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The Effects of Low Intensity Red Laser Irradiation on Hatching Eggs in Chicken and Quail

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Abstract: Low intensity red (λ=633 nm) light was used on hatching poultry and quail eggs to determine its influence on embryonic and post-embryonic development. Certain regimens of hatching egg irradiation (p=0.1 mW/cm^2, t=60 c) significantly increased hatching rates (3.66-4.05%, $p<0.001$) and decreased chick mortality (1.25-3.23%, $p<0.05$) for layer and broiler chickens compared to untreated controls (using industrial poultry farm conditions). Laser irradiation of hatching egg increased chick blood hemoglobin, changed (increased in embryo and decreased in postembryo period) liver peroxide levels and activated cytochrome P-450 enzyme system without adversely affecting liver energy metabolism.

Key words: Poultry, chicks, hatching eggs, low intensity laser light, embryo and postembryo development, hatch ability

Introduction

Although low intensity laser light, like sunlight, is non-ionizing, it has unique properties that can influence biological activities under certain conditions (Karu, 1988). Laser irradiation of fertilized poultry eggs has been reported to increase hatch ability rate and improve chick survival (Bessarabov *et al.*, 1986; Mamukaev, 1988; Popov *et al.*, 1984). The adoption of this procedure into standard poultry production has been hindered by variability in methods for egg exposure and a lack of understanding of the effects of low intensity laser light on biological mechanisms.

After years of research on the use of laser light in medicine and for enzyme modeling studies, there has been significant progress towards understanding its biological effects. The anti-oxidant enzymes (superoxide dismutase, catalase and ceruloplasmin) are among those having maximum light absorption in the red spectrum range and whose activities can be initiated by it (Gorbatenkova *et al.*, 1989; Aleksandrova, 1989; Grossman *et al.*, 1998;). Considering the important role anti-oxidant enzymes serve in maintaining cellular homeostasis, we can predict the importance of red laser light to the entire living organism. Another important biological effect of low intensity red laser light is to accelerate proliferative processes in irradiated tissues (Mester and Mester, 1985).

Our experiments were designed to examine the effects of laser irradiation on fertilized poultry eggs and to determine optimum conditions for improving hatching rates and chick survival.

Materials and Methods

The experiments were performed at the Laboratory of Physics of Bila Tserkva State Agrarian University using industrial Ukrainian poultry farm procedures over the period of 1990 to 2000. Fertilized eggs from both Japanese quail and two industrial breeds (Belarus-9 and Broiler-6) were used.

The eggs were collected from hens of the same age and breed and poultry house over a one to three day period. The quail hens were 4-5 months old while the industrial hens (layer and broiler breeds) were 9 months old.

Eggs within a group (95-100 quail eggs, 2650-2700 layer eggs, and 3250-3320 broiler eggs) were simultaneously incubated using the standard conditions required for each type. The quail eggs were incubated in the laboratory hatchery, while the layer and broiler eggs were incubated at industrial hatcheries.

The experimental eggs were irradiated prior to incubation. Laser light of 25-50 mW power and a wave length of 633 nm (red light) was generated using helium-neon lasers LHN-111 and LHN-602N (UkrLaser, Ukraine). In our earlier research it was estimated more then ten difference regimens of irradiation and we detected the most effective regimen of irradiation of hen hatching egg (Yakimenko *et al.*, 1997). This regimen was used in present research. The radiation density was 0.1 mW/cm^2 and duration of irradiation was 60 c. The radiation was performed under darkened conditions with background illumination not exceeding 3 lux 12–24 hours before the incubation after which the eggs were incubated in darkness. The eggs of control groups were not irradiated but were stored and incubated in the same conditions as eggs of experimental groups.

Biological incubation controls, hatching evaluation and analysis of wastage were evaluated using standard procedures for poultry industry. Next integrated parameters were used to assess the physiologic conditions of the chicks. Hematopoiesis was estimated from blood hemoglobin levels under the standard test using (Agat, Russia). Poultry liver energy metabolism was estimated using the electron spin resonance (ESR) method for measuring semiquinone free radicals giving a g-factor signal of 2.00 (Ajeepa, 1983). Liver detoxification system were estimated by using the same method to measure oxidized cytochrome p-450 level. Liver samples were prepared using liquid nitrogen (T = 77K). ESR spectrometer RE-1307 (Russian production) was used for ESR analyses. The thiobarbituric acid reaction method was used to measure liver peroxide levels (Andreeva *et al.*, 1988).

Seven embryos or birds in each experimental and control groups were chosen for analysis of metabolism indexes in each term of analysis. The terms of analysis was 19 day of incubation and 1, 30 and 90 days of breeding for chicks and 1 day, 3 weeks and 6 weeks of breeding for quail.

Statistical analysis was performed using Fisher's test for estimation of significance between percent indexes of incubation and Student's tests for estimation of significance between metabolic indexes of poultry of experimental and control groups.

Results and Discussion

The regimen employing a radiation density 0.1 mW/cm^2 and duration of hatching eggs irradiation 60 c decreased early embryo mortality and increased the quail hatching rate by 6.9 % compared to the untreated control group (Table 1).

This laser irradiation regimen for fertilized eggs using production conditions produced a significant increase in hatch ability of layer and broiler eggs (Tables 2 and 3). Decreased embryo loss at all stages of development led to marked increases in hatch ability.

The effectiveness of laser irradiation for improving egg hatch ability was of increased benefit for eggs whose condition had declined from long-term storage. For example, in the experiment using layer hen eggs stored for 20 days prior to incubation, the hatching rate for the control eggs (n = 38) declined to 47.22% while the treatment eggs (n = 83) receiving red laser light one day

Table 1: Embryonic development and hatch ability of Japanese quail eggs after irradiation with 6mJ/cm^2 of red laser light

Parameters	Treatment	Control
Number of eggs	99	100
Unfertilized eggs (%)	7	11
Lost embryos (%):		
at 1-5 days	0*	2.0
at 6-15 days	2.0	2.0
at 16-17 days	1.0	2.0
Hatched chicks, (%)	89 (89.9)	83 (83.0)
Hatch ability, %	96.7	93.3

Note: * - $p<0.05$

Table 2: Embryonic development and hatch ability of layer eggs (Belarus 9 cross) after irradiation with 6mJ/cm^2 of red laser light

Parameters	Treatment	Control
Number of eggs	2657	2693
Unfertilized eggs, %	3.4	2.5
Lost embryos (%)		
at 1-6 days	1.8	1.6
at 7 – 19 days	2.7**	4.1
at 19-21 days	1.5**	2.9
Weak chicks, %	0.6***	1.4
Normal chicks, %	90.1**	87.3
Hatch ability, %	93.2***	89.6

Note: ** - $p<0,01$; *** - $p<0,001$

Table 3: Embryonic development and hatch ability of broiler eggs (Broiler 6 cross) after irradiation with 6 mJ/cm^2 of red laser light

Parameters	Treatment	Control
Number of eggs	3272	3313
Unfertilized eggs, %	7.4	7.1
Lost embryos (%)		
at 1-6 days	1.2**	2.0
at 7 – 19 days	7.2*	8.4
at 19-21 days	2.29	3.0
Weak chicks, %	3.0*	4.1
Normal chicks, %	78.9**	75.4
Hatch ability, %	85.2***	81.1

Note: * - ð<0,05; ** - $p<0,01$; *** - $p<0,001$

prior to incubation was 74.32% ($p<0.001$). The negative correlation ($r = -0.98$) between the control groups hatching rate and difference of control and treatment groups hatching rates for each breed after irradiation using the optimal regimen is illustrated in Fig.1.

Early post-embryonic development was also improved by laser irradiation. The mortality of layer chicks hatched from irradiated eggs was 1.25 – 3.23% lower than that for the control group ($p<0.05$). In addition, the irradiated chickens were noticeably more resistant to contagious disease. While the monthly salmonellosis mortality rate at the egg production poultry farm was 29.84% for the control group (n = 3000 chickens), it was 17.72% (n = 3000 chickens) in the irradiated treatment group ($p<0.001$). These differences suggest a positive influence of laser irradiation on immune mechanisms of the birds.

Post-radiation examination of the hemopoietic system of the embryo and chicks who received the optimal dose of laser light revealed increased levels of blood hemoglobin compared to control. The hemoglobin level in 19 day old layer embryos was 15.7% higher, while the level in 1-90 day old chicks was 6.8 – 14.4% higher than for the controls ($p<0.0$l, Fig. 2).

There were no consistent differences in liver semiquinone free

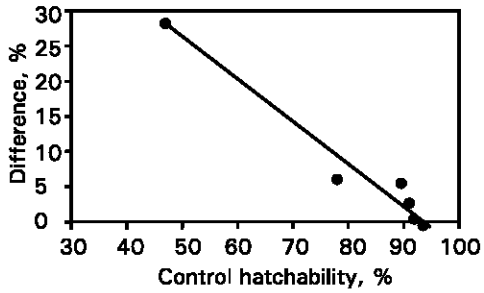

Fig. 1: Dependence of layer egg hatch ability after low-intensity laser irradiation (6 mJ/cm^2) on initial egg quality. X axis: hatch ability of control eggs (%), Y axis: hatch ability differences between analogous experimental and control groups (%).

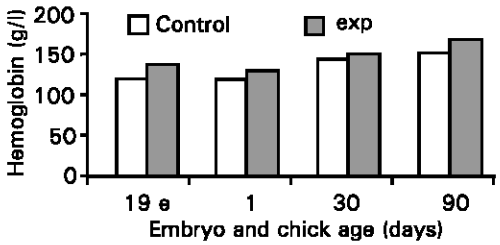

Fig. 2: Blood hemoglobin levels in embryos and chicks (Belarus 9) after laser treatment (6mJ/cm^2). X axis: embryo and chick age (days) Y axis: hemoglobin (g/l)

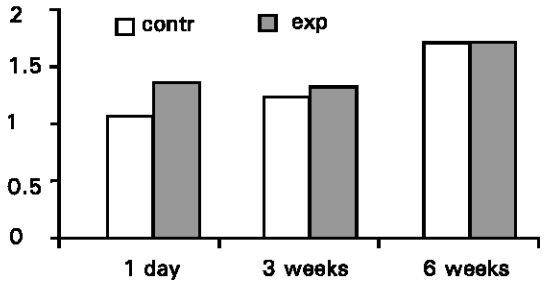

Fig. 3: Free radical levels in quail liver after low-intensity laser irradiation (6mJ/cm^2) of hatching eggs. X axis: poultry age Y axis: free radical concentration (relative units)

radical levels among the experimental and control groups, although a 20 % increase in free radical ESR signals was observed 13-15 days after irradiation. We studied liver semiquinone free radical dynamics in quail chicks hatched following laser irradiation and found 29.8% higher free radical levels in one day old chicks compared to controls. This difference was not seen when comparing groups at the next stage of development (Fig. 3).

It has shown (Ajeepa, 1983) that free radical ESR intensity is a reflection of the metabolic state of respiratory processes. The laser irradiation regimens we used did not appear to negatively affect cellular metabolism in the systems that we tested. There were higher amounts of oxidized cytochrome P-450 in poultry liver in the experimental compared to control groups at certain stages of embryonic development. Oxidized P-450 enzyme levels were 25-50% higher in 11-15 day old layer embryos after egg irradiation. Liver tissues in 15 day old quail embryos (two days prior to hatching) had 40% higher levels of oxidized P-450 enzymes. The cytochrome P-450 enzymes, positioned on the

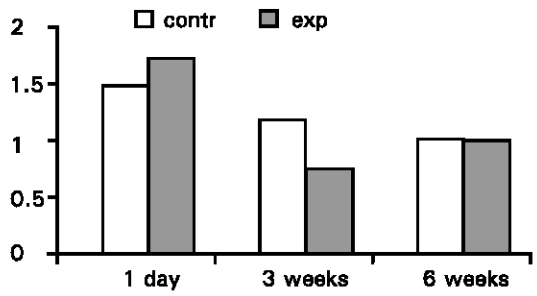

Fig .4: Peroxide levels in quail liver after low-intensity laser irradiation (6 mJ/cm^2) of hatching eggs. X axis: poultry age Y axis: peroxide concentration (mcmol/g)

inner membrane of liver cells, provide a potent hydroxylation system to detoxify endogenous materials and participate in lipid peroxidation reactions. Augmented amounts of oxidized (catabolically active) enzymes in the absence of pathologic processes demonstrate that the system has been activated and suggest a beneficial physiological state for the organism.

Liver tissues from one day old quail chicks after egg irradiation had 16.4% higher peroxide levels, while in 3 week old quail chicks, the levels were decreased 39.5 % ($p < 0.01$) compared to controls (Fig. 4). At six weeks, no difference between treatment and control groups was found. Interestingly, this same pattern has been observed in the blood of patients receiving low intensity red laser therapy (Yalchenko and Lagutin, 1996). So, this results can be interpret as an oxidant effect on the first stage after irradiation of biological system and significant antioxidant effect on the next stage.

Birds hatched from irradiated eggs had similar body mass (broilers), egg production (layers and quail) as contemporaries from the control groups. It appears that the laser treatment influenced only embryonic and early post-embryonic development. Our data demonstrated that laser irradiation of hatching eggs reliably decreased embryonic mortality, increased the proportion of eggs hatched, and improved chick survival. The efficiency of this method was dependent on the initial condition of the fertilized eggs. Laser treatment produced a positive effect on blood hemoglobin levels in chicks, increased the proportion of oxidized cytochrome P-450 enzymes in embryo liver tissues and changed peroxide levels during certain periods. No negative effects were seen on liver energy metabolism.

While the mechanism of the process is not clear, low intensity laser light probably is absorbed by enzymes such as catalase in the egg white, superoxide dismutase or/and other molecular complexes of the yolk and embryo cells. Research using model systems has shown that enzyme activation may change molecular complexes or alter lipid peroxidation levels (Dorovskich *et al.*, 1998). In an earlier laser irradiation experiment, we detected changes in the migration process of ions from egg white to embryo structures in the first five days of incubation after treatment (Yakimenko *et al.*, 1997). Red laser light has also been reported to accelerate passage of Fe^{3+}-complexes from the yolk to embryo structures (Yakimenko, 1997). These changes may impact hemopoiesis, energy metabolism, detoxification, and anti-oxidation systems during poultry embryonic and early post-embryonic development.

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