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RESEARCH PAPER

Effect of Probiotic *Lactobacillus fermentum* on Growth Performance, Bioaccumulation and Antioxidant Defenses of Zebrafish (*Danio rerio*) Under Cadmium Toxicity

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Abstract

This study aimed to evaluate the effect of *Lactobacillus fermentum* ATCC 14931 as a probiotic to improve immune function and reduce the adverse effects of cadmium (Cd) in zebrafish. This study was performed for 60 days of feeding and 72 hours exposure to 7 g/L Cd. The study groups included C: Control, B: Feeding with a basal diet and exposure to cd, T1 and T3: feeding with probiotic diet in concentrations of 1.5×10^4 and 1.5×10^5 CFU ml^{-1} and exposure to cd, respectively. The biometrical index, mortality rate, Cd accumulation in tissue, superoxide dismutase (SOD) activity, and concentration of malondialdehyde (MDA) were examined. According to the results, feeding with probiotics increased the length and weight growth of fish ($P<0.05$). The mortality rate in probiotic-fed groups was significantly reduced ($P<0.05$), while no significant difference was recorded in the cd accumulation rate of the gastrointestinal tract ($P>0.05$). Also, the SOD activity and MDA level decreased in T1 and T2 ($P<0.05$). Generally, the results showed that *L. fermentum* can be used as a suitable dietary supplement to prevent the adverse effects of acute Cd exposure in fish.

Introduction

Today, the increasing demand for seafood consumption has made aquaculture a very important industry in the world. This issue has increased the importance of identifying and introducing new strategies to increase the productivity of aquaculture (Madreseh et al., 2019). One of the strategies that have attracted the attention of researchers today is the use of health-improving food supplements in aquaculture (Kazempoor et al., 2022). In this regard, probiotics are among the most popular health-improving food supplements in food animal breeding. Probiotics are live microorganisms that provide health benefits to the host when administered in sufficient quantities (Salimi and Kazempoor, 2022).

Lactic acid bacteria are the most important group of probiotics involved in improving metabolism, immune function, and modulating intestinal microbiota in the host (Kazempour and Kazempoor, 2022; Loghmani *et al*., 2022; Mirabdollah Elahi *et al*., 2020; Mollanourozi *et al*., 2021). *Lactobacillus fermentum* (L. *fermentum*) is a unique species of Lactobacillus that improve immune response and antioxidant activity (Mikelsaar & Zilmer, 2009). So far, several studies have reported the effect of feeding with *L. fermentum* on improving growth performance, survival rate, and immune system function in fish (Ahmadifar et al., 2019; Madreseh et al., 2019; Krishnaveni et al., 2021). For example, Ahmadifar et al. (2019) reported improved antioxidative defense in common carp (*Cyprinus carpio*) fed L. *fermentum*. Also, Madreseh et al. (2019) observed that feeding with L.

fermentum reduces the accumulation of heavy metals in rainbow trout (*Oncorhynchus mykiss*) tissues.

In today's world, the development of industry has caused an increase in environmental pollution, which poses many risks for living organisms (Fallah et al., 2011). One of these living organisms is aquatic animals because many of these pollutants enter aquatic ecosystems in different ways and cause irreparable losses. On the other hand, they are transferred into the food chain by accumulating in aquatic tissues (Yi et al., 2011; Varol et al., 2017). Today, heavy metals are of great interest from the point of view of toxicology due to the increase in their concentration in fish tissues (Madreseh et al., 2019).

Cadmium (Cd) is one of the most abundant metal pollutants on earth (Gao *et al*., 2018), and is entering the environment and surface waters through industrial or urban wastes (Swarnalatha *et al*., 2015). The increased pollution of Cd in water systems has been reported (including in Iran) with the development of industry and population growth (Chahid et al., 2014; Rahimi *et al*., 2010). Cd contamination is a serious threat to the growth and health of organisms (Liu *et al*., 2014) because this metal is absorbed by aquatic tissues through contaminated water (Morgano *et al*., 2014). In addition to increasing mortality in aquatic species, it also enters the human body indirectly through the food chain (Copat *et al*., 2013; Kumar & Singh, 2010). Considering that the conventional strategies for decontamination of the environment with heavy metals are very expensive and have high energy requirements and have problems with the production of secondary pollutants, it is necessary to identify economic, environmentally friendly, and sustainable methods (Mishra et al., 2021). In this regard, studies have shown that some bacterial cells have a protective mechanism against the toxicity of heavy metals (Huang et al., 2018).

The zebrafish (*Danio rerio*) is a valuable laboratory model that is among the significant species in the study of poisoning among vertebrates (Dai et al., 2014). This fish has many advantages, including small size, the possibility of genetic changes, high reproduction, and rapid evolution (Arayesh et al., 2021). In this regard, many studies have investigated the effects of heavy metals on zebrafish *in-vivo* (Yin et al., 2018; Gao et al., 2019).

The effects of organisms' contamination with heavy metals include effects on aerobic metabolism, production of reactive oxygen species, disruption of the oxidation and reduction cycle, oxidative stress, and tissue damage (Javed *et al*., 2017; Suresh, 2009). In this regard, low molecular weight antioxidant enzymes and non-enzymatic antioxidants, such as superoxide dismutase (SOD) and malondialdehyde (MDA) are the most essential components of the immune system which protect organisms from damage caused by free radicals. As a result, scientists can measure the level of oxidative stress caused by exposure to a variety of pollutants by assessing the level of these factors (Souid *et al*., 2013). Also, heavy metals such as Cd have had negative effects on the intestinal microbial flora of living organisms (Liu *et al*., 2014). However gastrointestinal fluoro-bacteria play a critical role in the immune function and metabolism of fish (Gómez & Balcázar, 2008). As a result, enhancing the microbial flora of the gastrointestinal tract can effectively prevent the adverse effects of metallic contamination (Zhai *et al*., 2016). In this regard, numerous studies have shown that probiotics can effectively modulate intestinal bacterial flora and regulate immunity (Heo *et al*., 2013; Standen *et al*., 2015). Therefore, probiotics are very important in stabilizing the body's microbial population (Mikelsaar & Zilmer, 2009). On the other way, another significant effect of probiotic bacteria on contamination with metal contaminants is their ability to bind to metals such as Cd, which reduces the effects of poisoning and oxidative stress (Zhai *et al*., 2014; Zhai *et al*., 2015). According to the mentioned points, it has been important to recognize the effects and damage caused by metal pollutants in the diagnosis and prevention of their effects in aquatic animals due to the spread of pollution of aquatic ecosystems in recent years. Also, recognizing and introducing growth-promoting compounds and immunity has become very important to control the adverse effects of poisoning as food additives in farmed aquatic animals. In this study, the effect of feeding with *L. fermentum* probiotic on mortality, Cd accumulation, and oxidative stress indices in the zebrafish gastrointestinal tract in the presence of Cd is investigated.

Materials and Methods

Experimental Zebrafish

This study was performed in the Razaf laboratory complex, Islamic Azad University, Rudehen, Iran. 600 zebrafish (1.06±0.23 g) were purchased from an ornamental fish breeding center in Tehran and transferred to the research laboratory. Purchased fish were adapted for two weeks before the start of the experiments in the laboratory. The fish were placed in tanks containing 80 liters of water during the adaptation period and fed daily using Tetra commercial feed based on 2% of the fish's body weight. Brightness was set at 14:10 and the fish-keeping temperature was set at 25±1°C (Gao *et al*., 2018). 30% of the aquarium water was changed daily and two hours after feeding during the test.

Preparation and Culture of Probiotics

Lactobacillus fermentum ATCC 14931 was prepared in lyophilized form from the Iranian Biological Resource Center. It was first kept with a kettle on an MRS agar (Sigma-Aldrich) culture medium for 24 hours at 37°C to prepare the required concentration. After bacterial growth, a colony was selected and transferred

to an MRS broth culture medium for further study and incubated again for 20 hours at 37°C and 5% CO_2 (Wang *et al*., 2013). The prepared bacteria were centrifuged at 2500 rpm for 10 minutes at 4°C. Finally, a suspension with two concentrations of 1.5×10^5 CFU ml^{-1} and 1.5×10^4 CFU ml^{-1} was prepared using the half-McFarland comparison method (Qin *et al*., 2014), and 20% glycerol was used for storage at -20°C (Alavinezhad et al., 2020).

Diet Preparation

Feeding was done with a ration containing *L. fermentum* probiotic in fresh weight, by combining probiotic suspension of *L. fermentum* at a concentration of 1.5×10^4 CFU ml^{-1} and 1.5×10^5 CFU ml^{-1} with Tetra commercial feed at 2% of fish body weight and then incubated in ice for 15 minutes to absorb the bacteria (Yi *et al*., 2019). Feeding in the control group was done with commercial feed with equivalent amounts of PBS buffer (Wang *et al*., 2016). Feeding was done for 60 days (twice a day) at 9 and 16 o'clock.

Experiment Design

In this experiment, 600 zebrafish were randomly divided into four groups with three replications in 12 aquariums (50 fish per tank) containing 80 liters of water. The groups were as follows: Control group, Blank: Feeding with a basal diet for 60 days and 72 hours of exposure to cadmium (Cd) at a concentration of 7 g L^{-1}, T1: Feeding with probiotic diet (*L. fermentum* with a concentration of 1.5×10^4 CFU g^{-1}) for 60 days and 72 hours exposure to Cd at a concentration of 7 g L^{-1}, T2: Feeding with probiotic diet (*L. fermentum* with a concentration of 1.5×10^5 CFU g^{-1}) for 60 days and 72 hours of exposure to Cd at a concentration of 7 g L^{-1}.

Exposure to Cd

Cd poisoning was considered according to OECD Guideline 203 (Fish, acute toxicity test) (Organisation for Economic & Development, 1992). After 60 days of feeding with different diets under previous laboratory conditions (temperature 25 ±1°C and 14 hours light: 10 hours dark) Groups B, T1 and T2 were exposed to Cd at a concentration of 7 g L^{-1} for 72 hours (Yin *et al*., 2018). During the poisoning, 50% of the aquarium water was replaced with fresh water daily to keep the amount of Cd at the desired level and a new amount of Cd was added. During this period, the fish did not receive food (Gao *et al*., 2018).

Sample Collection

After 60 days of feeding with basic and probiotic diets, biometric indices including weight, total length, standard length, and fish fork length were measured. Cd accumulation in the gastrointestinal tract of fish was measured at zero, 24, 48, and 72 hours of poisoning. During each sampling, three fish were randomly caught from each treatment and examined after gastrointestinal isolation. The total number of fish deaths and survival rate after the onset of Cd poisoning were recorded daily and were reported at the end of the poisoning period. Three fish were randomly caught from each treatment to evaluate superoxide dismutase (SOD) and Malondialdehyde (MDA) at 0, 24, 48, and 72 hours of intoxication. The samples were immediately frozen in liquid nitrogen and stored in a freezer until -80°C (Yin *et al*., 2018).

Cd Accumulation

The gastrointestinal tract samples were first dried for 90 hours at 90°C. The samples were digested in 200 ml of HNO_3 for 12 hours at room temperature followed by 12 hours at 80°C to obtain a clear solution. After adding 1 ml of double-distilled water, the Cd content of the samples was determined by atomic absorption spectroscopy at a wavelength (λ) of 228: 8 nm (Hallare *et al*., 2005).

Antioxidant Response Analysis

After removing the samples from the freezer, the extracted gastrointestinal tracts were homogenized using 300 µl of ice-cold Tris-HCl buffer solution (containing 0.01M Tris-HCl, 0.1mM EDTA-2Na, 0.01M Sucrose, 0.8% sodium chloride solution, pH=7.4) to evaluate the activity of oxidative stress factors .The homogenized mixture was centrifuged at 3000 g for 15 minutes at 4 C° and the supernatant was quickly separated and analyzed by enzymatic analyses (Gao *et al*., 2018). Determination of MDA and SOD activity was performed according to the manufacturer's commercial kits (Zellbio) at 535 and 420 nm, respectively.

Statistical Analysis

The normality of the data was considered using the Kolmogorov-Smirnov test. The means were compared using Duncan's method at a significance level of 95% and factorial in a completely randomized design. The test results are shown as mean±standard deviation (Mean±SD) ($P<0.05$). Statistical analysis was performed using SPSS software version 21.0.

Results

Groth Rate

The results of the measured biometric indices at the end of 60 days are summarized in Table 1. The results of changes in fish weight (W) showed an increase in groups T1 and T3 compared to other groups ($P<0.05$). The standard length (SL) results showed an increase in groups T1 and T3 compared to other groups ($P<0.05$). The results of total length (TL) showed an increase in

groups T1 and T3 compared to other groups (P<0.05). The results of fork length (FL) also showed an increase in groups T1 and T3 compared to other groups (P<0.05).

Mortality Rate

Based on the results shown in Table 2, mortality and survival rate were observed in fish exposed to cadmium (Cd). The highest mortality rate was observed in group Blank, while the lowest mortality rate was recorded in group T1 (P<0.05).

Cd Accumulation

The results of the Cd accumulation in the fish gastrointestinal tract are summarized in Table 3. The results of Cd accumulation in different groups showed that there was no significant difference in different hours in group Control (P>0.05). A gradual increase was observed in group Blank at 48 hours (P<0.05) and a decrease in Cd was reported at 72 hours (P>0.05). A gradual increase in Cd accumulation was reported in group T1 (P<0.05). A gradual increase was observed in the T3 group at 48 hours (P<0.05) and a decrease in Cd accumulation was reported at 72 hours (P<0.05). Based on the results at different hours, no Cd was detected in the fish body at zero time. An increase in Cd accumulation was observed in the Blank, T1, and T2 at 24 and 72 hours (P>0.05). Also, the amount of Cd accumulation increased in the body of fish at 48 hours and the highest amount was recorded in groups Blank and T3 (P<0.05).

Superoxide Dismutase Activity

The results of superoxide dismutase (SOD) activity are summarized in Table 4. Based on the compared results in different groups, there was no significant difference in SOD activity of group Control at different hours (P>0.05). A gradual increase in SOD activity was observed in group Blank and the highest rate was recorded at 72 hours (P<0.05). A significant increase was reported in the T1 group (P>0.05). Also, a gradual increase was observed in group T3 at 48 hours (P<0.05), and a decrease in SOD activity was reported at 72 hours (P<0.05). Also, no significant differences were observed between the groups at zero time. There was an increase in SOD activity in the groups exposed to Cd at 24 hours and the highest amount was recorded in groups Blank and T3 (P<0.05). This increasing trend was continued in the groups exposed to Cd at 48 hours and the highest rate was recorded in the T3 group (P<0.05). Also, the highest SOD activity was recorded at 72 hours in group Blank (P<0.05), and no significant difference was observed between the other groups (P>0.05).

Table 1. Results of weight, total length (TL), standard length (SL), and fork length (FL) in different groups in zebrafish at the end of 60 days.

	FL	SL	TL	W
Blank	3.67 ± 0.08^{b}	3.46 ± 0.08^{b}	4.05 ± 0.07^{b}	0.87 ± 0.09^{b}
Control	3.47 ± 0.10^{b}	3.56 ± 0.07^{b}	4.10 ± 0.14^{b}	0.90 ± 0.04^{b}
T1	3.96 ± 0.05^{a}	3.85 ± 0.07^{a}	4.45 ± 0.07^{a}	1.12 ± 0.03^{a}
T3	3.92 ± 0.09^{a}	3.81 ± 0.05^{a}	4.37 ± 0.07^{a}	1.09 ± 0.01^{a}

Data are expressed as the mean±SD for each group. The letters a and b mean that for each parameter, groups with different letters differ significantly (P<0.05).

Table 2. Fish Mortality of the studied groups at the end of the cadmium exposure test in zebrafish.

	Mortality	Survival rate
Blank	12.00 ± 1.4^{a}	76%
Control	0.00 ± 0.00^{c}	100%
T1	7.50 ± 0.98^{b}	84%
T3	9.50 ± 0.7^{ab}	80%

Data are expressed as the mean±SD for each group. The letters a and b mean that for each parameter, groups with different letters differ significantly (P<0.05).

Table 3. Absorption of cadmium at different hours in groups.

	0	24	48	72
Blank	0.00 ± 0.00^{Ba}	1.00 ± 0.00^{Ba}	7.00 ± 2.82^{Aa}	3.50 ± 2.12^{ABa}
Control	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Aa}
T1	0.00 ± 0.00^{Ca}	1.00 ± 1.41^{Aa}	1.50 ± 2.12^{Ab}	2.00 ± 1.41^{Aa}
T3	0.00 ± 0.00^{Aa}	0.50 ± 0.70^{Aa}	7.00 ± 1.41^{Aa}	3.00 ± 1.41^{Ba}

Data are expressed as the mean±SD for each group. Different lowercase letters in each column and different uppercase letters in each row indicate significant differences (P<0.05).

Malondialdehyde

The results of the Malondialdehyde (MDA) test are summarized in Table 5. According to the results of the MDA, no significant difference was observed in group Control in different hours (P>0.05). Also, a gradual increase in MDA was observed in group Blank and the highest rate was recorded at 48 and 72 hours (P<0.05). A non-significant increase was reported in the T1 group (P>0.05). A gradual increase was observed in group T3 at 48 hours (P<0.05), and a decrease in MDA was reported at 72 hours (P<0.05). Also, no significant differences were observed between the groups at zero hours. A significant increase in MDA was observed in all groups exposed to Cd at 24 hours (P>0.05). The increasing trend continued in groups at 48 hours and the highest rate was recorded in groups Blank and T3 (P<0.05). The highest amount of MDA was recorded in group Blank at 72 hours (P<0.05), and no significant difference was observed between the other groups (P>0.05).

Discussion

Feeding with the *Lactobacillus fermentum (L. fermentum)* probiotic led to a significant increase in length and weight growth in fish. The increased growth rate is the first positive effect observed by the use of probiotics in the fish diet (Alavinezhad *et al*., 2020; Carnevali *et al*., 2017). The positive effect of probiotic nutrition on increasing fish growth has also been reported in the studies of Krishnaveni *et al*. (2021); Tachibana *et al*. (2020); Zang et al. (2019), Tachibana *et al*. (2020), and Krishnaveni *et al*. (2021). Effective mechanisms in improving growth by feeding on probiotics include detoxification of nutrients, production of enzymes to break down indigestible compounds in the diet such as amylase and protease, and production of vitamins (such as biotin and B12) by probiotic microorganisms that help improve host growth (Balcázar *et al*., 2006; Fuller, 1989; Suzer *et al*., 2008). The use of probiotics in fish feeding also increases the length of intestinal villi and thus increases the absorption level and leads to more efficient use of food sources (Pirarat *et al*., 2011).

Based on the results obtained, the fish loss rate was significantly reduced in the groups fed with a diet containing probiotics in the exposure to acute cadmium (Cd) concentrations. Similar results have been reported in fish exposed to Cd in the study by Guo *et al*. (2018). The effect of probiotic nutrition on reducing fish losses in the presence of cadmium can also be seen in the results of the study by Zhai *et al*. (2017), which is consistent with the present results. In this study, contamination with Cd led to the accumulation of this heavy metal in the gastrointestinal tract of fish, which is consistent with the earlier studies (Dang & Wang, 2009) .Previous studies have shown that Cd can be absorbed and accumulated in tissues, especially the intestine, from the earliest hours of exposure (Souid *et al*., 2013) because non-essential metals usually remain in the intestinal mucosa (Clearwater *et al*., 2002). In addition, the metal is uptake in other organs such as the liver and kidneys (Marijić & Raspor, 2006). This process of metal uptake needs further investigation in various organs of the studied zebrafish. The results of the study by Souid *et al*. (2013) showed that the Cd accumulation in different tissues is mainly dependent on the length of the metal exposure period, which is consistent with the results of the present study. Evans *et al*. (2005) showed that different diets can show obvious differences in the accumulation of contaminants. According to the results of this study, the Cd accumulation rate was lower compared to the blank group in the tissues of probiotic-

Table 4. Effect of probiotic *L. fermentum* on the superoxide dismutase (SOD) levels in the gastrointestinal tract of zebrafish at different times (hours).

	0	24	48	72
Blank	15.96 ± 1.07^{Ca}	30.59 ± 2.24^{Ba}	28.75 ± 1.76^{Bab}	44.66 ± 5.19^{Aa}
Control	15.50 ± 0.70^{Aa}	17.43 ± 2.72^{Ab}	17.43 ± 3.43^{Ac}	17.40 ± 1.97^{Ab}
T1	17.00 ± 1.41^{Aa}	21.16 ± 3.05^{Ab}	22.60 ± 1.97^{Abc}	19.50 ± 2.12^{Ab}
T3	14.00 ± 1.01^{Ba}	32.05 ± 2.05^{Aa}	35.00 ± 4.24^{Aa}	16.00 ± 1.41^{Bb}

Data are presented as the Mean±SD for each group. The same letters mean no difference and different letters mean a significant difference at the 5% level between experimental regions (P<0.05).

Table 5. Effect of probiotic *L. fermentum* on the Malondialdehyde (MDA) levels in the gastrointestinal tract of zebrafish at different times (hours).

	0	24	48	72
Blank	0.11 ± 0.014^{Ba}	$0.25\pm0.07A^{Ba}$	0.28 ± 0.04^{Aa}	0.32 ± 0.07^{Aa}
Control	0.144 ± 0.019^{Aa}	0.12 ± 0.06^{Aa}	0.13 ± 0.03^{Ab}	0.12 ± 0.03^{Ab}
T1	0.163 ± 0.018^{Aa}	0.18 ± 0.04^{Aa}	0.19 ± 0.05^{Aab}	$.08\pm0.04^{Ab}$
T3	0.113 ± 0.035^{Ba}	$0.20\pm0.03A^{Ba}$	0.25 ± 0.01^{Aa}	0.11 ± 0.04^{Bb}

Data are presented as the Mean±SD for each group. The same letters mean no difference and different letters mean a significant difference at the 5% level between experimental regions (P<0.05).

fed fish at the end of poisoning, although this difference was not significant. In the study of the probiotic's function in preventing the uptake of Cd by other methods, it was found that probiotics can inhibit Cd uptake by protecting the intestinal barrier and preventing tight junctions of the intestinal epithelium (Zhai *et al*., 2014; Zhai *et al*., 2013). This finding could be one of the factors involved in reducing metal uptake in the tissue of fish receiving probiotics. In addition to damaging the intestinal bacterial flora, Cd reduces the metabolism of short-chain carbohydrates and fatty acids (Liu *et al*., 2014), while probiotics multiply during feeding inside the host intestine and increase saccharolytic metabolism (Amaretti *et al*., 2013), and use sugars to make short-chain fatty acids, which are the main source of energy for intestinal epithelial cells.Thus they play an important role in improving the intestinal structure (Pelicano *et al*., 2005).

Based on the results, Cd can be absorbed by transcellular and paracellular in the intestine (Vesey, 2010; Zalups & Ahmad, 2003), which indicates the importance of intestinal barrier health to limit the absorption of this metal and is an acceptable reason for the results of the present study based on the positive antioxidant effects and reduction of Cd accumulation in tissues during feeding with probiotics. (Ayyat *et al*., 2017) examined the protective effect of a probiotic supplement on Cd-induced toxicity in rats and found that probiotics may have excreted Cd through feces by binding to the intestinal cell wall. Simultaneously, the amount of this metal in the liver, kidneys, and blood decreased compared to the group that received only Cd. This finding can be studied by fecal examination in future studies.

Reactive oxygen species (ROS) are produced continuously in fish during phagocytosis of neutrophils and macrophages by a mechanism called respiratory bomb activity and during oxidative metabolism (Martínez-Álvarez *et al*., 2005). Oxidative stress occurs when ROS production is greater than its removal rate (Firuzi *et al*., 2011). Antioxidant supplements are used to prevent excessive ROS and cell damage which affect the activity of enzymatic antioxidants such as superoxide dismutase (SOD) and non-enzymatic (such as Malondialdehyde (MDA)) (Seifried *et al*., 2007). Induction of oxidative stress is one of the toxic mechanisms of Cd on vital organs of the host body (Liu *et al*., 2009; Thijssen *et al*., 2007). As a result, antioxidant factors are the first defense line against Cd metal (Basha & Rani, 2003). SOD is one of the most common enzymes that can protect living organisms against free radical damage (Li *et al*., 2008; Seifried *et al*., 2007). Based on the results, exposure to Cd leads to increased SOD activity, which is consistent with the findings of Liu *et al*. (2006). They showed that SOD activity in Mudskippers could be cited as a biomarker of oxidative stress due to Cd contamination in fish. The results also showed that feeding with the *L. fermentum* probiotic reduced the SOD activity so that no significant increase in SOD activity was observed in the T2 group during the exposure to Cd. The results of studies by Amaretti *et al*. (2013); Mikelsaar and Zilmer (2009) on the antioxidant effect of probiotics including *L. fermentum* in mice and humans, and the study of (Wang *et al*., 2013; Wang *et al*., 2009) on the antioxidant effect of *Lactobacillus fermentum* in pigs are consistent with the results of this study. Zhai *et al*. (2013) investigated the protective effects of *L. Plantarum* on acute Cd toxicity in mice. The results showed that the use of this probiotic effectively reduces hepatic oxidative stress that can be used as a new treatment strategy in the exposure to acute Cd poisoning. Despite the differences in the host and the type of probiotic used, these studies confirm the antioxidant performance of probiotics in the exposure of Cd and are consistent with the results of the present study. Numerous studies have been performed on the mechanisms of probiotic action to reduce oxidative stress. According to (Zhai *et al*., 2017), exposure to Cd causes a severe reduction in intestinal microbial diversity and significant changes in the intestinal microbiota composition of fish. Therefore, considering the undeniable importance of the bacterial flora of fish intestines and its role in improving the function of the immune system, the effect of probiotic modulators on the microbial flora of the host intestine and improving the immune system function can be mentioned (Gómez & Balcázar, 2008; Zhai *et al*., 2017).

Also, the protection of probiotics from the intestinal barrier can inhibit the absorption of Cd and reduce the oxidative stress caused by exposure to Cd (Zhai *et al*., 2016). According to the results of Zhai *et al*. (2015), the binding of *L. Plantarum* to Cd is the reason for the antioxidant effect observed in intestinal cells *in-vitro*. The results also showed that Cd was removed from the culture medium using seven strains of *L. Plantarum* and *Lactobacillus fermentum* (Kirillova *et al*., 2017). Despite the differences in the type of probiotics and test conditions, these cases could be one of the reasons for the decrease in SOD activity in the groups receiving *L. fermentum* compared to the blank group. Initially, the activity of the SOD enzyme increased in the groups receiving a probiotic diet after Cd poisoning. However, according to the results of the last sampling, these amounts decreased at the end of the poisoning and reached a level close to the control group while the increasing trend of SOD activity in the Blank group continued until the end of the test. No similar report has been observed during the previous studies, but due to oxidative damage to biological molecules and impaired cellular function due to long-term increase in antioxidant enzymes (Guo *et al*., 2010), host immune function improves as a result of feeding with probiotics, which leads to a rapid and balanced response to oxidative stress and return the antioxidant factors to normal.

In the present study, the level of MDA indicated different levels of lipid peroxidation in the studied groups. Free radicals cause the peroxidation of lipids in

an organism MDA is one of the final products of polyunsaturated fatty acids peroxidation. The increase in free radicals causes the overproduction of MDA. As a result, the level of this factor is commonly known as an indicator of oxidative stress and antioxidant status (Gaweł *et al*., 2004). According to the results, the MDA production in the Blank group increased significantly after exposure to Cd. This result has also confirmed the study of Pandey *et al*. (2008); Souid *et al*. (2013). Acute Cd contamination causes lipid peroxidation and increases the levels of MDA, which may be due to redox metal ions or a decrease in glutathione content (Souid *et al*., 2013). Based on the study by (Souid *et al*., 2013), the probiotic *Lactobacillus fermentum* reduced the amount of MDA at the time of exposure to Cd compared to the blank group, which was consistent with the SOD changes in the present study. The study by Zhai *et al*. (2014) is one of the important studies on the probiotic nutrition effect on reducing MDA levels in the exposure to Cd.

Conclusions

Exposure to heavy metals causes interference in the health of organisms living in aquatic ecosystems, including fish. In addition, the consumption of fish contaminated with heavy metals is a potential threat to public health. In this regard, it is necessary to use economic, accessible, and sustainable strategies to reduce complications caused by heavy metal poisoning in aquaculture. The results of the present study revealed the ability of L. fermentum probiotic to growth performance, reduce mortality rate, increase survival rate, and improve the antioxidant defense in zebrafish in exposure to Cd. The prospect of using L. fermentum as an immune system stimulant in the exposure to Cd is revealed by the ability of this probiotic to modulate the oxidative parameters in fish. However, it is necessary to carry out additional investigations to identify the mechanism of action of this probiotic in improving the growth performance and immune system of fish under heavy metal poisoning.

Ethical Statement

Animal handling and tissue sampling procedures were carried out under the standard principles of laboratory animal care to reduce animal suffering; and the study was approved by the local ethics committee of the Faculty of veterinary Medicine, Islamic Azad University (reference number IR.IAU.SRB.REC.2949436). For euthanasia, the fish were first anesthetized with clove powder.

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Author Contribution

M.M.A.: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, R.K.: Conceptualization, Supervision, Writing – review & editing., N.H.S.: Conceptualization, Supervision, Formal analysis, Writing – review & editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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