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Original article

EFFECTS OF PRE-INCUBATION LASER IRRADIATION ON HATCHABILITY AND SMALL INTESTINE ENZYMES ACTIVITY IN POST-HATCHED BROILER CHICKENS

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Summary

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The aim of this study was to investigate effects of short-term laser application on hatchability, died embryo in eggs and effects on post-hatch enzyme activity in different sections of small intestine (1, 10, 30, 50, 70 and 90%) in broiler chickens at 21 and 42 days of age. Two experiments were carried out. In the first experiment, 900 fertilised eggs (Ross 308) were randomly divided into three experimental groups (three replications and 100 eggs per group), which were irradiated with laser (Helium-Neon) at 0, 6 and 10 mW power respectively for 90 s; 12 h prior to incubation. The eggs were randomly incubated at industrial hatcheries. At hatch day, rates of unfertilised eggs, hatched, unhatched, dead unhatched embryos were determined in different phase of incubation (1–6 and 7–18 days). In the second experiment, 234 one-day-old chickens where allocated into three experimental groups based on irradiation laser levels (three replications and 26 birds per group). At post hatch days 21 and 42, six birds were randomly selected from each group, slaughtered and various sections of small intestine (1, 10, 30, 50, 70 and 90%) were sampled to evaluate alkaline phosphates (ALP), leucine aminopeptidase (LAP) and sucrase activities. According to the results, laser irradiation had no significant difference on egg hatchability, unfertilised eggs, unhatched died and died embryos ($P>0.05$). A significant difference was observed in small intestine LAP and ALP levels at days 21 and 42 ($P<0.05$). These results suggested that laser irradiation altered small intestine enzyme activity in broilers.

Key words: alkaline phosphatase, broiler chicken, hatchability, laser irradiation, leucine amino peptidase, sucrase

INTRODUCTION

Recently, the effects of laser irradiation on animal reproduction, productivity as well as food safety were investigated (Yakimenko *et al.,* 2002; Iaffaldano *et al.,* 2005; Yakovlev *et al.,* 2007; Iaffaldano *et al.,* 2010; Maktabi *et al.,* 2011). New protocols have been suggested by researchers for laser therapy in medicine. Dose-dependent laser irradiation has both positive and negative biological effects. Formerly, it was reported that laser irradiation increased cell mitotic and proliferation processes (Mester & Mester, 1985; Yakovlev *et al.,* 2007). Also, it was reported that laser therapy increased anti-oxidant enzymes levels than other type of enzymes (Yakimenko *et al.,* 2002). Conversely, it was suggested that low-intensity laser irradiation had no efficacy. Laboratory studies indicated that gallium-aluminum-arsenide laser irradiation was unable to stimulate cell proliferation, migration and wound healing (Moore *et al.,* 2005). Helium-neon laser beam was shown to stimulate gametic, embryonic cells and sperm motility in birds (Iaffaldano *et al.,* 2005). Additionally, irradiation improved fibroblasts mitosis and growth factor production in chickens (Lubart *et al.,* 1992; Yu *et al.,* 1994). Yakimenko *et al.* (2002) reported that hatchability of irradiated egg increased and mortality rate decreased by using He-Ne laser (0.1 mW/cm^2 for 60 s) in meat and layer-type chickens, respectively.

It is proved that intestine villi and epithelial cell morphology are indispensable in intestine growth and function (Ruttanavut *et al.,* 2009). It is well known that a correlation exists between luminal cell proliferation and its turnover rate. Narrow and long intestinal villi are an indicator of rapid proliferation of crypts and short turnover rate of these epithelial cells whereas high villi indicate active intestine villi function with long turnover rate (Nordstrom & Dahlqvist, 1973; Langhout *et al.,* 1999). With this regard, Abolhassani *et al.* (2010) reported that He-Ne laser irradiation had positive effects on villus height, crypts depth and villus height/crypt depth ratio in various sections of small intestine in broilers. The effects of laser irradiation have not been completely studied in chickens (Yakovlev *et al.,* 2007). Leucine amino peptidase is one of the dominant peptidases in small intestine villus which degrades long-chain peptides of proteins to smaller peptides and amino acids to enhance nutrient absorption efficacy in animals (Ghiasi Ghalehkandi *et al.,* 2009). Alkaline phosphatase (ALP) is another prominent enzyme from the group of catalytic enzymes, frequently distributed in brush border of intestinal mucosal cytosol, degrading phosphate esters and separating phosphoric acid molecules (Ghiasi Ghalehkandi *et al.,* 2012). It is reported that low-level laser therapy significantly diminished carbon tetrachloride-increased ALP of rat liver (Oliveira-Junior *et al.,* 2013).

So far, there are few literature reports related to the effects of low-power laser irradiation on gastrointestinal enzymes in birds. Therefore, the recent study was designed to investigate the possible effects of pre-incubation laser irradiation (He-Ne) on egg fertility and hatchability. We also designed a second experiment to follow out the influence of laser irradiation on post hatch small intestinal function through the effects of He-Ne laser irradiation on enzyme activity (ALP, LAP and sucrase) in different sections of small intestine of post hatch broilers at 21 and 42 days of age.

MATERIALS AND METHODS

Laser irradiation

A single mode continuous (He-Ne), 633 nm wavelength laser irradiation (red light) made by Bonab Nuclear Research Institute (East Azarbayjan, Iran) was used. All doses of laser irradiation were calculated based on previous and pilot studies (Yakimenko *et al.,* 1997; 2002).

Experiment 1

This experiment was performed at the Bonab Animal and Agriculture Nuclear Research Center, East Azarbayjan, Iran. One thousand and eight-hundred fertilised eggs were purchased from Eshragh Co., Iran. Nine-hundred fertilised eggs (Ross 308) were selected, sorted, graded and randomly divided into three experimental groups (three replications and 100 eggs per group). Experimental eggs were irradiated with He-Ne laser irradiation (red light) at zero, 6 and 10 mW power, 633 nm wavelength for 90 s, 12 h prior to incubation. The irradiation procedure was performed under darkened conditions (Yakimenko *et al.,* 2002). Eggs collected

Table 1. Ingredient and nutrient compositions of experimental diets (1–42 days of age)

Ingredient (%)	Starter (1–21 days of age)	Grower (22–35 days of age)	Finisher (36–42 days of age)
Corn grain	52.01	58.75	71.1
Soybean meal (44%)	35.50	28.35	25.94
Wheat	5.50	8	0
Barley	1.53	0	0
Oil	1.75	1	0.1
DCP	1.4	0	0
Oyster	1.27	0.77	0.76
Bone meal	0	2.14	1.8
Mineral premix	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25
DL-methionine	0.24	0.12	0.02
Lysine monohydro-chloride	0	0.08	0
Salinomycin	0.1	0.1	0.1
Salt	0.02	0.02	0.02
Calculated analysis			
ME, kcal/kg	2900	2941	3032
Crude protein (%)	20.84	18.38	17.05
Calcium (%)	0.9063	1.02	0758
Available phosphorus	0.4028	0.4	0.285
ME/protein	139.130	160	177.77
Calcium/phosphorus	2.22	2.56	2.66

DCP=dicalcium phosphate, ME=metabolisable energy. Per 2.5 kg feed, the mineral supplement contains 99,200 mg magnesium, 84,700 mg zinc, 50,000 mg iron, 10,000 mg copper, 990 mg iodine, 200 mg selenium, 250,000 ml g choline chloride. Per 2.5 kg feed, the vitamin supplement contains 900,000 IU of vitamin A, 200,000 IU of vitamin D_3, 190,00 IU of vitamin E, 2,000 mg vitamin K_3, 18,050 mg vitamin B1, 49,000 mg vitamin B_2, 9,800 mg vitamin B_3, 29,650 mg vitamin B_5, 2,940 mg vitamin B_6, 1,000 mg vitamin B_9, 15 mg vitamin B_{12}, 100 mg biotin, 190,000 mg choline chloride, 1,000 mg antioxidant.

on plastic flats, cooled to room temperature (18.6 °C, humidity 75%) were randomly incubated in an automated incubator (37.2 °C, humidity 60%) and at day 18 were transferred in industrial hatcheries (37.5 °C, air speed 0.2 m/sec, humidity 75%). Egg sets were candled twice during incubation to remove infertile and dead embryo eggs. On hatch day, rates of unfertilised eggs, hatched eggs, unhatched eggs, dead embryos during different phase of incubation (1–6 and 7–18 days) and unhatched dead eggs were determined.

Experiment 2

Two-hundred and thirty four of hatched chickens from experiment one were randomly allocated into three experimental groups (completely randomised design) based on irradiation laser levels and transferred into heated batteries (three replications and 26 birds per group) with continuous lighting at temperature 22±1 °C with 50% humidity, on litter floor (Olanrewaju *et al.,* 2006). Chickens received diets (starter, grower and finisher) formulated using User Friendly Feed Formulation Done Again (UFFDA) (Pesti *et al.,* 1992). Chemical composition of experimental diets is presented in Table 1. During the study all birds had free access to food and fresh water.

At 21 and 42 days of age, three hours prior to intestinal sample collection, birds were deprived from food. Six birds were randomly selected from each replication, slaughtered and the entire gastrointestinal tract was removed. Various sections of small intestine – A, B, C, D, E and F (1, 10, 30, 50, 70 and 90%, respectively) were sampled (a 7-cm sample was taken), rinsed with phosphate buffer solution (PBS, pH=7) and stored at –80 ºC (Ghiasi Ghalehkandi *et al.,* 2014). All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (USA) and the current laws of the Iranian government.

Enzyme assays

In the laboratory, 0.01 g of small intestine mucosa was weighed using a sensitive scale (OHAUS) and along with 10 mL PBS was homogenised using sonic vibracell device (VCX 130 TE USA) to cell degradation and achieve brush border total protein (TP), ALP, LAP and sucrase activity. Total protein level was determined according to the pyrogallol method (Wantanabe *et al.,* 1986); LAP level according to Nigel *et al.* (1964) and Hill (1971); sucrase enzyme activity according to the Dahlqvist procedure (1964) and ALP activity – colorimetrically by measuring the rate of p-nitrophenol formation (Forstner *et al.,* 1968) on an auto analyzer (Mindray BS-200, Germany). Enzyme activities of samples were divided to the total protein concentrations and calculated as IU per g protein (Teshfam, 1984).

Statistical analysis

Data were analysed by multivariate analysis of variance by the linear model using SAS statistical software (SAS v.9.1). Comparative analysis of the means of treatments was performed using Duncan's multiple tests at $P<0.05$ as significant difference between treatments.

RESULTS

The effect of short-term laser irradiation on hatchability, unfertilised eggs, unhatched dead and dead embryos in chickens is presented on Fig. 1. As seen from the figure, there was no significant difference on studied hatchability traits of eggs after laser irradiation (6 and 10 mW, 90 s)

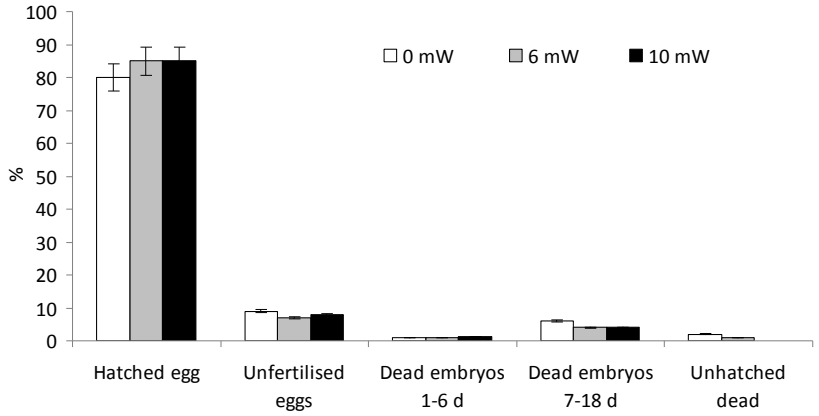

Fig. 1. Effects of different levels of laser irradiation (He-Ne, 90 sec.) on hatchability, unfertilised eggs, unhatched dead and dead embryos during different days of incubation in broiler chickens.

in experimental groups compared to the control group (P>0.05).

In experiment 2, ALP activity was significantly increased after 6 mW He-Ne irradiation on 1% section of small intestine in broilers aged 21 days (P<0.05). Furthermore, a significant difference was observed between ALP activity of irradiated groups in various small intestine sections (30, 50 and 70%) in 21-day-old broilers (P<0.05), but there was no difference compared to controls (P>0.05) (Table 2). It seemed that 6 mW pre-incubation He-Ne irradiation had a better effect on small intestine ALP activity in birds. Also, there was a significant decrease in ALP activity in 50 and 90% of small intestine in 10 mW He-Ne irradiated birds at 42 days of age (P<0.05). In addition, no significant differences were observed in ALP activity in the other sections of small intestine (P>0.05) (Table 2).

A significant difference was detected in small intestine LAP activity (30% of small intestine length) in 6 mW He-Ne group vs controls (P<0.05). Moreover, the same results were obtained for LAP activity in different sections of small intestine length (50, 70 and 90%) using 10 mW He-Ne for 90 s in 21-day-old broilers (P<0.05) (Table 3). According to the data, at 42 days of age a significant decrease was found out on LAP activity in different sections of small intestine (10, 30, 50, 70 and 90) in birds receiving 10 mW He-Ne laser irradiation (P<0.05) (Table 3). There was no significant difference between the 6 mW group and the control group (P>0.05). It seemed that 10 mW He-Ne pre-incubation irradiation had an adverse effect on small intestine LAP activity in birds.

In this study, there was no significant difference between sucrase enzyme activity in various sections of small intestine (1, 10, 30, 50, 70 and 90 small intestine length) in experimental birds compared to control group both at 21 and 42 days of age (P>0.05) (Table 4).

DISCUSSION

To our knowledge, this is the first study to investigate the possible effects of He-Ne laser irradiation on small intestine enzyme

Table 2. Effects of different levels of laser irradiation (He-Ne, 90 s) on alkaline phosphatase activity (IU/g protein) in various sections of the small intestine in broiler chickens at 21 and 42 days of age. Data are presented as mean±standard deviation (n=6)

	Laser irradiation	Small intestine section					
		[A]	[B]	[C]	[D]	[E]	[F]
post hatch day 21	0 mW	1127.46±618.30^{ab}	1098±607.65^{a}	997.1±399.82^{ab}	435.7±91.61^{b}	312.57±80.62^{b}	158.96±60.91^{a}
	6 mW	1289.20±291.92^{a}	610±173.82^{b}	871.6±365.78^{b}	785.44±676.08^{a}	511.09±546.77^{a}	143.15±70.42^{a}
	10 mW	899.85±424.73^{b}	1075±635.48^{a}	345.02±267.06^{a}	237.24±128.65^{b}	237.24±93.10^{b}	237.86±64.09^{a}
post hatch day 42	0 mW	1154.26±286.34 ^{a}	933.83±302.15^{a}	1025.40±395.11^{a}	907.36±653.10^{a}	980.27±623.19^{a}	330.30±73.14^{a}
	6 mW	889.41±263.97^{a}	980.76±344.37^{a}	850.25±381.02^{a}	697.29±510.64^{ab}	331.55±84.65 ^{a}	219.12±184.36^{b}
	10 mW	783.34±298.31^{a}	887.05±321.33^{a}	809.98±370.61^{a}	585.04±131.05^{b}	264.34±97.35^{a}	194.40±164.38^{b}

A: 1% of small intestine length, B: 10% of small intestine length, C: 30% of small intestine length, D: 50% of small intestine length, E: 70% of small intestine length, F: 90 % of small intestine length. There are significant differences between groups with different letters within a column ($P<0.05$).

Table 3. Effects of different levels of laser irradiation (He-Ne, 90 s) on leucine aminopeptidase activity (IU/g protein) in various sections of the small intestine in broiler chickens at 21 and 42 days of age. Data are presented as mean ± standard deviation (n=6)

	Laser irradiation	Small intestine section					
		[A]	[B]	[C]	[D]	[E]	[F]
post hatch day 21	0 mW	1514.0±324.21^{a}	1512.9±362.47^{a}	1707.83±452.36^{a}	1830.6±591.64^{a}	2175.7±989.36^{a}	2306.2±1263.68^{a}
	6 mW	1542.9±365.15^{a}	1301.2±304.57^{a}	1421.17±399.84^{b}	1781.1±537.12^{a}	2251.2±1065.94^{ab}	1739.2±362.95^{b}
	10 mW	1729.7±361.75^{a}	1478.01±370.94^{a}	1782.60±431.46^{a}	1504.6±436.79^{b}	1727.5±469.78^{b}	1650.2±412.36^{b}
post hatch day 42	0 mW	1864.2±656.23^{b}	1744.4±549.88^{a}	2254.80±677.77^{a}	2920.9±1164.46^{a}	3641.9±1112.98^{a}	3750.6±1014.30^{a}
	6 mW	1759.8±420.14^{ab}	1766.0±349.02^{a}	1748.70±411.18^{ab}	2306.0±414.68^{ab}	3859.2±1111.02^{a}	3360.4±1223.02^{ab}
	10 mW	1330.8±341.84^{a}	1226.0±285.59^{b}	1599.10±233.26^{b}	1950.9±265.74^{b}	2357.3±352.87^{b}	2148.6±308.88^{b}

A: 1% of small intestine length, B: 10% of small intestine length, C: 30% of small intestine length, D: 50% of small intestine length, E: 70% of small intestine length, F: 90 % of small intestine length. There are significant differences between groups with different letters within a column ($P<0.05$).

Table 4. Effects of different levels of laser irradiation (He-Ne, 90 s) on sucrase activity (IU/g protein) in various sections of the small intestine in broiler chickens at 21 and 42 days of age. Data are presented as mean ± standard deviation (n=6)

	Laser irradiation	Small intestine section					
		[A]	[B]	[C]	[D]	[E]	[F]
post hatch day 21	0 mW	0.07596±0.012	0.03479±0.011	0.03479±0.013	0.03481±0.012	0.04495±0.013	0.03645±0.021
	6 mW	0.07555±0.017	0.04285±0.016	0.06400±0.021	0.04070±0.016	0.04909±0.02	0.03861±0.017
	10 mW	0.07490±0.016	0.05437±0.021	0.03447±0.012	0.04287±0.015	0.04761±0.017	0.04787±0.016
post hatch day 42	0 mW	0.06605±0.014	0.04252±0.013	0.05193±0.010	0.02186±0.012	0.04597±0.012	0.05888±0.020
	6 mW	0.07852±0.020	0.045435±0.01	0.03299±0.011	0.05552±0.019	0.04823±0.016	0.06219±0.018
	10 mW	0.07640±0.019	0.030467±0.01	0.05471±0.015	0.04713±0.017	0.04764±0.014	0.05077±0.021

A: 1% of small intestine length, B: 10% of small intestine length, C: 30% of small intestine length, D: 50% of small intestine length, E: 70% of small intestine length, F: 90% of small intestine length.

activity in broilers. According to the obtained results from experiment 1 (Fig. 1), laser irradiation (6 and 10 mW, 90 s) had no effects on hatchability, unfertilised eggs, unhatched dead and dead embryo rates in broiler eggs. Previously, it is reported that 0.1 mW/cm^2 He-Ne laser irradiation significantly improved hatchability and decreased mortality rates in broiler eggs (Yakimenko *et al.,* 2002). Moreover, Ivanov *et al.* (1989) was reported increased hatchability rate consequently to pre-incubation He-Ne laser irradiation. Also, similar findings were reported on duck eggs by Melnikova *et al.* (1985). According to our data, irradiation tended to improve hatchability and decreased mortality rate but the effects were not significant. Low-level red light laser irradiation increases cell proliferation. It seems that irradiation increases Fe^{3+} absorption from yolk sack during the embryonic phase; promotes haemoglobin synthesis and improves subsequent survival (Yakimenko *et al.,* 2002). An alternative mechanism was suggested for the mitogenic effects of low power laser irradiation – it is supposed that the infrared light can be absorbed through the mitochondrial respiratory chain to promote cellular proliferation via stimulating adenosine triphosphate or cyclic AMP, inducing photo-activation of calcium channels (Moore *et al.,* 2005; Gao & Xing, 2009).

According to our results, a significant increase was observed in ALP activity using He-Ne irradiation in various sections of small intestine (30, 50 and 70 %) in broilers at 21 days of age. Low-energy laser irradiation has positive effects on bone formation, osteoblast proliferation and ALP activity (Ninomiya *et al.,* 2003). According to evidence, the lumen of broiler's embryo is ALP free during the first week of embryonic life and it gently increases form day 9 (Moog, 1950). In this regard, Ninomiya *et al.* (2003) reported that low level laser therapy amplified cell proliferation rate and bone ALP activity in rat. Conversely, a decrease was detected on small intestine ALP activity in 10 mW He-Ne irradiated birds on the 42nd post hatch day. There is a difference in duodenal, jejunal and ileal villus growth rate in broilers. After hatching, duodenal villi growth rate is much faster than that of jejunal and ileal villi (Obst & Diamond, 1992; Shin *et al.,* 2013). Dynamic cell proliferation increases villus number (Antheony *et al.,* 1999). A range of factors affects small intestine villi growth rate and secretion e.g. age, food deprivation, luminal microflora, chemicals and irradiation (Ghiasi Ghalehkandi *et al.,* 2011; Ouf *et al.,* 2012). Additionally, recent findings suggest that a prominent physiological effect of low power laser irradiation is cell proliferation. In mammalian cells, low power laser irradiation amplifies cell proliferation by stimulating signal-regulated protein kinase, growth-factor-induced proliferation e.g. epidermal growth factor (EGF) stimulation or GTPase Ras protein, the Raf-1, the mitogen-activated protein kinase (MAPK) pathways (Gao & Xing, 2009). With this regard, Abolhassani *et al.,* (2010) reported that the application of 10 mW He-Ne laser for 90 s significantly increased villous length and crypt depth in irradiated broiler eggs at post hatched day 42. Also, similar findings were reported in rats (Carr *et al.,* 1996). To our belief, laser irradiation was able to improve small intestine secretory cell proliferation which caused increased digestive enzyme production. In this study, a significant decrease was found out on LAP activity in different sections of small intestine in birds receiving 10 mW He-Ne laser irradiation. Huge villi height and excessive

mitosis in intestine indicate active function of intestinal villi. On the other hand, the narrow and long intestinal villus is an emblem of rapid crypt proliferation. During cell evolution, these cells rapidly migrate to villus apex (Langhout *et al.,* 1999; Awad *et al.,* 2008). Rapid cell proliferation through multipotent stem cells in the crypts of Lieberkuhn, increases terminally differentiated cells which anatomically are more columnar absorptive cells interspersed with minute secretory cells (Van Der Flier & Clevers, 2009). According to evidence and our observations it seems that laser irradiation stimulated intestinal cell proliferation rate and decreased cell turnover rate which results in diminished enzyme secretion in broilers (Nordstrom & Dahlqvist, 1973; Langhout *et al.,* 1999). The suggested mechanism is that light laser stimulates cyclic adenosine monophosphate elevation and increases both DNA and RNA synthesis which may terminate to increase enzyme secretion (Gao & Xing, 2009). To our knowledge, there is no former study on the effects of laser irradiation on post-hatched intestinal enzyme activity in broilers, so there are no previous reports investigating the effects of laser therapy on small intestine enzyme activity to compare our results with. It seems that laser irradiation enhanced small intestine secretory cell number and increased digestive enzyme production. So laser treatment helped promoting digestion and absorption functions and subsequently, improves bird's efficacy for better nutrients utilisation, hence faster growth rate and/or production. Also, it may decrease nutritional costs.

Finally, we recommend further investigations to clarify effects and/or side effects of pre-incubation laser irradiation of eggs on hatchability and post-hatched small intestine enzymes activity in broiler chickens in order to distinguish their potential for use in clinical trials.

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