

# Prenatal effects of red and blue light on physiological and behavioural parameters of broiler chickens

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**Abstract:** Light during incubation can influence embryonic and postembryonic development of chickens, but the underlying mechanisms are poorly understood. Previous studies have demonstrated that red and blue lights during incubation had opposite effects on the development of embryonic melatonin biosynthesis; red light results in the highest and blue light in the lowest amplitude of the daily rhythm. Therefore, in this study, we investigated if exposure to monochromatic red (632 nm) and blue (463 nm) light during incubation can differently influence growth, selected biochemical (glucose, cholesterol, triacylglycerols) and endocrine (corticosterone and thyroid hormones) traits and behavioural parameters during postembryonic development in broiler chickens. For analysis, we used 10 and 11 hatchlings incubated in red and blue light, respectively and 10 birds per each group (six males and four females) in 3-weeks-old broilers. During the rapid growth phase (days 18, 20 and 21 of age), higher body weight was recorded in broilers incubated under red compared to blue light, whereas endocrine and metabolic traits did not differ between the treatments. The improved growth rate was related to behavioural traits, mainly because chickens incubated in red light exhibited more passive (resting, standing, preening, dust bathing) and less active behaviours (walking, foraging, fighting, wing-flapping) than the blue-light incubated birds. The time spent for eating and drinking and the results of the tonic immobility test did not differ between both groups. Our results suggest that red and blue monochromatic light during incubation can differently program the postembryonic development of broilers, with possible consequences for their growth and welfare.

**Keywords:** behavioural traits; birds; incubation; melatonin; monochromatic light

Environmental conditions during embryonic life can shape not only embryonic development but also they can have long-lasting effects on postembryonic development and behaviour later in life. Birds are important model organisms for understanding these “programming” effects because of their extra-uterine development. Moreover, the programming of developmental trajectories

may be important for poultry practice in relation to production efficiency and welfare, especially in broiler chickens that are produced in enormous numbers over the world.

Temperature and humidity are major environmental factors influencing embryonic development and hatching success in birds (Bruzual et al. 2000). Possible effects of lighting conditions during in-

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cubation have been studied in the field of poultry science only recently, and the results are not conclusive. In poultry practice, eggs are usually incubated in darkness due to concerns about possible adverse effects of light stimulation on physical activity and dehydration of chicks in hatcheries, which can affect performance later. Nevertheless, photostimulation during incubation can have positive effects on physiological processes and behaviour, although the results are still limited and often contradictory. Initial studies exploring the consequences of light during incubation used white incandescent or full-spectrum fluorescent light, finding significant effects of light on the hatching process and post-hatching performance (Ozkan et al. 2012a; Zhang et al. 2012); however, other studies failed to prove these positive effects (Archer et al. 2009; Archer 2017). They were introducing light-emitting diodes, which minimised the contribution of heat to the incubator, improved repeatability of studies and enabled to explore the effects of selected wavelengths on embryonic and postembryonic development and behaviour (Huth and Archer 2015). Positive effects of intermittent green light during incubation were first documented by Rozenboim et al. (2004), who showed an accelerated embryonic development and post-hatch growth in broilers incubated in green light. Moreover, this light increases feed intake and improves feed efficiency compared with chicken incubated in the dark (Zhang et al. 2012). Further studies aimed at mechanisms mediating the effects of photostimulation revealed the stimulation of pectoral muscle growth (Halevy et al. 2006) and the upregulation of the somatotrophic axis (Zhang et al. 2014a).

In addition to growth stimulation, light during incubation can improve the stress responsiveness of the chicken after hatching, reduce fear (Archer and Mench 2014), and contribute to chickens that are better adapted to the new environment compared to broilers incubated in the dark (Ozkan et al. 2012a, b). Although green light improves growth, it does not reduce fear and stress susceptibility, whereas white and red light might be more effective in influencing chicken behaviour (Archer 2017).

Mechanisms that can mediate the effects of light during incubation on postembryonic development and behaviour are not well understood, and programming of the neuroendocrine system is plausible. In our previous studies, we focused on the development of circadian rhythms in the neurohormone melatonin. Melatonin transmits infor-

mation on the ambient photoperiod to the internal milieu of organisms because its biosynthesis is low during the day and high at night in all diurnal and nocturnal species studied until now. Moreover, melatonin exhibits pleiotropic effects and can influence different physiological and behavioural processes (Hardeland et al. 2011). Our first study revealed that chicken embryos could perceive light during incubation (Zeman and Illnerova 1990); we also found a high activity of the melatonin-biosynthesising pathway, which mirrors the duration of the dark phase. Moreover, our recent results show that the daily profile of melatonin biosynthesis reflects different wavelengths of light in incubators (Drozdova et al. 2019). The highest amplitude of pineal melatonin levels was found in embryos incubated under red and white light, with lower levels under green light. Incubation under blue light resulted in the lowest melatonin biosynthesis. In another study (Drozdova et al. 2020), we showed that incubation in warm and cold white light did not significantly influence embryonic melatonin biosynthesis, and therefore, it is possible that distinct wavelengths may selectively influence postembryonic development and behaviour.

In this context, in our present study, we explored the effects of incubation under monochromatic red and blue light on postembryonic growth rates and behaviour of broiler chickens reared under conditions used in poultry farming (nutrition, lighting, temperature, humidity, etc.). We analysed possible effects of different wavelengths of light during incubation on metabolism evaluated via plasma glucose, cholesterol, triacylglycerols and thyroid hormones, which were measured immediately after hatching and during the rapid growth phase in 3-week-old broiler chickens. Moreover, between the second and the third week of age, behavioural traits reflecting passive (resting, standing, preening, dust bathing) and active behaviours (walking, foraging, fighting, wing-flapping) were assessed.

## MATERIAL AND METHODS

We used broiler breeder eggs ( $n = 120$ ; weight  $54 \pm 4$  g) from a 27-week-old parent flock Ross 308 (Mach Hydina Budmerice, s.r.o., Budmerice, Slovakia) with a light-brown eggshell. Eggs were incubated in two MIDI F500 incubators (PL Maschine KFT, Tárnok, Hungary), at standard temperature

( $37.5 \pm 0.2$  °C) and humidity (50% to 60%), with automatic egg turning. Eggs were incubated in the light-dark (L-D) regime of 12:12 h, provided by LED strips (Slov-Led Plus, Zvolen, Slovakia) mounted on incubator ceilings and emitting monochromatic red light at 632 nm or blue light at 463 nm. The light intensity was adjusted to  $0.04 \text{ W/m}^2$  and measured with a CL-500A illuminance spectrophotometer (Konica Minolta, Tokyo, Japan).

After hatching, 10 chickens per light treatment (six males and four females) were weighed, individually labelled with a leg ring and kept in two pens according to light exposure. Birds were reared for three weeks in pens sized  $180 \times 100 \text{ cm}$  ( $1\,800 \text{ cm}^2/\text{bird}$ ) with wood shavings under standard conditions used in poultry farming (nutrition, lighting, temperature, humidity, etc.). The broiler starter diet (20.5% crude protein and  $11.5 \text{ MJ/kg}$  of metabolisable energy; Energys® Hobby; De Heus, Bučovice, Czech Republic) and water were available *ad libitum*. The temperature was gradually lowered from the initial  $33$  °C to  $25$  °C at the end of the experiment. Birds were exposed to fluorescent white light (Osram GmbH, Munich, Germany) with an intensity of 20 lux and a lighting regime of 23L:1D. Two experiments were performed.

### Experiment 1

After hatching, 10 birds incubated in red and blue light were sacrificed. The blood samples were collected into heparin-coated tubes and kept on ice. After centrifugation at  $2\,000 \text{ g}$  for 15 min, plasma was stored at  $-20$  °C for further analysis. Another 10 birds per embryonic light treatment were followed for the next three weeks, during the rapid growth phase. They were weighed twice a week, and feed consumption was recorded on a group basis. At 21 days, both groups of chickens were sacrificed, and blood was collected and centrifuged. In plasma, we measured glucose, cholesterol and triacylglycerol concentrations using commercially available kits according to the manufacturer's instructions (Erba Lachema s.r.o., Brno, Czech Republic). Plasma levels of the thyroid hormones triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) (Institute of Isotopes Co., Ltd., Budapest, Hungary), as well as the stress hormone corticosterone (DRG Instruments GmbH, Marburg, Germany) were determined by commercial radioimmunoassay kits according to the manufacturer's instructions (Drozdova et al. 2020). Hormones were

measured in one assay and the coefficient of intra-assay variation was lower than 5%.

### Experiment 2

From days 14 to 21, the behaviour was continuously video-recorded, and selected behavioural traits were later evaluated from the recording. We used IP cameras Hikvision DS-2CD2020F-I with a wide-angle lens, connected to a network video recorder Hikvision DS-7616NI-E2/16P/A (Hikvision, Hangzhou, China). The following parameters were quantified: resting (sitting or lying on the floor, without any other activity), standing, preening (tidying and cleaning the feathers with a beak), dust-bathing (driving dust into its feathers by rolling and moving in the wood shavings), walking, foraging (pecking and scratching the floor, walking with head down), fighting, wing-flapping (rapid movement of both wings), eating and drinking. The number of chicks involved in these activities was counted every 30 minutes. Behavioural observations were carried out continuously during eight days from video records. In the analysis, "day" was used as a repeated factor since the same birds were observed repeatedly.

Tonic immobility (TI) test was performed in a separate room to avoid possible interferences. The chicken was placed on the back in a U-shaped wooden cradle with one hand restraining the animal by a light press on the chest. After 5 s, the hand was slowly removed, and the stopwatch started. The researcher then remained silent and stationary while watching the bird. The duration of TI was defined as the time until the bird righted itself. If a bird righted itself within 10 s after removing the hand, we repeated the induction process up to a maximum of three times. If TI was not induced after three trials, zero TI duration was recorded. If a bird remained in TI for 10 min, the process was terminated, and the bird was assigned the maximum duration of 600 seconds.

### Statistical analysis

Statistical analysis was performed by SigmaPlot 13 (Systat Software, San Jose, CA, USA) and GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Body weight was analysed by two-way repeated measures analysis of variance (ANOVA), with the factors

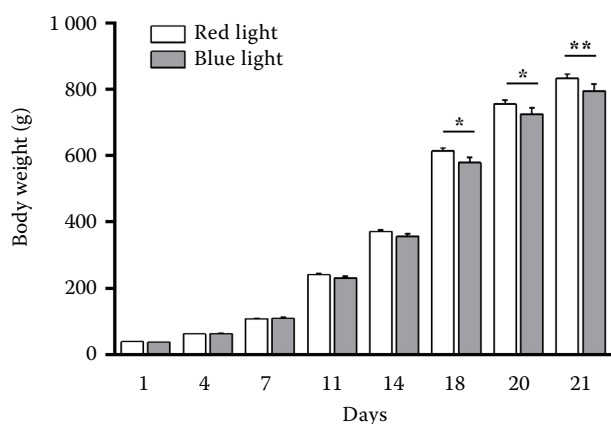


Figure 1. Body weight in broiler chickens

Eggs were incubated under red and blue light with light-dark cycle of 12:12 h. Data are given as mean  $\pm$  SEM,  $n = 18$  to 19 per day

\* $P < 0.05$  and \*\* $P < 0.01$  for differences between red- and blue-light groups

light treatment and age, using the Holm-Sidak post hoc test. An unpaired two-tailed  $t$ -test was used to compare biochemical and endocrine parameters. Behavioural observations were analysed using one-way repeated measures ANOVA. Tonic immobility duration and the number of inductions were analysed using the Mann-Whitney  $U$ -test. Shapiro-Wilk was used as a normality test and Brown-Forsythe to test the equality of variance.

The experiment was approved by the State Veterinary and Food Administration of the Slovak Republic (1464/19-221/3a).

## RESULTS

Hatchability was not different between groups. We found a significant interaction effect of age

and light treatment on the body weight of chickens ( $F_{1,7} = 2.630$ ;  $P = 0.012$ ). Chickens incubated under red light were significantly heavier in comparison with the blue light group at days 18 ( $P = 0.016$ ), 20 ( $P = 0.031$ ) and 21 ( $P < 0.008$ ) of age (Figure 1). The feed conversion ratio was recorded on a group basis and was similar in both groups for the 21-day growing period (1.66 and 1.71 for red and blue light-incubated birds, respectively).

No significant differences were found between light treatments in the plasma glucose levels in neither age group (hatchlings:  $F_{1,19} = 1.494$ ;  $P = 0.152$ ; 21-day-old chickens:  $F_{1,18} = 0.430$ ;  $P = 0.672$ ) (Figure 2A). No significant differences were found in plasma cholesterol levels in hatchlings and 21-day-old chickens incubated under red and blue light (hatchlings:  $F_{1,18} = 0.259$ ;  $P = 0.798$ ; 21-day-old chickens:  $F_{1,18} = 2.011$ ;  $P = 0.059$ ) (Figure 2B). No changes between light treatments were found in plasma triacylglycerol concentrations (hatchlings:  $F_{1,18} = 0.879$ ;  $P = 0.392$ ; 21-day-old chickens:  $F_{1,18} = 1.151$ ;  $P = 0.265$ ) (Figure 2C).

Light conditions during incubation did not influence the levels of corticosterone (hatchlings:  $F_{1,17} = 0.669$ ;  $P = 0.512$ ; 21-day-old chickens:  $F_{1,18} = 0.048$ ;  $P = 0.963$ ) (Figure 3A) and had no effect on plasma  $T_3$  concentrations (hatchlings:  $F_{1,14} = 0.069$ ;  $P = 0.946$ ; 21-day-old chickens:  $F_{1,18} = 0.140$ ;  $P = 0.890$ ) (Figure 3B). Plasma  $T_4$  levels did not differ between hatchlings and 21-day-old chickens from both groups (hatchlings:  $F_{1,17} = 0.414$ ;  $P = 0.684$ ; 21-day-old chickens:  $F_{1,18} = 2.047$ ;  $P = 0.056$ ) (Figure 3C).

Analysis of video recordings did not indicate the presence of rhythmic changes over the 24-h cycle (data not given); hence, we used the average number of animals performing defined behaviours during 24 h (Figure 4). Days were treated as repeated measures. The most frequently observed behaviour

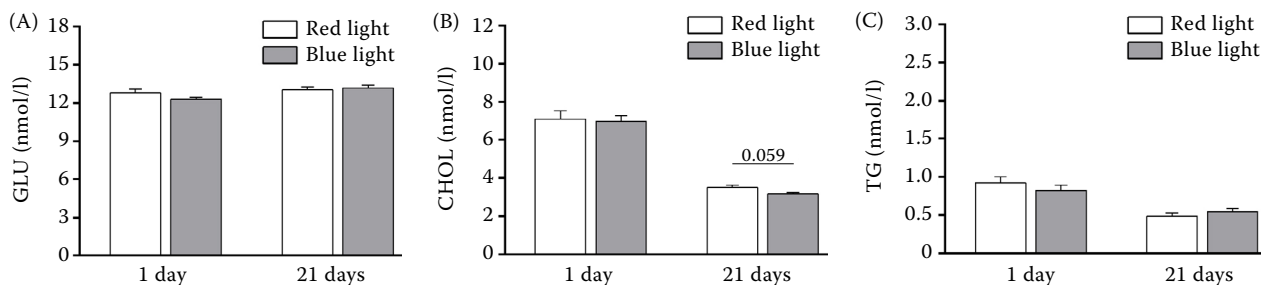


Figure 2. Concentrations of glucose (GLU), cholesterol (CHOL) and triacylglycerols (TG) in hatchlings and 21-day-old chickens

Eggs were incubated under red and blue light with the light-dark cycle of 12:12 h. Data are given as mean  $\pm$  SEM,  $n = 8$  to 11 per group

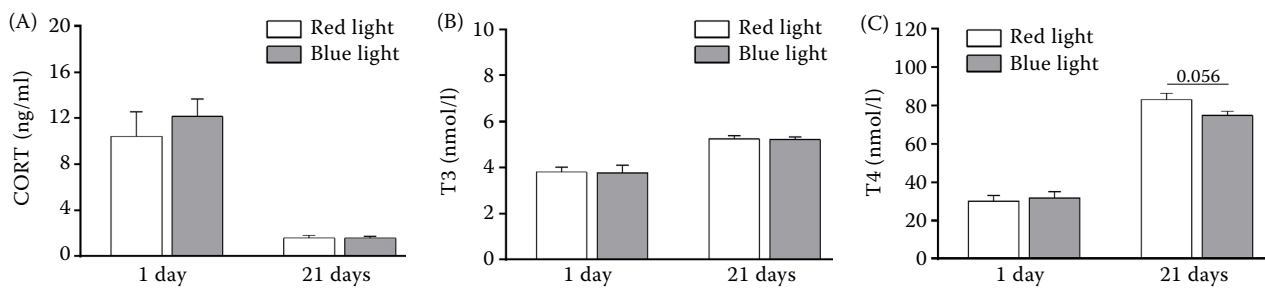


Figure 3. Concentrations of corticosterone (CORT), triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) in hatchlings and 21-day-old chickens

Eggs were incubated under red and blue light with the light-dark cycle of 12:12 h. Data are given as mean  $\pm$  SEM,  $n = 8$  to 11 per group

was resting, where significantly more animals from the red-light group were observed resting in comparison with the blue-light group ( $F_{1,8} = 10.365$ ;

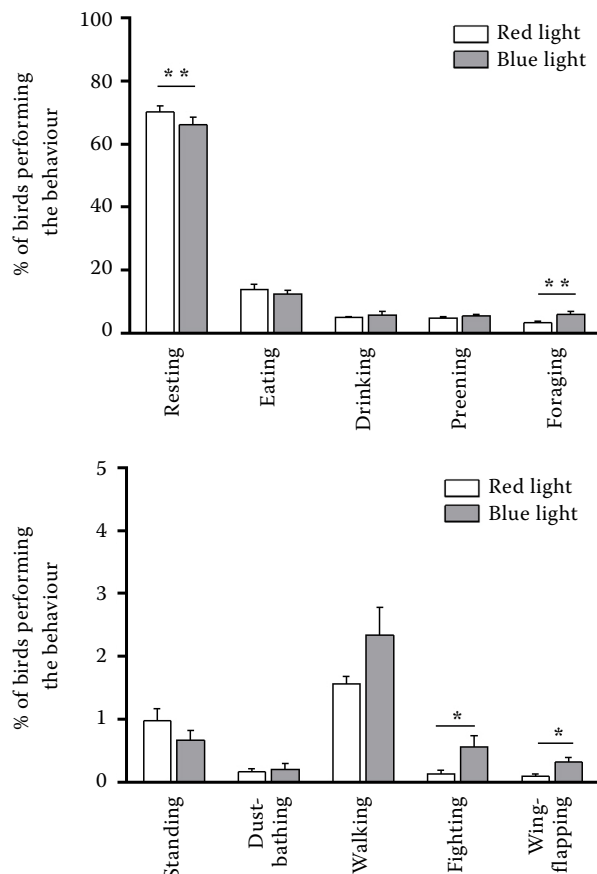


Figure 4. Percentage of chickens performing selected behaviours during the period of 14 to 21 days of age

Eggs were incubated under the light-dark cycle of 12:12 h, using red and blue light during the light phase. Data are given as mean  $\pm$  SEM

\* $P < 0.05$  and \*\* $P < 0.01$  for differences between red- and blue-light groups

$P = 0.01$ ). On the other hand, the blue-light group showed significantly more foraging ( $F_{1,8} = 11.670$ ;  $P = 0.009$ ), fighting ( $F_{1,8} = 5.327$ ;  $P = 0.05$ ) and wing-flapping ( $F_{1,8} = 9.405$ ;  $P = 0.015$ ) behaviour.

To better illustrate the differences between groups, we pooled the behaviours into three categories: passive (resting, standing, preening, dust bathing), active (walking, foraging, fighting, wing-flapping) and alimentary (eating, drinking) (Figure 5). We found no difference between groups in alimentary behaviours. The red-light group showed significantly more passive behaviours ( $F_{1,8} = 6.109$ ;  $P = 0.039$ ), whereas the blue-light group performed more active behaviours ( $F_{1,8} = 13.839$ ;  $P = 0.006$ ).

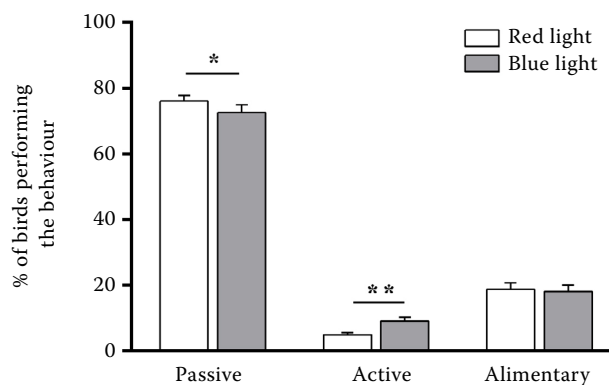


Figure 5. Percentage of chickens performing passive (resting, standing, preening and dust-bathing), active (walking, foraging, fighting and wing-flapping) and alimentary (eating and drinking) behaviours during the period from 14 to 21 days of age

Eggs were incubated under the light-dark cycle of 12:12 h, using red and blue light during the light phase. Data are given as mean  $\pm$  SEM

\* $P < 0.05$  and \*\* $P < 0.01$  for differences between red- and blue-light groups



The tonic immobility test indicates fear and fearfulness in the flock. The number of inductions needed to induce TI for the red light (median = 1.50) and blue light (median = 1.75) group did not differ ( $U = 162$ ,  $n_{\text{red}} = 19$ ,  $n_{\text{blue}} = 18$ ,  $P = 0.788$ ). Neither did TI duration for red light (median = 115.50) and blue light (median = 93.25) show a statistically significant difference ( $U = 132$ ,  $n_{\text{red}} = 19$ ,  $n_{\text{blue}} = 18$ ,  $P = 0.242$ ).

## DISCUSSION

Our results demonstrated that incubation under red or blue light differently influenced the growth and behavioural parameters of 3-week-old broiler chickens. Broilers incubated in red light had a higher body weight compared to those incubated under blue light from day 18 onwards. However, the body weight of hatchlings did not differ between light treatments. Therefore, the changes in body weight do not reflect changes in the speed of embryonic development or the time of hatching. Instead, we hypothesise that different quality of light during incubation programmed neural mechanisms which control the physiology and behaviour of chickens during development. Indeed, when we evaluated the behavioural traits of broilers during the last (third) week of the experiment, we found significant differences between both groups in terms of behaviour. Broilers from the red light-incubated group exhibited more passive, energy-saving traits, such as resting, as compared with the blue light-incubated chickens, which were more active and had a higher frequency of foraging, fighting and wing-flapping.

A stimulatory effect of monochromatic light on body weight has been observed after embryonic exposure to green light (Rozenboim et al. 2004, 2013; Zhang et al. 2014b), although some studies did not find this stimulatory effect (Dishon et al. 2018; Tong et al. 2018). Studies exploring mechanisms mediating possible stimulatory effects of green light have suggested changes in the activity of the somatotrophic axis (Halevy et al. 2006; Dishon et al. 2021). In our experiment, concentrations of thyroid hormones did not differ between groups. In chickens, the somatotrophic and thyreotropic axes are closely interconnected (Darras et al. 1992; Vasilatos-Younken et al. 2000) and influenced by the growth hormone, especially via deiodination (conversion of  $T_4$  to  $T_3$ ). Since the concentrations of  $T_3$ , the active form of thyroid hormones involved in growth control, were not sig-

nificantly affected by light during incubation, we believe that the stimulatory effect of red light on growth rate during the rapid growth phase was not mediated by the somatotrophic/thyreotropic axis.

Instead, we propose that different behaviour in both groups is responsible for a higher growth rate in red compared to blue light-incubated birds. Indeed, published data indicate that hatchlings incubated under the LD cycle may better cope with stressful conditions after hatching than chickens incubated in darkness. Light during incubation can affect the stress responsiveness of chickens during development and can reduce fear in broilers after hatching (Archer and Mench 2014; Archer 2017), significantly contributing to a better adaptation to the postnatal environment and welfare (Ozkan et al. 2012b). Moreover, the same LD during embryonic and postembryonic development may further improve their adaptation to environmental conditions after hatching (Ozkan et al. 2012a), suggesting a programming effect of the photoperiod during embryonic development. However, in our study, incubation in blue or red light did not result in differences in either tonic immobility test or plasma corticosterone concentration, which are considered indicators of an acute stress response. Chick quality could also be improved by exposing eggs to white or red light during embryogenesis. It has been suggested that red light may be the key spectrum to reduce fear and stress susceptibility, whereas the green light is not so effective (Archer 2017). The absence of differences in stress response in hatchlings is in line with data from a study exposing broilers to white light treatment (Shafey et al. 2005; Zhang et al. 2012).

Thus, if the acute parameters of stress response were not changed, we expect that the behavioural phenotype of broilers incubated in red or blue light was programmed at least partially via a different melatonin profile. Circadian rhythmicity of melatonin biosynthesis develops during the last (third) week of embryonic development, and the daily pattern in the melatonin-synthesising pathway reflects the length of the photoperiod to which broiler embryos were exposed during incubation (Zeman and Illnerova 1990). Moreover, our recent study shows that the amplitude of the melatonin rhythm reflects not only the duration of the photoperiod but also the quality of light to which eggs were exposed during incubation. The maximum amplitude of the pineal melatonin rhythm was determined in 20-day-old embryos incubated under red and white light and

the lowest amplitude in blue light-incubated embryos (Drozdova et al. 2019). The consequences of these differences in the melatonin rhythm on postembryonic life are not known, and therefore, we investigated them in our present study. Interestingly, melatonin receptors develop earlier than rhythmic melatonin biosynthesis, and they were first identified at the beginning of week 2 (day 8) of embryonic development (Chong and Sugden 1992). Melatonin receptors are expressed in many parts of the avian brain, especially in structures connected with the visual system (Siuciak et al. 1991). Their highest density was identified on day 18 of embryonic development, and receptor density decreases during postembryonic life (Dubocovich and Markowska 2005). The high presence of melatonin receptors during embryonic life suggests their important role, especially in the development of the brain. Indeed, melatonin was found to increase the mitotic activity of astrocytes in the brain (Paulose et al. 2009) and may be involved in brain lateralisation. Moreover, recently, melatonin has been suggested to be involved in developmental programming (Chen et al. 2013; Hsu and Tain 2020). Interestingly, a recent study indicates that the critical period for the green light to influence the somatotropic axis occurs in the final stages of embryonic development, after day 18 (Dishon et al. 2021), when the melatonin biosynthesis steeply increases.

## CONCLUSION

In conclusion, our results suggest that monochromatic red and blue light during embryonic development differently affects and programs processes involved in controlling the growth and behaviour of chickens. Red light during incubation resulted in a less active behavioural phenotype, whereas blue light during incubation may program a pro-active behavioural phenotype. The less active chickens may exhibit better growth, whilst the pro-active phenotype can show higher physical activity with contrasting consequences on the postembryonic development and welfare of broilers.

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## Conflict of interest

The authors declare no conflict of interest.

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