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Effect of cold and warm white light on selected endocrine and immune parameters of broiler embryos and hatchlings

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Abstract: Lighting conditions during incubation can influence embryonic development, post-hatching ontogeny and production efficiency. Previous studies revealed that different light colours differently affect pineal melatonin biosynthesis in embryos and postembryonic development of broiler chickens, but physiological mechanisms mediating these effects are not known. Cold and warm white light consists of different wavelengths and therefore the aim of the present study was to explore if these two lights can differently influence the development of circadian melatonin biosynthesis, production of thyroid hormones and corticosterone, concentration of metabolites (glucose, cholesterol and triacylglycerols) as well as expression of two important immune genes, presenilin 1 and avian beta-defensin 1 (*AvBD-1*). We evaluated these traits in embryos before hatching (456, 460, 465, 468 and 472 h of incubation) and in hatchlings. The rhythmic profile of pineal melatonin with higher concentrations during the dark time was determined in both treatment groups. Melatonin levels increased considerably in hatchlings in comparison with embryos, but we found no difference in rhythm characteristics between groups. We did not identify any daily rhythms in plasma corticosterone and thyroid hormone levels in either studied age and no differences were found between light treatments in concentrations of thyroid hormones, corticosterone, metabolites and expression of presenilin and *AvBD-1*. The expected developmental increase of thyroid hormones was proved. Gene expression of presenilin increased in the duodenum of hatchlings in comparison with embryos, but the expression did not change in the bursa of Fabricius. On the other hand, expression of *AvBD-1* decreased in hatchlings compared to embryos in both tissues. Based on these results, we can conclude that the colour temperature of white light did not influence endocrine and immune parameters determined in this study and probably monochromatic rather than polychromatic light should be used to influence embryonic development and postembryonic ontogeny of broiler chickens.

Keywords: chicken; incubation; polychromatic light

Environmental conditions during embryonic and early postembryonic development can influence the performance and health of animals in adulthood. Several variables acting during prenatal and perinatal life (parental nutrition, stress, hypoxia, immune load, etc.) can affect the future development

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of individuals (Dixon et al. 2016). Understanding the effects of environmental conditions during embryonic development in poultry should optimise not only hatchability but also post-hatching development and production efficiency, especially in broiler chickens which are raised in enormous numbers around the world.

When considering incubation conditions, temperature and humidity have usually been studied because they are well known as being able to influence embryonic development, the hatching process and even post-hatch performance. Moreover, recently, lighting conditions during incubation have attracted attention, as they have the potential to influence embryonic development and production efficiency (Ozkan et al. 2012; van der Pool et al. 2017; Archer 2018; van der Pool et al. 2019; Hannah et al. 2020). In addition to the intensity of illumination, the duration of the photoperiod and the quality of light have been considered but until now no generally accepted standards or recommendations related to light in incubators have been available. Moreover, physiological mechanisms mediating the effects of lighting conditions on embryos are not known, and their understanding could promote optimisation of these conditions.

Rhythmic production of melatonin is the primary signal, which transmits information about environmental lighting conditions on the internal milieu of animals. Previous studies proved that the circadian rhythm in melatonin biosynthesis develops during the last third of the embryonic period in chicken embryos, much earlier than in mammals (Zeman and Herichova 2011). Moreover, a recent study demonstrated that monochromatic light of different wavelengths differently affects the development of circadian melatonin production with the highest efficiency in embryos incubated in red and less in blue light (Drozdova et al. 2019). Melatonin production during embryonic and early postembryonic life can be important, because this hormone was suggested as playing a role in growth control (Zeman et al. 1999) and bone development (van der Pool et al. 2019), key processes affecting welfare and production performance of broiler chickens.

The profitability of poultry production is determined by the health of the flocks, which is related to the activity of the immune system. Knowledge about the effects of lighting during incubation on the development and maturation of the immune system is limited. The immune system in avian species develops rapidly during the final days of embryonic development and during critical periods after hatching (Bar-Shira and Friedman 2006; Meade et al. 2009; Hincke et al. 2019). This maturation also concerns the local immune system in the gastrointestinal tract (GIT) of embryos and newly hatched chickens (Bar-Shira et al. 2003; Bar-Shira and Friedman 2006), and can be critically important for future performance. This maturation of the intestinal immune system has not been investigated from the aspect of lighting conditions during incubation, and therefore in our present experiments, we focussed on this aspect.

Recent studies have suggested that exposure to cold and warm light during embryonic development can affect performance traits of broiler chickens (Archer 2018) but the underlying mechanisms are not understood. Therefore, the aim of the present study was to explore if cold or warm white light during incubation can influence the development of the circadian, endocrine and immune system of embryos and hatchlings of broiler chickens. We evaluated melatonin biosynthesis, production of thyroid hormones critical for perinatal development and corticosterone, the important cue for immune system functioning, as well as expression of essential genes (presenilin 1, *PSEN-1* and avian beta-defensin 1, *AvBD-1*), which can indicate development and maturation of the immune system in the bursa of Fabricius and gastrointestinal tract.

MATERIAL AND METHODS

This study was conducted using Ross 308 broiler breeders' (age 40 weeks) eggs (n = 150; weight 60 ± 4 g), which have a light-brown eggshell (Mach Hydina, Budmerice, Slovakia). Eggs were incubated in two incubators, MIDI F500 (PL Maschine KFT, Tárnok, Hungary), at a standard temperature of 37.5 ± 0.2 °C and humidity levels of 50% to 60% with automatic egg turning. In our experiment, eggs were exposed to the light-dark (LD) regime of 12 : 12 h with polychromatic cold white light in one and warm white light in the other incubator. Incubators were illuminated by light-emitting diode (LED) strips (Slov-Led Plus, Zvolen, Slovakia), emitting light with an average intensity of ± 500 lux. The strips were mounted on the top of the incubators and emitted polychromatic cold white light

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(± 5 000 K) and warm white light (± 2 700 K). Light intensity and chromatic temperature were measured using an illuminance spectrophotometer (CL-500A; Konica Minolta, Tokyo, Japan).

Experiment 1

In the first experiment, 19-day-old embryos (ED19) (456, 460, 465, 468 and 472 h of incubation) were used. Embryos were taken from the eggshell and decapitated under dim red light during the dark time. Blood was collected in heparinised ice cold test tubes. Samples were centrifuged at 2 000 × *g* for 10 min at 4 °C, plasma was removed and stored at 4 °C until analyses. Pineal glands were quickly excised, immediately frozen on dry ice (–70 °C) and stored at –20 °C until melatonin measurement. Tissues for gene expression analyses (the duodenum and the bursa of Fabricius) were dissected immediately after the decapitation of chicken embryos during the light phase after 456 h of incubation, and frozen in sterile tubes in liquid nitrogen. Tissues were stored at –80 °C until analyses.

Experiment 2

In the second experiment, hatchlings were kept in incubators after hatching under identical lighting conditions as during incubation. The chickens had no access to food and water, simulating the situation prevalent in the poultry industry. Chickens were killed by decapitation during the light phase at ZT10 and during the dark phase (ZT14, ZT19 and ZT22). Samples of biological material were taken in the same way as described in Experiment 1.

Hormonal and biochemical analyses

Melatonin from the pineal glands was extracted with methanol and melatonin levels were measured by a radioimmunoassay (RIA) according to the methods validated for pineal extracts (Drozdova et al. 2019). Intra- and inter-assay coefficients of variation were 4.3% and 14.1%, respectively. The sensitivity of the assay was 9.0 pg/pineal gland. Plasma thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), were measured using a commercial RIA kit (Institute of Isotopes Co., Ltd., Budapest, Hungary). Plasma corticosterone concentrations were measured by RIA kit (DRG Instruments GmbH, Marburg, Germany) according to the supplier's instructions.

Plasma glucose, cholesterol and triacylglycerol concentrations were measured using a commercial kit (Erba Lachema s.r.o., Brno, Czech Republic). Total plasma antibody levels were determined by an enzyme-linked immunosorbent assay as described previously (Grindstaff et al. 2005).

Gene expression analysis

The duodenum and the bursa of Fabricius were homogenised in 1 ml and 0.5 ml of TRI Reagent (Molecular Research Centre, Inc., Cincinnati, OH, USA), respectively, using FastPrep-2 (MP Biomedicals, Santa Ana, CA, USA; 6.0 m/s; 40 s). Messenger RNA was isolated using the phenol chloroform extraction according to the manufacturer's instructions. The concentration and purity of isolated mRNA were evaluated with a NanoDrop spectrophotometer (NanoDrop One spectrophotometer; Thermo Fisher Scientific, Waltham, MA, USA). The integrity of mRNA was tested on agarose gel using electrophoretic separation of the mRNA samples. Isolated mRNA was treated before the synthesis of cDNA with DNase I (Thermo Fisher Scientific, Waltham, MA, USA). The synthesis of cDNA was performed with the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions (amount of mRNA: 500 ng; final reaction volume: 20 µl). The prepared cDNA was diluted at a ratio of 1 : 20 and stored at –20 °C before running the real-time polymerase chain reaction (PCR). The analysis of gene expression was performed on the CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), with a final reaction volume of 10 µl. Primers for avian beta-defensin 1 (*AvBD-1*; NM_204993.1, F 5'-GAGAAACCATTGTCAGCCCTGT-3', R 5'-CAATCTGACTTCCTTCCTAGAGCC-3', product length: 138 bp), presenilin 1 (*PSEN-1*; NM_204163.2, F 5'-GGGCAGCGTCAAGATCCATT-3', R 5'-TATCATTGGTGTTGCTGAGGTG-3') and housekeeper gene *RPL13* (NM_204999.1, F 5'-AGCTTA-

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AACTGGCGGGCAT-3', R 5'-GAGCGATACTCC-TTCAGCCG-3', product length: 127 bp) were used in a concentration of 300 nM. The real-time PCR programme consisted of an initial denaturation step for 30 s at 95 °C, 40 cycles of 15 s at 95 °C, 30 s at 59 °C and 30 s, followed by a melting curve step of 15 s at 95 °C and 5 s at 65–95 °C, as recommended by the manufacturer. Data were analysed by the LinReg PCR program, v2016.0 (Ruijter et al. 2009). Gene expression was calculated using an average assay efficiency (average efficiency^–Cq), normalised to the expression of the housekeeping gene *RPL13*. The final values were expressed as a ratio of individual values to the average of all studied samples.

Statistical analysis

Data were analysed by the statistical program STATISTICA v12.5 software (StatSoft CR s.r.o., Prague, Czech Republic). All data were checked for normal distribution and, if needed, normalised using a Box–Cox transformation to meet the requirements for parametric statistics. The results were evaluated using analysis of variance with light regime and age of animals as categorical factors. If the interactions were significant, the differences between the individual groups were evaluated using the Fisher's least significant difference post hoc test.

The experiment was approved by the Ethical Committee at the Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences and State Veterinary Authority of the Slovak Republic.

RESULTS

Hormonal and biochemical data

During the final stages of embryonic life, pineal melatonin levels of chicken embryos incubated under LD 12 : 12 h exhibited a 24-h rhythm with higher concentrations during the dark phase (Figure 1A). This increase was substantial especially during the dark time also in hatchlings (Figure 1B). No differences were found between warm and cold white light incubated embryos (Figure 1A) ($F_{1,46}$ = 0.000 1; P = 0.99) and hatched chickens (Figure 1B) ($F_{1,42}$ = 0.004 9; P = 0.94). Pineal melatonin concentrations significantly increased postnatally (Figure 1B) compared with pre-hatching levels in the dark time (Figure 1A) ($F_{1,80}$ = 191.8; P < 0.001).

No significant differences were found in the levels of T_3 (Figure 2A) ($F_{1,39}$ = 0.57; P = 0.45) and T_4 (Figure 2B) ($F_{1,37}$ = 0.04; P = 0.83) in chicken embryos incubated under warm and cold white light. Additionally, we did not find any significant differences in the levels of T_3 (Figure 2C) ($F_{1,29}$ = 0.12; P = 0.74) and T_4 (Figure 2D) ($F_{1,29}$ = 0.006; P = 0.94) in chickens kept under both kinds of white light.

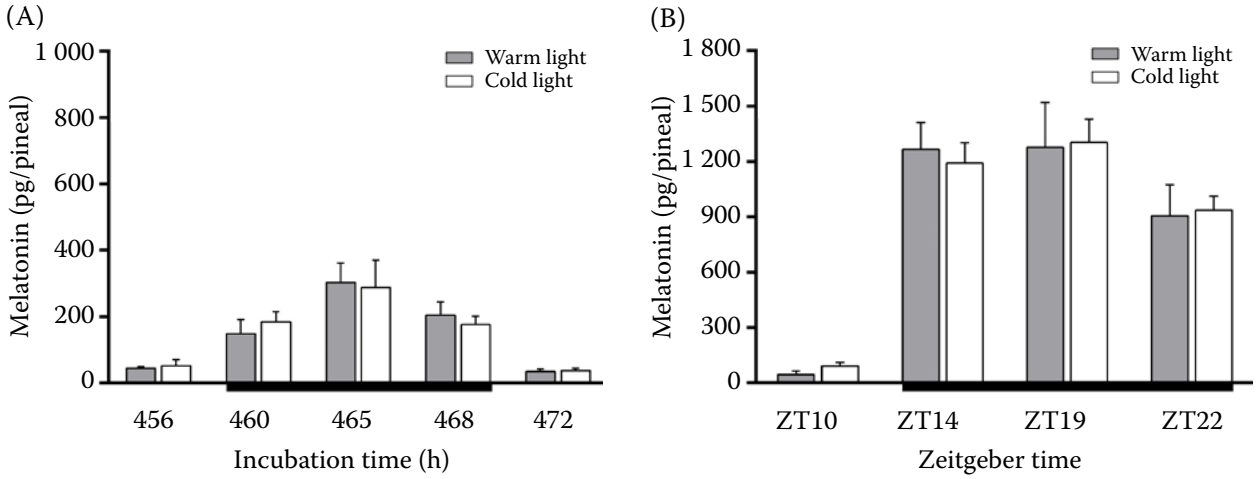

Figure 1. Pineal melatonin concentrations in chicken embryos (A) and hatchlings (B)
Eggs and chickens were incubated and kept under the light-dark cycle 12 : 12 h using warm and cold white light during the light phase. Time is expressed as incubation time (hours) with n = 5 to 6 per time point and zeitgeber time (ZT; ZT0 = light on) with n = 6 to 10 per time point. Black bars at the bottom line represent the dark phase. Data are given as mean ± SEM

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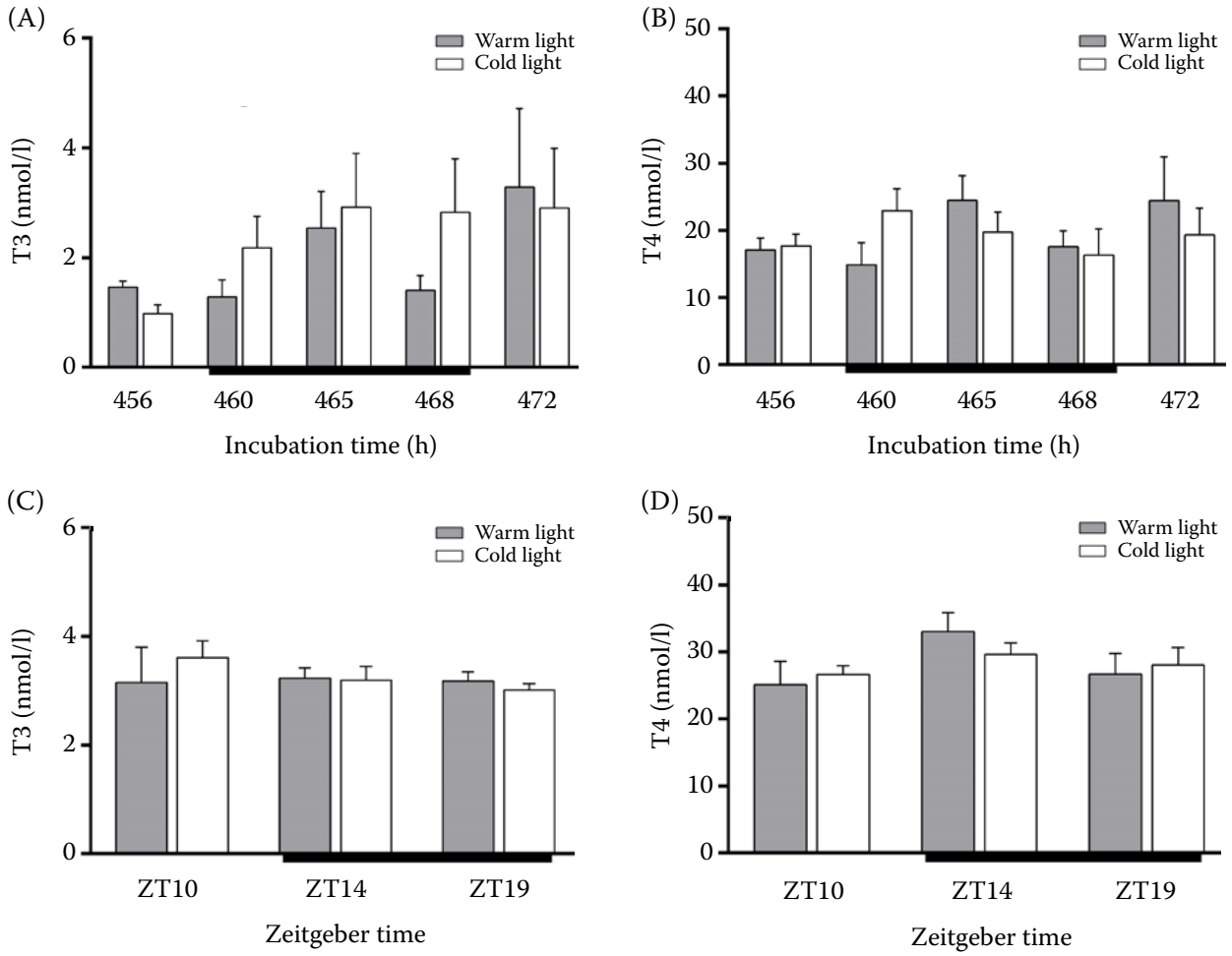

Figure 2. Concentrations of triiodothyronine (T_3) (A) and thyroxine (T_4) (B) in chicken embryos and concentrations of T_3 (C) and T_4 (D) in hatchlings

Eggs and chickens were incubated and kept under the light-dark cycle 12 : 12 h using warm and cold white light during the light phase. Time is expressed as incubation time (hours) with n = 4 to 6 per time point and zeitgeber time (ZT; ZT0 = light on) with n = 5 to 9 per time point. Black bars at the bottom line represent the dark phase. Data are given as mean ± SEM

No changes were found in plasma concentrations of corticosterone (Figure 3A) ($F_{1,29}$ = 3.6; P = 0.06) and plasma IgY antibodies (Figure 3B) ($F_{1,45}$ = 0.20; P = 0.65) in chicken embryos between the light treatments. The levels of plasma corticosterone (Figure 3C) ($F_{1,47}$ = 0.23; P = 0.63) and IgY antibodies (Figure 3D) ($F_{1,49}$ = 2.31; P = 0.13) in chickens after hatching were not affected by the light treatment.

The concentration of plasma metabolites, such as glucose ($F_{1,47}$ = 0.45; P = 0.50) (Figure 4A), cholesterol ($F_{1,47}$ = 0.000 3; P = 0.98) (Figure 4B) and triacylglycerols ($F_{1,46}$ = 0.37; P = 0.54) (Figure 4C) in chickens was not affected by lighting in incubators.

Expression of immune genes

The expression of *PSEN-1* was not affected by light during incubation either in the bursa of Fabricius ($F_{1,30}$ = 0.25; P = 0.618) or in the duodenum ($F_{1,30}$ = 0.21; P = 0.654). The age of chickens influenced the expression, but only in the duodenum ($F_{1,30}$ = 94.71; P < 0.001). The expression of *PSEN-1* increased after hatching in comparison with the last day of incubation (Figure 5A). There were no changes in the expression of *PSEN-1* in the bursa of Fabricius after hatching compared to embryos ($F_{1,30}$ = 1.32; P = 0.261) (Figure 5C). Statistical analysis did not reveal any interaction between light during incubation and age of chickens.

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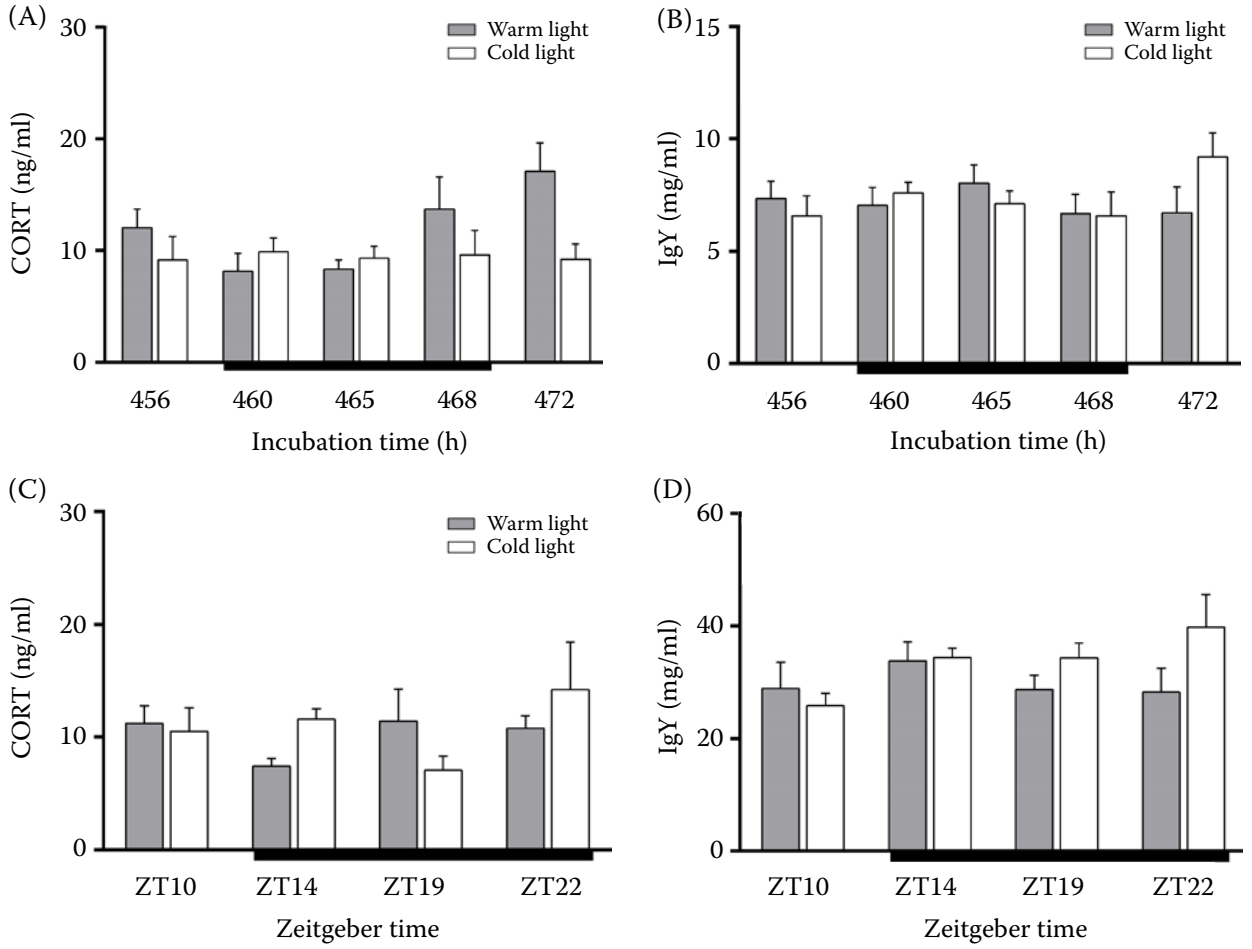

Figure 3. Concentrations of corticosterone (CORT) (A) and IgY antibodies (IgY) (B) in chicken embryos and concentrations of corticosterone (C) and IgY antibodies (D) in hatchlings

Eggs and chickens were incubated and kept under the light-dark cycle 12 : 12 h using warm and cold white light during the light phase. Time is expressed as incubation time (hours) with n = 4 to 6 per time point and zeitgeber time (ZT; ZT0 = light on) with n = 5 to 10 per time point. Black bars at the bottom line represent the dark phase. Data are given as mean ± SEM

Warm light tended to increase the expression of *AvBD-1* in the bursa of Fabricius ($F_{1,30}$ = 2.71; P = 0.110) but not in the duodenum ($F_{1,30}$ = 0.805; P = 0.377). The gene expression of *AvBD-1* decreased after hatching in the bursa of Fabricius ($F_{1,30}$ = 11.23; P < 0.01) (Figure 5D) as well as in the duodenum ($F_{1,30}$ = 71.22; P < 0.001) (Figure 5B) in comparison with the embryonic stage. There were no interactions between light during incubation and age.

DISCUSSION

Light conditions during incubation were reported to positively influence chicken quality and their performance after hatching (Archer 2016; Zhang et al. 2016; van der Pol et al. 2017; 2019). Spectral composition of light can stimulate the postnatal growth of broiler chickens (Rozenboim et al. 2004) and therefore, in the present study, we used cold (5 000 K) and warm (2 700 K) light, which differ in their light quality.

In our experiment, we identified a rhythmic profile of pineal melatonin levels in chicken embryos with expected higher concentrations during the dark time in both treatment groups. These results are in line with previously published results (Zeman and Herichova 2011). However, we found no difference in the amplitude of the rhythm between the groups incubated in different lighting conditions. In contrast, when embryos were incu-

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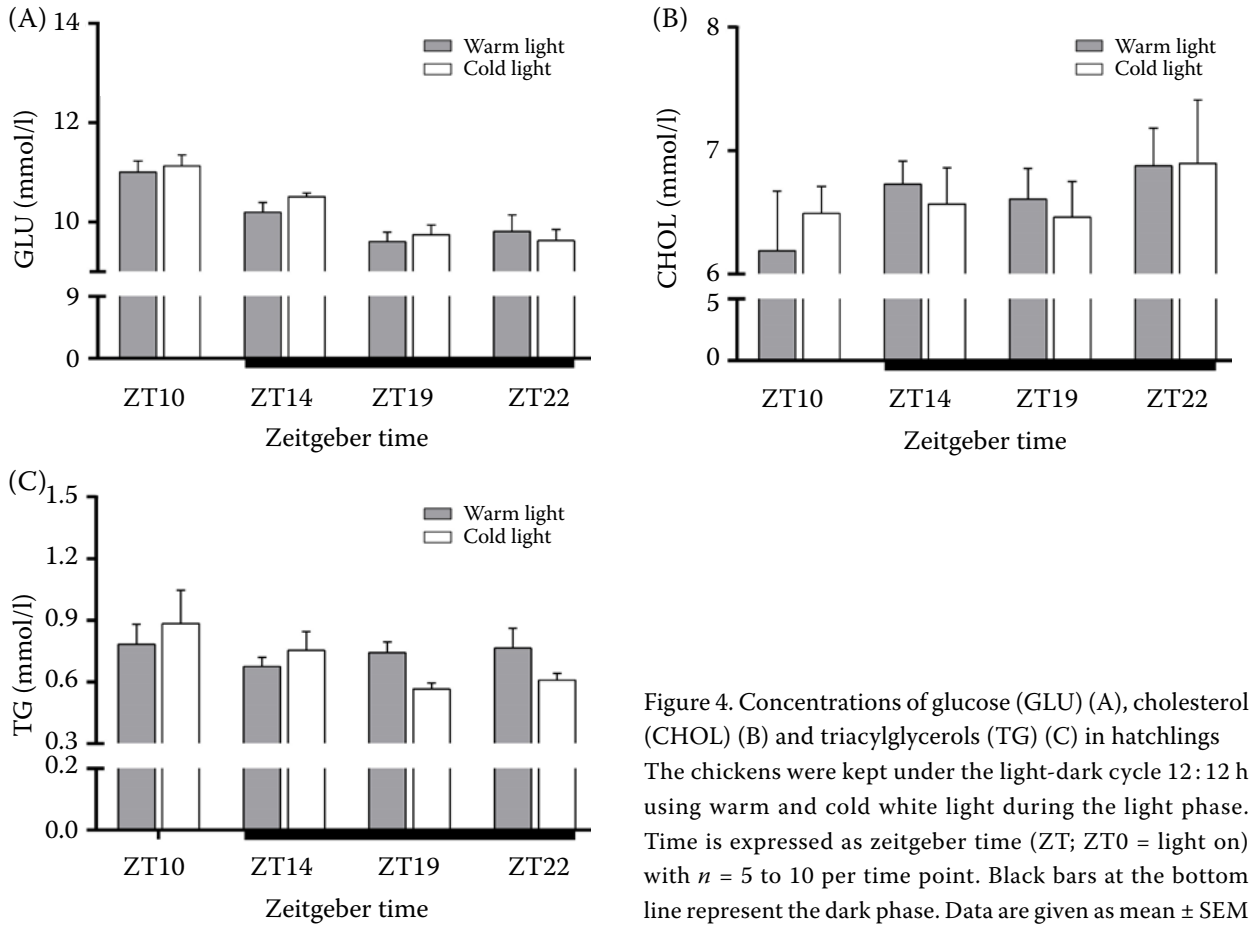

Figure 4. Concentrations of glucose (GLU) (A), cholesterol (CHOL) (B) and triacylglycerols (TG) (C) in hatchlings
The chickens were kept under the light-dark cycle 12 : 12 h using warm and cold white light during the light phase. Time is expressed as zeitgeber time (ZT; ZT0 = light on) with n = 5 to 10 per time point. Black bars at the bottom line represent the dark phase. Data are given as mean ± SEM

bated in monochromatic light, the highest pineal melatonin levels were determined in chicken embryos incubated under red and white light, lower levels under green light and the incubation under blue light resulted in the lowest melatonin biosynthesis (Drozdova et al. 2019). Apparently, since both light qualities contained the whole spectrum of wavelengths, their different composition was not sufficient to influence differently the development of melatonin synthesising pathways, even though warm light contains a higher proportion of the red component. Moreover, the correlated colour temperature of lights used in our experiment is similar to colour temperatures used in common practice (2 700 and 5 000 K). It is possible that if we apply very cold and very warm lights, a significant difference might appear. Pineal melatonin concentrations increased substantially post-hatching compared with pre-hatching levels in the present study, and the results corroborate the previously published data (Drozdova et al. 2019) and indicate that for melatonin production, the developmental pattern is firmly conserved and depends less on acute lighting conditions. Like in embryos, we did not find any differences in melatonin concentration related to lighting conditions during incubation.

We did not identify any rhythms in the concentration of corticosterone or thyroid hormones either in chicken embryos or in chickens after hatching. Plasma corticosterone concentrations showed a tendency of an increase at the end of ED19 in embryos incubated in warm compared to cold light, and further studies are needed to evaluate a possible physiological significance of this difference, especially in relation to development of the immune system. A tendency to suppressed plasma antibody concentrations was found during this time in our experiment. In the literature, plasma corticosterone levels of 17- and 18-day-old embryos decreased during the dark period and increased during the light period (Tong et al. 2018). High corticosterone is often connected with impaired humoral immunity since it can decrease antibody production in an antigen-specific manner (El-Lethey et al. 2002). Chickens exposed to light during incubation can be less susceptible to stress.

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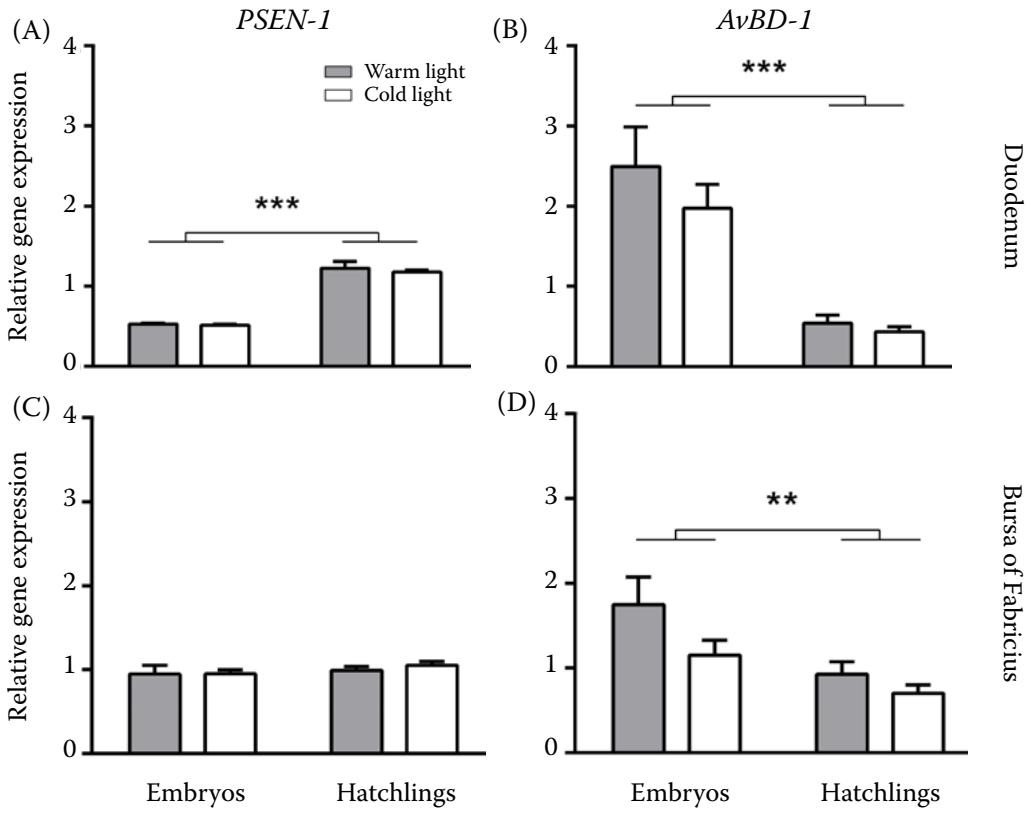

Figure 5. Expression of the immune genes presenilin-1 (*PSEN-1*) and avian beta-defensin 1 (*AvBD-1*) in the duodenum and in the bursa of Fabricius in chicken embryos (n = 5) and hatchlings (n = 12) incubated in warm or cold white light
Values are expressed as means ± SEM
$^{**}P < 0.01$; $^{***}P < 0.001$ for differences between warm and cold white light groups

Chicken embryos incubated in white light exhibited a decreased stress response post-hatch in comparison with chickens incubated in constant darkness (Archer 2016). Clearly, data concerning the effects of light during incubation on corticosterone levels and stress responsiveness are not consistent, and further research is needed in this field.

Thyroid hormones are involved in the regulation of most physiological processes and represent a key factor in the control of development and growth. Circulating T_3 concentrations increase at the end of embryonic development, especially at the time of the internal pipping, which is related to the transition of embryos to pulmonary respiration (Tona et al. 2003). For chicken embryos, this period is critical for survival because they need a massive supply of oxygen and energy to hatch. In our study thyroid hormones exhibited the expected developmental increase in plasma concentrations between embryos and hatchlings in line with published data (Tona et al. 2003). However, the pattern did not differ between the light treatments. The developmental increase in T_3 and T_3/T_4 conversion during day 19 of incubation is related to the dominant role of triiodothyronine during hatching (for a review see Darras 2019), but again they did not differ between embryos incubated in cold and warm light. In our study, we recorded 16 embryos in the stage of internal pipping and eight embryos prior to pipping in cold light, 13 embryos with internal pipping and 12 embryos prior to pipping in warm light. We found lower T_3 levels in embryos prior to pipping compared with internal pipping in both light treatments.

The immune system of chickens undergoes rapid maturation in the last days of incubation (Bar-Shira and Friedman 2006; Meade et al. 2009; Hincke et al. 2019). Despite this rapid embryonic development, chickens are still immunologically naive after hatching, and need up to approximately two weeks to mature and launch at least elementary adaptive immune responses (Mast and Goddeeris 1999; Bar-Shira et al. 2003; Smits and Bortolotti 2008). This pre- and post-hatch maturation also takes

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place in the gastrointestinal tract. After hatching, the intestinal tissues are colonised by immature cells of the innate as well as adaptive part of the immune system (Bar-Shira et al. 2003; Bar-Shira and Friedman 2006).

Bar-Shira and Friedman (2006) have demonstrated an extramedullary granulopoietic process taking place in the small intestine. Their finding was supported by histological observations and increased expression of the *PSEN-1* and *AvBD-1* genes. Published data indicate that *PSEN-1* is related to differentiation and maturation of cells from the myeloid cell line. In mammals, the gene is expressed during development of neutrophils, and it is co-localised with myeloperoxidase within the azurophil granules. *PSEN-1* is also expressed in the avian DT40 B-cell line (Mirnics et al. 2002).

We detected the expression of *PSEN-1* in the duodenum and bursa of Fabricius in both embryos and hatchlings with a significant increase in the duodenum of hatchlings in comparison with 19-day-old embryos. The expression of this gene did not change in the bursa of Fabricius. Previously, *PSEN-1* was detected in peritoneal heterophils and in the thymus but not in the peripheral blood and bursa of Fabricius in newly hatched chickens (Bar-Shira and Friedman 2006). The increase in *PSEN-1* expression after hatching, observed in our experiment in the duodenum, is in line with the findings of Bar-Shira and Friedman (2006).

Changes in expression of *PSEN-1* indicate maturation processes in heterophils. Therefore, the rising expression in the duodenum might reflect the continuing maturation of the GIT immune system in chickens. Since light itself, as well as its colour temperature during incubation, can influence several characteristics of developing chickens (Huth and Archer 2015; Archer 2018), we expected that the quality of white light during incubation might influence the development of the immune system and its preparedness to act on the gut and bursa of Fabricius. The colour temperature of the light did not influence *PSEN-1* expression, but we found a tendency to higher expression of another important immune gene, namely *AvBD-1* in the warm-light group in the bursa of Fabricius.

AvBD-1 belongs to β-defensins with anti-microbial and anti-fungal properties (Cuperus et al. 2013) and it is crucial for coping with neonatal infection in chickens (Meade et al. 2009). Beta-defensins are synthesised and stored in granules of heterophils (Harwig et al. 1994). *AvBD-1* is constitutively expressed in the lungs, bursa of Fabricius, in low levels in the small intestine and large intestine. Ramasamy et al. (2012) described constitutive as well as immunologically stimulated expression of β-defensins in the GIT of chickens. During the first three days of embryonic development, the constitutive expression of *AvBD-1* is at very low levels, rises at day 6 of embryonic development and then declines until day 12 of embryonic development (Meade et al. 2009). Constitutive expression of *AvBD-1* might be a preparation of chickens' gut for an invasion by new pathogens and other antigens after hatching since the maturation of granules is independent of previous bacterial colonisation (Bar-Shira and Friedman 2006).

In our study, the *AvBD-1* expression decreased in hatchlings compared to embryos in both tissues. This decrease is in line with the findings of Bar-Shira and Friedman (2006), who found that expression of β-defensins decreased during the first week after hatching and increased during the second week of life (Bar-Shira and Friedman 2006). A similar pattern was also found by Crhanova et al. (2011), who observed an increased expression of avian β-defensins in three-day hatchlings, followed by a decrease from day 4 of life. These changes in expression of β-defensins were accompanied by a transient increase in IL-8 and IL-17 expression, indicating physiological inflammation and maturation of the gut (Crhanova et al. 2011).

There was a slight tendency ($P = 0.110$) to higher levels of expression of *AvBD-1* in chickens of the warm-light group in the bursa of Fabricius. We found no other significant differences in studied immune genes and therefore we presume that the colour temperature of white light during incubation has no substantial effect on the innate part of the immune system in the developing GIT. However, since we studied only the constitutive expression of both genes in chickens which were not fed after hatching, we cannot exclude a potential effect of warm/cold light on chickens challenged with new antigens and pathogens in food.

We did not observe different effects of the two light qualities during incubation on the hormonal and immune status of embryos at the end of incubation and immediately after hatching. Filtration of light by the eggshell might have contributed to this fact because cold (7 500 K) and warm (3 250 K) LEDs with a light intensity of 250 lux

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are filtered similarly by the eggshells, making them virtually the same light (Archer 2016).

In summary, our study proved the expected developmental pattern of melatonin and thyroid hormone during perinatal development of broiler chickens. The relationship between plasma corticosterone and antibody concentrations before and shortly after hatching requires further analysis. Expression of the analysed immune genes exhibited a different developmental pattern, but there were no significant differences suggesting alterations of immune development between the light groups. Cold and warm white light did not affect the endocrine and immune parameters determined in this study, and probably monochromatic rather than polychromatic light should be used to influence embryonic development and postembryonic ontogeny of broiler chickens.

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Conflict of interest

The authors declare no conflict of interest.

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