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Influence of extremely low frequency electromagnetic fields on growth performance, innate immune response, biochemical parameters and disease resistance in rainbow trout, *Oncorhynchus mykiss*

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Abstract The effects of extremely low frequency electromagnetic fields on rainbow trout growth performance, innate immunity and biochemical parameters were studied. Rainbow trout (17–18 g) were exposed to electromagnetic fields (15 Hz) at 0.01, 0.1, 0.5, 5 and 50 μT, for 1 h daily over period of 60 days. Growth performance of fish improved in different treatment groups, especially at 0.1, 0.5, 5 and 50 μT. Immunological parameters, specifically hemagglutinating titer, total antiprotease and α_1-antiprotease levels in treatment groups, were also enhanced. Total protein and globulin contents in the serum of fish exposed to 0.1, 0.5, 5 and 50 μT were significantly higher than those in the control group. No significant differences were found in serum enzyme activities, namely aspartate aminotransferase and alanine aminotransferase of fish in all treatment groups. Conversely, alkaline phosphatase level decreased in fish exposed to 0.01 and 50 μT electromagnetic fields. Meanwhile, electromagnetic induction at 0.1, 0.5, 5 and 50 μT enhanced fish protection against *Yersinia ruckeri*. These results indicated that these specific electromagnetic fields had possible effects on growth performance, nonspecific immunity and disease resistance of rainbow trout.

Keywords Extremely low frequency electromagnetic fields · Rainbow trout · Growth performance · Nonspecific immunity · Biochemical parameters · *Yersinia ruckeri*

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Introduction

The electromagnetic spectrum comprises ionizing radiation, optical radiation and non-ionizing radiation. 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 (Schüz and Ahlbom 2008).

Extremely low frequency electromagnetic fields (ELF-EMFs) originated from many sources such as household electric wiring, high voltage transmission lines and appliances have increased due to the high demand for electrical energy (Canseven et al. 2008). Anthropogenic sources of EMFs in the aquatic environment, such as subsea cables, are also increasing

(Gill et al. 2012). With increase in EMF-producing equipment and environmental exposure, the hypothesis that EMF might have biological effects on human and/or on animal health has 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, stimulates ATP production, produces changes in the flow of ions through the membranes and increases 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 μT) for 12 h per day during 120 days had negative effects on mice marrow stem cells. Occupational exposure to ELF-MF exceeding 1 μT induced a reduction in NK activity in workers (Gobba et al. 2009). Meanwhile, long-term (4 days), continuous exposure of chick embryos to 60 Hz, 8 μT ELF-EMF caused lower HSP70 levels resulting in decline in cytoprotection, whereas short-time (20 min) exposure induced protection against hypoxia (Di Carlo et al. 2000). Exposure to ELF-EMF (50 Hz) at 0.5–1.5 mT for 45 min led to stimulation of murine macrophages (Simkó et al. 2001). Significantly elevated phagocytic activity, free radical release and IL-1β production of mouse macrophages by ELF-EMF (50 Hz and 1.0 mT) were also demonstrated (Frahm et al. 2006).

Effects of ELF-EMF on antioxidant system and biochemical parameters such as liver enzymes and metabolic products were also noted. ELF-EMF of 180–195 Hz and 120 μT decreased antioxidative enzyme activities and increased lipid peroxidation in 3T3-L1 preadipocyte cultures (Zwirska-Korczala et al. 2005). Electromagnetic fields of 50 Hz and 1.4 mT for 30 days caused body weight loss, lower glucose and total protein in mouse serum. Meanwhile, increase in lactate dehydrogenase activity was shown in mouse serum and liver (Hashish et al. 2008). Conversely, exposure to 50 Hz, 2 mT ELF-EMF for 2 months, 8 h/day, caused an increase in antioxidant enzyme levels in accordance with lower lipid peroxidation mouse red blood cells, liver and lungs (Singh et al. 1999). Exposure to ELF-EMF at 5 mT and 60 Hz attenuated insulin secretion from an islet-derived insulinoma cell line, RIN-m, by affecting calcium influx through calcium channels (Miyakoshi 2006).

There is some information regarding the biological effects of ELF-EMF exposure on fish species. Exposure to sinusoidal magnetic fields delayed the development of zebrafish (*Danio rerio*) embryos at 60 Hz and 0.1 mT (Cameron et al. 1985), and 50 Hz and 1 mT (Skauli et al. 2000). The lateral line of *Anguilla anguilla* showed an electrophysiological response to changes in EMF (Vriens and Bretschneider 1979; Moore and Riley 2009). Activity of locomotor muscles in *Salmo salar* altered with exposure to LF EMF (Richardson et al. 1976). Positive effects on growth and immune system in fantail goldfish caused by exposure to LF EMF signals (200–5000 Hz) between 0.15 and 50 μT intensities were also shown (Cuppen et al. 2007). In fact, anthropogenic EMF within the aquatic environment has only relatively recently come to be of interest, and scientific understanding of the consequences to species individuals, populations and ecosystem is slowly being identified and addressed (Gill et al. 2012). Considering previous studies, little is known regarding the possible effects of ELF-EMF exposure on fish growth, biochemical and immune parameters. Therefore, the present study was designed to delineate the possible roles of ELF-EMF with lower frequency (15 Hz) at field strengths 0.01, 0.1, 0.5, 5 and 50 μT, for 60 days on growth performance, innate immunity, serum-specific marker enzymes besides some serum metabolites and disease resistance against *Yersinia ruckeri* (*Y. ruckeri*) in juvenile rainbow trout.

Materials and methods

Fish and husbandry conditions

One hundred and forty-day-old rainbow trout (mean weight 17–18 g) obtained from a fish farm in Urmia, Iran, were used in this study. After health examination, they were distributed in 18 glass aquaria (45 cm × 95 cm × 35 cm) continuously supplied with aerated free-flowing river water with the flow rate set at 0.5 lit s^{-1}, water temperature 11 ± 1 °C and dissolved oxygen 5.2 ppm under natural photoperiod (10L:14D). Adaptation to these tanks was performed for 14 days with pelleted diet. The formulation of the diet was the same as reported in a previous study

(Nootash et al. 2013). Briefly, it contained 40 % protein, 14 % crude lipid, 3.5 % crude fiber, 10 % crude ash and 1.2 % phosphorous.

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 composed of a pair of rectangular Helmholtz coils (100 cm × 40 cm) with copper wire (300 turns). The coils were mounted on both sides of each glass aquarium (Fig. 1). Both DC and AC power supply were employed. An electric current of 0–3 A (I_{eff} in case of AC) passed through each coil generating a uniform magnetic flux density of 0–1.8 mT over a large volume in the space between the coils. The magnetic flux density was measured by a Leybold 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

Four hundred and fifty fish were distributed equally into six groups. Each group contained 25 fish in triplicates reared in individual glass aquarium. In treatment groups, fish were exposed to electromagnetic fields (15 Hz) in a range of 0.01 μT (T1 group), 0.1 μT (T2 group), 0.5 μT (T3 group), 5 μT (T4 group) and 50 μT (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 knowing the dip angle that was about 0.245 ± 0.003 Gauss (Maus et al. 2010). All groups were fed pelleted diet three times a day, 7 days a week at a rate of 2.5 % body weight. Uneaten food was siphoned out after feeding but not weighed to determine feed efficiency.

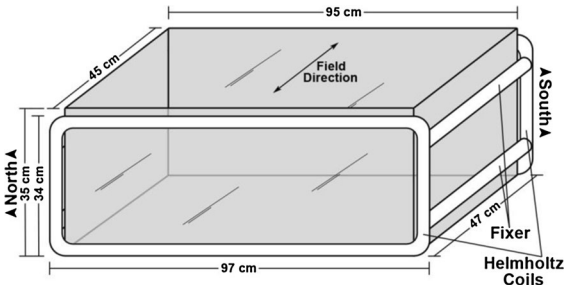

Fig. 1 Low frequency electromagnetic field exposure system. The exposure aquarium and rectangular Helmholtz coils located on both sides of the glass aquarium

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:

$$\text{SGR} = 100 \times \ln(W_2 - W_1)/T;$$

where W_1 and W_2 are the initial and final weight (g), respectively, and T is the number of days in the feeding period.

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

Post-mortem examination

On day 60, two fish in each aquarium were randomly selected. After complete gross examination, tissues, namely liver, kidney, heart, spleen, gills, skeletal muscle, intestine, and pyloric caeca samples, were fixed in 10 % buffered formalin for 48 h. Tissues were dehydrated in alcohols and xylene and then embedded in paraffin. Five micron subsamples were then rehydrated in alcohol and stained with hematoxylin-eosin.

Blood collection

On days 30 and 60, four fish from each aquarium were sampled. The fish were anaesthetized with 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.

Nonspecific immune parameters

Serum total antiprotease assay

The level of serum total antiprotease was measured according to Rao and Chakrabarti (2005) with slight

modification. In tubes, 10 µl of serum was diluted with 20 µg of trypsin dissolved in 100 µl of PBS (pH 7.4). All tubes were incubated at room temperature for 30 min. Then, 1 ml of casein dissolved in PBS (2.5 mg/ml) was added to all tubes and incubated for a further 15 min. Finally, the color change was stopped by adding 500 µl of 10 % trichloroacetic acid. All tubes were centrifuged at 3800 rpm for 10 min to remove the precipitate. The optical density was read at 280 nm in a Biophotometer, Eppendorf.

Serum α_1-antiprotease assay

The level of serum α_1-antiprotease was measured according to Rao and Chakrabarti (2005). In tubes, 10 µl of serum was diluted with 20 µg of trypsin dissolved in 100 µl of Tris–HCl (50 mM, pH 8.2). In the serum blank, trypsin was replaced with 100 µl of Tris–HCl, and in the positive control, no serum was added to trypsin. All tubes were made up to 200 µl with Tris–HCl and incubated at room temperature for 1 h. Then, 2 ml of 0.1 mM substrate, BAPNA (Na-benzoyl-DL-arginine-*p*-nitroanilide HCl, Sigma) dissolved in Tris–HCl (containing 20 mM calcium chloride), was added to all tubes and incubated for a further 15 min. Finally, the color change was stopped by adding 500 µl of 30 % acetic acid, and the optical density was read at 410 nm in a UV–visible spectrophotometer, Spectronic 2OD.

The OD of the positive control, i.e., the activity of trypsin without the addition of any serum, was taken as 100 % activity. The OD values of the serum blank were deducted from trypsin plus serum, and the percentage trypsin inhibition by the serum was calculated.

Serum peroxidase content

The total peroxidase content in fish serum was measured according to Cuesta et al. (2005). Serum samples (15 µl) were placed in each well of a 96-well plate. HBSS without Ca^{+2} or Mg^{+2} (135 µl) was then added to each well. Finally, 50 µl of 20 mM 3,30,5,50-tetramethylbenzidine hydrochloride (TMB) (Sigma) and 5 mM H_2O_2 was added. The color-change reaction was stopped after 2 min by adding 50 µl of 2 M sulfuric acid, and the optical density was read at 450 nm by ELISA reader.

Hemagglutination assay

Chicken red blood cell (C-RBC, 2.5 %) suspension in TBS was prepared. In round-bottomed microplates, 50 µl of serum sample was added to first well, followed by serial dilution in TBS buffer from the second to eighth wells. Equal volume of 2.5 % C-RBC was added to all the wells. After incubation at room temperature for 1 h, the reciprocal of the highest dilution with visible agglutination was considered as the hemagglutinating titer.

Serum-specific marker enzymes and organic compositions

Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum were determined using enzymatic colorimetric methods by previously reported method (Sheikhzadeh et al. 2012) and expressed as U L^1 of serum. Serum total protein and albumin contents were determined by Lowery and bromocresol green methods, respectively. Serum globulin level was estimated by subtracting these two amounts. Triglyceride and cholesterol levels were estimated according to the enzymatic colorimetric method of Fossati and Prencipe (1982) and Allain et al. (1974), respectively.

Challenge test

After the 60-day trial, the remaining fish from each group were injected intraperitoneally with 0.1 ml of a fresh culture *Y. ruckeri* (BCCM/LMG3279) (0.5×10^7 CFU ml^{-1}). The challenged fish were kept under observation for 10 days and fed a maintenance diet. Dead fish were removed daily, and tissues samples were used for bacteriological culture on trypticase soy agar (TSA) (Merck, Darmstadt, Germany) plates. An agglutination test was carried out to confirm the bacteria isolated.

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. For fish in different experimental groups, fish length was also without any 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 in comparison with control group. SGR was also enhanced in T2 group compared with control group (Table 1).

In postmortem examination, no relevant gross lesions or microscopic changes were noticed in all groups. During this study before challenging, no mortality of fish was observed in different groups. After challenge with *Y. ruckeri*, average cumulative mortality was 62.07 % in control group, 58.33 % in T1 group, 57.01 % in T2 group, 52.13 % in T3 group, 57.29 % in T4 group and 46.82 % in T5 group, respectively. These data showed that the fish in T2, T3, T4 and T5 groups had significantly lower cumulative mortality than the fish in the control group (Fig. 2). Mortality started in challenged fish 3 days post-injection with some clinical signs such as darkening, exophthalmia and hemorrhaging.

On day 30, total antiprotease showed a similar pattern in all groups, but on day 60, a significant elevation was noted in T2, T3, T4 and T5 groups in comparison with control group. On the other hand, percentage trypsin inhibition due to α_1-antiprotease did not show any significant difference ($P > 0.05$) between different groups on day 30 during the trial, while on day 60 significant elevations were noted in T3, T4 and T5 groups compared with the control group. Fish in T4 group exhibited higher peroxidase content on day 30. On day 60, peroxidase content was higher in T4 and T5 groups. On day 30, no significant differences in hemagglutination antibody titer were observed among all groups. However, antibody titer on day 60 of trial was significantly higher in T4 and T5 groups compared with control group (Table 2).

On day 30, serum total protein and globulin levels were similar between all groups, but on day 60, these metabolites showed significant increase in T2, T3, T4 and T5 groups compared with the control group. In the current study, triglyceride and cholesterol levels in fish

Table 1 Growth performance in rainbow trout during exposure to ELF-EMF at 15 Hz frequency and 0.01 μT (T1 group), 0.1 μT (T2 group), 0.5 μT (T3 group), 5 μT (T4 group) and 50 μT (T5 group) induction for 1 h per day

Group	Growth performance							
	Weight			Length			Feed conversion ratio	Specific growth rate
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60		
control	18.06 ± 0.45	26.25 ± 0.41	37.12 ± 0.39	8.35 ± 0.25	9.79 ± 0.18	14.03 ± 0.31	1.40 ± 0.22	2.17 ± 0.07
T1	17.93 ± 0.30	27.42 ± 0.30	38.69 ± 0.37	7.95 ± 0.18	10.39 ± 0.18	14.33 ± 0.21	1.31 ± 0.08	2.19 ± 0.15
T2	17.89 ± 0.32	27.84 ± 0.31	39.61 ± 0.29	8.10 ± 0.17	10.62 ± 0.18*	14.68 ± 0.20*	1.28 ± 0.09*	2.60 ± 0.37*
T3	18.12 ± 0.31	28.64 ± 1.92	41.68 ± 0.31	8.07 ± 0.18	11.01 ± 0.10*	15.04 ± 0.25*	1.22 ± 0.07*	2.29 ± 0.32
T4	17.71 ± 0.40	32.68 ± 0.37*	44.72 ± 4.41*	7.91 ± 0.19	11.69 ± 0.22*	15.71 ± 0.08*	1.27 ± 0.12*	2.49 ± 0.09
T5	17.65 ± 0.29	29.68 ± 0.41*	43.96 ± 0.35*	7.92 ± 0.15	11.45 ± 0.20*	15.52 ± 0.17*	1.17 ± 0.03*	2.36 ± 0.11

Data represent the mean ± SEM

* $P < 0.05$

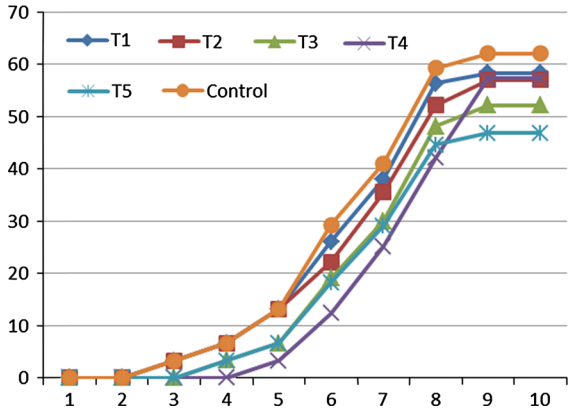

Fig. 2 Cumulative mortality after challenge with *Yersinia ruckeri* in rainbow trout exposed to ELF-EMF at 15 Hz frequency and 0.01 μT (T1 group), 0.1 μT (T2 group), 0.5 μT (T3 group), 5 μT (T4 group) and 50 μT (T5 group) induction for 1 h per day. Data represent the mean ± SEM. $^*P < 0.05$

serum did not show any significant differences between different groups on day 30, whereas on day 60, triglyceride level of fish in T1 group was significantly lower than that of control group (Table 3).

AST and ALT levels of the fish in different groups were almost similar with control groups on days 30 and 60. ALP level decreased significantly in T5 and T1 groups on days 30 and 60, respectively, compared with control group (Table 4).

Discussion

In the present study, ELF-EMF exposure in treatment groups, especially in T4 and T5 groups, improved growth parameters. Similarly, continuous electromagnetic field of 361 Gauss per cm^2 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 in comparison with control group. 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). Conversely, electromagnetic fields of 50 Hz and 1.4 mT for 30 days caused body weight loss in mice (Hashish et al. 2008). Some authors have attributed body weight differences in EMF-exposed animals to changes in eating habitat or metabolic changes (Hashish et al. 2008). Since in our

Table 2 Immunological parameters in rainbow trout serum during exposure to ELF-EMF at 15 Hz frequency and 0.01 μT (T1 group), 0.1 μT (T2 group), 0.5 μT (T3 group), 5 μT (T4 group) and 50 μT (T5 group) induction for 1 h per day

Group	Total antiprotease (280 nm)		α_1-Antiprotease (%)		Peroxidase (450 nm)		Hemagglutination titer	
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60
Control	24.80 ± 2.59	21.50 ± 7.50	2.85 ± 0.17	2.80 ± 0.15	0.221 ± 0.006	0.206 ± 0.008	1.16 ± 0.16	1.01 ± 0.16
T1	20.31 ± 5.29	18.25 ± 4.48	2.68 ± 0.20	3.02 ± 0.18	0.229 ± 0.016	0.229 ± 0.006	1.16 ± 0.40	1.55 ± 0.17
T2	22.83 ± 1.79	47.01 ± 13.02*	2.46 ± 0.18	2.71 ± 0.26	0.201 ± 0.008	0.233 ± 0.026	1.83 ± 0.47	1.11 ± 0.13
T3	20.03 ± 2.10	37.40 ± 7.54*	2.49 ± 0.11	3.46 ± 0.13*	0.190 ± 0.003	0.192 ± 0.005	1.66 ± 0.49	1.33 ± 0.16
T4	20.05 ± 3.05	34.14 ± 1.56*	2.67 ± 0.14	3.40 ± 0.22*	0.284 ± 0.038*	0.258 ± 0.020*	1.83 ± 0.16	2.09 ± 0.40*
T5	24.50 ± 2.50	34.50 ± 2.51*	2.55 ± 0.14	3.96 ± 0.19*	0.216 ± 0.010	0.255 ± 0.023*	1.50 ± 0.22	2.17 ± 0.76*

Data represent the mean ± SEM

* $P < 0.05$

Table 3 Serum metabolites in rainbow trout during exposure to ELF-EMF at 15 Hz frequency and 0.01 μT (T1 group), 0.1 μT (T2 group), 0.5 μT (T3 group), 5 μT (T4 group) and 50 μT (T5 group) induction for 1 h per day

Group	Total protein ($g\ dl^{-1}$)		Globulin ($g\ dl^{-1}$)		Triglyceride ($mg\ dl^{-1}$)		Cholesterol ($mg\ dl^{-1}$)	
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60
control	5.05 ± 0.17	3.95 ± 0.59	3.70 ± 0.76	2.80 ± 0.53	251.22 ± 11.56	355.77 ± 21.47	257.80 ± 5.23	264.66 ± 14.77
T1	5.40 ± 0.92	4.91 ± 0.24	3.60 ± 0.36	3.55 ± 0.20	220.66 ± 62.69	284.88 ± 23.51*	234.01 ± 48.79	250.44 ± 27.94
T2	4.43 ± 0.13	5.47 ± 0.14*	3.43 ± 0.09	4.05 ± 0.19*	282.66 ± 25.45	400.25 ± 19.73	242.01 ± 18.62	283.25 ± 17.48
T3	4.35 ± 0.78	5.35 ± 0.26*	3.55 ± 0.72	3.66 ± 0.35*	315.20 ± 17.74	347.11 ± 29.23	262.80 ± 17.96	247.77 ± 30.24
T4	4.76 ± 0.39	5.04 ± 0.31*	3.44 ± 0.51	3.80 ± 0.24*	214.33 ± 28.53	306.22 ± 31.35	240.09 ± 18.28	295.77 ± 32.59
T5	5.68 ± 0.42	5.52 ± 0.39*	3.56 ± 0.13	3.77 ± 0.21*	214.40 ± 6.85	298.75 ± 20.98	256.01 ± 6.66	309.50 ± 20.93

Data represent the mean ± SEM

* $P < 0.05$

study no differences were noted in eating habitat between all groups, it seems that metabolic changes might occur in treatment groups which lead to improved growth performance. In fish species, higher growth performance can happen by different mechanisms (Heidarieh et al. 2012). Influencing nutrient, especially protein digestibility by maintaining the function and structure of the small intestine, leads to an increased digestive capacity of the gut. Meanwhile, improved digestive enzymes, including lipase, amylase and protease, could result in better growth performances. In the present study, pathological examination did not show any differences in small intestine structure. Further studies to assess the digestive enzyme activities in fish exposed to ELF-EMF are warranted to elucidate the mechanisms through which ELF-EMF can affect the growth in fish species.

Bacterial pathogens produce proteolytic enzymes to aid in the breakdown of host tissues. Protease inhibitors in sera and other body fluids are involved in acute phase reactions and in defense against these pathogens (Magnadottir 2006). Fish plasma contains a number of protease inhibitors, principally a1-antiprotease, a2-antiplasmin and a2-macroglobulin, which have a role in restricting the ability of bacteria to survive in vivo (Ellis 2001). In our study, fish in all treatment groups except T1 group showed higher levels of total and α_1-antiprotease activities as compared with control group. The results of this study indicate that exposure to ELF-EMF might enhanced nonspecific factors of the immune system by enhancing the level of natural antiproteases in the serum. Possibly, these may have provided some defense against infection by the pathogen (Newaj-Fyzul et al. 2007).

Phagocytosis in activated leukocytes is known to be associated with the production of toxic radicals such as O_2^- and NO^-. At the end of respiratory burst activity, myeloperoxidase and eosinophil peroxidase use H_2O_2 and halide ions to form chlorides and chloramines to help in the fight. After full activation, these enzymes present inside phagocytic cells are released. Therefore, peroxidase content released by degranulation from phagocytic cells can be a good indicator of leukocyte activation (Cuesta et al. 2007). In the present study, serum peroxidase content was increased in T4 and T5 groups in comparison with control group. In previous studies, different effects of EMF on leukocytes were shown. For example, Simkó et al.

Table 4 Serum enzyme activity in rainbow trout during exposure to ELF-EMF at 15 Hz frequency and 0.01 μT (T1 group), 0.1 μT (T2 group), 0.5 μT (T3 group), 5 μT (T4 group) and 50 μT (T5 group) induction for 1 h per day

Group	ALP ($U\ lit^{-1}$)		AST ($U\ lit^{-1}$)		ALT ($U\ lit^{-1}$)	
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60
control	508.80 ± 20.72	378.03 ± 44.01	126.80 ± 17.44	117.11 ± 10.88	4.08 ± 0.93	4.10 ± 0.08
T1	599.50 ± 93.83	221.50 ± 46.44*	124.50 ± 10.70	112.01 ± 10.30	4.23 ± 0.16	4.50 ± 0.50
T2	351.66 ± 48.57	469.42 ± 34.27	109.03 ± 10.19	106.25 ± 19.70	5.08 ± 1.36	4.75 ± 0.52
T3	521.60 ± 81.96	428.08 ± 53.26	133.60 ± 18.63	130.01 ± 16.10	4.05 ± 0.18	4.88 ± 0.58
T4	434.40 ± 50.32	274.25 ± 43.80	128.66 ± 14.55	122.50 ± 15.39	4.09 ± 0.16	4.44 ± 0.40
T5	307.50 ± 10.70*	341.50 ± 38.85	108.80 ± 12.62	123.14 ± 18.64	5.60 ± 0.48	4.25 ± 1.94

Data represent the mean ± SEM

* $P < 0.05$

(2001) showed that 50 Hz, 1 mT EMF activated murine bone marrow-derived macrophages to release superoxide radicals. Frahm et al. (2006) also observed that ELF-MF (50 Hz at different flux densities) exposure resulted in increased ROS formation, enhanced phagocytotic activity and increased IL-1β release in macrophages. It is noteworthy to mention no genotoxic effects in mouse macrophages or anomaly in mitotic events was found. Conversely, Nakajima and Nishimura (1998) reported that 14 mT, 50 Hz MFs had no significant effect on the phagocytic activity in prestimulated peritoneal mouse macrophages (Frahm et al. 2006).

Serum total protein is divided into two parts, albumin and globulin. The γ globulin fraction contains almost all the immunoglobulin of blood. In the present study, serum total protein and globulin contents showed significant increase in treatment groups. Significant increase in protein level was also found in different cell lines exposed to LF-MF (Murray and Farndale 1985; Rodemann et al. 1989; Goodman 1991). Conversely, 50 Hz ELF-EMF exposure made a significant decrease in serum total protein level in mice (Hashish et al. 2008). This result was in accordance with overproduction of ROS and significant increase in lactate dehydrogenase and alkaline phosphatase activities as indicator of tissue damage in the exposed group, indicating hepatic injury (Hashish et al. 2008). Considering the lack of liver damage and nonsignificant changes in liver enzyme activities as well as higher antibody titer in most treatment groups, mainly T4 and T5 groups, it can be assumed that higher protein synthesis might happen after ELF-EMF exposure in rainbow trout.

In the current study, higher survival rate against *Y. ruckeri* was also observed in rainbow trout exposed to ELF-EMF in T2, T3, T4 and T5 groups. Therefore, the stimulation of specific immune system and consequently protection against disease agents also occurred. Similar results were obtained by Maniu and Hritcu (2010) who noted higher antibody titer in rats exposed to ELF pulse electromagnetic field (50 Hz and 2.7 mT). Markov et al. (2006) also observed that EMF may enhance immune responses as evidenced by increased antibody levels and faster maturation of B lymphocytes.

Even though positive effects of ELF-EMF on immune system were shown in some studies, immune dysfunction by exposure to EMF should also be considered (Johansson 2009). Specific findings from studies on exposure to various types of EMFs report overreaction of the immune system, morphological alterations of immune cells, changes in lymphocyte viability, decreased count of natural killer cells and decreased count of T lymphocytes (Johansson 2009). For example, Cetin et al. (2006) showed that pulsed EMFs (60 Hz and 3 μT) for 12 h per day during 120 days negatively affected the hematological parameters of mice by reducing proliferation and differentiation of marrow stem cells.

Simkó and Mattsson (2004) presented a hypothesis of a possible initial cellular event by exposure to ELF-EMF. Based on their research, EMF exposure can cause both acute and chronic effects that are mediated by increased free radical levels. Short exposure to EMF leads to free radical production by phagocyting cells that positively lead to higher cytokine production. This mechanism can trigger both innate and

specific immune system activations as indicated in previous studies. Conversely, an increase in the lifetime of free radicals by EMF leads to persistently elevated free radical production, subsequently causing negative effects such as DNA damage, tumor development and immune dysfunctions.

The effects of ELF-EMF exposure on serum triglyceride level showed significant decrease in T1 group compared with control group. Similarly, exposition for 14 days to ELF electric fields (50 Hz, 17,500 V/m intensity for 15 min per day) of ischemic rats decreased free fatty acids and triacylglycerol plasma levels (Harakawa et al. 2005). Conversely, no changes were observed in total cholesterol and triacylglycerol serum levels in rats exposed to ELF-EMF (60 Hz and 2.4 mT) for 2 h. Triglycerides as the main energy source in many organisms are synthesized in the liver and digested into fatty acids or glycerol by lipase distributed in various tissues (Harakawa et al. 2005). It seems that in our study, triglyceride metabolism was enhanced by the EMF-induced increase in energy metabolism, including fat metabolism (Harakawa et al. 2005).

Liver-specific enzymes such as ALT and AST are sensitive measures of hepatotoxicity and histopathological changes. During cell injury, by higher permeability of hepatocyte membrane, these enzymes penetrate to sinusoids and then enter into the peripheral blood (Friedel et al. 1979). In the current study, serum activity of ALT and AST did not differ between all groups. On the other hand, significant decrease in ALP level was noted in T1 and T5 groups. Therefore, it can be proposed that ELF-EMF in these intensities might be safe for exposed fish. This can be partly proven by no relevant gross and microscopic lesions in liver of all treatment groups following postmortem examination. Meanwhile, no major pathological signs including inflammation or tumors related to exposure were observed in different tissues of exposed fish in all treatment groups.

It appears that there are many factors that influence the effects of ELF-EMF on animal performance, immune and biochemical values. 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 general, besides numerous studies on disturbing the immune system and thus increasing disease after EMF exposure, the positive side of EMF use, which could be useful for specific therapeutic applications, should also be considered. In conclusion, this preliminary study showed that square wave ELF-EMF exposure at 15 Hz and intensities more than 0.5 μT may have beneficial effects in rainbow trout, thus affecting parameters such as growth performance, immunity and biochemical values. Further investigations are needed to fully understand the interaction between ELF-EMFs and different fish species even on the molecular level.

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