

# Monochromatic Light Stimuli During Embryogenesis Enhance Embryo Development and Posthatch Growth

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**ABSTRACT** Photostimulation with green light accelerated BW and muscle development of broilers. In experiment 1, temperature sensors were inserted into 50 broiler eggs. The eggs were placed under 5 green light-emitting diode (LED) lamps at an intensity of 0.1 W/m<sup>2</sup> at eggshell level for 5, 10, 15, 20, and 25 min (n = 10). Egg temperatures were recorded continuously. A high correlation was found between lighting period and egg temperature elevation, and an intermittent light regimen of 15 min on and 15 min off was found to eliminate light-induced egg overheating. In experiment 2, the effect of in ovo green light photostimulation on embryonic development was studied. Five hundred fertile eggs were divided into 2 groups: the first was photostimulated with green light from 5 d of incubation until hatch (0.1 W/m<sup>2</sup> intensity)

and the second was incubated in the dark. In ovo green light photostimulation caused a significant elevation in BW and breast muscle weight during embryo development and posthatch until 6 d of age. In experiment 3, 240 fertile broiler eggs were divided into 2 groups as described in experiment 2. At hatch, chicks from each in ovo light treatment were divided into 2 subgroups: the first was reared under green light and the second under white light. In ovo photostimulation with green light enhanced BW and breast muscle weight. However, rearing under green light did not have any synergistic effect on BW. Collectively, the results suggest that stimulation with green light enhances development and growth in chicks and that the best effect is achieved when this stimulus is provided during incubation.

(Key words: broiler embryo, growth, monochromatic light, photostimulation)

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## INTRODUCTION

In today's commercial poultry industry, birds are only exposed to artificial light; therefore, light quality, determined by photoperiod, spectrum, and intensity has become a major environmental factor affecting bird performance (Andrews and Zimmerman, 1990). Light source and spectrum are determinant factors in manipulating the growth of meat-type birds. For example, broilers reared under fluorescent lamps have been found to have equal or slightly better growth than birds reared under incandescent lamps (Zimmerman, 1988; Andrews and Zimmerman, 1990; Scheideler, 1990). Furthermore, broilers (Wabeck and Skoglund, 1974) and quail (Phogat et al., 1985) reared under blue or green fluorescent lamps gained significantly more weight than birds reared under red or white light, whereas feed conversion and mortality were not affected.

Recently, our group has shown similar results utilizing a pure monochromatic light source based upon light-

emitting diodes (LED; Rozenboim et al., 1999). Broilers reared under green or blue light exhibited greater body and muscle growth than birds reared under red or white light (Rozenboim et al., 1999). These parameters are highly correlated with the number of skeletal muscle satellite cells at 5 d of age (Halevy et al., 1998), which are crucial for muscle growth in the early posthatch period (Halevy et al., 2000). The significant effects of blue and in particular green light on BW and satellite cell number in the first days posthatch led us to hypothesize that monochromatic green light stimuli during embryogenesis would accelerate embryo development and enhance posthatch body and muscle growth.

In commercial hatcheries, broiler eggs are set in the dark; however, several studies have shown that photostimulation accelerates embryonic development. Early studies showed that stimulation with white light increases BW in avian embryos including broilers, White Leghorn chickens, turkeys, and quail (Shutze et al., 1962; Siegel et al., 1969; Cooper, 1972; Walter and Voitle, 1972, 1973; Coleman and McNabb, 1975). Moreover, embryos that had been exposed to light hatched earlier than those

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**Abbreviation Key:** LED = light-emitting diode.

kept in the dark. White light stimulation of White Leghorn and broiler eggs accelerated hatching by approximately 1 d (Siegel et al., 1969; Walter and Voitle, 1973; Coleman and McDaniel, 1976). A similar acceleration has been observed in turkey embryos, with no effect on hatchability or BW on hatching day (Fairchild and Christensen, 2000), suggesting embryonic photostimulation as a tool for shortening the hatching period. A recent study has shown that broiler embryos stimulated with fluorescent green light hatch earlier and are heavier at hatch than those kept in the dark (Shafey and Al-Mohsen, 2002). However, the positive effects on embryonic development and hatching observed in these studies could have been due to an effect of overheating, in addition to the illumination effect (Romanoff, 1960), because the eggs were incubated under continuous illumination. In addition, none of these studies analyzed the effect of pure monochromatic light on embryonic development.

Recently, we reported that photostimulation of turkey eggs with intermittent monochromatic green light provided by LED lamps enhances posthatch body and muscle weight of turkey females (Rozenboim et al., 2003). The aim of the present study was to elucidate the effect of stimulation with monochromatic green light during incubation on embryonic and early posthatch broiler development. In addition, we studied the effect of pre- and posthatch green light stimulation combinations on posthatch body and muscle growth in broilers.

## MATERIALS AND METHODS

### Experiment 1

Fifty fertile broiler eggs (Cobb strain) were preweighed and selected for an average weight of 68 g (range = 65 to 70 g). Eggs were divided randomly into groups of 10 eggs each. In every study, we used 10 eggs (total of 5 replicates), which were placed in various locations in setter trays in a commercial incubator.<sup>2</sup> The eggs were placed under LED<sup>3</sup> lamps that provided 560 nm of light (half band of 535 to 585 nm) at an intensity of 0.1 W/m<sup>2</sup> at eggshell level.<sup>4</sup> Temperature sensors<sup>5</sup> were inserted into the eggs via small pores and placed in the yolk. A thermometer<sup>6</sup> was laid parallel to the trays to monitor incubator temperature. The core temperature of each egg, as well as the temperature of the incubator, was monitored on a per minute basis until it reached a steady state (time zero). The eggs were illuminated for various periods and temperatures were recorded continuously before, during, and after illumination. Once the illumination was switched off, we de-

termined the time needed for the egg temperature to return to its level at time zero.

### Experiment 2

**Embryos.** Five hundred fertile broiler eggs were preweighed and selected for an average weight of 68 g (range = 65 to 70 g). Eggs were divided randomly into 2 groups: the first (n = 250) received monochromatic green light (5 LED lamps of 560 nm, half band of 535 to 585 nm), at an intensity of 0.1 W/m<sup>2</sup>; light was provided intermittently—15 min light, 15 min dark, according to the results of experiment 1, from embryonic d 5 (E5) until hatch; the second, control group (n = 250) was kept in the dark (as measured by 0 W/m<sup>2</sup> light intensity) for the entire period. At E10, all eggs were candled, and infertile eggs were removed. Incubating procedure was as dictated by the Cobb's manual.

Autopsies were conducted daily, starting at E10, until hatch. At each embryonic age, 10 eggs from each group were weighed, broken open, and albumen and yolk sac were weighed. Embryos were removed, cleaned of external membranes, and weighed. Pectoralis muscles attached to the sternum were removed, cleaned of connective tissue, and weighed.

**Hatching and Posthatch Period.** Eggs from the different treatments were observed for hatching time every 6 h between E19 and E21. Chicks were considered hatched when they had completely emerged from the shell. Upon hatching, each chick was weighed, sexed, and wing-banded. Birds were transferred to brooders with free access to commercial diet and water and grown under normal light. All chicks were maintained in temperature-controlled brooders. All experimental procedures were approved by the Animal Welfare Committee of the Faculty of Agriculture, The Hebrew University of Jerusalem.

### Experiment 3

Two hundred and forty fertile broiler eggs (Cobb strain) were divided into 2 in ovo light stimulation groups (green light and dark) and were incubated under the conditions described in experiment 2. At hatch, birds were weighed, wing-banded, and sexed. Chicks from each in ovo light treatment were then divided into 2 subgroups (equal number of males and females in each subgroup). The first subgroup was housed in an environmentally and light-controlled room (2 × 3 m) previously installed with LED lamps providing 560 nm at an intensity of 0.1 W/m<sup>2</sup> at birds' head level (green room). The second subgroup was housed in a similar room which was installed with white light provided by an incandescent lamp at the same intensity (white room). Lighting schedule was 23L:1D during the entire experiment, as described in Rozenboim et al. (1999). The light treatments are summarized in Table 1. Autopsies were performed at 42 d of age and pectoralis muscles

<sup>2</sup>Setter 84, Petersime, Zulte, Belgium.

<sup>3</sup>Lite On, Bangkok, Thailand.

<sup>4</sup>Li-Cor Pyranometer Sensor, Lincoln, NE.

<sup>5</sup>Thermocoupling, Knick, Model Berlin 37

<sup>6</sup>YSI Precision 400A Thermometer, Dayton, OH. [Au: please provide model #, Supplier, City, Country]

TABLE 1. Light regimen for embryos and posthatch chicks

Group	Treatment	
	Embryo <sup>1</sup>	Posthatch <sup>2</sup>
Dark to green	Dark	Green
Dark to white	Dark	White
Green to green	Green	Green
Green to white	Green	White

<sup>1</sup>Dark = control group, eggs kept in dark (0 W/m<sup>2</sup>) for embryonic period; Green = monochromatic green light (560 nm, at 0.1 W/m<sup>2</sup>) from E5 to hatch.

<sup>2</sup>Green = chicks reared in green room (LED lamps providing 560 nm at 0.1 W/m<sup>2</sup> at birds' head level) from d 0 to d 42; White = chicks reared in white room (incandescent lamps at 0.1 W/m<sup>2</sup>) from d 0 to d 42. [AUTH QUERY: footnotes OK at written?]

(major and minor) were removed from the sternum, cleaned of connective tissue and weighed.

**Statistical Analysis**

Data were analyzed by one-way ANOVA using the GLM procedures of SAS for effect of light. Data differences between means were tested by t-test and significance was *P* < 0.05 unless otherwise stated (SAS Institute, 1987).

**RESULTS**

**Experiment 1**

As a first step in eliminating the illumination-dependent heating effect, we conducted an experiment in which we evaluated egg heating in response to illumination by monochromatic green light. Photostimulation of broiler eggs with monochromatic green light caused a significant, time-dependent elevation in yolk temperature (Figure 1A). The highest elevation was detected after 25 min of green light photostimulation and was 0.24% of the initial temperature, corresponding to 0.25°F. Figure 1B demonstrates the high correlation between the photostimulation period and the elevation in yolk temperature, suggesting that 1 min of photostimulation increases yolk temperature by 0.01% of its initial value (approximately 0.01°F).

In eggs illuminated for periods longer than 15 min, the temperature did not decline to zero-time temperatures, even 60 min after the light had been turned off, suggesting the need for intermittent lighting. Setting the eggs under an intermittent light regimen (15 min on, 15 min off) eliminated the rise in egg yolk temperature, and as a result, prevented early hatch associated with in ovo photostimulation (Figure 1C).

**Experiment 2**

**Body and Breast Muscle Weight During Embryonic Development and Hatchability.** Embryo BW, calculated as percentage of egg weight, increased similarly during the incubation period in both dark and green

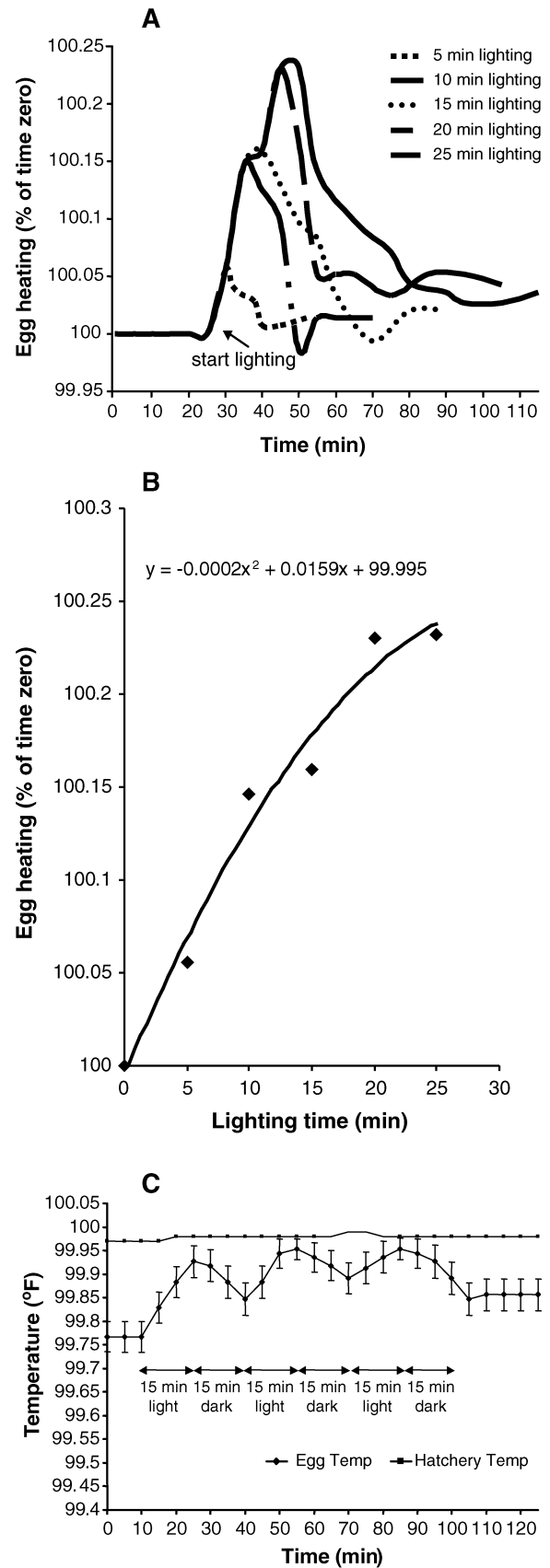


FIGURE 1. In ovo green light photostimulation affects the core temperature of incubated eggs. (A) Time-course effect of lighting period on egg temperature and (B) correlation between lighting period and temperature elevation. Data are presented as percentage of temperature at time zero. (C) Egg core and hatchery temperature changes by intermittent light (15 min on and 15 min off). Results are means ± SEM.

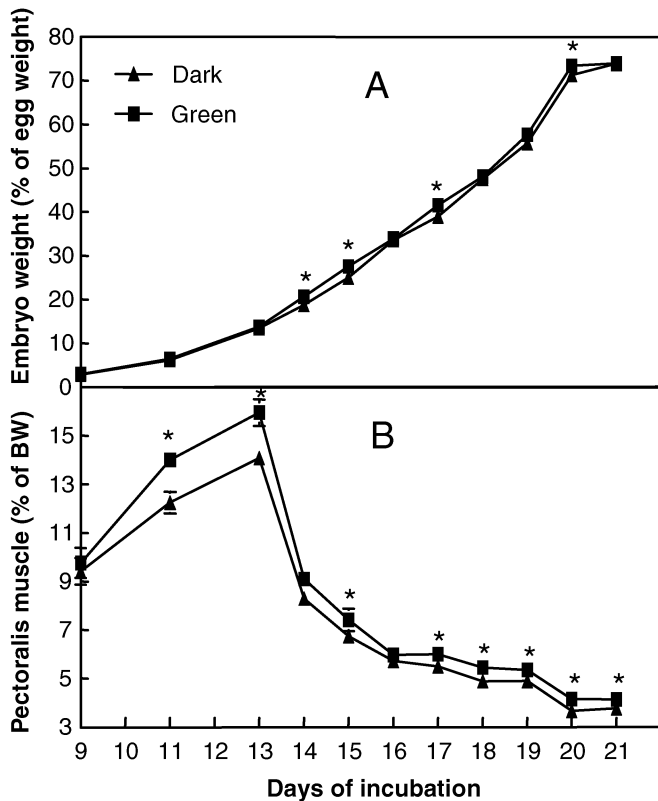


FIGURE 2. Percentage BW of egg weight (A) and pectoralis muscle weight as percentage of BW (B) of chick embryos incubated under green light or kept in the dark on various days during incubation. Results are means  $\pm$  SEM and asterisks mark significant differences between treatments ( $P < 0.05$ ).

groups (Figure 2A). Significantly higher BW were seen in the green group than in the dark one on embryonic days E14, E15, E17, and E20. The pectoralis muscle percentage of embryo BW increased between E9 and E12 to approximately 13 and 15% in the dark and green groups, respectively, and then declined in both groups, reaching approximately 5% on E21. On nearly all days between E9 and E21, the muscle percentage was significantly higher in the green group than in the dark group (Figure 2B). There were no significant differences between treatments with respect to body length, or albumen and egg yolk weights (data not shown). No major differences in hatching time or percentage hatchability were observed between the 2 groups (Figure 3).

**Body and Muscle Growth in Posthatch Chicks.** At hatch (d 0), BW of the male chicks did not significantly differ between the 2 groups. However, during the first week posthatch, the BW of chicks that hatched under green light was significantly higher than that of those hatched in the dark (Figure 4A). The pectoralis muscle weight as percentage of BW of the chicks that hatched under green light was significantly higher than that of the chicks that hatched in the dark, both on hatching day and at 6 d of age (Figure 4B).

### Experiment 3

Previous studies have shown that illuminating chicks from d 1 of age under monochromatic green and blue

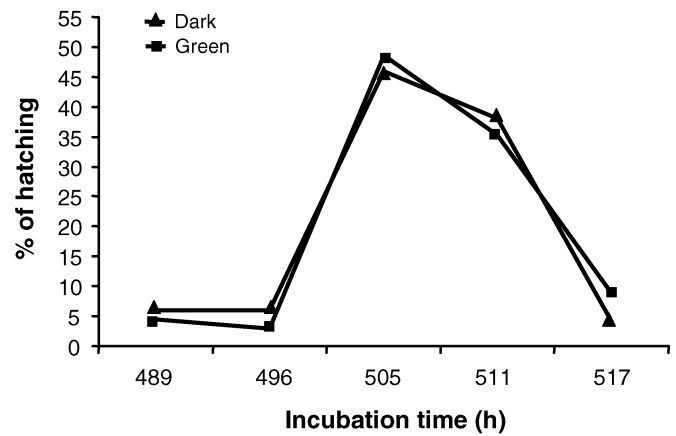


FIGURE 3. Percentage hatching relative to hatching time of chicks incubated under green light or dark conditions.

light enhances their BW and muscle growth relative to chicks reared under white light (Rozenboim et al., 1999). Therefore, in the present experiment we evaluated chicken performance as affected by combinations in lighting regimen during the embryonic and posthatch periods. Weekly BW results are presented in Figure 5.

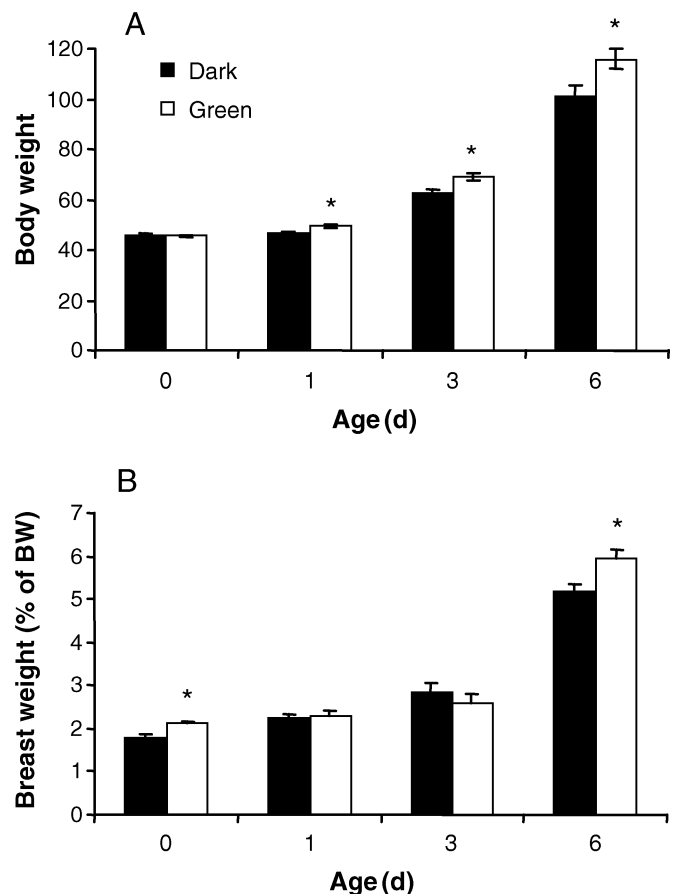
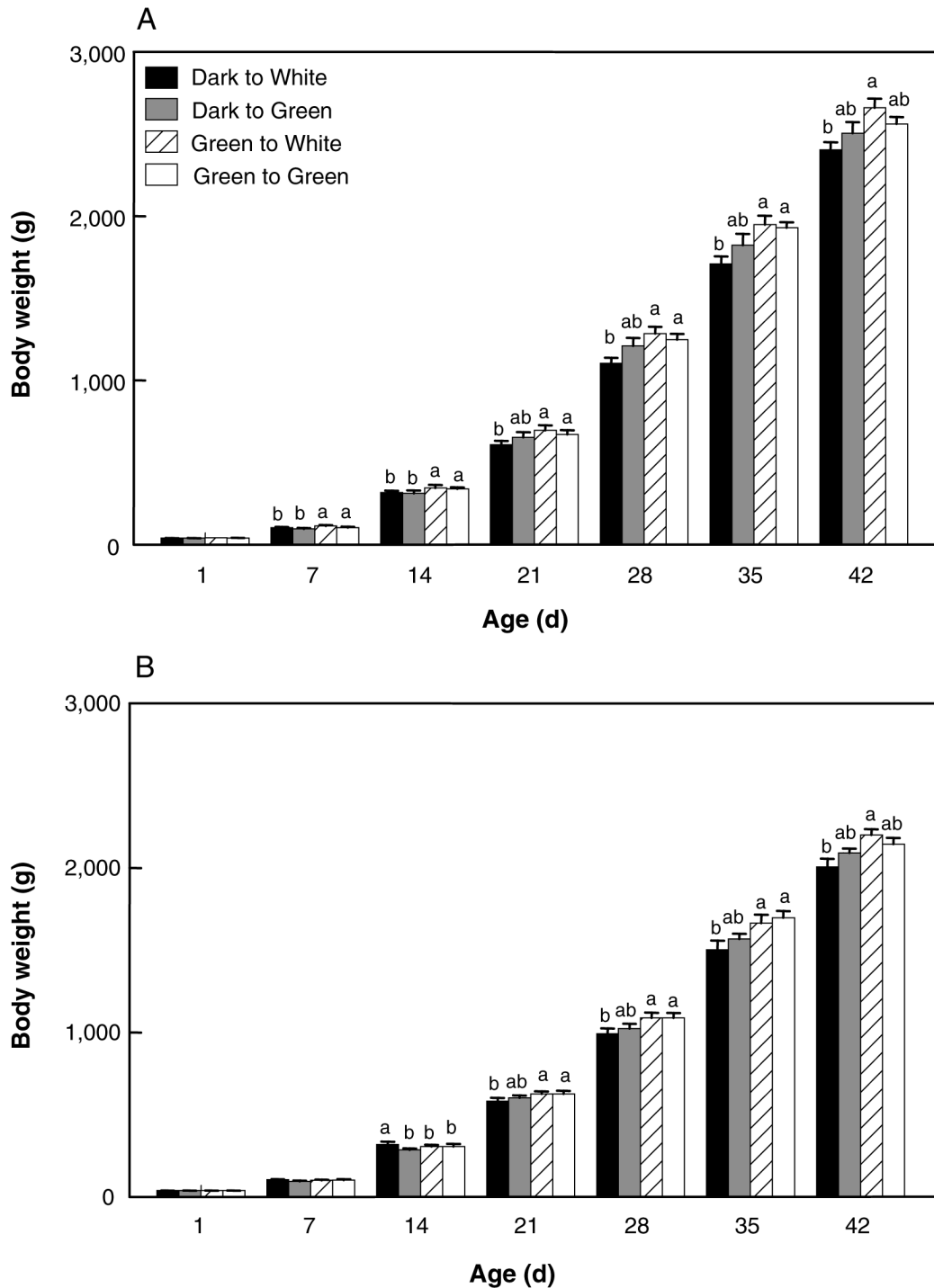


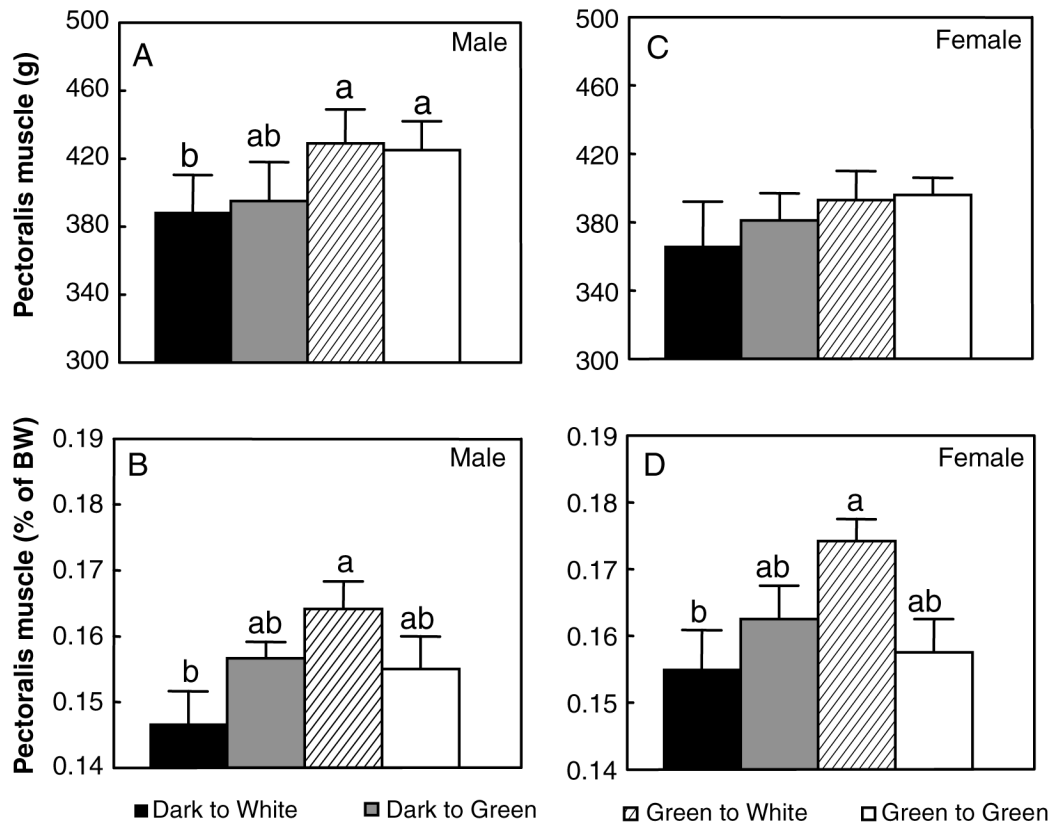
FIGURE 4. Body weight (A) and pectoralis muscle weight as percentage of BW (B) of chicks incubated under green light or kept in the dark on various days posthatch. Results are means  $\pm$  SEM and asterisks mark significant differences between treatments ( $P < 0.05$ ).



**FIGURE 5.** Body weights of male (A) and female (B) chicks incubated under green light and reared under white (hatched bars) or green (open bars) light, or incubated in the dark and reared under either white (black bars) or green (broadly hatched bars) light. Bars represent means  $\pm$  SEM. Values marked with different letters are significantly different ( $P < 0.05$ ).

Body weights of male chicks (Figure 5A) that were photostimulated in ovo with green light (green to white) were significantly higher than those of control chicks (dark to white) until 42 d of age. Green to white female birds were significantly heavier than dark to white female birds from 21 to 42 d of age (Figure 5B). In both males and females, in ovo photostimulation with green light followed by rearing under green light (green to green) had no additive or synergistic effects on BW.

Pectoralis muscle weight of green to white and green to green males at 42 d of age was significantly heavier than that of the dark to white birds (Figure 6A). When pectoralis muscle was compared as percentage of BW (Figure 6B), only that of the green to white birds was significantly higher relative to that of dark to white birds. No difference in muscle weight was found in the females (Figure 6C); however, when analyzing the data as percentage of BW, a significant elevation in percent-



**FIGURE 6.** Pectoralis muscle weight (A, C) and pectoralis muscle weight as percentage of BW (B, D) of male (A, B) and female (C, D) birds at 42 d of age. Birds were reared as described in Figure 5. Bars represent means  $\pm$  SEM. Values marked with different letters are significantly different ( $P < 0.05$ ).

age muscle was found in the green to white females compared to the dark to white birds (Figure 6D), similar to the percentage elevation in males.

## DISCUSSION

In this study, we found that in ovo photostimulation with monochromatic green light augments embryonic development and posthatch BW and muscle growth in broilers. The higher BW of the embryos stimulated with green light, compared with that of the dark-treated embryos, was evident after 2 wk of incubation and remained as such until d 42 posthatch, in both male and female chicks.

Continuous photostimulation during incubation augments embryonic development and accelerates hatching in birds (Siegel et al., 1969; Walter and Viotle, 1973; Coleman and McNabb, 1975; Coleman and McDaniel, 1976). Moreover, a recent study has shown that continuous stimulation with green light at an intensity of 20 W enhances embryo development and BW (Shafey and Al-Mohsen, 2002). Our results show that illuminating eggs with monochromatic green light at an intensity as low as  $0.1 \text{ W/m}^2$  increases egg temperature, even after short periods. It is well established that a slight increase in incubation temperature, which increases egg core temperature by approximately  $0.2^\circ\text{F}$  ( $0.11^\circ\text{C}$ ), accelerates embryonic development, whereas a high increase in

temperature causes abnormalities in the embryos and higher mortality (Romanoff, 1960). Indeed, continuous photostimulation of turkey embryos with white light causes early hatch and increased mortality (Rozenboim et al., 2003). Thus, intermittent lighting (15 min on and 15 min off) in which the rise in egg temperature does not exceed  $0.2^\circ\text{F}$  ( $0.11^\circ\text{C}$ ) eliminates the heat effect. Indeed, our results indicate that hatching period was not affected by intermittent photostimulation, in agreement with a similar study in turkeys (Rozenboim et al., 2003). We propose that the accelerated embryo development and increased posthatch growth observed in this study were due solely to the green light photostimulation.

In ovo photostimulation with green light had a pronounced effect on BW compared with dark conditions. This was observed at all ages until 42 d of age in both males and females (Figure 5). In ovo photostimulation had a significant enhancing effect on muscle growth as percentage of BW until d 42 in both males and females. However, this effect was limited to the group that was reared under white light (Figure 6). These results are in agreement with a previous study reporting an increase in BW and muscle growth due to green light stimulation in turkey embryos, although in that study it was only in females (Rozenboim et al., 2003). Taken together, these findings suggest that sexual dimorphism in growth during embryo development is not associated with light stimulation in broilers.

Growth of the pectoralis muscle in the embryo as well as in posthatch chicks was enhanced by the green light photostimulation, as evidenced by its higher percentage of BW. Moreover, the positive effect of light stimulation on muscle growth preceded its effect on BW in embryos and was evident on almost all days during incubation, suggesting a specific effect of green light on muscle growth. To the best of our knowledge, this is the first study that shows this phenomenon in the embryo. In a recent study, we showed that green and blue light stimuli given as early as 1 d posthatch enhance muscle growth, and that this is due to an increase in the number of satellite cells (Halevy et al., 1998). Moreover, we have observed a higher number of myoblasts in embryos that were incubated under green light compared with that in embryos kept in the dark (Halevy et al., submitted). Therefore, it is plausible that in ovo green light stimulation enhances the proliferation and differentiation of embryonic myoblasts and subsequent muscle hypertrophy. Similarly, it may be that the effect of light stimuli lasts long enough to increase satellite cell proliferation in the embryo and posthatch. However, the mechanism underlying these specific effects is still unknown and might involve a direct effect on myoblast proliferation and differentiation, as well as an indirect effect on these processes via systemic or locally produced growth factors.

Previously, we showed that green light photostimulation posthatch enhances body and muscle weight in broilers (Halevy et al., 1998; Rozenboim et al., 1999). However, in the present study, in ovo photostimulation with green light increased BW and muscle growth with no additive or synergistic effect of green light photostimulation during the rearing period. One explanation could be that the effects caused by photostimulation during incubation are maximal, making any additional photostimulation at a later time ineffective. Indeed, it was only when chicks were incubated in the dark that green light photostimulation posthatch had any positive effect on BW (Figure 5 and Rozenboim et al., 1999). In addition, it may be that signals produced during embryonic development persist long enough to affect posthatch growth. Further studies will have to be conducted in order to clarify this phenomenon.

Overall, we suggest that green light stimulation enhances development and growth in chicks and that the strongest effect is achieved when this stimulus is given during incubation.

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