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THE EFFECTS OF THERMAL AND LIGHT EXPOSURE ON THE DEVELOPMENT OF BROILER CHICKEN LEG MUSCULATURE

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Abstract – Modified incubation factors affect skeletal musculature development of broiler chickens during embryonic and postnatal periods of development. These changes appear to have great influence on the proliferation of myoblasts and muscle growth after hatching. In this study, the histomorphological and -morphometric parameters of broiler chicken leg musculature, after thermal and light treatments during embryogenesis, were examined. The applied treatments included thermal manipulation (exposure of fertilized eggs to increased temperature), light manipulation (exposure of fertilized eggs to monochromatic green light) and simultaneous thermal and light manipulations. Following the specific parameters that were observed, it was determined that in the late postnatal period of development, treated groups have a larger diameter and smaller nucleocytoplasmic ratio of muscle cells compared to those of the control group, in which fertilized eggs were incubated at a constant temperature of 37.8°C, without light. Diameter was increased by 4.20%, 3.77% and 4.55% on day 42 after hatching in thermal, light and combined thermal and light treated groups, respectively, compared to the control. The nucleocytoplasmic ratio was increased by 37.5% on day 42 after hatching in the control group compared to all treated groups. The volume density of the muscle connective tissue was approximately at the same level between the groups. It can be concluded that thermal and light treatments stimulate the proliferation of myoblasts and enhance development and growth of leg muscles in broiler chickens.

Key words: skeletal musculature; broiler; modified incubation factors

INTRODUCTION

In the previous studies it was shown that some different feed treatments and additives affect the growth performance of chickens (Shashidhara and Devegowda, 2003; Li et al., 2007; Amad et al., 2011; Zikic et al., 2011). Halevy et al. (2006c) reported that environmental manipulations during embryogenesis appear to have important relevance for muscle development and also affect chicken growth. Thermal treatments of the fertilized eggs, from the 16th to 18th embryonic days, resulted in higher myoblast proliferation (Piestun et al., 2009), prolonged cell cycle of satellite cells (Halevy et al., 2006b) and higher body weight (Collin et al., 2005), all of which caused enhanced meat production. Illumination of chicken embryos with monochromatic green light resulted in higher breast and body weight at the later posthatch stages (Rozenboim et al., 2004), and a two-fold higher number of adult myoblasts in the muscles (Halevy et al., 2006a).

Duration and timing of thermal manipulations, as well as the exposure of fertilized eggs to some

different part of light spectrum, have a key role in chicken development. Previous studies showed that the most appropriate duration and timing of thermal manipulations in order to reach higher growth is 3 h per day, during the period from the 16th to 18th embryonic day (Yahav et al., 2004). The exposure of fertilized eggs to white and green monochromatic light increases embryo weight, which positively reflects embryonic and postnatal development (Halevy et al., 2006c). The most appropriate temperatures that can be used for thermal manipulations are from 38.5°C to 39.5°C (Piestun et al., 2008), while temperatures above 40°C, due to them causing a lower proliferative response of myoblasts (Halevy et al., 2006b), are not of choice.

The aim of this study was to examine the histomorphological and morphometric parameters of broiler chicken leg musculature after thermal and light manipulations during incubation.

MATERIALS AND METHODS

Experimental design

Eight hundred fertilized broiler eggs (*Ross 308*) of approximately the same weight (65.0±2.0 g), from one parent flock (47 weeks of age), were used in the study. The eggs were divided into 4 groups (control, thermally treated, light treated and simultaneously thermally and light treated), and incubated in 200-egg incubators (type ISC32, *Fenil*, Beska, Serbia). Control eggs were maintained at 37.8°C and 58% relative humidity during the whole incubation period. Thermal manipulation at 39.0°C was applied to the second group, for 3 h/day (09:00 – 12:00) on days 16, 17 and 18 of the embryonic period. Eggs from the third group, which were light treated, received monochromatic green light provided by LED (light-emitting diode) lamps of 560 nm light, at an intensity of 0.1 W/m^2 at eggshell level. Lighting was intermittent (15 min light, 15 min dark) from embryonic days 6-15; thereafter, lighting was continuous. Eggs from the fourth group were simultaneously thermally and light treated according to the regime of the second group (for thermal manipulations) and the third group (for light manipulations).

On the tenth day of incubation, unfertilized eggs and dead embryos were identified by candling and removed from the experiment. Upon hatching, chickens were transferred to brooders with free access to commercial diet and water, and were grown to 42 days of age.

From each of four groups, 10 embryos were randomly selected on the embryonic days 9, 12, 15, 19 and 20, as well as 10 chickens at days 1, 3, 7, 14, 21 and 42 of age. From these embryos and chickens, samples of leg skeletal muscle tissue (M. biceps femoris) were taken.

Histological analysis

Histological analysis was performed using classical light, scanning electron and transmission electron microscopy. To perform classical light microscopy analysis, the muscle tissues were fixed in a 10% buffered formalin solution, followed by a sequence of dehydration and clearing. After that, the samples were embedded in Paraplast and cut into 5-μm thick sections using a microtome Leica RM 2145 (Leica Microsystems, Germany). Histological preparations were stained with hematoxylin and eosin (H&E) and Mallory method. H&E-stained preparations were used for determining diameter and nucleocytoplasmic ratio of muscle cells, while the Mallory method-stained preparations were used for showing connective tissue (Bancroft and Gamble, 2008a, 2008b; Grizzle, 2009).

Classical light microscopy was performed using a light microscope Leica DMLS with a Leica DC 300 digital camera, and the software package IM 1000 (Leica Imaging Systems Ltd, Germany). The diameter of muscle cells was measured as the average of the longest lines drawn across the length and width of their cross-sections. For the analyses of the volume density of connective tissue and the nucleocytoplasmic ratio of muscle cells, the M_{42} testing system described elsewhere was used (Burity, 2004;

Weibel, 2007; Uscebrka et al., 2010), which basically consists of 21 line segments and 42 points in a testing area. The volume density (%) of connective tissue of leg muscle was calculated using the following formula:

$$Vv(ct) = \frac{P(ct)}{P(m)} \cdot 100\ (\%) \qquad (1)$$

where Vv(ct) is the volume density of connective tissue of leg muscle, P(ct) the number of test points lying over the connective tissue of leg muscle, and P(m) the number of test points lying over the muscle. The nucleocytoplasmic ratio of muscle cell was calculated using the following formula:

$$N / C = \frac{P(n)}{P(c)} \qquad (2)$$

where N / C is the nucleocytoplasmic ratio of muscle cell, P(n) the number of test points lying over the cell nucleus, and P(c) the number of test points lying over the cell cytoplasm.

For electron microscopy histological analysis, the muscle tissue samples were initially fixed in 2.5% glutaraldehyde, followed by washing in sodium cacodylate buffer. The samples were then post-fixed in 2% osmium tetroxide, followed by washing in distilled water and dehydration in a series of rising ethanol concentrations. After these processes, for scanning electron microscopy the samples were subjected to critical point drying, mounted on aluminum stubs and coated with gold-palladium, while for the transmission electron microscopy, samples were embedded in epoxy resin, cut into 1-μm thick sections, placed on copper grids and stained with uranyl acetate and lead citrate (Silva et al., 1992).

Scanning electron microscopy was performed using a scanning electron microscope Jeol JSM-6460LV (JEOL, USA). Tissue samples were critical point dried in a CPD 030 "Critical Point Dryer" (BAL-TEC, *Liechtenstein)*. Transmission electron microscopy was performed using a transmission electron microscope *Morgagni 268 (FEI, USA)*.

Statistical analysis

Statistical significance of differences obtained in measurements was determined using two-way ANOVA and the *post hoc* Tukey test for each of the parameters measured. It was considered significant at $p \leq 0.05$. Statistical processing of data was carried out using the software package Statistica for Windows®, ver. 10.0 (StatSoft, Tulsa, OK, USA).

RESULTS

Diameter of leg muscle cells

Comparisons of the diameter of leg muscle cells showed no significant differences ($p > 0.05$) between chicken groups during the embryonic period of development as well as on the first day post hatching (Table 1). During the postnatal period of development, significant differences in the diameter of leg muscle cells were determined between the control group and all treated groups on days 7, 21 and 42 after hatching ($p < 0.05$). Namely, a greater diameter was measured in the muscle cells of the control on day 7 after hatching compared to all treated groups (diameter in the control group was increased by 13.29% compared to the thermal and simultaneously thermally and light treated group; and by 13.63% compared to the light treated group), while in muscle cells of all treated groups greater diameter was measured on days 21 and 42 after hatching in comparison with the control group (Table 2). The diameter was increased by 8.76%, 8.56% and 9.19% on day 21 after hatching (by 4.20%, 3.77% and 4.55% on day 42 after hatching) in the thermally-, light- and the simultaneously thermally and light-treated groups, respectively, as compared to the control group.

Comparing the values of the diameter of leg muscle cells (Table 1), significant differences ($p < 0.05$) were determinated in the control and thermally treated group between days 15 and 19 of embryonic development (on day 19 the diameter was increased by 7.55% and 7.06% in the control and thermally treated groups, respectively), and in the control group between day 20 of embryonic develop-

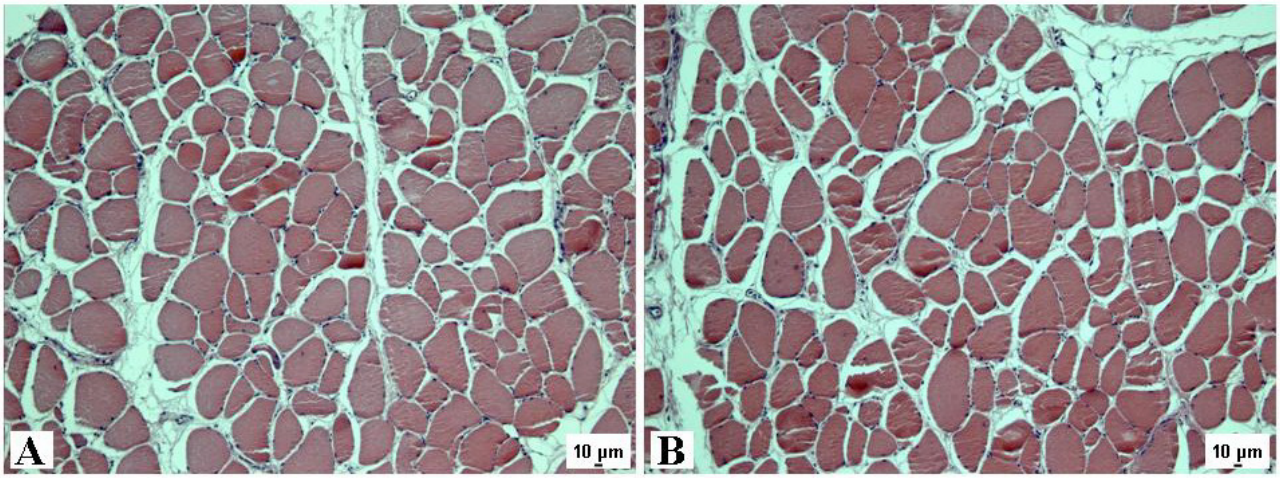

Fig. 1. M. biceps femoris of broiler chickens of control group (**A**) and simultaneously thermally and light treated group (**B**) on day 42 post hatching stained with H&E. Scale bar = 10 µm.

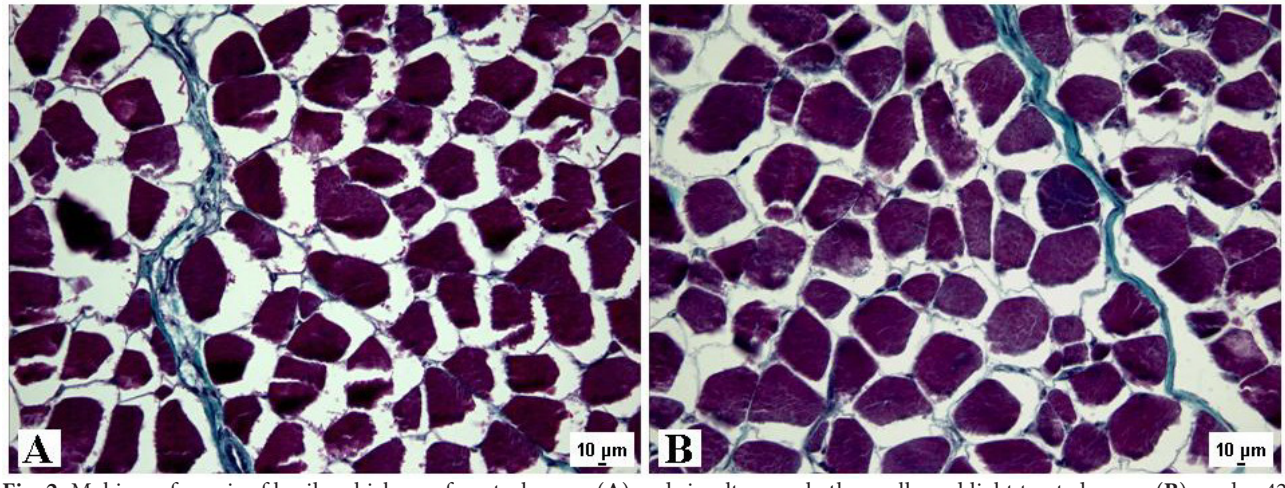

Fig. 2. M. biceps femoris of broiler chickens of control group (**A**) and simultaneously thermally and light treated group (**B**) on day 42 post hatching stained with the Mallory method. Scale bar = 10 µm.

ment and the first day post hatching (diameter was increased by 8.75% on the first day post hatching). During the postnatal period of development, in all chicken groups significant differences ($p < 0.05$) in the diameter of leg muscle cells were determined (Table 2) between days 3 and 7, 7 and 14, 14 and 21, and 21 and 42 after hatching (in all groups, diameter was increased by 32.34-45.42% on day 7 compared to day 3 after hatching, by 40.12-59.22% on day 14 compared to day 7 after hatching, by 28.69-40.26% on day 21 compared to day 14 after hatching, and by 131.95-142.68% on day 42 compared to day 21 after hatching).

The differences in the diameter of muscle cells of the M. biceps femoris between control and treated groups on day 42 after hatching can be seen in Fig. 1. In the same area, in muscle of the treated group a smaller number of muscle cells of greater diameter can be observed, while in muscle of the control group, a greater number of muscle cells of smaller diameter can be seen.

Volume density of connective tissue in leg muscle

Comparison of the volume density of connective tissue in the leg muscle showed no significant differ-

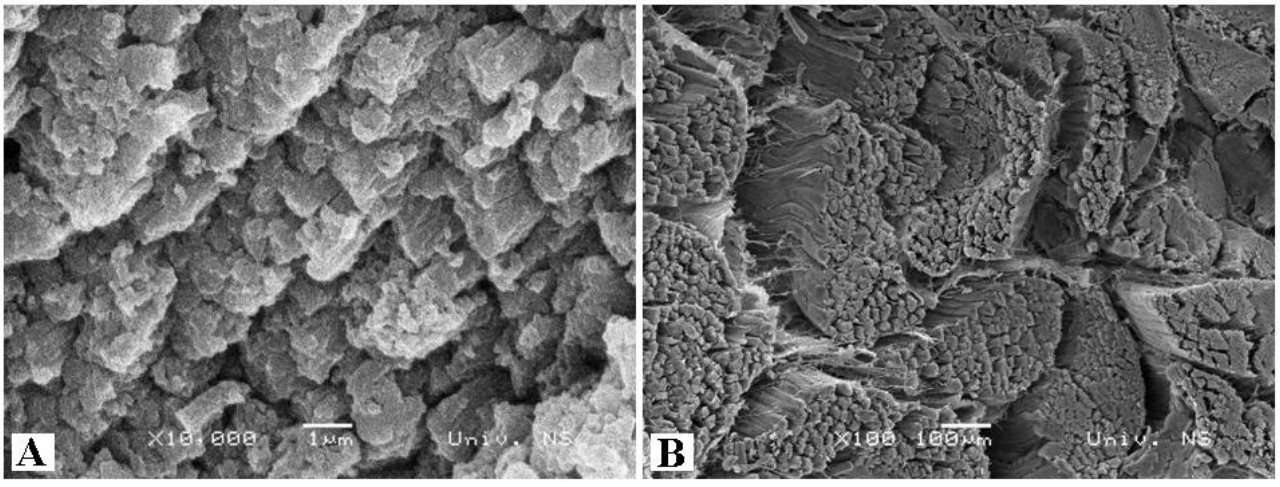

Fig. 3. Scanning electron micrograph of M. biceps femoris of simultaneously thermally and light treated group of chickens. Figure shows initial phase (**A**, day 15 of embryonic development, scale bar = 1 μm) and mature stage (**B,** day 42 after hatching, scale bar = 100 μm) of the formation of skeletal muscle cells bundles.

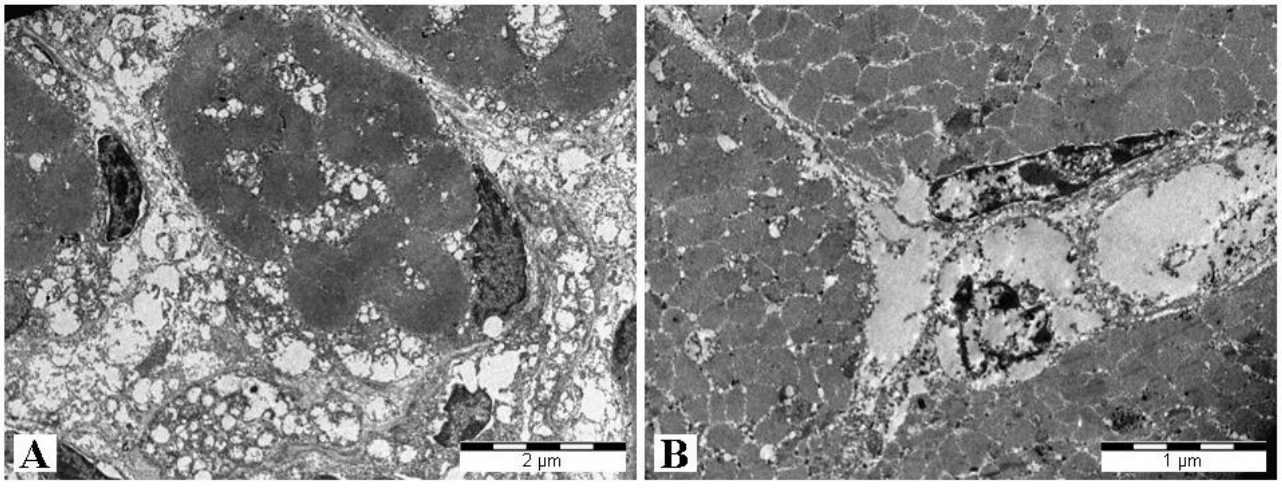

Fig. 4. Transmission electron micrograph of M. biceps femoris of chickens of simultaneously thermally and light treated group. In leg muscle cell, myofibrils show little differentiation on day 15 of embryonic development (**A**, scale bar = 2 μm), while on day 42 post hatching (**B**, scale bar = 1 μm) myofibrils are fully differentiated.

ences ($p > 0.05$) between chicken groups during the embryonic period of development, the first day post hatching (Table 1), and during the postnatal period of development (Table 2).

In all chicken groups, significant differences ($p < 0.05$) in the volume density of connective tissue in the leg muscle were determined (Table 1) between days 9 and 12, 15 and 19, and 19 and 20 of embryonic development (in all groups, volume density of connective tissue was decreased by 7.97%-8.74% on day 12 compared to day 9 of embryonic development, by 10.16-12.62% on day 19 compared to day 15 of embryonic development, and by 10.34-13.52% on day 20 compared to day 19 of embryonic development).

An approximately equal amount of muscle and connective tissue in M. biceps femoris between control and treated groups on day 42 after hatching can be seen in Fig. 2. For detection of muscle (coloured

Table 1. Examination of samples of leg muscle tissue during the embryonic period of development and first day post hatching: diameter of muscle cells (μm), volume density of connective tissue of leg muscle (%) and the nucleocytoplasmic ratio of muscle cells. Values are means ± SE.

Day of embryonic development	Diameter (μm)			
	Control	Thermal treatment	Light treatment	Simultaneously thermal and light treatment
9	5.32 ± 0.16^{a}	5.29 ± 0.16^{a}	5.43 ± 0.14^{a}	5.42 ± 0.22^{a}
12	5.51 ± 0.10^{ab}	5.52 ± 0.11^{ab}	5.64 ± 0.12^{ab}	5.62 ± 0.12^{ab}
15	5.83 ± 0.14^{b}	5.81 ± 0.16^{b}	5.96 ± 0.21^{bc}	5.92 ± 0.23^{bc}
19	6.27 ± 0.14^{c}	6.22 ± 0.12^{c}	6.24 ± 0.15^{cd}	6.23 ± 0.16^{cd}
20	6.40 ± 0.12^{c}	6.48 ± 0.16^{cd}	6.43 ± 0.19^{de}	6.45 ± 0.17^{de}
First day post hatching	6.96 ± 0.29^{d}	6.74 ± 0.06^{d}	6.72 ± 0.07^{e}	6.72 ± 0.12^{e}
Source	p value			
Treatment (T)	NS			
Age (A)	< 0.05			
T x A	NS			
Day of embryonic development	Volume density (%)			
	Control	Thermal treatment	Light treatment	Simultaneously thermal and light treatment
9	31.10 ± 1.05^{a}	30.62 ± 0.69^{a}	30.66 ± 0.90^{a}	31.37 ± 0.68^{a}
12	28.52 ± 0.77^{bc}	28.18 ± 0.62^{bc}	27.98 ± 0.80^{bc}	28.76 ± 0.65^{bc}
15	28.33 ± 0.86^{c}	28.04 ± 0.91^{c}	27.86 ± 0.96^{c}	28.69 ± 1.14^{c}
19	24.98 ± 0.74^{d}	24.57 ± 0.76^{d}	25.03 ± 0.90^{d}	25.07 ± 1.06^{d}
20	21.83 ± 0.77^{ef}	22.03 ± 0.96^{ef}	22.05 ± 0.87^{ef}	21.68 ± 0.52^{ef}
First day post hatching	21.33 ± 0.53^{f}	21.42 ± 0.59^{f}	20.98 ± 0.65^{f}	21.55 ± 0.61^{f}
Source	p value			
Treatment (T)	NS			
Age (A)	< 0.05			
T x A	NS			
Day of embryonic development	Nucleocytoplasmic ratio			
	Control	Thermal treatment	Light treatment	Simultaneously thermal and light treatment
9	0.40 ± 0.02^{a}	0.39 ± 0.02^{a}	0.41 ± 0.02^{a}	0.40 ± 0.03^{a}
12	0.37 ± 0.02^{ab}	0.38 ± 0.01^{ab}	0.38 ± 0.02^{a}	0.39 ± 0.03^{a}
15	0.36 ± 0.02^{ab}	0.35 ± 0.02^{ab}	0.36 ± 0.02^{ab}	0.37 ± 0.03^{a}
19	0.32 ± 0.03^{b}	0.33 ± 0.02^{b}	0.32 ± 0.02^{b}	0.31 ± 0.03^{b}
20	0.26 ± 0.01^{c}	0.27 ± 0.02^{c}	0.26 ± 0.02^{c}	0.25 ± 0.01^{c}
First day post hatching	0.19 ± 0.02^{d}	0.20 ± 0.01^{d}	0.20 ± 0.02^{d}	0.19 ± 0.01^{d}
Source	p value			
Treatment (T)	NS			
Age (A)	< 0.05			
T x A	NS			

Means within a column, with no common superscript small letter (a, b, c, d, e, f) differ significantly ($p < 0.05$)

Table 2. Examination of samples of leg muscle tissue during the postnatal period of development: diameter of muscle cells (μm), volume density of connective tissue of leg muscle (%) and nucleocytoplasmic ratio of muscle cells. Values are means ± SE.

Day of postnatal development	Diameter (μm)			
	Control	Thermal treatment	Light treatment	Simultaneously thermal and light treatment
3	7.97 ± 0.26^{a}	7.72 ± 0.28^{a}	7.68 ± 0.38^{a}	7.73 ± 0.27^{a}
7	11.59 ± 0.09^{bA}	10.23 ± 0.04^{bB}	10.20 ± 0.05^{bB}	10.23 ± 0.08^{bB}
14	16.24 ± 0.08^{c}	16.26 ± 0.10^{c}	16.24 ± 0.12^{c}	16.27 ± 0.09^{c}
21	20.90 ± 0.26^{dA}	22.73 ± 0.32^{dB}	22.69 ± 0.24^{dB}	22.82 ± 0.65^{dB}
42	50.72 ± 1.45^{eA}	52.84 ± 0.64^{eB}	52.63 ± 0.24^{eB}	53.03 ± 0.97^{eB}
Source	p value			
Treatment (T)	< 0.05			
Age (A)	< 0.05			
T x A	< 0.05			
Day of embryonic development	Volume density (%)			
	Control	Thermal treatment	Light treatment	Simultaneously thermal and light treatment
3	20.60 ± 0.54^{a}	21.04 ± 0.82^{a}	20.47 ± 0.79^{a}	21.02 ± 0.94^{a}
7	19.50 ± 0.68^{ab}	19.55 ± 0.66^{ab}	19.25 ± 0.72^{ab}	20.04 ± 0.84^{ab}
14	18.47 ± 0.60^{bc}	18.36 ± 0.59^{bc}	18.54 ± 0.65^{bc}	18.61 ± 0.68^{bc}
21	17.21 ± 0.60^{cd}	16.99 ± 0.62^{cd}	17.30 ± 0.49^{cd}	16.97 ± 0.67^{cd}
42	16.02 ± 0.77^{d}	15.73 ± 0.70^{d}	15.56 ± 0.76^{d}	15.69 ± 0.65^{d}
Source	p value			
Treatment (T)	NS			
Age (A)	< 0.05			
T x A	NS			
Day of embryonic development	Nucleocytoplasmic ratio			
	Control	Thermal treatment	Light treatment	Simultaneously thermal and light treatment
3	0.18 ± 0.01^{a}	0.18 ± 0.02^{a}	0.19 ± 0.02^{a}	0.18 ± 0.02^{a}
7	0.15 ± 0.008^{bA}	0.18 ± 0.004^{aB}	0.18 ± 0.008^{aB}	0.18 ± 0.005^{aB}
14	0.15 ± 0.01^{b}	0.14 ± 0.01^{b}	0.15 ± 0.01^{b}	0.15 ± 0.02^{b}
21	0.14 ± 0.004^{bA}	0.11 ± 0.004^{cB}	0.11 ± 0.008^{cB}	0.11 ± 0.008^{cB}
42	0.11 ± 0.005^{cA}	0.08 ± 0.008^{dB}	0.08 ± 0.007^{dB}	0.08 ± 0.007^{dB}
Source	p value			
Treatment (T)	< 0.05			
Age (A)	< 0.05			
T x A	< 0.05			

Means within a column, with no common superscript small letter (a, b, c, d, e) differ significantly ($p < 0.05$)
Means within a row, with no common superscript capital letter (A, B) differ significantly ($p < 0.05$)

red-purple on photomicrographs) and connective tissue (coloured green on photomicrographs) the Mallory staining method was used.

The distribution of connective tissue within the M. biceps femoris is shown in Fig. 3. Bundles of muscle cells exhibit low levels of differentiation on day 15 of embryonic development, with small amounts of connective tissue between them. On the other hand, on day 42 after hatching, bundles of muscle cells are fully differentiated with a large amount of connective tissue between them, while inside them clear boundaries between individual muscle cells exist.

Nucleocytoplasmic ratio of leg muscle cells

Comparison of the nucleocytoplasmic ratio of leg muscle cells showed no significant differences ($p > 0.05$) between chicken groups during the embryonic period of development as well as on the first day post hatching (Table 1). During the postnatal period of development, significant differences in the nucleocytoplasmic ratio of leg muscle cells were determined between the control group and all treated groups on days 7, 21 and 42 after hatching ($p < 0.05$). Namely, a higher nucleocytoplasmic ratio was measured in the muscle cells of all treated groups than on day 7 after hatching (nucleocytoplasmic ratio in all treated groups was increased by 20% compared to the control group), while a lower nucleocytoplasmic ratio was measured on day 21 and 42 after hatching compared to the same parameter in the control group (Table 2). The nucleocytoplasmic ratio was increased by 27.27% on day 21 after hatching (by 37.5% on day 42 after hatching) in the control group compared to all treated groups.

Comparing the values of nucleocytoplasmic ratio of leg muscle cells (Table 1), significant differences ($p < 0.05$) were determined in all chicken groups between days 19 and 20 of embryonic development and between day 20 of embryonic development and the first day post hatching (in all groups, the nucleocytoplasmic ratio was decreased by 18.18-19.35% on day 20 compared to day 19 of embryonic development, and by 23.08-26.92% on the first day post hatching compared to day 20 of embryonic development), as well as in the group that was simultaneously thermally and light treated during incubation between days 15 and 19 of embryonic development (nucleocytoplasmic ratio was decreased by 16.21% on day 19 of embryonic development). During the postnatal period of development, significant differences ($p < 0.05$) in the nucleocytoplasmic ratio of leg muscle cells were determined (Table 2) in all chicken groups between days 21 and 42 after hatching (in all groups nucleocytoplasmic ratio was decreased by 21.43-27.27% on day 42 after hatching), in all groups except the control between days 7 and 14, and 14 and 21 after hatching (in all groups except the control, the nucleocytoplasmic ratio was decreased by 16.67-22.22% on day 14 compared to day 7 after hatching, and by 21.43-26.67% on day 21 compared to day 14 after hatching), as well as in the control group between days 3 and 7 after hatching (the nucleocytoplasmic ratio was decreased by 16.67% on day 7 after hatching).

The position of the nuclei inside the muscle cell of M. biceps femoris is shown in Fig. 4. The nucleus is peripherally positioned inside the muscle cell on day 15 of embryonic development. On day 42 after hatching, the nucleus retains a peripheral position, while the muscle cell diameter is significantly enlarged, which causes the nucleus to take up a much smaller part of the muscle cell area compared to the embryonic period.

Effects of incubation treatments on morphometric parameters

There were no significant differences ($p > 0.05$) regarding the diameter and nucleocytoplasmic ratio of leg muscle cells between the treatments, and volume densities of connective tissue in leg muscle, during the embryonic period of development and the first day post hatching (Table 1). During the postnatal period of development, there were significant differences ($p < 0.05$) between the treatments in terms of diameter and nucleocytoplasmic ratio of the leg muscle cells (as described before, significant differences ($p < 0.05$) in the diameter and nucleocytoplasmic ratio

of leg muscle cells were determined between the control group and all treated groups on days 7, 21 and 42 after hatching), but not for the volume density of connective tissue in leg muscle (Table 2).

Statistical analysis showed differences ($p < 0.05$) among the ages when comparing the diameter and nucleocytoplasmic ratio of the leg muscle cells and volume density of connective tissue in the leg muscle during the embryonic period of development and the first day post hatching (Table 1), as well as during the postnatal period of development (Table 2). Generally, the diameter of leg muscle cells increased, while the volume density of connective tissue of the leg muscle as well as the nucleocytoplasmic ratio of leg muscle cells decreased from the early embryonic days to the late postnatal period of development.

Significant interactions were not detected between treatment and age regarding the diameter and nucleocytoplasmic ratio of leg muscle cells, as well as the volume density of connective tissue in leg muscle during the embryonic period of development and the first day post hatching (Table 1). During the postnatal period of development, significant interactions ($p < 0.05$) were detected between treatment and age in terms of diameter and nucleocytoplasmic ratio of the leg muscle cells, but not the volume density of connective tissue in the leg muscle (Table 2).

DISCUSSION

The results of our study show that on day 7 after hatching the leg muscle cells of the control group have a greater diameter compared those of all treated groups. The smaller diameter of the muscle cells in the treated groups could be explained by thermal and light exposure during incubation, which caused prolonged proliferation of myoblasts (Rozenboim et al., 2004; Collin et al., 2007). We observed that during the time when the proliferation of myoblasts was still underway in the treated groups, an increase occurred in the diameter of muscle cells of the control group. On day 14 after hatching, no significant differences in the diameter of leg muscle cells between all chicken groups were determined. Obviously, the accelerated growth of skeletal muscle cells in the treated groups occurs upon myoblast proliferation. The thermal and light treatments applied in our study affected not only the prolonged proliferation of myoblasts, but also accelerate growth of the leg muscle cells in the late postnatal development. Precisely, we observed a larger diameter of leg muscle cells in the treated groups compared to those of the control group on days 21 and 42 after hatching. Enhanced growth of muscle cells of broiler chickens at market age caused by thermal manipulation during embryogenesis was also noticed by Halevy et al. (2006c) who pointed out that increased incubation temperature had a delayed effect on satellite cell proliferation and differentiation, which also contributes to muscle growth in the postnatal period.

We detected no significant differences in the volume density of connective tissue in the leg muscle between the control and all treated groups. This could be due to the equal quantity of muscle tissue per unit area within the leg muscle in all chicken groups at the same age. On day 7 after hatching, the thermal and light treatments induced prolonged proliferation of myoblasts, causing the formation of a higher number of leg muscle cells of smaller diameter per unit area in all treated groups, while within the leg muscles of the control group, a smaller number of muscle cells of greater diameter was formed per the same unit area. On days 21 and 42 after hatching, the applied treatments caused an increase in the diameter of leg muscle cells, which was reflected in the formation of a smaller number of leg muscle cells of greater diameter per unit area. Within the leg muscles of the control group, a higher number of muscle cells of smaller diameter was formed per the same unit area. On the other days measurements were performed, approximately the same number of leg muscle cells of approximately the same diameter were formed per unit area in all chicken groups. The described relation between diameter and number of leg muscle cells per unit area is in agreement with the observations of other authors (Halevy et al., 2006a; Halevy et al., 2006b), who detected an increased number of myoblasts and augmented muscle cell proliferation caused by environmental manipulations during

embryogenesis of broilers. These processes did not cause significant differences in the volume density of connective tissue in leg muscle between the control and treated groups.

The results of our study show that on day 7 after hatching, the leg muscle cells of the treated groups had a higher nucleocytoplasmic ratio compared to those of the control group. This is supposedly the consequence of the greater diameter of leg muscle cells of the control group, as well as the greater amount of cytoplasm within these cells, where the nucleus takes up a smaller part of the cell. Namely, the leg muscle cells of all treated groups had a smaller diameter and smaller amount of cytoplasm, where the nucleus takes up a bigger part of the cell. The nuclei had the same diameter in all chicken groups on day 14 after hatching, which was reflected in the absence of significant differences in the nucleocytoplasmic ratio of leg muscle cells between all groups. On days 21 and 42 after hatching, the nucleocytoplasmic ratio of leg muscle cells was higher in the control group compared to all treated groups. This is related to the smaller diameter and smaller amount of cytoplasm of the leg muscle cells of the control group compared to all treated groups, which is similar to results previously reported by Piestun et al. (2009). In that study, in broiler chickens that were thermaly manipulated during late-term embryogenesis, a higher diameter of muscle cells in the late postnatal period was also detected, which is related to the smaller nucleocytoplasmic ratio of these cells.

As the result of thermal and light treatments during embryogenesis, beside prolonged proliferation of myoblasts Ghatpande et al. (1995) detected accelerated morphogenesis and growth of broiler chickens, which is in agreement with our results. Namely, we observed that during the time that myoblasts proliferated in the treated groups, an increase in the diameter of leg muscle cells of the control group occurred, which was supported by the following results of our study: a significant increase in the diameter of leg muscle cells of the control group between day 20 of embryonic development and the first day post hatching, as well as a significant decrease in the nucleocytoplasmic ratio of lthe eg muscle cells of the same group between day 3 and day 7 after hatching. The accelerated growth of the broiler chickens in the treated groups was related to a significant decrease in the nucleocytoplasmic ratio of leg muscle cells of treated groups between days 7th and 4, as well as between days 14 and 21 after hatching.

In this study, a decrease in amount of connective tissue in the leg muscle from day 9 of embryonic development to day 42 after hatching was determined in all chicken groups. This could be explained by several processes that occur during muscle tissue development from the early embryonic to late postnatal period, i.e. growth and differentiation of leg muscle cells (Bellairs and Osmond, 2005), formation of bundles of these cells (Junqueira et Carneiro, 2003), as well as the distribution of connective tissue in and outside the formed bundles of muscle cells (Eurell and Frappier, 2006; Samuelson, 2007). These processes lead to a decrease in the amount of connective tissue, which is highly present in the early embryonic period, and an increase in the amount of muscle tissue toward the end of incubation and the period after hatching.

Finally, it can be concluded that thermal and light manipulations during incubation cause changes in the development of the skeletal musculature of broiler chickens, as shown by the described differences in the diameter and nucleocytoplasmic ratio of leg muscle cells, as well as the approximately equal volume density of connective tissue in the leg muscle between the control and all treated groups. Applied thermal and light treatments caused prolonged proliferation of myoblasts and accelerated growth of the leg muscle cells in the late postnatal development, which was reflected by the values of the observed parameters. These findings emphasize the possibility of enhancing skeletal muscle growth of broiler chickens using thermal and light manipulations during incubation.

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REFERENCES

Amad, A.A., Manner, K., Wendler, K.R., Neumann, K. and *J. Zentek* (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poult. Sci.* **90**, 2811-2816.

Bancroft, J.D. and *M. Gamble* (2008a). The hematoxylin and eosin, In: *Theory and practice of histological techniques*, 6th edition, 121-134. Churchill Livingstone Elsevier ltd., Philadelphia.

Bancroft, J.D. and *M. Gamble* (2008b). Connective tissues and stains, In: *Theory and practice of histological techniques*, 6th edition, 135-160. Churchill Livingstone Elsevier Ltd., Philadelphia.

Bellairs, R. and *M. Osmond* (2005). Skeleton and muscles, In: *The atlas of chick development*, 2nd edition, 91-l04. Elsevier Academic Press, London.

Burity, C.H.F., Pissinatti, A. and *C.A. Mandarim-de-Lacerda* (2004). Stereology of the liver in three species of *Leontopithecus* (Lesson, 1840) Callitrichidae – Primates. *Anat. Histol. Embryol.* **33**, 183-187.

Collin, A., Picard, M. and *S. Yahav* (2005). The effect of duration of thermal manipulation during broiler chick embryogenesis on body weight and body temperature of post-hatched chicks. *Anim. Res.* **54**, 105-111.

Collin, A., Berri, C., Tesseraud, S., Rodon, R.F.E., Skiba-Cassy, S., Crochet, S., Duclos, M.J., Rideau, N., Tona, K., Buyse, J., Bruggeman, V., Decuypere, E., Picard, M. and *S. Yahav* (2007). Effects of thermal manipulations during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. *Poult. Sci.* **86**, 795-800.

Eurell, J.A. and *B.L. Frappier* (2006). Muscle, In: Dellmann's textbook of veterinary histology, 6th edition, 79-90. Blackwell Publishing, Oxford.

Ghatpande, A., Ghatpande, S. and *M.Z. Khan* (1995). Effect of different intensities of fluorescent light on the early development of chick embryos *in ovo*. *Cell. Mol. Biol. Res.* **41**, 613-621.

Grizzle, W.E. (2009). Special symposium: fixation and tissue processing models. *Biotech. Histochem.* **84**(5) 185-193.

Halevy, O., Piestun, Y., Rozenboim, I. and *Z. Yablonka-Reuveni* (2006a). *In ovo* exposure to monochromatic green light promotes skeletal muscle cell proliferation and affects myofiber growth in posthatch chicks. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**, 1062-1070.

Halevy, O., Rozenboim, I., Yahav, S. and *I. Piestun* (2006b). Muscle development-Could environmental manipulations during embryogenesis of broilers change it? Paper presented at XII European Poultry Conference, Verona, Italy, September 10-14. *World's Poult. Sci. J.* **62** (Suppl.), 251.

Halevy, O., Yahav, S. and *I. Rozenboim* (2006c). Enhancement of meat production by environmental manipulations in embryo and young broilers. *World Poult.* **62**, 485-497.

Junqueira, L.C. and *J. Carneiro* (2003) Muscle Tissue, In: *Basic histology*, 10th edition, 191-214. The McGraw-Hill Companies, New York.

Li, X.J., Piao, X.S., Kim, S.W., Liu, P., Wang, L., Shen, Y.B., Jung, S.C. and *H.S. Lee* (2007). Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. *Poult. Sci.* **86**, 1107-1114.

Piestun, Y., Shinder, D., Ruzal, M., Halevy, O., Brake, J. and *S. Yahav* (2008). Thermal manipulations during broiler embryogenesis: effect of acquisition of thermotolerance. *Poult. Sci.* **87**, 1-10.

Piestun, Y., Harel, M., Barak, M., Yahav, S. and *O. Halevy* (2009). Thermal manipulations in late-term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. *J. Appl. Physiol.* **106**, 233-240.

Rozenboim, I., Piestun, Y., Mobarkey, N., Barak, M., Hoyzman, A. and *O. Halevy* (2004). Monochromatic light stimuli during embryogenesis enhance embryo development and posthatch growth. *Poult. Sci.* **83**, 1413-1419.

Samuelson, D.A. (2007) Muscle, In: *Textbook of veterinary histology*, 1st edition, 159-178. Saunders Elsevier, St. Louis, Missouri.

Shashidhara, R.G. and *G. Devegowda* (2003). Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. *Poult. Sci.* **82**, 1319-1325.

Silva, T.J., Bracker, C.E., Orcutt, M.W., Forrest, J.C. and *M.D. Judge* (1992). Preparation of muscle samples for comparative electron microscopy. *J. Anim. Sci.* **70**, 2452-2461.

Uscebrka, G., Stojanovic, S., Zikic, D. and *Z. Kanacki* (2010). Morphodynamics of embryonic development of skeletal musculature of broiler and layer chickens. *Avian Biol. Res.* **3** (4), 179-186.

Weibel, E.R., Hsia, C.C.W. and *M. Ochs* (2007). How much is there really? Why stereology is essential in lung morphometry. *J. Appl. Physiol.* **102**, 459-467.

Yahav, S., Collin, A., Shinder, D. and *M. Picard* (2004). Thermal manipulations during broiler chick embryogenesis: effects of timing and temperature. *Poult. Sci.* **83**, 1959-1963.

Zikic, D., Peric, L., Uscebrka, G., Stojanovic, S., Milic, D. and *L. Nollet* (2011). Influence of dietary mannanoligosaccharides on histological parameters of the jejunal mucosa and growth performance of broiler chickens. *Afr. J. Biotechnol.* **10** (32), 6172-6176.