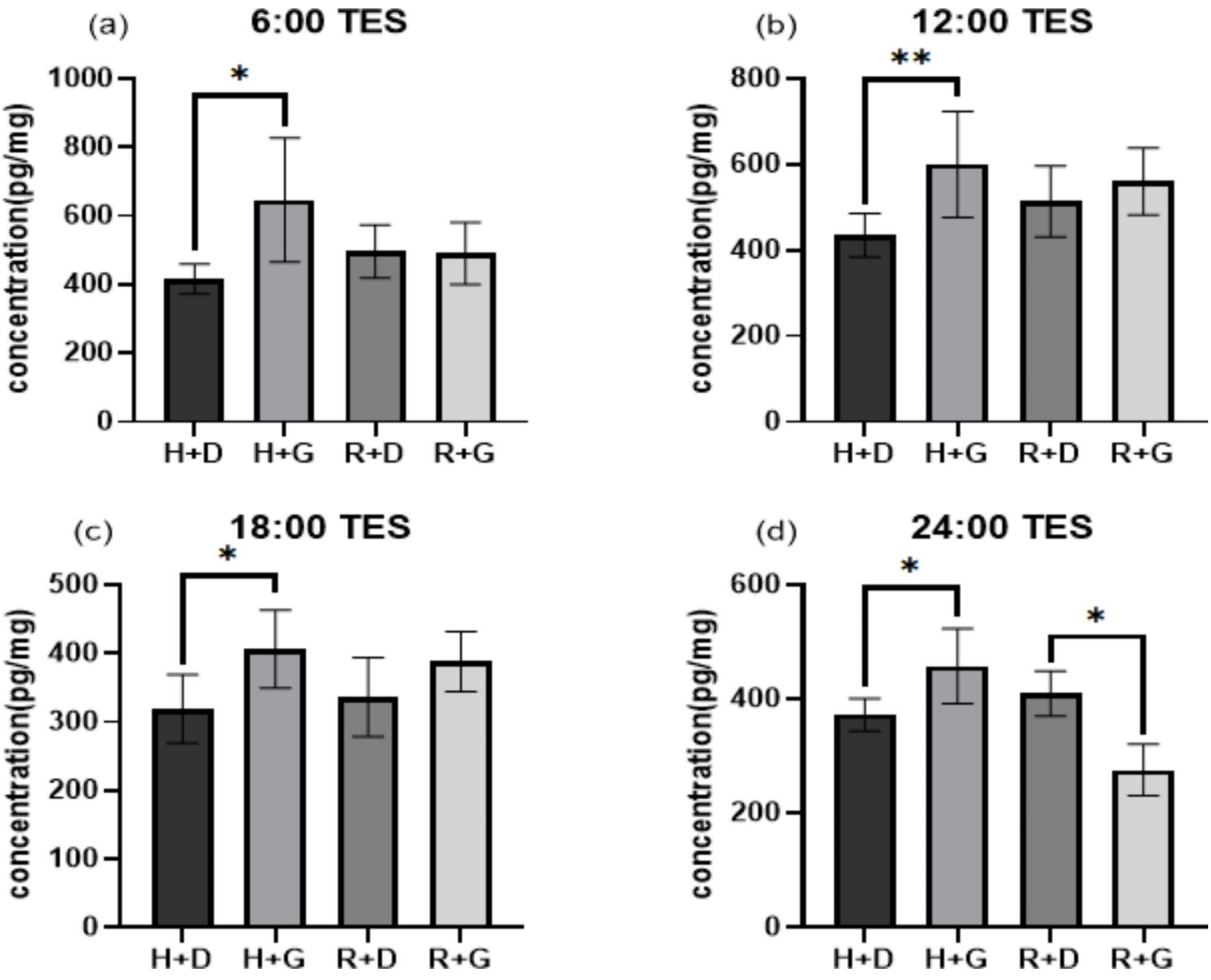

Fig. 4 The content of TES in the liver of Hy-line Brown/Roman Pink embryos at the 18th day of embryo hatching. (H + D: Hy-line Brown hatched in the dark; H + G: Hy-line Brown hatched in green light; R + D: Roman pink hatched in the dark; R + G: Roman pink hatched in green light, 6:00, 12:00, 18:00, 24:00 are the time points of the day. Note: * Represent the significance difference; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

significant difference in plasma 5-HT concentrations between UI and PI chicks.

Plasma CORT content analysis

As shown in Fig. 7, at HD1, the plasma CORT concentrations of CI chicks hatched in the dark group were significantly higher than those of UI chicks at 6:00, 12:00 and 24:00 sampling points. While LED green light incubation significantly reduced the CORT concentration in the CI group and neutralize the effect of CORT injection at 6:00, 12:00 and 24:00 sampling points. Plasma CORT concentrations of dark-hatched CMI chicks were significantly higher than those of UI chicks at 18:00 while non-significant at other sampling points. However, LED green light incubation reduced the CORT concentration at this sampling point. The UI and PI chicks had no significant difference in plasma CORT concentrations.

Analysis of plasma MLT rhythms

There was a significant difference in plasma MLT concentrations in dark hatched CI chicks at 24:00 and 6:00, while the plasma MLT circadian rhythm of PI and UI chicks remained stable. The plasma MLT content of CMI chicks in the green light group showed a stable circadian rhythm. The circadian rhythm of MLT regulated by LED green light was consistent in PI and UI chicks (Fig. 8).

Discussion

Analysis of different hormones during the incubation period

Our research explores the physiological and behavioural dynamics of chickens, emphasising the importance of LED green light incubation on hormone modulation and its influence on behaviours. Building on previous research, physiological factors are

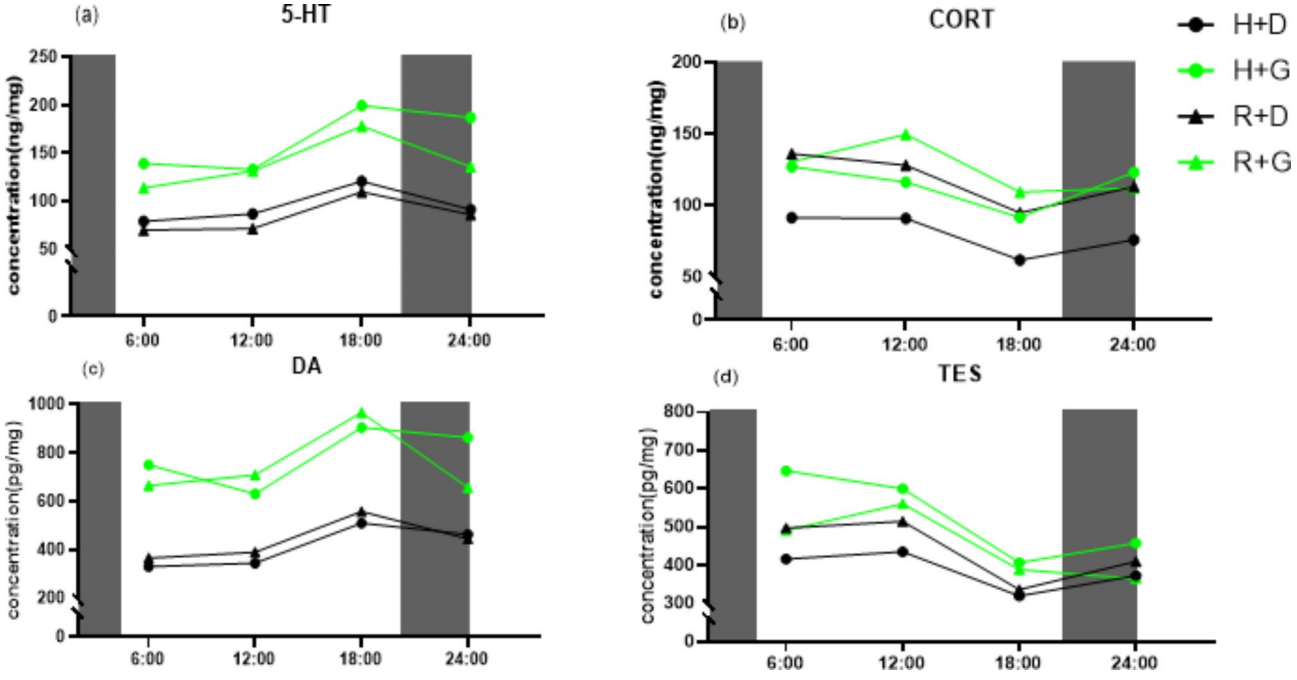

Fig. 5 The circadian rhythm of pecking-related hormones in the liver of Hy-line Brown/Roman Pink embryos at the 18th day of embryo hatching. (5-HT): serotonin, (CORT): corticosterone, (DA): dopamine, (TES): testosterone, 6:00, 12:00, 18:00, 24:00 are the time points of the day

recognised as central to feather pecking, with variations in hormonal concentrations such as 5-HT and CORT reflecting underlying causes of such behaviour, such as compulsive and redirected foraging behaviours [21–23]. Our findings reinforce that certain light wavelengths, particularly green light, might influence certain hormonal states. Our findings showed that exposure to green light during incubation significantly elevates 5-HT levels, a neurotransmitter essential for controlling damaging feather-pecking behaviours [24, 25]. This is in line with previous research, which showed that stress and damaging behaviours in chickens are associated with dysregulated 5-HT levels [21]. Therefore, it is possible that by controlling the 5-HT system, LED green light can reduce the pecking behaviour of Roman pink chickens. The 5-HT system consists of central neurotransmitters and peripheral signalling molecules, which can modulate DA levels in the brain [26]. The results of the current study demonstrated that the impact of LED green light significantly raised the DA levels in Roman pink and Hy-line brown embryos, suggesting greater foraging behaviour, which can be a natural antidote to damaging pecking [27, 28]. Furthermore, our research showed that Roman pink under green light incubation had significantly lower liver CORT (a hormone strongly associated with stress) levels, which may lessen stress-related pecking behaviours. This reduction in CORT could lessen the activity of tryptophan hydroxylase, impacting 5-HT synthesis and consequently behaviour [29, 30]. Previous research confirms that exposure to particular light wavelengths, such as green light, dramatically reduces the levels of stress-related hormones in the plasma [31]. Moreover, CORT can disrupt the 5-HT system by raising the sensitivity of the 5-HT1A receptor (a kind of 5-HT receptor) and decreasing SLC6A4 expression (the genetic code for tryptophan), resulting in more damaging behaviour [30]. Studies on broilers have demonstrated that high levels of plasma CORT can considerably enhance damaging behaviour [32]. Previous research found that monochromatic green light significantly lowered maternal ovarian steroid hormone (CORT) concentrations in the plasma [31], consistent with our findings. Furthermore, LED green light incubation decreased liver testosterone (TES) levels, linked to increased damaging pecking behaviour [23].

Effect of LED green light incubation under hormonal stress on breeder eggs

In the current study, the regulating effect of green light on CORT and MLT during incubation highlights the potential of implementing green light incubation to mitigate the adverse effects of prenatal stress. This was evaluated through the injection of the CORT hormone to produce artificial parental stress in the eggs. Results demonstrated that MLT and green light have a synergistic beneficial effect on preserving hormonal balance and reducing damaging behaviours [32–34]. It was found that CORT injection abruptly elevated

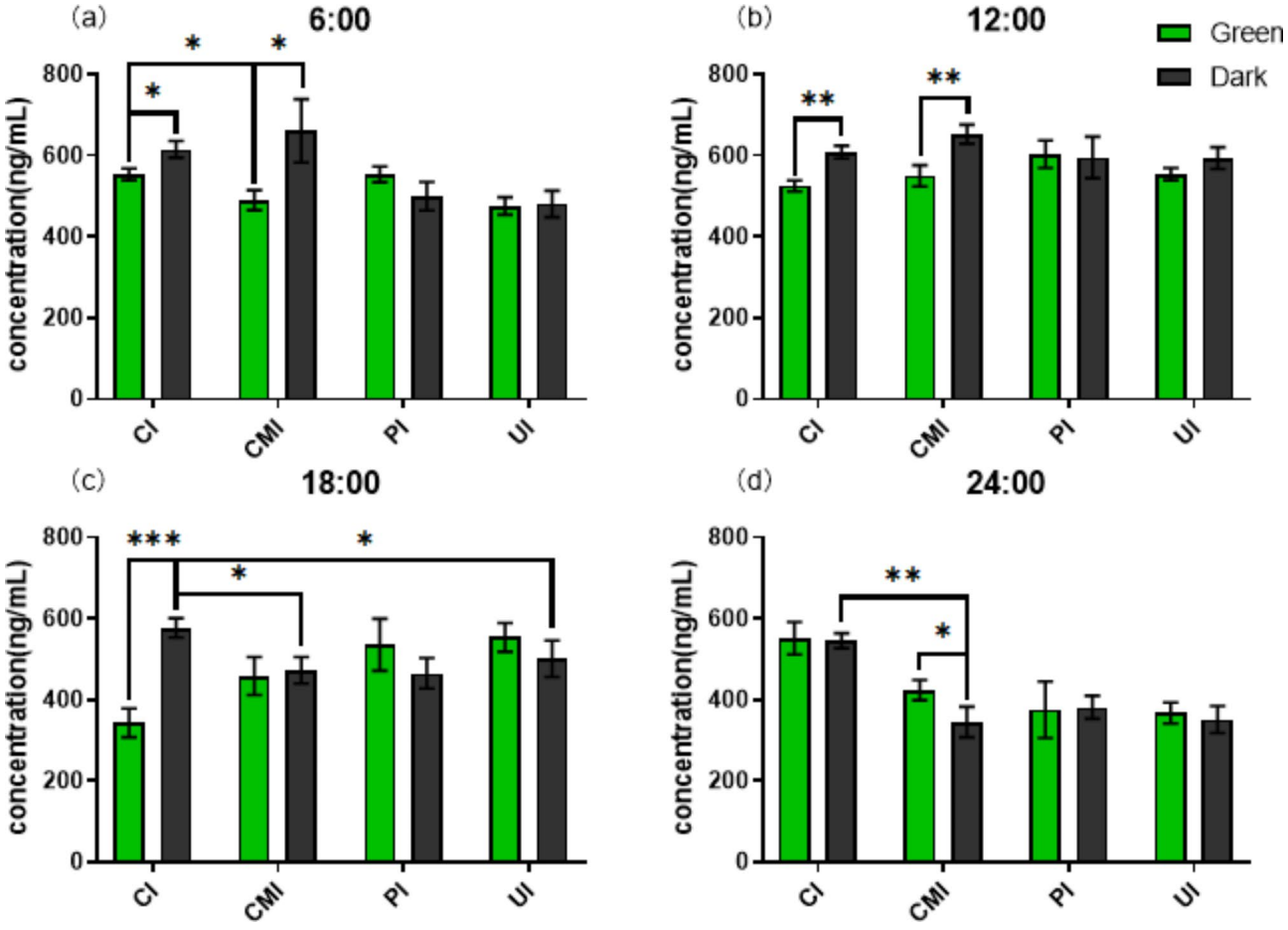

Fig. 6 Plasma 5-HT concentration of chicks on the first day after hatching (CI: CORT injected; CMI: CORT + MLT injected; PI: PBS injected; UI: uninjected), 6:00, 12:00, 18:00, 24:00 are the time points of the day. Note: * Represent the significance difference; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

plasma 5-HT concentrations in the dark group, which can cause stress and damaging behaviour. LED green light incubation significantly reduced chick plasma 5-HT content, demonstrating the calming effect of LED green light. CORT injection to egg significantly increased plasma CORT concentrations in offspring chicks, in line with previous findings that CORT injections in chickens at embryonic stages led to decreased progeny quality, elevated plasma CORT concentrations, and increased damaging behaviour [32]. Studies have also shown that exposure to corticosteroids during pregnancy inhibits foetal growth in mice [33] and permanently increases plasma CORT concentrations in offspring [34]. It was found that embryonic CORT injection led to a circadian rhythm disorder at the time of hatching. Another study showed that circadian rhythm disorder led to an increase in aggressive behaviour in mice [35]. Our study indicated that the mixed injection of MLT and CORT also induced regular rhythm changes in MLT in chicks and reduced the negative effects caused by circadian rhythm disorders in chicks due to CORT exposure. This further characterized the synergistic effect of MLT injection and LED green light-mediated incubation to reduce the hormonal stress caused by CORT injection, indicating that LED green light-mediated incubation and MLT can reduce the negative effects caused by prenatal stress and limit the occurrence of damaging offspring behaviour. The circadian rhythm of the animal body is regulated by MLT. The incubation process of poultry eggs is different from that of mammals, as eggs cannot directly obtain melatonin secretion signals from the mother, so exogenous signals are needed to determine circadian rhythms such as light [36]. When eggs hatch naturally, the mother will turn the eggs or leave the nest, causing the eggs to receive a regular "light-dark" periodic signal. MLT in poultry is mainly secreted from the pineal gland which is sensitive to light, and even at low light intensity, the pineal gland can still secrete MLT normally and produce rhythms [37]. In the present study, monitoring the daily change in MLT concentration in the plasma of chick embryos, it was discovered that incubation with LED green light significantly improves rhythm recovery in chicks

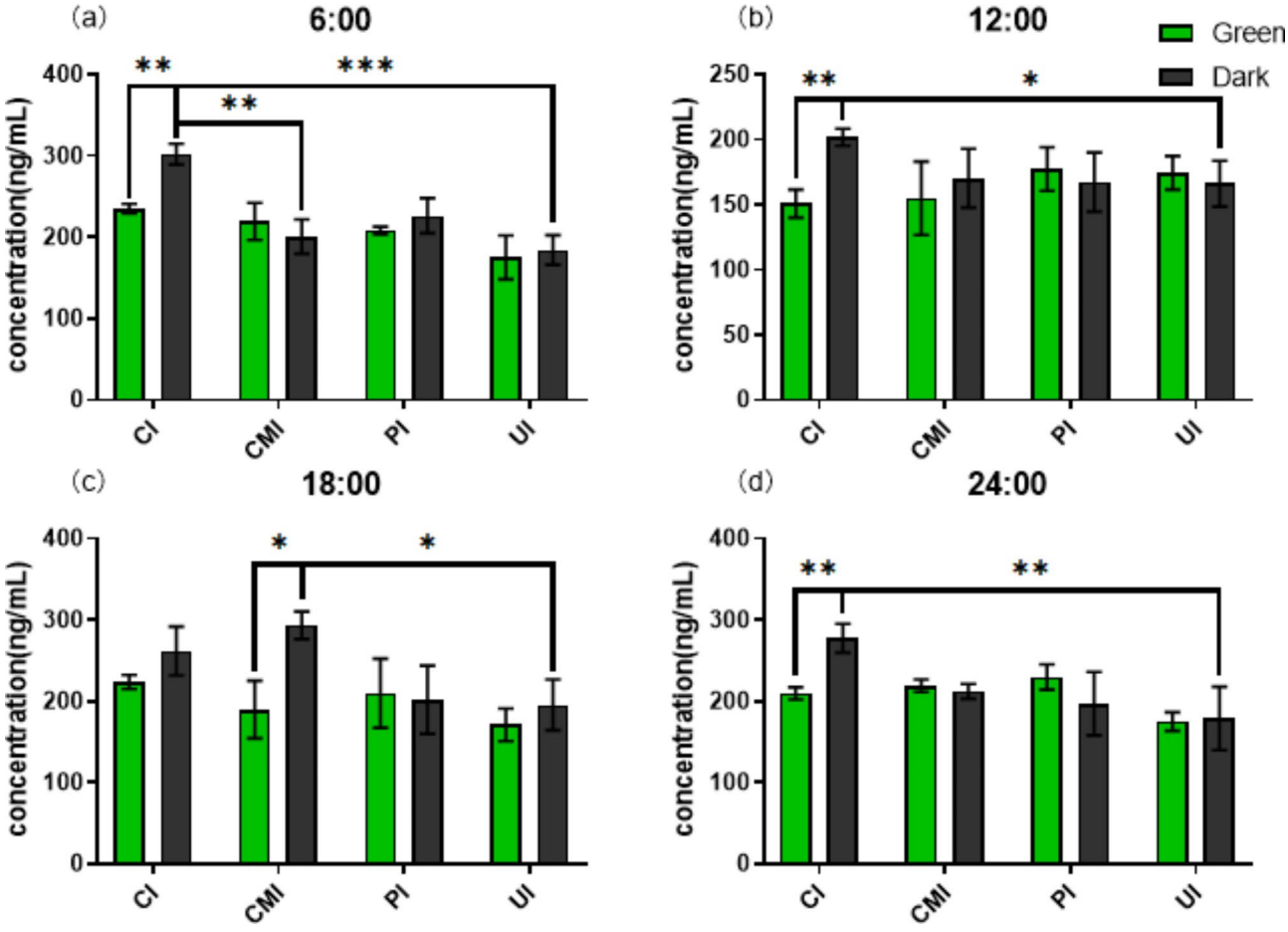

Fig. 7 Plasma CORT concentration of chicks on the first day after hatching. (CI: CORT injected; CMI: CORT + MLT injected; PI: PBS injected; UI: uninjected), 6:00, 12:00, 18:00, 24:00 are the time points of the day. Note: * Represent the significance difference; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

undergoing prenatal stress compared to the control group. Additionally, prenatal supplementation of melatonin (MLT) aids in rhythm recovery. This effect may be because chicks hatched in darkness only experience endogenous circadian rhythm changes, and prenatal stress further disrupts these changes. LED green light provides an external circadian rhythm signal to chicken embryos during incubation, which helps mitigate the disruptive effects of prenatal stress. Our findings are aligned with the findings that the pineal gland, responsive even to low light intensities, can significantly affect MLT secretion and thereby influence circadian rhythms [36, 37]. Our study underscores the beneficial effects of green light on behavioural and physiological parameters and improving circadian rhythms evidenced by the studies indicated beneficial effects of LED green light during incubation on poultry [13, 38]. While our findings are promising, they call for further experimental studies to fully understand the complex interactions between light exposure, hormonal regulation, and behavioural outcomes. This comprehensive exploration could provide a clearer direction for leveraging LED green light during incubation to enhance poultry welfare and productivity in sustainable livestock systems.

Conclusions

Our findings indicate that LED green light incubation effectively regulates the hormonal profiles associated with behaviour in chicken embryos. This intervention could improve behaviour in chickens by regulating serotonin and corticosterone levels and maintaining melatonin rhythms, which are crucial for circadian regulation. This is a step towards maintaining a sustainable livestock system by minimising behavioural activities in poultry. However, the rise in DA levels caused by LED green light incubation, as well as the possibility of varied responses among breeds or individual birds, may challenge the universal application of LED green light incubation in different poultry environments. Therefore, further research into the broader applications of this strategy could help optimise poultry welfare and productivity in diverse farming environments.

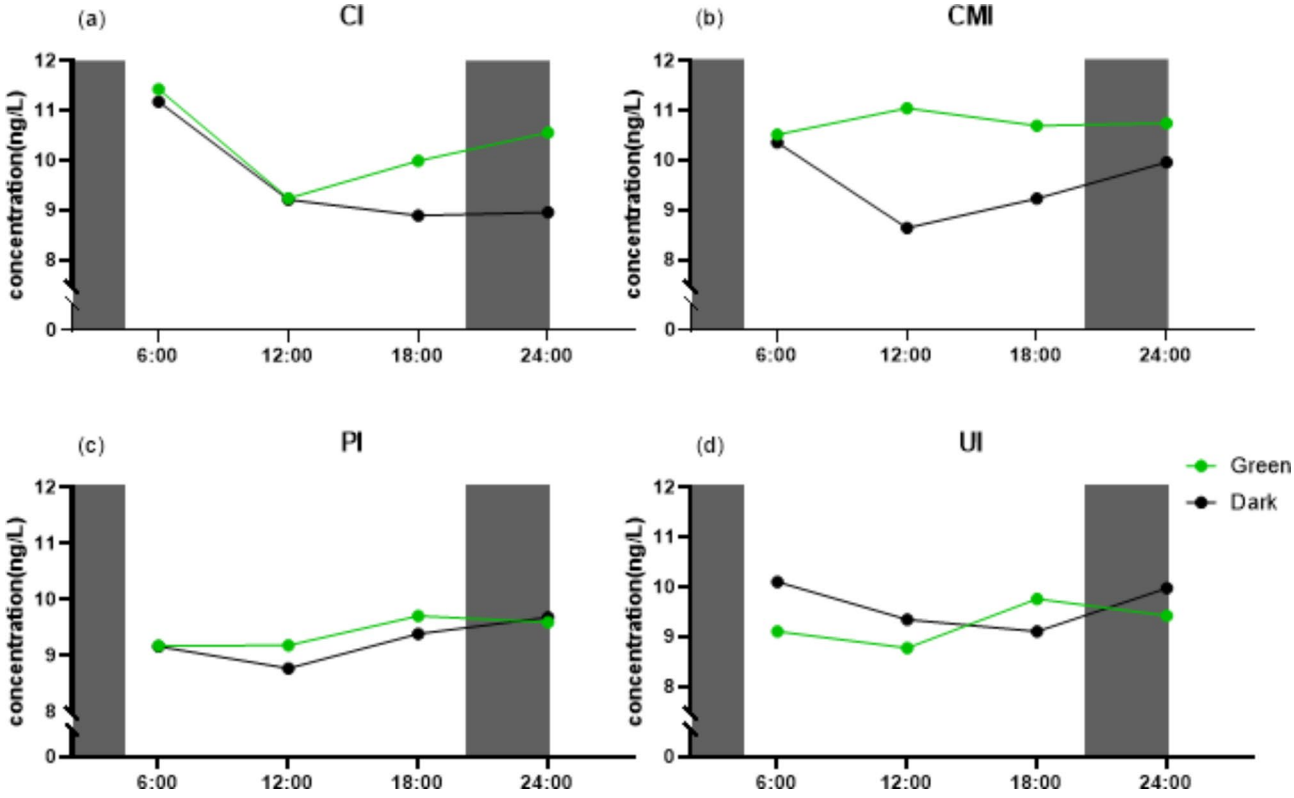

Fig. 8 Rhythm changes in plasma MLT concentration of chicks on the first day after hatching. (CI): corticosterone solution injection, (CMI): corticosterone + melatonin mixed solution injection, (PI) phosphate buffer solution injection, (UI) no injection, 6:00, 12:00, 18:00, 24:00 are the time points of the day

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Author contributions
KH, and TW: Designed the experiment, wrote the first draft, and revised the manuscript. RZ and JP: Handled project administration, acquired funding, revised the manuscript and help in technical writing improvement. LZ and ZY: Contributed to data analysis and manuscript writing. MA and BHS revise and improved the manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval
The current study followed the all-ethical standard of bird welfare and was approved by the ethical committee of Zhejiang University, Hangzhou, China (approval number: ZJU20220004). Moreover, all applicable rules and regulations of the organization and Government were followed regarding the ethical use of experimental animals.

Consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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