

Effects of low frequency electromagnetic fields on growth, total antioxidant activity and morphology of the intestine in rainbow trout (*Oncorhynchus mykiss*)

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Keywords

extremely low-frequency electromagnetic field, rainbow trout, growth performance, serum antioxidant power, gastrointestinal structure

Abstract

For many years it was believed that extremely low-frequency electromagnetic field (ELF-EMF) do not have any significant biological effects. In this study, the influence of extremely low-frequency electromagnetic fields on the growth performance, serum antioxidant power and gastrointestinal structure of rainbow trout were evaluated. Rainbow trout (17-18 g) were exposed to electromagnetic fields (15 Hz) at the range of 1 h daily and 0.01, 0.1, 0.5, 5 and 50 μ T, for a period of 60 days. Growth performance of the trout improved in different treatment groups especially at 0.1, 0.5, 5 and 50 μ T. Serum total antioxidant activity was significantly enhanced with different doses of electromagnetic induction at 0.5, 5 and 50 μ T. Meanwhile, higher density of goblet cells per villus in fish intestines and pyloric caeca at 0.5 μ T induction was observed. These

results indicate that application of extremely low-frequency electromagnetic fields with a frequency of 15 Hz and induction of more than 0.5 μ T might improve the growth performance, total antioxidant power and gastrointestinal structure in rainbow trout.

Abbreviations

Hz: Hertz

μ T: Microtesla

ELF-EMF: Extremely Low Frequency

Electromagnetic Field

LF-EMF: Low Frequency Electromagnetic Field

SGR: Specific Growth Rate

FCR: Feed Conversion Ratio

FRAP: Reducing Ability of Plasma

MDA: Malondialdehyde

IGF-1: Insulin-like Growth Factor 1

SOD: Superoxide Dismutase

Introduction

The electromagnetic spectrum comprises ionizing, optical, and non-ionizing radiations. Non-ionizing radiation is further subdivided into static fields (0 Hz), extremely low frequency (ELF; 0 to ~300 Hz), intermediate frequency (300 Hz up to ~100 kHz) and radio frequency (RF; 100 kHz to ~300 GHz) fields (Schuz and Ahlbom, 2008).

The electromagnetic application in biotechnological processes is relatively recent and still very limited in industrial scale. For many years it was believed that low-frequency electromagnetic fields had not had any significant biological effects. In recent decades, several researchers demonstrated that heat was not the only potentially important property of these radiations.

With increase of EMF producing equipment and environmental exposure, the hypothesis that EMF might have biological effects on human and/or on animal health have motivated scientists to direct their efforts toward understanding the biological influences of EMF. Many scientific studies verified that ELF-EMF can influence the biological systems, could involve principal changes in the cellular proliferation, stimulate ATP production, produce changes in the flow of ions through the membranes, and increase CO₂ formation in cellular cultures (Justo et al. 2006). In fact, biological effects of ELF-EMF have shown contradictory results. Several studies indicated an association between the exposure to ELF-EMF and suppression of immune system. For example, Cetin et al (2006) showed that exposure to EMFs (60 Hz and 3 μ T) for 12 hours per day during 120 days had negative effects on mice bone marrow stem cells. Occupational exposure to ELF-MF exceeding 1 μ T induced a reduction of NK activity in workers (Gobba et al. 2008). Meanwhile, long-term (4 days), continuous exposure of chick embryos to 60 Hz, 8 μ T ELF-EMF caused lower HSP70 levels resulting in a decline in cytoprotection, whereas short time (20 min) exposure induced protection against hypoxia (Di Carlo et al., 2002). Exposure to ELF-EMF (50 Hz) at 0.5 - 1.5 μ T for 45 min led to stimulation of murine macrophages (Simkó et al., 2001).

Since some available data showed positive effects of ELF-EMF on antioxidant system in animals and human, the present study was designed to delineate the possible roles of ELF-EMF (15 Hz) at field strengths of 0.01, 0.1, 0.5, 5 and 50 μ T for 60 days on growth performance, serum total antioxidant power and gastrointestinal structure in juvenile rainbow trout.

Materials and Methods

Fish and husbandry conditions

Rainbow trout weighing 17-18 g obtained from a fish farm in Urmia, Iran were used in this study. They were distributed in 18 glass aquaria (45 cm \times 95 cm \times 35 cm) continuously supplied with well water with a flow rate set at

0.5 lit s⁻¹, water temperature 13 ± 1 °C, pH 7.68, ammonia 0.06 ppm, nitrate 0.01 ppm and dissolved oxygen 8.82 ppm under natural photoperiod (10L:14D). Adaptation to these tanks was performed for 14 days. Fish were fed commercial pelleted diet (Chineh, Iran).

ELF-EMF exposure system

Approximately uniform square wave electromagnetic fields were generated around each glass aquarium. Uniformity deviation over the volume of the aquaria was less than 8%. Briefly, the system was composed of a pair of rectangular Helmholtz coils (100 cm \times 40 cm) with copper wire (300 turns). The coils were mounted on both sides of each glass aquarium (Figure 1). Both DC and AC power supply were employed. An electric current of 0-3A was passed through each coil generating a uniform magnetic flux density of 0-1.8 μ T over a large volume in the space between the coils. The magnetic flux density was measured by a Laybold Hall effect EMF meter located at the center of chamber. In AC mode, the frequency of applied voltage could be varied from 1 Hz to 5,000 Hz and magnetic flux density could be adjusted.

ELF-EMF exposure

One hundred and eighty fish were distributed equally into six groups. Each group contained 10 fish in triplicate reared in individual glass aquariums. In treatment groups, fish were exposed to electromagnetic fields (15 Hz) in the range of 0.01 μ T (T1 group), 0.1 μ T (T2 group), 0.5 μ T (T3 group), 5 μ T (T4 group) and 50 μ T (T5 group) induction for 1 h daily during 60 days. It must be noted that all aquariums were positioned in equally similar conditions regarding light intensity, temperature and background magnetic field intensity. No background AC noise was detected. Local earth magnetic field in location of all aquaria was measured using a Helmholtz coil and the dip angle, which was about 0.245 ± 0.003 Gauss, was known. All groups were fed pelleted diet three times a day, seven days a week at a rate

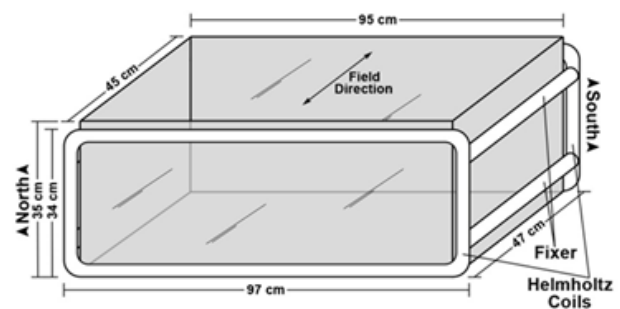


Figure 1
The low frequency electromagnetic field exposure system. The exposure aquarium and rectangular Helmholtz coils located on both sides of the glass aquarium

of 2.5% body weight. Uneaten food was siphoned out after feeding but not weighed.

Fish growth performance

Before experiment and at the end of 60-day trial, all fish from each individual aquarium were weighed and factors such as specific growth rate (SGR) and feed conversion ratio (FCR) were calculated as follows:

$SGR = 100 / (\ln W_2 - W_1) / T$; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the trial;

$FCR = \text{dry feed supplied (g)} / \text{weight gain (g)}$.

Post-mortem examination

On day 60, two fish in each aquarium was randomly selected. After gross examination, tissues samples from liver, kidney, heart, spleen, gills, skeletal muscle, intestine, pyloric caeca were fixed in 10% buffered formalin for 48 h. Tissues were dehydrated in alcohol and xylene and then embedded in paraffin. A 5 micron subsample was then rehydrated in alcohol and stained with haematoxylin-eosin. Length and thickness of proximal intestinal villi and pyloric caeca folds were measured using a graded ocular lens. The percentage of goblet cells in fish proximal intestine and pyloric caeca was also defined.

Blood collection and antioxidant power assay

On days 30 and 60, four fish from each aquarium were sampled. The fish were anaesthetized with a solution containing clove powder (200 mg L⁻¹). Blood samples were collected from the caudal vein and allowed to clot at 4 °C for 5 h. After centrifugation, serum was removed and frozen at -80 °C until use. Total antioxidant activity (Randox Laboratories Ltd.) was determined in the serum specimens using ferric reducing ability of plasma (FRAP) assay. The malondialdehyde (MDA) level in the serum was measured using the thiobarbituric acid test based on previously established procedure. MDA level was expressed as nmol / dl of serum (Sheikhzadeh et al., 2012).

Statistical analysis

Analysis of variance (ANOVA) and LSD tests were run to compare different treatments using the SPSS 19. The mean and standard errors were calculated for each treatment. The accepted level of significance was $p \leq 0.05$.

Results

Before the trial, no significant differences in weight were observed among fish of different groups. On days 30 and 60, fish in groups T4 and T5 had significantly higher weight than fish in control group. Fish lengths in different experimental groups had no significant differences on day 0. Conversely, fish in T2, T3, T4 and T5 groups showed significantly higher length than fish in control group on days 30 and 60. Moreover, FCR was significantly enhanced in T2, T3, T4 and T5 groups as compared to control groups. SGR appeared to be the same in all groups in this experiment (Table 1).

In post-mortem examination, no relevant gross lesions or microscopic changes were noticed in all groups. Light microscopy of fish intestine and pyloric caeca also showed normal appearance in all groups. In fish intestine and pyloric caeca, the epithelial goblet cell percentage appeared to be higher in T3 treatment group, while significant decrease was noted in T1 group as compared to control group. Villus thickness and length in fish intestine did not change during the experiment. In pyloric caeca, fold length and thickness appeared to be in the same condition in all groups (Table 2). In fish intestine and pyloric caeca, the epithelial goblet cell percentage appeared to be higher in T3 treatment group, while significant decrease was noted in T1 treatment group as compared to control group. There was no change in the villus thickness and length in fish intestine during this experiment. In pyloric caeca, fold length and thickness appeared to be the same in all groups (Figure2-3).

Antioxidant activity in all treatment groups except fish in T4 group remained unchanged on day 30. Meanwhile, fish in T3, T4 and T5 groups showed significant increase in

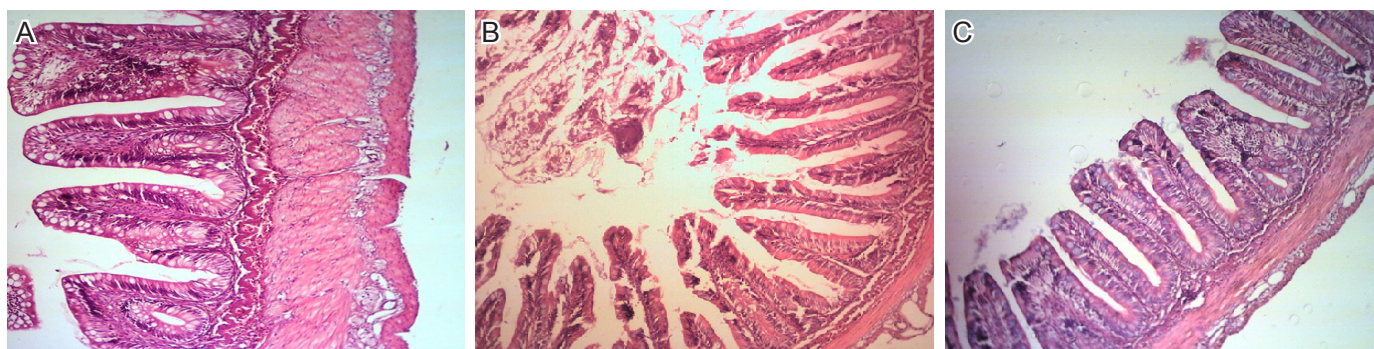


Figure 2

(A) Normal intestine of rainbow trout in control group without exposure to ELF-EMF (staining with H & E, 200×). (B) The intestine of rainbow trout with exposure to ELF-EMF at 15Hz frequency and 0.01 μT induction for one hour per day (staining with H & E, 200×). (C) The intestine of rainbow trout with exposure to ELF-EMF at 15Hz frequency and 0.5 μT induction for one hour per day (staining with H & E, 200×).

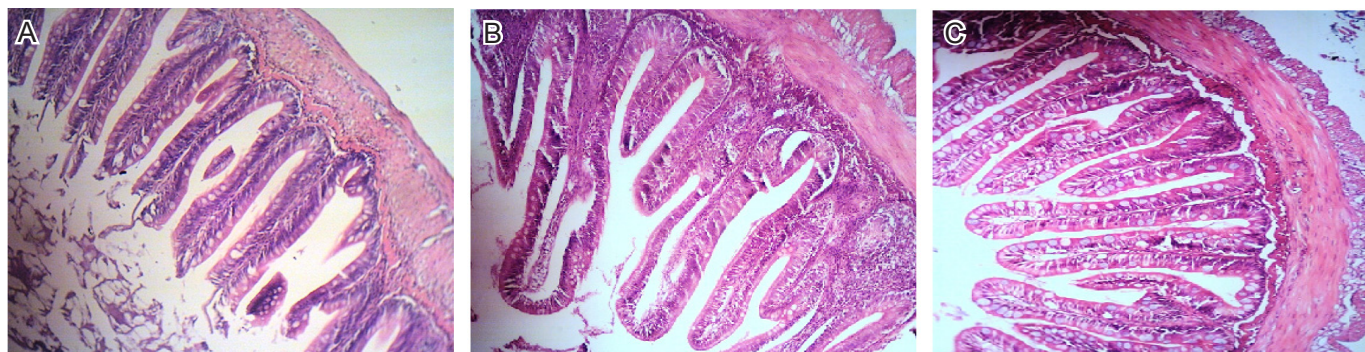


Figure 3
 (A) Normal pyloric caeca of rainbow trout in control group without exposure to ELF-EMF (staining with H & E, 200×). (B) The pyloric caeca of rainbow trout with exposure to ELF-EMF at 15 Hz frequency and 0.01 μ T induction for one hour per day (staining with H & E, 200×). (C) The pyloric caeca of rainbow trout with exposure to ELF-EMF at 15 Hz frequency and 0.5 μ T induction for one hour per day (staining with H & E, 200×).

total antioxidant activity on day 60 (Table 3). Similarly, serum lipid peroxidation in T4 group was considerably lower than other groups on day 30 of the trial. Fish in T3, T4 and T5 groups had significantly lower lipid peroxidation product on day 60 of the trial (Table 3).

Discussion

In the present study, ELF-EMF exposure in the treatment groups especially in T4 and T5 improved growth parameters. In previous study, ELF-EMF exposure enhanced the keratinocyte growth in cell line (Vianale et al., 2008). This effect is consistent with those reported for other cell lines (Wei et al., 2000; Sul et al., 2006). Similarly, in vivo studies also showed the positive effects of EMF on animal growth performance. For example, continuous electromagnetic field of 361 Gauss per cm² increased chick embryos weight at 15-days of age (Piera et al., 1992). Cuppen et al. (2007) also observed that broiler chickens exposed to ELF-EMF had improved feed conversion as compared to

control group (Cuppen et al., 2007). An increase in body weight after 10 weeks of exposure to a 0.5 mT magnetic field was also demonstrated in rats (Gerardi et al., 2008). Even though no common mechanism has been implicated for growth performance in all animals, in a study by Rodriguez et al (2002) increased level of insulin-like growth factor 1 (IGF-1) and higher weight gain were shown in dairy cattle exposed to 60-Hz EMF. IGF-1 stimulates systemic body growth, and has growth promoting effects on almost every cell in the body, especially skeletal muscle, cartilage, bone, liver, skin, hematopoietic cell, nerves, and lungs (Rodriguez et al., 2002).

Several factors such as nutritional components, stress and disease affect intestinal morphology. Intestinal morphology affects the fish physiology and metabolism of nutrient absorption (Vechklang et al., 2011). In the current study, fish exposure to ELF-EMF in T3 group enhanced the density of the goblet cells in fish intestine and pyloric caeca. Conversely, exposure to lower intensity ELF-EMF in T1 group caused significant decrease in these parameters.

Table 1

Growth performance in rainbow trout exposed to ELF-EMF. Exposure was at 15 Hz frequency and induction of 0.01 μ T (T1 group), 0.1 μ T (T2 group), 0.5 μ T (T3 group), 5 μ T (T4 group) and 50 μ T (T5 group) for one hour per day. Data represent the mean \pm SEM. Those within a column superscripted by different letters are significantly different ($p < 0.05$).

Group	Growth Performance						Feed conversion ratio
	Weight (g)			Length (cm)			
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60	
Control	18.06 \pm 0.45	26.25 \pm 0.41 ^a	37.12 \pm 0.39 ^a	8.35 \pm 0.25	9.79 \pm 0.18 ^a	14.03 \pm 0.31 ^a	1.40 \pm 0.22 ^a
T1	17.93 \pm 0.30	27.42 \pm 0.30 ^a	38.69 \pm 0.37 ^a	7.95 \pm 0.18	10.39 \pm 0.18 ^a	14.33 \pm 0.21 ^a	1.31 \pm 0.08 ^a
T2	17.89 \pm 0.32	27.84 \pm 0.31 ^a	39.61 \pm 0.29 ^a	8.10 \pm 0.17	10.62 \pm 0.18 ^b	14.68 \pm 0.20 ^b	1.28 \pm 0.09 ^b
T3	18.12 \pm 0.31	28.64 \pm 1.92 ^a	41.68 \pm 0.31 ^a	8.07 \pm 0.18	11.01 \pm 0.10 ^b	15.04 \pm 0.25 ^b	1.22 \pm 0.07 ^b
T4	17.71 \pm 0.40	32.68 \pm 0.37 ^b	44.72 \pm 4.41 ^b	7.91 \pm 0.19	11.69 \pm 0.22 ^b	15.71 \pm 0.08 ^b	1.27 \pm 0.12 ^b
T5	17.65 \pm 0.29	29.98 \pm 0.41 ^b	43.96 \pm 0.35 ^b	7.92 \pm 0.15	11.45 \pm 0.20 ^b	15.52 \pm 0.17 ^b	1.17 \pm 0.03 ^b

Table 2

Intestinal and pyloric caeca morphology of rainbow trout during exposure to ELF-EMF. Exposure was at 15 Hz frequency and induction of 0.01 μ T (T1 group), 0.1 μ T (T2 group), 0.5 μ T (T3 group), 5 μ T (T4 group) and 50 μ T (T5 group) for one hour per day. Data represent the mean \pm SEM. Those within a column superscripted by different letters are significantly different ($p < 0.05$).

Group	Intestinal morphology			Pyloric caeca morphology		
	Goblet cell percentage	Villus length (μ m)	Villus thickness (μ m)	Goblet cell percentage	Fold length (μ m)	Fold length (μ m)
Control	5.50 \pm 1.97 ^a	498.33 \pm 31.72	121.67 \pm 5.42	9.50 \pm 2.52 ^a	675.01 \pm 30.84	675.01 \pm 30.84
T1	1.23 \pm 0.09 ^b	480.02 \pm 41.63	136.67 \pm 31.79	3.03 \pm 0.15 ^b	683.33 \pm 60.09	683.33 \pm 60.09
T2	4.33 \pm 0.55 ^a	461.67 \pm 18.33	116.67 \pm 6.66	5.83 \pm 0.60 ^a	661.67 \pm 26.25	661.67 \pm 26.25
T3	10.67 \pm 1.76 ^c	450.01 \pm 28.86	113.33 \pm 24.03	16.33 \pm 0.88 ^c	673.33 \pm 14.53	673.33 \pm 14.53
T4	4.83 \pm 0.60 ^a	448.33 \pm 25.61	133.33 \pm 18.91	5.17 \pm 0.87 ^a	640.10 \pm 30.22	640.10 \pm 30.22
T5	4.33 \pm 1.45 ^a	473.33 \pm 46.66	150.01 \pm 11.54	6.01 \pm 0.57 ^a	790.05 \pm 95.39	123.33 \pm 12.01

Goblet cells distributed along the villi play important roles in synthesizing and secreting mucin to destroy pathogens (Vechklang et al., 2011). It can be assumed that increased mucus cell content in T3 treatment group could partially result in enhanced mucosal immunity.

In this study, ELF-EMF showed beneficial effects by significant increase in antioxidant activity. Similarly, Singh et al. (1999) reported that levels of antioxidant enzymes of catalase, glutathione reductase, glutathione peroxidase, and superoxide dismutase (SOD) were significantly increased in mice exposed to a 2 μ T MF (Singh et al., 1999). Zwirska-Korcza et al. (2004) also suggested that ELF-MF play an important role in regulation of some antioxidant enzyme activities, namely MnSOD, CuSOD and ZnSOD in AT478 murine squamous cell carcinoma culture

(Zwirska-Korcza et al., 2004). A significant reduction of nitric oxide level was also noted in rats after exposure for 10 months, 2 h a day to ELF-MF of 100 and 500 μ T intensities (Akdag et al., 2007). Exposure to ELF-EMF leads to generation of ROS and reactive nitrogen species (RNS). On the other hand, these metabolites can alter antioxidant enzyme activities. Moderate levels of these free radicals can induce the antioxidant system, whereas overproduction of these metabolites is shown to attenuate the antioxidant system activities (Zwirska-Korcza et al., 2005). It seems that in our study, the moderate level of free radicals was generated following the mild stress during ELF-MF exposure. This resulted in higher total antioxidant activity, especially in higher intensities. It must be noted that, more studies are needed to fully understand the exact mechanism of ELF-

Table 3

Antioxidant activity in serum of rainbow trout during exposure to ELF-EMF at 15Hz frequency and 0.01 μ T (T1 group), 0.1 μ T (T2 group), 0.5 μ T (T3 group), 5 μ T (T4 group) and 50 μ T (T5 group) induction for one hour per day. Data represent the mean \pm SEM. Those superscripted by different letters are significantly different ($p < 0.05$).

Group	Antioxidant activity (mmol/l)		serum lipid peroxidation (mmol/l)	
	Day 30	Day 60	Day 30	Day 60
Control	0.17 \pm 0.02 ^a	0.13 \pm 0.01 ^a	45.02 \pm 2.23 ^a	49.90 \pm 1.89 ^a
T1	0.12 \pm 0.01 ^a	0.16 \pm 0.01 ^a	48.43 \pm 3.78 ^a	42.19 \pm 0.78 ^a
T2	0.13 \pm 0.03 ^a	0.12 \pm 0.02 ^a	46.73 \pm 2.56 ^a	46.89 \pm 1.20 ^a
T3	0.16 \pm 0.04 ^a	0.21 \pm 0.02	40.16 \pm 2.87 ^a	18.10 \pm 2.18 ^b
T4	0.27 \pm 0.03 ^b	0.22 \pm 0.04 ^b	19.90 \pm 1.27 ^b	12.19 \pm 2.90 ^b
T5	0.16 \pm 0.01 ^a	0.21 \pm 0.01 ^b	42.93 \pm 2.81 ^a	20.13 \pm 1.08 ^b

MF on different aspects of fish antioxidant system.

Many factors appear to influence the effects of ELF-EMF on animal performance and antioxidant system. They include the type of EMF, frequency, amplitude, timing and length of exposure. Therefore, inconsistent results have been achieved from studies on fish and other animals. However, EMF penetrates the animal body and acts on all organs, altering the cell membrane potential and distribution of dipoles and ions. These alterations influence all processes in animal body.

In conclusion, we provide evidence that ELF-EMF exposure at 15 Hz and intensities more than 0.5 μ T may have beneficial effects in rainbow trout, thus affecting parameters like growth performance, total antioxidant power and gastrointestinal structure. The precise explanation of these findings requires further investigation to fully understand their mechanisms of action.

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اثرات میدان مغناطیسی با فرکانس ضعیف بر روی رشد، فعالیت آنٹی اکسیدانی تام و مورفولوژی دستگاه گوارش ماهی قزل آلائی رنگین کمان (*Oncorhynchus mykiss*)

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چکیده

سالیان متمادی، دانشمندان بر این باور بودند که میدان الکترومغناطیسی با فرکانس ضعیف هیچ گونه اثرات بیولوژیک ندارد. در این مطالعه، اثرات میدان الکترومغناطیسی با فرکانس بسیار ضعیف بر روی تظاهرات رشد، قدرت آنٹی اکسیدانی سرم و ساختار دستگاه گوارش مورد بررسی قرار گرفته است. ماهی‌های قزل‌آلائی رنگین‌کمان با وزن متوسط ۱۷-۱۸ گرم، تحت تاثیر میدان الکترومغناطیسی ۱۵ هرتز به مدت یک ساعت در روز و با طیف ۰/۱، ۰/۱، ۰/۵، ۵ و ۵۰ میکروتسلا در طی ۶۰ روز قرار گرفتند. رشد ماهی در تیمارهای مختلف به ویژه در ۰/۱، ۰/۵ و ۵۰ میکروتسلا افزایش یافت. فعالیت آنٹی اکسیدانی تام سرم، در شدت جریان‌های مختلف ۰/۵، ۵ و ۵۰ میکروتسلا به طور معنی‌داری افزایش داشت. در عین حال، تراکم بالاتر سلول‌های جامی در هر ویلی در روده ماهی و زوائد پیلوریک، در میدان ۰/۵ میکروتسلا مشاهده شد. نتایج این تحقیق بیانگر این مساله می‌باشد که میدان الکترومغناطیسی با فرکانس ضعیف ۱۵ هرتز و شدت جریان بیش از ۰/۵ میکروتسلا ممکن است اثرات مفیدی روی رشد ماهی، عملکرد آنٹی اکسیدانی و ساختار دستگاه گوارش ماهی قزل‌آلائی رنگین‌کمان داشته باشد.

واژگان کلیدی: میدان الکترو مغناطیسی با فرکانس بسیار ضعیف، ماهی قزل‌آلائی رنگین‌کمان، رشد، سیستم آنٹی اکسیدانی، ساختار دستگاه گوارش