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Influence of modified incubation factors on meat characteristics of broiler chickens*

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Monochromatic green light and thermal conditioning during embryonic development of broiler chickens may stimulate muscle growth and affect characteristics of the meat. Green light can penetrate the eggshell and act on muscle tissue. Thermal conditioning, at an early age, leads to a temporary inhibition of growth followed by rapid, presumably compensatory growth. The aim of this paper was to evaluate the influence of these incubation factors and their combination on the level of growth factor, secondary cellular messenger systems, body and breast muscle weight, chemical composition of meat and progressive moisture loss (drip loss). Four hundred broiler chicken eggs (Ross 308) were divided into four groups of 100 eggs each and set in separate incubators that allowed selective and simultaneous application of monochromatic green light and thermal conditioning at precise stages of embryonic development. The level of growth factor and secondary cellular messenger systems were monitored during the prenatal and postnatal development and the meat characteristics were determined at the end of the production cycle. In conclusion, monochromatic green light, thermal conditioning, and their combination significantly affected meat characteristics by increasing the body and breast muscle weight, the percentage of dry matter, total protein content, and reducing progressive moisture loss.

KEY WORDS: broiler / incubation factors / meat characteristics

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During embryonic development of broiler chickens, muscle growth can be stimulated, resulting in increased meat production, by applying monochromatic green light and by thermal conditioning. Thermal conditioning is achieved by increasing the incubation temperature during certain critical phases of development. Green light and thermal conditioning act both directly and indirectly. Indirect effects are mediated via the endocrine system or locally produced mediators [Halevy *et al*. 2006]. Both of these types of mediators act at the molecular and cellular level and lead to increased proliferation and differentiation of satellite cells and then, as a consequence, hypertrophy of muscle cells [Uščebrka *et al*. 2010, Zhang *et al*. 2014].

Monochromatic green light stimulates growth early in postnatal development, during the embryonic stage [Rozenboim *et al.* 2013]. Direct effects of the light on muscle are due to the light's ability to penetrate the eggshell. Indirect effects, which act through the endocrine system or through growth factors produced in adjacent tissues, are the result of the interaction of the light with photoreceptors in the retina of the eye or receptors in the pineal gland. Light energy can also reach the hypothalamus by passing through the skull and soft tissues [Lewis and Morris 2000].

Developmental and physiological modifications associated with a slight increase in incubation temperature have presumably evolved to improve thermotolerance in broilers [Nichelmann and Tzschentke 2002]. Embryos and newly hatched chickens, which possess an immature thermoregulatory system, are exposed to thermal conditioning during critical stages in the development of this system [Yahav and McMurtry 2001] leading to increased tolerance of heat stress. This thermal conditioning treatment resulted in a temporary inhibition of growth, followed by a rapid growth rate, presumably compensatory [Yahav 2000]. The main mediators of muscle cell response to the moderate increased temperature are locally produced growth factors, primarily those produced in the muscles themselves [Halevy *et al*. 2001].

Insulin-like growth factor-1 (IGF-1) is essential for normal growth and development of birds [Proudman et al. 1994]. IGF-1 in muscle may be derived from two different sources: it may be synthesized in the liver, where it is regulated by growth hormone and, of greater importance, it may be of local origin, and act as an autocrine or paracrine [Halevy et al. 2001]. Local production of IGF-1 in muscle tissue can be under the influence of exogenous factors [Paul and Rosenthal. 2002]. The effects of IGF-1 may be, at least partly, mediated by hormones such as the thyroid hormones [Adams et al. 2000]. Among these effects, it has been shown that IGF-1 stimulates proliferation of satellite cells in chickens [Hodik *et al*. 1997].

Growth factors in muscle tissue regulate development of muscle cells, affecting both cell proliferation and differentiation. The same growth factor can affect both of these processes, but acts through different second-messenger systems in the cell. For example, the activation of the phosphoinositide 3-kinase (PI3K) pathway stimulates the differentiation of myoblasts, while activation of the extracellular signal-regulated kinases (ERK), also called mitogen-activated protein kinase (MAPK), pathway stimulates the proliferation of myoblasts but inhibits their differentiation [Halevy and Cantley 2004].

IGF-1 plays an important role in the metabolism of carbohydrates, fats, and protein in adipose tissue, liver, and skeletal muscle. In skeletal muscle cells, it stimulates protein synthesis and the uptake of glucose [LeRoith and Yakar 2007]. Through this effect, IGF-1 can have a large impact on the chemical composition and progressive loss of moisture from the meat.

The aim of this study was to determine the influence of monochromatic green light, thermal conditioning, and their combination on the protein expression of IGF-1 and ERK in muscle tissue and on the body and breast muscle weight, chemical composition and progressive loss of moisture (drip loss) from the meat.

Material and methods

Eggs, incubators, broilers, housing system and diet

Four hundred broiler chicken eggs (Ross 308) from 49-week-old parents were analyzed. After the rest period, the eggs were divided into four groups of 100 eggs each and set in separate incubators that allowed selective and simultaneous application of monochromatic green light and thermal conditioning (TC) at precise stages of embryonic development.

Temperature and relative humidity were monitored by an analog device (Thermometer-higrometer Veb, Berlin, Germany). The temperature was regulated by an electronic thermostat (Temperature controller ET-01, Pro-Elektro, Novi Sad, Srbija). Monochromatic green light was applied by a light-emitting diode (Led Jcdr 18, Vito Industrial Limited, Zhuhai, China). Light intensity and homogeneity of the light were controlled with a digital luxmeter (Teck Peak 5025, Peak Technologies, Inc., Old Columbia Rd., Columbia, USA). The humidity in all incubators was maintained at 58%.

The experimental groups were formed on the following principles: the experimental group A was the control group (temperature of 37.8°C, no light); the experimental group B was under thermal conditioning treatment (during the 16th, 17th and 18th day of the embryonic development the incubation temperature was increased to 39°C for 3 hours per day from 9:00 AM until 12:00 PM); the experimental group C was subjected to the influence of monochromatic green light wavelengths from 450 to 550 nm and intensity 0.1 W/m2 (from 6th to 15th day of embryonic development under the regime of intermittent light 15 minutes and 15 minutes of darkness and under constant regime from 16th day until the end of incubation); the experimental group D was a combination of experimental treatments applied in the experimental groups B and C.

After hatching, drying, and resting, the broilers were sexed and packed into transport boxes and transferred to a facility, which used the floor system with boxes, for fattening. The hatching results were monitored in all groups. The conditions (temperature, humidity, lighting, density) were adapted to the needs of this hybrid [Halevy *et al.* 2001]. Chickens were vaccinated based on a regular vaccination program

against Newcastle disease and infectious bursal disease. Throughout the experiment they were under veterinary supervision and the chicken mortality was monitored.

During the production cycle broilers were fed *ad libitum* in three phases. For days 1-13, their feed was 22% proteins (starter), for days 14-34 it was 19% protein (grower), and for days 35 until the end of the production cycle (day 42) it was 18% protein (finisher).

During the postnatal development the samples were taken after cervical dislocation.

Western blot analysis

Breast muscle (*m. pectoralis superficialis*) samples were analyzed by Western blot. Samples were taken on days 15 and 19 of embryonic development and on days 3, 21, and 42 of postnatal development (equal number of males and females). On each day ten samples were taken from each group (A, B, C and D) – in total 200 samples. Samples were taken from the right side of the breast and frozen in liquid nitrogen. The tissue was macerated three times in ten seconds intervals with ten second pauses, homogenized in radioimmune precipitation buffer with protease inhibitors (F. Hoffmann-La Roche Ltd. Basel, Swiss), sonicated and centrifuged. The resultant supernatants were used for Western blot analysis.

Western blot was performed in a quantitative manner [Tienrungroj *et al*. 1987]. Antibodies and concentrations used in this experiment were: antibody to IGF-1 (ab9572, the concentration of 0.2 mg/ml., Abcam, Cambridge, UK) and antibody to ERK1+ERK2 (ab79853, the concentration of 0.2 mg/ml, Abcam, Cambridge, UK). The secondary antibody was obtained by immunization of goats with a rabbit immunoglobulin G (IgG) (Santa Cruz Biotechnology, Inc., Santa Cruz, USA). The average protein expression of IGF-1 and ERK on day 15 of prenatal development was taken as 100%.

Meat chemistry and progressive loss of moisture

Meat was chemically analyzed and the progressive loss of moisture from it was assessed. For these measurements samples of breast muscle (*m. pectoralis superficialis*) were taken from ten (5 males and 5 females) 42-day-old broilers from each group. Before sampling, body weight and breast muscle weight were measured. The moisture was determined with the rapid microwave drying method for moisture in meat and poultry products (AOAC International, 2000). Fat, protein, and ash were also determined (AOAC International. 2000) and calculated as percentage of dry matter (%DM). Drip loss was measured from breast samples at 24 and 48 hours postmortem. For drip loss measurements, breast meat was separated from the bone and both breasts from each broiler were weighed separately, enclosed in a plastic bag to collect liquid, and placed in a refrigerator at 4°C. After 24 hours, one breast portion from each bird was assessed for drip loss and after 48 hours the second breast portion was assessed [Perić *et al*. 2009].

Statistical inference

Statistical tests were performed using two-way analysis of variance (ANOVA) to determinate the effect of treatments (modified incubation factors) and age (days of development) on protein expression of IGF-1 and ERK in muscle tissue. One-way ANOVA was used to determinate the effect of treatments on body and breast muscle weight, chemical composition of meat and drip loss. The significance of differences between mean values was determined with the Tukey's test. Differences were considered significant at $P < 0.05$. Statistical tests were carried out using the software package Graph Pad Software 3.03 program (GraphPad Prism 3.03, GraphPad Software Inc., San Diego, CA, USA).

Results and discussion

There were no differences between groups in the hatching results (94%, 93%, 95% and 94% in groups A, B, C and D respectively) and chicken mortality (below 2 % in all groups).

The applied treatments had significant effect on local production of IGF-1, in breast muscle tissue, during the prenatal and early stage of postnatal development. The protein expression of IGF-1 in breast muscle tissue is shown in Table 1.

Table 1. Expression of IGF-1 in muscle tissue (%)

Day of development	Experimental group							
	A (Control)		B (TC)		C (Light)		D (TC + Light)	
	mean	SD	mean	SD	mean	SD	mean	SD
Prenatal								
day 15	100^{bC}	11	102^{bC}	9	228^{a}	19	220^{aC}	5
day 19	132^{cB}	13	232^{bA}	24	229^{b}	18	280^{Aa}	37
Postnatal								
day 3	232^{aA}	21	205^{bB}	21	235^{a}	25	236^{aC}	21
day 21	242^{A}	22	233^{A}	22	232	27	250^{B}	47
day 42	245^{A}	22	238^{A}	20	230	25	248^{B}	22

$^{aA...}$Within columns means bearing different superscripts differ significantly at: small letters –$P<0.05$; capitals – $P<0.01$.

There was no significant difference in the amount of IGF-1 in thermal conditioned embryos on 15th day of development compared to the control, but thermal conditioned embryos had more of this protein by day 19 than the control ($P < 0.05$). Monochromatic green light treatment resulted in more IGF-1 than was detected in the control on 15th day of embryo development and the enhancement was still apparent by day 19 ($P<0.05$). Although the light effect was observed earlier than the thermal effect, by day 19 the levels of IGF-1 in both thermal conditioned and light-treated groups were similar. The effect of light may be due to either direct or indirect effects of light, as discussed earlier [Halevy *et al.* 2001]. Also, the experimental group D, which received both

treatments, had significantly more IGF-1 than the untreated control group ($P<0.05$) and more than either single treatment at day 19 ($P<0.05$).

By day 3 of postnatal development, IGF-1 in breast muscle tissue had declined compared to 19-day-old embryos in both groups receiving thermal conditioning ($P<0.05$), while the protein level increased during this time period in the light-treated group. It may be that thermal conditioning causes enhanced accumulation of IGF-1 followed by a transient decrease. Study of Yahav [2000] suggested that thermal conditioning at an early age of postnatal development also led to a transient delay in growth that is followed by a compensatory increased growth rate. This pattern may reflect the levels of IGF-1 in this early period of postnatal development.

IGF-1 expression in breast muscle in 21-day-old broilers is crucial, because more than two-thirds of final body weight accumulates in the second half of the production cycle. Between days 3 and 21 of postnatal development, IGF-1 in both groups that received thermal conditioning accumulated more IGF-1 than the control ($P<0.05$). In contrast, IGF-1 in breast muscle of the group receiving only light declined slightly during this time period. The increase of IGF-1 levels during the later stages of postnatal development in the thermal conditioned groups may, in fact, represent a compensatory increase following the decreased accumulation during the early postnatal period [Halevy et al. 2006]. Higher expression of IGF-1 in the experimental group in which both treatments were applied is evidence for a synergistic effect. Finally, there were no statistically significant differences between the experimental groups by day 42 of postnatal development.

A transient decrease in IGF-1 in muscle may be essential for achieving increased muscle mass at the end of the production cycle. IGF-1 in muscle tissue induces the differentiation of myoblasts, while some factors, such as fibroblast growth factor (FGF) and hepatocyte growth (HGF), inhibit this process and stimulate cell proliferation [Barton-Davis *et al*. 1999]. Low levels of IGF-1 in muscle early in development lead to proliferation of satellite cells, while increased levels of IGF-1 in later stages of development stimulate differentiation and hypertrophy. This may be the basis of the mechanism through which the treatments imposed here resulted in increased mass and protein content of muscle tissue.

The applied treatments had significant effect on the protein expression of ERK in muscle during the prenatal and postnatal development. The protein expression of ERK in breast muscle tissue is shown in Table 2.

ERK protein expression was significantly higher ($P<0.05$) on day 15 of embryonic development in both groups that received green light compared to the two groups that did not. After the application of thermal conditioning and the transition from intermittent to continuous light regime on the 19th day of embryonic development and on the 3rd day of postnatal development, there was significant increase ($P<0.05$) of ERK in all of the treated groups compared to the control group. By day 3 of postnatal development, ERK levels began to decline in all of the groups, and continued to decline through the rest of the production cycle. This decline was especially pronounced in

the control group, in which the level was significantly lower (P<0.05) than in the other groups at days 21 and 42. The decline of ERK protein expression during postnatal development was significant in all groups (P<0.05).

Table 2. Protein expression of ERK in muscle tissue (%)

Day of development	Experimental group							
	A (Control)		B (TC)		C (Light)		D (TC + Light)	
	mean	SD	mean	SD	mean	SD	mean	SD
Prenatal								
day 15	100^{Ab}	5	103^{Bb}	8	122^{Aa}	10	125^{Aa}	5
day 19	98^{Ab}	7	135^{Aa}	12	133^{Aa}	14	137^{Aa}	6
Postnatal								
day 3	89^{Ab}	6	131^{Aa}	10	129^{Aa}	11	135^{Aa}	6
day 21	25^{Bb}	3	53^{Ca}	7	48^{Ba}	4	46^{Ba}	5
day 42	10^{Cc}	2	42^{Ca}	3	31^{Cb}	6	45^{Ba}	4

$^{aA...}$Within columns means bearing different superscripts differ significantly at: small letters –P<0.05; capitals – P<0.01.

As a result of light and thermal conditioning the proliferation of myoblasts, first, and differentiation, later, was detected in muscle tissue [Halevy *et al*. 2006]. This raises the possibility that treatments resulted in retention of myoblasts in the proliferation phase. The treatments may have immediate effects during and immediately after application, as well as delayed effects that would be apparent in the postnatal period [Stojanović *et al*. 2013]. The decline in protein expression of ERK and increased protein expression of IGF-1 in the control group during embryonic and postnatal periods indicate a shift from the proliferation phase to the differentiation phase during development of muscle tissue. Growth factors can stimulate proliferation as well as differentiation and hypertrophy in muscle tissue [Halevy and Cantley 2004], and it may be that these processes are controlled by the protein expression of ERK, as part of the secondary messenger system in the muscle cells.

The results suggests that IGF-1 plays an important role in metabolism in skeletal muscles, especially of proteins as described earlier by LeRoith and Yakar [2007]. Thus, its activity affects the quality of meat. The body and breast muscle weight, chemical composition of meat from the different treatment groups, as well as the drip loss, are shown in Table 3. Body weight and breast muscle weight showed differences between control group and all treated groups (P<0.05). Body weight and breast muscle weight were higher in all treated groups which can be explained by the influence of higher protein expression of ERK in this stage of development (Tab. 2). The breast muscle of all treated groups had significantly (P<0.05) more dry matter, less moisture, and a greater percentage protein than the control group. The percentages of protein in all treated groups were above 19%. There were no statistically significant differences between any groups in the lipid and ash content in the breast meat. Because actin and myosin are the most common proteins in muscle tissue, they are also the most responsible for the moisture holding capacity of meat [Barton-Davis *et al.* 1999]. Our

Table 3. Body and breast muscle weight (g), chemical composition of meat (% DM) and drip loss (%)

Item	Experimental group							
	A (Control)		B (TC)		C (Light)		D (TC + Light)	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight	2489.3[b]	43.01	2575.7[a]	43.73	2566.9[a]	40.70	2590.6[a]	50.52
Breast muscle weight	363.8[b]	13.92	388.3[a]	10.24	380.6[a]	12.74	393.3[a]	14.66
Dry matter	27.47[b]	0.91	30.71[a]	1.52	29.04[a]	1.28	30.49[a]	1.92
Moisture	72.62[a]	0.91	69.38[b]	1.52	70.06[b]	1.28	69.60[b]	1.92
Total proteins	18.07[b]	0.56	19.49[a]	0.77	19.45[a]	0.44	19.56[a]	0.36
Fat	2.25	0.13	2.14	0.36	1.88	0.62	1.95	0.18
Ash	1.03	0.13	1.05	0.06	1.04	0.07	1.03	0.03
Drip loss								
after 24 hours	1.89[a]	0.81	1.12[b]	0.34	1.21[b]	0.19	1.20[b]	0.29
after 48 hours	1.95[a]	0.35	1.42[b]	0.46	1.50[b]	0.15	1.37[b]	0.11

[ab,..] Within columns means bearing different superscripts differ significantly at $P<0.05$.

results show, unambiguously, that the treatments imposed retarded drip loss. The drip loss in all treated groups was significantly ($P<0.05$) lower than in the control group after both 24 and 48 hours. This can be regarded as a consequence of the greater protein content in the breast meat of the treated broilers.

Thermal conditioning and green monochrome light increased the protein expression of IGF-1 in muscle during the late embryonic development. Both treatments and their combination increased the protein expression of ERK in the late embryonic stages and early postnatal stages. Thermal conditioning and green monochrome light increased the dry matter in the meat due to increased percentage of total protein but not to an increased percentage of fat or ash. All applied treatments reduced drip loss. These results indicate that modified incubation factors can improve meat characteristics by stimulating the protein expression of IGF-1 and ERK in muscle tissue.

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