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Stimulation with monochromatic green light during incubation alters satellite cell mitotic activity and gene expression in relation to embryonic and posthatch muscle growth of broiler chickens

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Previous studies showed that monochromatic green light stimuli during embryogenesis accelerated posthatch body weight (BW) and pectoral muscle growth of broilers. In this experiment, we further investigated the morphological and molecular basis of this phenomenon. Fertile broiler eggs (Arbor Acres, n = *880) were pre-weighed and randomly assigned to 1 of the 2 incubation treatment groups: (1) dark condition (control group), and (2) monochromatic green light group (560 nm). The monochromatic lighting systems sourced from light-emitting diode lamps and were equalized at the intensity of 15 lx at eggshell level. The dark condition was set as a commercial control from day 1 until hatching. After hatch, 120 male 1-day-old chicks from each group were housed under incandescent white light with an intensity of 30 lx at bird-head level. No effects of light stimuli during embryogenesis on hatching time, hatchability, hatching weight and bird mortality during the feeding trial period were observed in the present study. Compared with the dark condition, the BW, pectoral muscle weight and myofiber cross-sectional areas were significantly greater on 7-day-old chicks incubated under green light. Green light also increased the satellite cell mitotic activity of pectoral muscle on 1- and 3-day-old birds. In addition, green light upregulated MyoD, myogenin and myostatin mRNA expression in late embryos and/ or newly hatched chicks. These data suggest that stimulation with monochromatic green light during incubation promote muscle growth by enhancing proliferation and differentiation of satellite cells in late embryonic and newly hatched stages. Higher expression of myostatin may ultimately help prevent excessive proliferation and differentiation of satellite cells in birds incubated under green light.*

Keywords: chick embryo, monochromatic green light, muscle regulatory factor, myostatin, satellite cell mitotic activity

Implications

In broiler chickens, stimulation with monochromatic green light during embryogenesis accelerates posthatch body weight and pectoral muscle growth. Therefore, it is important to explore the specific mechanism of muscle growth induced by green light regime. The present study showed that monochromatic green light stimuli promotes muscle growth by enhancing proliferation and differentiation of satellite cells in late embryonic and newly hatched stages. The higher expression of myostatin may ultimately help prevent excessive proliferation and differentiation of satellite cells in birds incubated under green light. These findings will provide a theoretical basis for chicken hatcheries choosing a scientific light-stimulation technology.

Introduction

Chickens are light-sensitive species, and their visible light spectrum is broader than livestock and humans (Prescott and Wathes, 1999; Lewis and Morris, 2000). In addition, chickens have special extra-retinal photoreceptors in the hypothalamus or in other sites of the brain, which is sensitive to different wavelengths (colors) of light and is involved in the transduction of photostimulation to biological signals, and then affect physiological response and growth performance (Foster and Follett, 1985; Lewis and Morris, 2000).

In commercial poultry hatcheries, the fertilized chicken eggs are normally incubated in artificial incubators under dark condition. However, several studies have shown that

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photostimulation during embryogenesis accelerates chick embryo development. Shutze *et al.* (1962) first reported that continuous incandescent treatment during embryogenesis accelerated chick embryo development and shortened incubation time compared with continuous colored light treatment and commercial dark condition. Some other studies also found the similar phenomenon in White Rock chickens (Siegel *et al.*, 1969), Rhode Island Red domestic fowl (Adam and Dimond, 1971), quail and broilers (Walter and Voitle, 1973). However, Lauber (1975) observed that the White Leghorn eggs stimulated with green light or blue–violet light showed a significantly higher embryo weight. Rozenboim *et al.* (2003) speculated that these contradictory findings are probably because of wide variability in the types of light sources, and the embryonic development accelerated by white light maybe because of an effect of overheating caused by incandescent lamps. Thus, to avoid the effect of overheating, Rozenboim *et al.* (2003, 2004) carried out intermittent light regime (15 min on and 15 min off) in turkey and broilers by using a pure monochromatic light source based on light-emitting diode (LED) lamps. They found that green LED light photostimulation during embryogenesis enhanced embryo development and posthatch growth in broilers and turkeys (Rozenboim *et al.*, 2003, 2004). Recently, Zhang *et al.* (2012) also found that continuous monochromatic green LED light stimuli during embryogenesis accelerated body weight (BW) and posthatch pectoral muscle growth of broilers.

However, the exact mechanisms of growth-promoting effect caused by green light stimulation during embryogenesis remain unclear. Halevy *et al.* (2006) demonstrated that monochromatic green light stimuli during embryogenesis enhances myofiber growth by promoting the skeletal muscle cell proliferation and differentiation during the first few days of posthatch chicks. However, the changes of myoblast proliferation and differentiation in the embryonic stage are still unknown. We thus investigated the effect of stimulation with monochromatic green light during incubation on pectoral muscle weight, satellite cell mitotic activity (SCMA) and gene expression in relation to embryonic and posthatch muscle growth of broiler chickens.

Material and methods

All the experimental procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

Light treatments and animal management

Fertile broiler eggs (Arbor Acres; $n = 880$) were pre-weighed and selected for an average weight of 68 g (range = 65 to 70 g). Eggs were randomly assigned to 1 of the 2 treatment groups in 2 modified commercial incubators: (1) control group (in dark condition) and (2) monochromatic green light group (560 nm). The continuous green light system was provided by LED lower-power lamps ($IF_{(forward\ current)} = 20$ mA, $VF_{(forward\ voltage)} = 2.1–2.5$ V, $\lambda p_{(peak\ wavelength)} = 565$ nm, $IV_{(luminous\ intensity)} = 240$ mcd, $VR_{(reverse\ voltage)} = 5$ V; Zhongshan Silsmart Optoelectronics Co., Ltd, Guangzhou, China), and was equalized with an average intensity of 15 lx (13 to 18 lx from the middle to the air cell end of the eggs) at eggshell level from the 1st incubation day until hatching. The illumination was measured with a digital luxmeter (Mastech MS6610, Precision Mastech Enterprises, Hong Kong, China). Two identical, microcomputer automatic incubators (K12SS-1-BO7, Dezhou Zhicheng Incubation Equipment Co., Ltd, Shandong, China) were calibrated before hatching the experimental eggs. There were no differences between the two incubators, as evidenced by the growth-promoting effect of green light stimulation. Incubation conditions were set at 37.8 ± 0.1°C of temperature and 60% of relative humidity (RH) from the 1st day until hatching, and the temperature and RH were recorded every 4 h. Eggs in the incubators were turned through 270° every 1.5 h until embryonic day 19. On embryonic day 10, all the eggs were candled, and the infertile eggs were removed. Eggs from the different treatment groups were recorded for hatching time every 5 h between E19 and E21. Chicks were considered hatched when they had completely emerged from the eggshell (Rozenboim *et al.*, 2004). Hatchability was calculated as (%) = 100 × (number of chicks hatched/number of fertile eggs set), where each set layer was taken as a replicate, and 6 layers of each incubator means 6 replicates per treatment.

After pipping, we weighed all the birds individually and then moved them from the brooder to a chicken house. All birds from the same incubator were mixed until the hatching period completed. Then, each bird was sexed, and 120 healthy male 1-day-old chicks from each incubation group were randomly placed in 6 replicates with 20 birds each. All birds were housed under incandescent white light (30 lx at bird-head level) with a light schedule of 23 h light and 1 h dark according to Rozenboim *et al.* (2004) and lights off from 2100 to 2200 h as described by Zhang *et al.* (2012). All the birds were allowed *ad libitum* access to a commercial diet and water. The diet met the nutrient requirements of broilers recommended by the National Research Council (1994). The bird management was consistent with the recommendations of Arbor Acres Broiler Commercial Management Guide.

Sampling procedure

On embryonic days 15, 17 and 19 (E15, E17 and E19), 1 embryo from each set layer was randomly selected. Eggs were opened from air chamber, the entire embryos were removed, and were cleaned of yolk sac and membrane to obtain the yolk-free body mass weight. They were then euthanized with sodium pentobarbital (20 mg/kg of BW; Beijing Chemical Co, Beijing, China). Immediately after death, the right pectoral muscle (~200 mg) was collected and stored in a 1.5-ml RNase-free centrifuge tube and was quickly frozen in liquid nitrogen and then kept at −80°C until mRNA analysis. The entire left pectoral muscle was removed from the sternum and fixed in 4% paraformaldehyde in

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0.1 M PBS (pH 7.4) for 48 h, and paraffin sections were made for H.E. staining.

On 1, 3, 5 and 7 days of hatch (H1, H3, H5 and H7), 1 bird with a BW close to the replicate average BW was selected, weighed and then killed. Immediately after death, the pectoral muscle samples for mRNA analysis (~200 mg) and for H.E. staining (~0.3 cm × 0.3 cm × 0.5 cm in size) were obtained. In addition, another embryo or chick from each replicate was weighed and killed on age of E15, E17, E19, H1, H3, H5 and H7, respectively. Their entire pectoral muscle was removed and weighed to calculate the pectoral muscle percentage of BW.

Measurement of myofiber area in pectoral muscle

For fixed sample, 8-μm thick muscle sections were cut and adhered to glass slides. H.E staining was performed to determine the cross-sectional area (CSA). At least 5 random fields in 5 sections of each muscle sample were photographed at a magnification of ×400 with microscope (Nikon 80i, Nikon, Tokyo, Japan) equipped with version 4.0.8 Spot RTke software (Spot Diagnostics Instruments, Sterling Heights, MI, USA). Myofiber CSA was then determined using Image-Pro Plus version 5.0 software (Media Cybernetics Inc., Bethesda, MD, USA). The criteria for concluding myofiber CSA analysis for each muscle were analyzing 150 fibers per sample and a total of 900 fibers per treatment.

Immunohistochemical staining for SCMA analysis

On age of H1, H3, H5 and H7, one bird from each replicate with a BW close to the average BW of the replicate was selected (six birds per treatment). All of these selected birds were injected with 5-bromo-2′-deoxyuridine (BrdU; 10 mg/ml; 100 mg/kg of BW) according to the method of Moore *et al.* (2005). After injection with BrdU, the birds were allowed to survive 2 h before sampling to allow for the incorporation of the BrdU into the nuclei entering the S-phase of the cell cycle, and then these birds were euthanized. Immediately after death, the left pectoral muscle (~0.5 cm × 0.5 cm × 0.5 cm) of each chick was harvested and fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) overnight, and then dehydrated, cleared and embedded in paraffin. Eight-micrometer-thick muscle sections were cut and adhered to glass slides. The tissue sections were deparaffinized, rehydrated and treated with 3% (vol/vol) hydrogen peroxide in methanol for 10 min, and incubated in 0.1 M PBS (pH = 7.4) at 95°C for 15 min in a thermostatic incubator (PYX-DHS 500 BS, Shanghai BaiDian Instrument Co. Ltd, Shanghai, China), and then cooled at room temperature until a temperature of 40°C was reached. Sections were blocked with normal goat serum for 20 min at room temperature, and incubated overnight with a mouse monoclonal anti-BrdU antibody (Sigma-Aldrich, 3050 Spruce Street, St Louis, MO, USA) that was diluted 1 : 100 in 0.1 M PBS. Slices were incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1 : 200 dilution; Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd, Beijing, China) for 30 min at 37°C, and then stained with a DAB (3,3′-diaminobenzidine) kit (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd) for 10 min. Nuclei were counterstained with hematoxylin. Control slides without primary antibody were examined in all cases.

Five photographs of each section were taken at a magnification of ×400. In each photograph, black dots are nuclei stained positive for BrdU, indicating newly divided cells. At least 1000 myofiber nuclei were counted for each sample. An index of SCMA was expressed as [BrdU-labeled nuclei/(BrdU-labeled nuclei + non-BrdU-labeled nuclei)] × 100 (Dangott *et al.*, 2000).

Analysis of MyoD, myogenin and myostatin gene expression by real-time PCR

RNA extraction was performed using Trizol reagent according to the protocol of the manufacturer (Invitrogen, Carlsbad, CA, USA). The isolated total RNA was resuspended in 40 μl of diethylpyrocarbonate-treated water. The RNA was quantified by using Ultrospec 2100 pro UV/Visible Spectrophotometer (Amersham Bioscience, Freiburg, Germany), and was quality-checked by running 1 μl of each RNA sample on a 1% agarose gel stained with ethidium bromide. To avoid genomic contamination, the RNA samples were treated with DNAse (Invitrogen). One microgram of total RNA of each sample was used for first-strand cDNA synthesis with TIANGEN Quantscript RT Kit following the manufacturer's instructions (TIANGEN Biotech Co. Ltd, Beijing, China). The cDNA samples were stored at −20°C. The gene expression of MyoD, myogenin and myostatin was performed with a real-time PCR Kit following the manufacturer's protocol (RealMasterMix-SYBR Green; TIANGEN). The PCR reaction was conducted on an iCycler iQ5 multicolor real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA), and the protocol used was as follows: 95°C for 5 min; 40 cycles of 95°C for 10 s, 60°C for 30 s, 72°C for 30 s; 72°C for 5 min. The melting curve was recorded at 60°C. Pairs of oligonucleotide primers used are shown in Table 1. The amplification efficiency of each gene was validated by constructing a standard curve through four serial dilutions of cDNA. β-actin was used as the housekeeping gene for normalization. The sample of birds from E15 in dark condition was used as the calibrator sample. All samples were run in triplicate, and the relative expression levels of MyoD, myogenin and myostatin were quantified using the $2^{-\triangle\triangle C_t}$ method (Livak and Schmittgen, 2001).

Statistical analysis

Data were presented as mean ± s.e.m. All statistical analyses were performed by a Student's *t*-test using JMP version 5 software (SAS, 2002). The significance level was defined as $P < 0.05$.

Results

Hatching time, hatching weight, hatchability and bird mortality

No effects of light stimuli during embryogenesis on hatching time (Figure 1), hatchability, hatching weight and bird

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Table 1 *Primers for RT-qPCR analysis*

Genes	Primer sequences	Fragment size (bp)	Annealing (°C)	GenBank number	References
MyoD	F: GACGGCATGATGGAGTACAG R: AGCTTCAGCTGGAGGCAGTA	201	60	NM_204214	Li and Velleman (2009)
myogenin	F: GGCTTTGGAGGAGAAGGACT R: CAGAGTGCTGCGTTTCAGAG	184	60	NM_204184	Li and Velleman (2009)
myostatin	F: CTTTTGATGAGACTGGACGAG R: AGCGGGTAGCGACAACATC	173	60	NM_001001461	Guernec *et al.* (2004)
β-actin	F: ATCCGGACCCTCCATTGTC R: AGCCATGCCAATCTCGTCTT	120	60	NM_205518	Our own lab

mortality (Table 2) during the feeding trial period were observed in the present study.

BW and myofiber growth

At 7 days of age, significantly greater BW (Figure 2a) and pectoral muscle percentage (Figure 2b) were observed in birds incubated under green light than those in dark condition ($P < 0.05$). Birds in green light group also showed an increasing trend in BW on day H5 ($P = 0.08$). We further analyzed the changes in the myofiber area of the pectoral muscle (Figure 3). On days H5 and H7, the myofiber CSAs in green light group (H5: $136 \pm 5.47\ \mu m^2$; H7: $202 \pm 10.0\ \mu m^2$) were significantly greater than those in dark condition (H5: $118 \pm 4.73\ \mu m^2$; H7: $175 \pm 7.66\ \mu m^2$; $P < 0.05$). No significant difference in myofiber CSA was observed between the two treatment groups from days E15 to H3 ($P > 0.05$).

SCMA of newly hecthed broilers

SCMA of the pectoral muscle was evaluated by the index of SCMA in Figure 4. On days H1 and H3, the index of SCMA in the green light group (H1: 10.8%; H3: 14.3%) was significantly greater than that in dark condition (H1: 7.98%; H3: 10.4%; $P < 0.05$). In both treatment groups, the index of SCMA reached peaks on day H3 (14.3% *v.* 10.4%), and markedly decreased to low levels on day H7 (3.65% *v.* 3.02%).

MyoD, myogenin and myostatin gene expression

The mRNA expression levels of MyoD, myogenin and myostatin are shown in Figure 5. Compared with the broilers in dark condition, the MyoD mRNA levels of the pectoral muscle in the green light group were significantly upregulated from days E17 to H3 with a highest level on day H1 ($P < 0.05$, Figure 5a), whereas the myogenin mRNA levels were upregulated from days H1 to H5 with a peak on day H3 ($P < 0.05$, Figure 5b). No significant differences were observed in the muscle myogenin mRNA levels in the late embryo stage ($P > 0.05$). The myostatin mRNA levels in birds of green light were also significantly upregulated from days E17 to E19 and H5 to H7 ($P < 0.05$, Figure 5c).

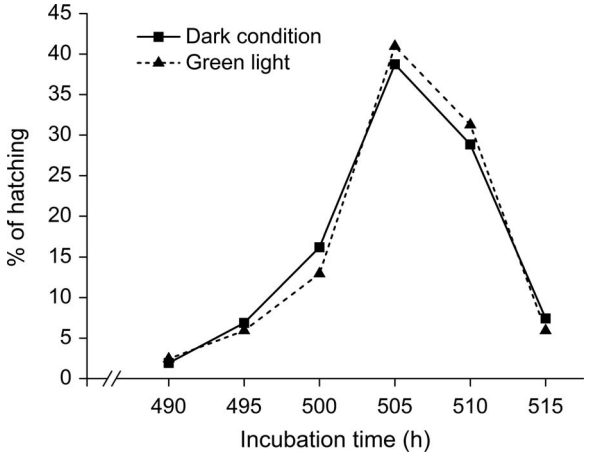

Figure 1 Percentage hatching relative to the hatching time of broiler chicks incubated under green light or dark condition.

Table 2 *Effect of monochromatic light stimuli during embryogenesis on hatchability, hatching weight and mortality of broiler chickens*

Items	Dark	Green	Probability
Hatchability (%)	90.59 ± 1.25	89.80 ± 1.19	0.675
Hatching weight[1] (g)	46.83 ± 0.46	47.12 ± 0.21	0.597
Mortality[2] (%)	6.67 ± 1.05	7.50 ± 1.71	0.687

BW = body weight.
Values are presented as mean ± s.e.m.
[1]Average BW of all hatching chicks (both male and female) of each treatment group.
[2]Chick mortality during the feeding trial (from day 0 to 7 of age).

Discussion

In the present study, green light stimuli during incubation did not affect the hatching time and hatchability, which is in agreement with studies on broilers (Rozenboim *et al.,* 2004; Zhang *et al.,* 2012) and turkeys (Rozenboim *et al.,* 2003). Rozenboim *et al.* (2004) found that BW and pectoral muscle percentage in the embryos, newly hatched birds, and post-hatch male and female Cobb broilers incubated under monochromatic green light were significantly greater than those incubated under dark condition. However, the present results showed that AA broilers incubated under green light

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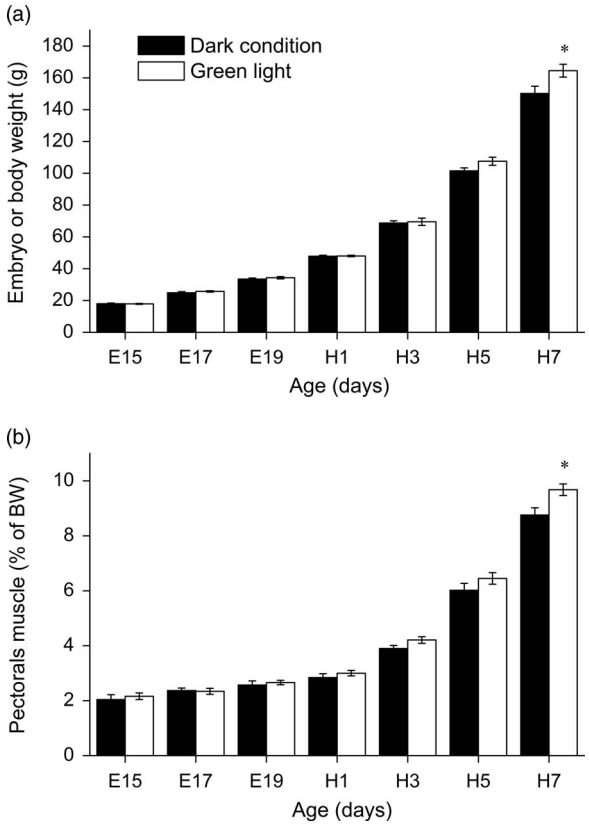

Figure 2 Chick embryo (yolk-free body mass) or body weight (a), and pectoral muscle weight percentage of body weight (BW) (b) of chick embryos (E15, E17 and E19 days of age) and newly hatched male chicks (H1, H3, H5 and H7 days of age) incubated under green light or dark condition. Values are presented as means ± s.e.m. ($n = 6$). *Indicates a significant difference between treatments at the same age ($P < 0.05$).

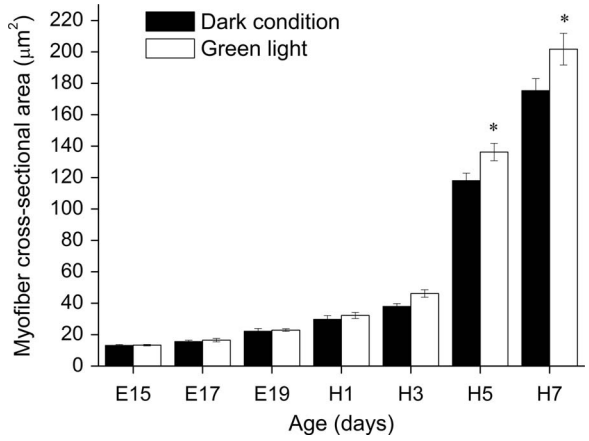

Figure 3 Myofiber cross-sectional area (CSA) of the pectoral muscle of late embryos (E15, E17 and E19 days of age) and newly hatched male chicks (H1, H3, H5 and H7 days of age) and incubated under green light or dark condition. Values are presented as means ± s.e.m. ($n = 6$). *Indicates a significant difference between treatments at the same age ($P < 0.05$).

showed a significant increase in BW and pectoral muscle percentage starting from age of H7, but not starting from the embryonic or early posthatch stage as reported by Rozenboim *et al.* (2004). We speculate that these different

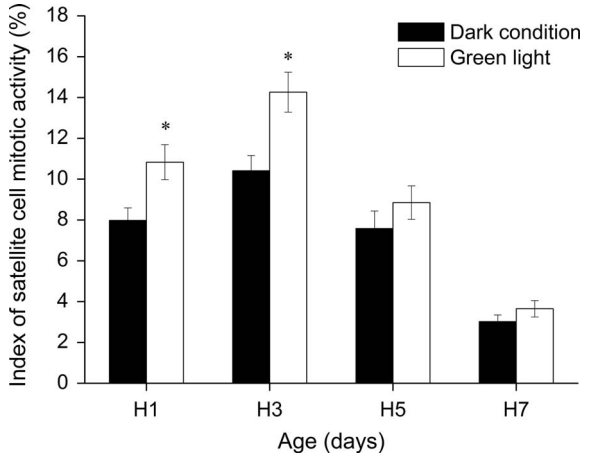

Figure 4 Index of satellite cell mitotic activity in the pectoral muscle of newly hatched chicks (H1, H3, H5 and H7 days of age) and incubated under green light or dark condition. Index of satellite cell mitotic activity = (BrdU-labeled nuclei/[BrdU-labeled nuclei + non-BrdU-labeled nuclei]) × 100. Values are presented as means ± s.e.m. ($n = 6$). *Indicates a significant difference between treatments at the same age ($P < 0.05$).

findings may be because of the Cobb strain that is more sensitive to green light stimuli. Further studies are needed to investigate whether genetic factors are closely related to the poultry response during green light stimulation.

Posthatch skeletal muscle growth is accompanied by the growth of individual myofibers, because the total number of muscle fibers is fixed before hatching (Smith, 1963). Growth of myofibers is considered to be controlled by two factors: (1) enlargement by an increase in size (area or diameter), mainly because of the augmentation of myofibrillar proteins; and (2) elongation because of the addition of newly formed sarcomeres to the ends (Williams and Goldspink, 1978). Thus, the measurement of myofiber CSA can reflect the growth of muscle fibers. Greater myofiber CSA of the pectoral muscle in poultry contributes to greater pectoral muscle weight (Moore *et al.*, 2005; Liu *et al.*, 2010). In our present study, a greater myofiber CSA of the pectoral muscle in green light is highly consistent with greater BW and pectoral muscle weight on day H7.

Skeletal muscle satellite cells, also known as myoblasts, are a mitotically active stem-cell population residing between the sarcolemma and the basal lamina of the myofiber (Muir *et al.* 1965). In newly hatched chicks, satellite cells proliferate, differentiate and fuse with the adjacent myofibers, which increase myofiber DNA contents and ultimately result in the increase in protein synthesis and muscle fiber hypertrophy (Allen and Goll, 2003). In poultry, SCMA is an important aspect of muscle development, and the higher SCMA in newly hatched birds will result in an increase in muscle size at maturity (Mozdziak *et al.*, 1997; Moore *et al.*, 2005). It has been reported that early hatched nutrition increased myogenic SCMA during immediate posthatch period, and subsequently promoted BW and pectoral muscle weight during the growth phase (Mozdziak *et al.*, 2002; Moore *et al.*, 2005). Newly hatched broiler chicks reared

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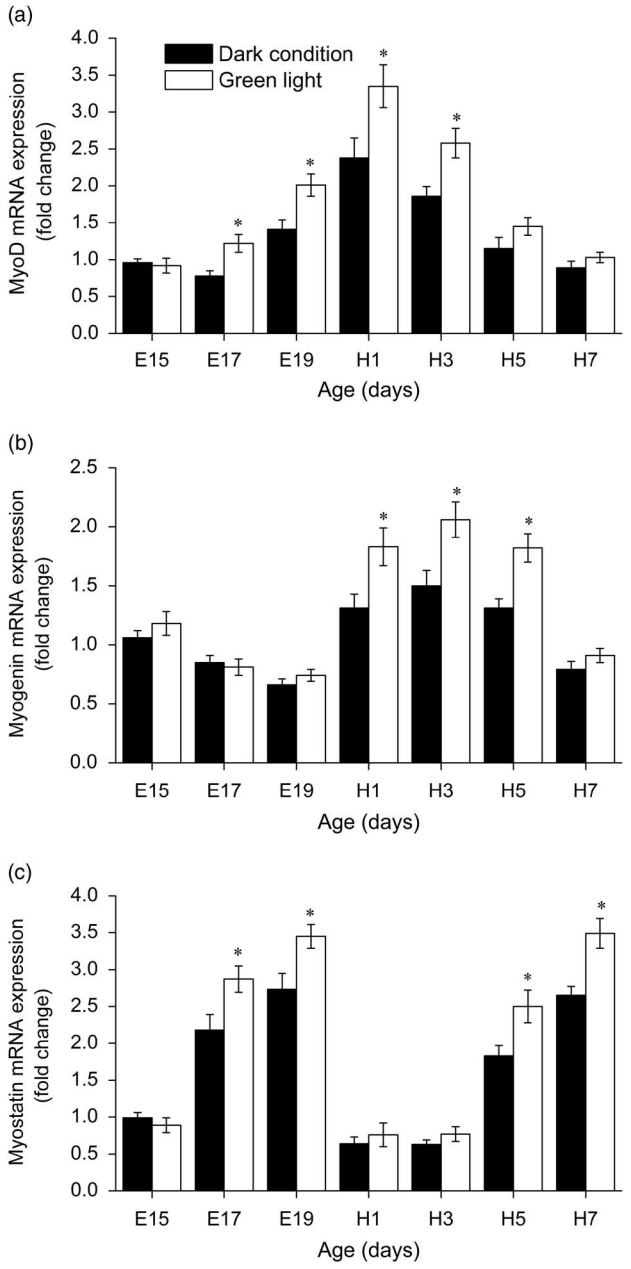

Figure 5 Relative mRNA expressions of MyoD (a), myogenin (b) and myostatin (c) in the pectoral muscle of late chick embryos (E15, E17 and E19 days of age) and newly hatched chicks (H1, H3, H5 and H7 days of age) and incubated under green light or dark condition. Values are presented as means ± s.e.m. ($n = 6$). *Indicates a significant difference between treatments at the same age ($P < 0.05$).

under monochromatic green light also showed a higher SCMA during early posthatch stage, and followed by a greater myofiber CSA, pectoral muscle weight and BW during the later growth phase (Liu *et al*., 2010). In the present study, we found that birds incubated under monochromatic green light showed a significant increase in SCMA on days H1 to H3, which subsequently led to the greater myofiber CSA on days H5 and H7, as well as a greater pectoral muscle weight and BW on day H7. These data suggested that myofiber growth caused by green light stimuli during embryogenesis is largely dependent on the contribution of new nuclei because of the increase in the SCMA during early posthatch stage.

The development of the skeletal muscle during embryogenesis is mediated by myogenic regulatory factors (MRFs), including myogenic factor 5, myogenic differentiation 1 (MyoD), myogenin and MRF4, which is a family of basic helix–loop–helix transcription factors essential for the activation, proliferation and differentiation of satellite cells (Schultz and McCormick, 1994). Numerous studies have demonstrated that MyoD was a marker of satellite cell activation and proliferation, whereas myogenin was a marker of cells entering the terminal differentiation program (Smith *et al*., 1994; Seale and Rudnicki, 2000).

In both treatment groups of our present study, the MyoD mRNA levels of the pectoral muscle significantly increased from day E17 to H3 and reached the highest level on day H1, and myogenin mRNA levels increased from day H1 to H5 and reached the peaks on day H3, indicating skeletal muscle satellite cell proliferation before differentiation. Moreover, during the period of day E17 to E19, an increase in MyoD mRNA level accompanying a decrease in myogenin mRNA level in the pectoral muscle in both the green light and the control groups demonstrated that skeletal muscle satellite cell proliferation with simultaneous repression of muscle differentiation. This phenomenon confirmed that the proliferation and differentiation of myoblasts are mutually exclusive (Olson, 1992; Lassar *et al*., 1994). Halevy *et al*. (2006) reported that posthatch muscle growth promoted by green light was mainly because of the light illumination that enhanced satellite cell proliferation and differentiation during the first days of age. In the present study, the higher MyoD mRNA levels from day E17 to H3 and higher myogenin from day H1 to H5 in embryos or birds incubated under the green light group suggested that in ovo green light enhanced the skeletal muscle satellite cell proliferation and differentiation in the late embryonic and newly hatched stage.

It is well established that myostatin is a potent negative regulator of skeletal muscle growth (Thomas *et al*., 2000). In the present study, myostatin is expressed in the pectoral muscle from day E15 to H7, indicating that myostatin regulates both embryonic myogenesis and early posthatch skeletal muscle growth in poultry. Higher myostatin mRNA levels accompanying lower MyoD and myogenin mRNA levels partly confirmed that myostatin inhibits muscle growth by downregulating the expression of MyoD, Myf5 and myogenin (Thomas *et al*., 2000; Langley *et al*., 2002). However, the higher expression of myostatin in birds of green light indicate that myostatin may play a role in counteracting muscle growth and preventing excessive proliferation and differentiation of satellite cells. In a cell-culture experiment, Morissette *et al*. (2009) found that myostatin inhibits IGF-I-induced C2C12 myotube hypertrophy through Akt. In addition, Herichová *et al*. (2008) demonstrated that embryonic avian pineal pacemaker is light-sensitive and can generate melatonin rhythmic output. Therefore, we recommended that further studies are needed to elucidate whether green light stimuli during incubation mediate the proliferation and differentiation

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of satellite cells and embryo development by affecting secretion of some related hormones.

In the current experiment, embryos were not separated on the basis of sex during the hatching period, similar to other previous studies (Rozenboim *et al.*, 2004; Halevy *et al.*, 2006). With regard to the sexual dimorphism in the embryos incubated under monochromatic light, there is little literature available. Rozenboim et al. (2004) deduced that there was no significant sexual dimorphism in broilers incubated under green light, as they observed that BW and pectoral muscle percentage in the embryos, newly hatched birds, and posthatch male and female Cobb broilers incubated under monochromatic green light were significantly greater than those incubated under dark condition. In addition, Lu *et al.* (2007) reported that there was no difference in the dynamic change of sex hormone levels between the male and female chick embryos in the normal dark incubation condition. However, another recent study on posthatch broilers reported that green and blue monochromatic lights promote growth and development of male broilers via stimulating testosterone secretion (Cao *et al.*, 2008). It seemed that embryos and posthatch chicks may respond differently in different sexes. Therefore, further studies are warranted to explore the relationship between the muscle growth and changes in sex hormones in birds incubated under green monochromatic light.

In summary, stimulation with monochromatic green light during incubation enhances posthatch muscle growth by promoting satellite cell proliferation and differentiation in both late embryonic and newly hatched stages. The higher expression of myostatin may ultimately help prevent excessive proliferation and differentiation of satellite cells in birds incubated under green light.

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References

Adam JH and Dimond SJ 1971. Influence of light on the time of hatching in the domestic chick. Animal Behaviour 19, 226–229.

Allen RE and Goll DE 2003. Cellular and developmental biology of skeletal muscle as related to muscle growth. In Biology of growth of domestic animals (ed. CG Scanes), pp. 148–169. Iowa State Press, Ames, USA.

Cao J, Liu W, Wang Z, Xie D, Jia L and Chen Y 2008. Green and blue monochromatic lights promote growth and development of broilers via stimulating testosterone secretion and myofiber growth. Journal of Applied Poultry Research 17, 211–218.

Dangott B, Schultz E and Mozdziak PE 2000. Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. International Journal of Sports Medicine 21, 13–16.

Foster RG and Follett BK 1985. The involvement of a rhodopsin-like photopigment in the photoperiodic response of the Japanese quail. Journal of Comparative Physiology A 157, 519–528.

Guernec A, Chevalier B and Duclos MJ 2004. Nutrient supply enhances both IGF-I and MSTN mRNA levels in chicken skeletal muscle. Domestic Animal Endocrinology 26, 143–154.

Halevy O, Piestun Y, Rozenboim I and Yablonka-Reuveni Z 2006. In ovo exposure to monochromatic green light promotes skeletal muscle cell proliferation and affects myofiber growth in posthatch chicks. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 290, R1062–R1070.

Herichová I, Monošíková J and Zeman M 2008. Ontogeny of melatonin, Per2 and E4bp4 light responsiveness in the chicken embryonic pineal gland. Comparative Biochemistry and Physiology, Part A 149, 44–50.

Langley B, Thomas M, Bishop A, Sharma M, Gilmour S and Kambadur R 2002. Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. Journal of Biological Chemistry 277, 49831–49840.

Lassar AB, Skapek SX and Novitch B 1994. Regulatory mechanisms that coordinate skeletal muscle differentiation and cell cycle withdrawal. Current Opinion in Cell Biology 6, 788–794.

Lauber JK 1975. Photoacceleration of avian embryogenesis. Comparative Biochemistry and Physiology Part A 51, 903–907.

Lewis PD and Morris TR 2000. Poultry and coloured light. World's Poultry Science Journal 56, 189–207.

Li X and Velleman SG 2009. Effect of transforming growth factor-β1 on embryonic and posthatch muscle growth and development in normal and low score normal chicken. Poultry Science 88, 265–275.

Liu WJ, Wang ZX and Chen YX 2010. Effects of monochromatic light on developmental changes in satellite cell population of pectoral muscle in broilers during early posthatch period. Anatomical Record 293, 1315–1324.

Livak KJ and Schmittgen TD 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle\triangle Ct}$ method. Methods 25, 402–408.

Lu JW, McMurtry JP and Coon CN 2007. Developmental changes of plasma insulin, glucagon, insulin-like growth factors, thyroid hormones, and glucose concentrations in chick embryos and hatched chicks. Poultry Science 86, 673–683.

Moore DT, Ferket PR and Mozdziak PE 2005. The effect of early nutrition on satellite cell dynamics in the young turkey. Poultry Science 84, 748–756.

Morissette MR, Cook SA, Buranasombati C, Rosenberg MA and Rosenzweig A 2009. Myostatin inhibits IGF-I-induced myotube hypertrophy through Akt. American Journal of Physiology, Cell Physiology 297, C1124–C1132.

Mozdziak PE, Schultz E and Cassens RG 1997. Myonuclear accretion is a major determinant of avian skeletal muscle growth. American Journal of Physiology 272, C565–C571.

Mozdziak PE, Walsh TJ and Mccoy DW 2002. The effect of early posthatch nutrition on satellite cell mitotic activity. Poultry Science 81, 1703–1708.

Muir AR, Kanji AH and Allbrook D 1965. The structure of the satellite cells in skeletal muscle. Journal of Anatomy 99, 435–444.

National Research Council (NRC) 1994. Nutrient requirements of poultry, 9th edition. National Academy Press, Washington, DC, USA.

Olson EN 1992. Interplay between proliferation and differentiation within the myogenic lineage. Developmental Biology 154, 261–272.

Prescott NB and Wathes CM 1999. Spectral sensitivity of domestic fowl (*Gallus g. domesticus*). British Poultry Science 40, 332–339.

Rozenboim I, Huisinga R, Halevy O and El Halawani ME 2003. Effect of embryonic photostimulation on the posthatch growth of turkey poults. Poultry Science 82, 1181–1187.

Rozenboim I, Piestun Y, Mobarkey N, Barak M, Hoyzman A and Halevy O 2004. Monochromatic light stimuli during embryogenesis enhance embryo development and posthatch growth. Poultry Science 83, 1413–1419.

SAS Institute Inc. (SAS) 2002. JMP–Statistics and Graphics Guide, Version 5. SAS, Cary, North Carolina, USA.

Schultz E and McCormick KM 1994. Skeletal muscle satellite cells. Reviews of Physiology, Biochemistry and Pharmacology 123, 213–257.

Seale P and Rudnicki MA 2000. A new look at the origin, function, and "stem-cell" status of muscle satellite cells. Developmental Biology 218, 115–124.

Shutze J, Lauber J, Kato M and Wilson W 1962. Influence of incandescent and coloured light on chicken embryos during incubation. Nature 196, 594–595.

Siegel PB, Isakson ST, Coleman FN and Huffman BJ 1969. Photoacceleration of development in chick embryo. Comparative Biochemistry and Physiology 28, 753–758.

https://doi.org/10.1017/S1751731113001882 Published online by Cambridge University Press

Smith CK II, Janney MJ and Allen RE 1994. Temporal expression of myogenic regulatory genes during activation, proliferation, and differentiation of rat skeletal muscle satellite cells. Journal of Cellular Physiology 159, 379–385.

Smith JH 1963. Relation of body size to muscle cell size and number in the chicken. Poultry Science 42, 283–290.

Thomas M, Langley B, Berry C, Sharma M, Kirk S, Bass J and Kambadur R 2000. Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. The Journal of Biological Chemistry 275, 40235–40243.

Walter JH and Voitle RA 1973. Effects of photoperiod during incubation on embryonic and post-embryonic development of quail and chickens. British Poultry Science 14, 533–540.

Williams P and Goldspink G 1978. Changes in sarcomere length and physiological properties in immobilized muscle. Journal of Anatomy 127, 459–468.

Zhang L, Zhang HJ, Qiao X, Yue HY, Wu SG, Yao JH and Qi GH 2012. Effect of monochromatic light stimuli during embryogenesis on muscular growth, chemical composition, and meat quality of breast muscle in male broilers. Poultry Science 91, 1026–1031.

https://doi.org/10.1017/S1751731113001882 Published online by Cambridge University Press