**PROTECTIVE EFFECTS OF NON-ENCAPSULATED
AND MICROENCAPSULATED *LACTOBACILLUS DELBRUECKII* SUBSP.
BULGARICUS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)
EXPOSED TO LEAD (PB) VIA DIET**

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Abstract

The present study was designed to investigate the effects of dietary non-encapsulated and microencapsulated *Lactobacillus delbrueckii* subsp. *bulgaricus* on growth performance, intestinal enzymatic activities, antioxidant capacity and hepato-biochemical parameters of rainbow trout before or after exposure to lead via diet. Fingerling fish (16 ± 4 g) were divided into four groups: negative control (NC), positive control (PC), probiotic (PR) and encapsulated probiotic (EN-PR). During the pre-exposure period (days 0–45), fish in the NC and PC groups received the basal diet, whereas fish in the PR and EN-PR groups were fed with basal diet containing 10^8 CFU g⁻¹ feed of non-encapsulated and microencapsulated probiotic, respectively. During the exposure period (days 46–66), the fish in the probiotic and PC groups were co-treated with $500 \mu\text{g g}^{-1}$ feed of lead nitrate. Blood, liver and gut samples were taken at days 0, 45, 52, 59 and 66. The results revealed that growth performance and intestinal enzymatic activities were significantly ($P < 0.05$) improved in the probiotic groups compared to the NC group (day 45). Dietary exposure to lead resulted in the highest levels of liver aspartate aminotransferase (AST), liver alkaline phosphatase (ALP) and serum malondialdehyde (MDA), and the lowest activities of serum superoxide dismutase (SOD) and catalase (CAT) in the PC group (day 66). The levels of liver ALP were significantly ($P < 0.05$) lower in the probiotic groups compared to the NC and PC groups prior to and after exposure to dietary lead. Serum levels of total protein, albumin, SOD, CAT and glutathione (GSH) were significantly increased in fish fed with both non-encapsulated and microencapsulated probiotics ($P < 0.05$). However, microencapsulated probiotic showed the greatest potential for alleviation of the disturbed activities of intestinal and hepatic enzymes, and improvement of serum biochemical and antioxidant parameters. Our findings suggest that *L. delbrueckii* subsp. *bulgaricus*, particularly in the microencapsulated form, can be used as a potential probiotic to protect rainbow trout from dietborne lead toxicity.

Key words: *Lactobacillus delbrueckii* subsp. *bulgaricus*, growth, lead, antioxidant, serum, rainbow trout

Rapid development of industrialization and urbanization has contributed to contamination of the environment (Bi et al., 2018). Environmental contaminants are ubiquitous and exposure to them is unavoidable. Contaminants that can cause detrimental effects on the environment are regarded as environmental pollutants (Masindi and Muedi, 2018). Among others, heavy metals are well-studied environmental pollutants that have potential toxicity, non-biodegradable properties, bioaccumulative nature and high persistence in the environment (Ali et al., 2019). Many heavy metals at trace levels are essential to maintain the body metabolism, but they are toxic at higher concentration. However, certain heavy metals such as lead have no known biological function (Zoghi et al., 2014). As a long-lasting pollutant, lead can cause several dysfunctions and pathologies in humans and aquatic animals (Puzas et al., 2004; Bhakta et al., 2012; Kirillova et al., 2017; Lee et al., 2019). Exposure to toxic concentrations of lead can result in damage to several tissues and leakage of cellular enzymes to the blood circulation (Li et al., 2017). The mechanism of lead toxicity has been attributed to direct damage to mitochondria, disturbance in calcium homeostasis, competition for zinc binding sites, disruption of the intracellular sulfhydryl homeostasis and induction of oxidative stress (Dabrowska-Bouta et al., 1996; Ahamed et al., 2007; Zhai et al., 2015). In general, heavy metals tend to accumulate in fish body which can result in chronic toxicity (Bhakta et al., 2012). Clinical signs related to chronic lead poisoning in fish have been reported previously. These include lordoscoliosis, darkening in caudal-peduncle region, growth retardation, anemia, hyperactivity, loss of equilibrium, muscular atrophy, hemorrhage at the base of the caudal fin and paralysis (in severe cases) (Mager, 2012).

Therapeutic and preventive strategies for control of heavy metal poisoning have been investigated in previous studies. For example, chelation therapy, as the commonly used therapeutic strategy, has been reported to enhance metal elimination (Bhattacharya, 2019). However, clinical use of chelating agents has been limited with respect to their potentially adverse effects, non-specific removal of heavy metals and loss of essential metals, and uncertainty for their safety and efficacy (Tian et al., 2012). On the other hand, dietary supplements have high potential for prevention or alleviation of metal toxicity. In this regard, the protective effects of dietary trace elements (zinc, selenium, magnesium, calcium and iron), vitamins (vitamins C, E, B₁, B₆), pigments (β -carotene), medicinal plants, phytochemicals, probiotics and microalga (*Spirulina*) have been demonstrated against exposure to heavy metals in various animal models (Ahamed et al., 2007; Zhai et al., 2015). Metal-binding probiotics, among other supplements, have high abilities for direct removal of heavy metals from liquid food and water (Mrvčić et al., 2012). Moreover, administration of these kinds of probiotics can improve the detrimental effects induced by heavy metal exposure in the body. Therefore, application of metal-removing probiotics is considered to be a promising method for decontamination of heavy metals (Kirillova et al., 2017).

The effectiveness of probiotics belonging to the genus *Lactobacillus* has been previously demonstrated against waterborne metal toxicity in fish models (Zhai et al., 2016; Yu et al., 2017; Giri et al., 2018). On the other hand, fish can be exposed to impermissible or intolerable levels of heavy metals such as lead from different

sources of the diet including fish meal and feed additives (binders and vitamin/mineral pre-mixtures) (Dai et al., 2016; Adamse et al., 2017; Janbakhsh et al., 2018). However, to our knowledge, little is known about the protective effects of *Lactobacillus* species (spp.) against dietborne lead toxicity in fish.

Regarding the fact that survivability of functional probiotics may be affected prior to their colonization in the host intestinal tract, encapsulation of probiotics has been suggested as a useful strategy for protection of probiont cells (Kailasapathy, 2002; Anal and Singh, 2007). In this context, encapsulated *L. rhamnosus* more effectively improved the health status of rainbow trout compared to non-encapsulated probiotic (Hooshyar et al., 2020). Nevertheless, there exists a paucity of studies pertaining to the potential effects of encapsulated probiotics against dietary exposure to heavy metals in fish. The aim of this study therefore was to investigate the possible effects of non-encapsulated and microencapsulated *L. delbrueckii* subsp. *bulgaricus* on growth performance, intestinal enzymes activity, antioxidant capacity, hepatic enzymes activity and serum biochemical parameters of rainbow trout before or after chronic toxicity of lead via the diet. Our findings suggest that dietary supplementation with *L. delbrueckii* subsp. *bulgaricus*, particularly in the microencapsulated form, can positively alter physiological responses of rainbow trout against dietborne lead toxicity.

Material and methods

Probiotic preparation and microencapsulation

Lactobacillus delbrueckii subsp. *bulgaricus* used in the present study was isolated from the intestine of a freshwater fish (*Tor grypus*) and identified using species-specific primers as described previously (Mohammadian et al., 2016). The beneficial effects of the strain on fish performance and its protective effects against lead toxicity were demonstrated in previous studies (Mohammadian et al., 2016, 2019 a, b). Overnight cultures of the bacterial strain in deMan, Rogosa, and Sharpe (MRS) broth were centrifuged at 3000 g for 15 min, and then the obtained pellets were washed three times, resuspended in sterile normal saline and adjusted at final concentration of 3×10^9 CFU ml⁻¹ by the spread plate method. The probiont strain was encapsulated according to the method described by Song et al. (2013) with modification. Briefly, 3.5 ml of a suspension containing 5 g calcium carbonate (Sigma-Aldrich, Germany) in 100 ml of distilled water was added to 55 ml of sodium alginate solution (2%, viscosity: 200–400 centipoise l⁻¹, Sigma-Aldrich, Germany). Thereafter, 10 ml of the resultant solution was combined with 5 ml of the bacterial suspension, and the alginate-cell mixture was emulsified in 45 ml of olive oil containing Span 80 (0.5%, Sigma-Aldrich). Afterwards, a total of 10 ml of olive oil and 0.5 ml of acetic acid were combined and then added dropwise to above mixture by stirring at 200 rpm for 30 min (Ahmed et al., 2021). After separation of the oil phase from the mixture by centrifugation for 5 min, 15 ml of chitosan solution (0.4%, Sigma-Aldrich, pH: 4) was gently added and the mixture was stirred at 800 rpm for 30 min. Finally, the encapsulated bacteria were harvested by centrifugation at 1000 g for 15 min.

Preparation of experimental diets

A commercial pellet feed (proximate composition on a dry matter basis: 53% crude protein, 17% crude lipid, 11% ash, 1.6% total phosphorus, 22 KJ g⁻¹ feed gross energy; Biomar, France) was used in the present study. In order to prepare the experimental diets, the pellets were crushed and the feed particles were passed gently through a 150 µm mesh sieve. The appropriate volume of sterile distilled water was added to the uniform feed particles to prepare stiff dough. It was subsequently passed through a laboratory pellet maker to obtain the feed pellets with a 2 mm diameter (Noor-Ul et al., 2020). The pellets were dried in an oven at 37°C for overnight and used as the basal diet. Fish in the NC group were fed with the basal diet throughout the experimental period (66 days). Fish in the PC group were fed with the basal diet for 45 days, and then received the basal diet containing 500 µg g⁻¹ feed of lead nitrate (Pb(NO₃)₂, Sigma, Germany) for another 21 days (Alves et al., 2006). Prior to administration of dietary lead, lead nitrate was dissolved in sterile distilled water and then added to the feed particles of the basal diet at final concentration of 500 µg g⁻¹ feed (Alves et al., 2006). To prepare the probiotic diets, the required volume of the suspensions containing 3×10^9 CFU ml⁻¹ of *L. delbrueckii* subsp. *bulgaricus*, either microencapsulated or non-encapsulated, were incorporated to the feed particles of the basal diet. This allowed the final concentrations of the probiotics to be adjusted at 1×10^8 CFU g⁻¹ feed (Mohammadian et al., 2019 a). Fish in the PR and EN-PR groups were then fed with diets supplemented with 1×10^8 CFU g⁻¹ feed of *L. delbrueckii* subsp. *bulgaricus* and microencapsulated *L. delbrueckii* subsp. *bulgaricus* for 45 days, respectively. Subsequently, lead nitrate (500 µg g⁻¹ feed) was also incorporated into the diets of the probiotic treatments for a period of 21 days. Administration of lead in the diet of the probiotic groups was similar to that of the positive control group. The experimental design has been summarized in Table 1. In order to ensure the inoculation of lead into diets, the food pellets were heat-digested by nitric acid (HNO₃) and the digested samples were then centrifuged at 10,000 g for 25 min. The resultant supernatant was used to determine the levels of lead in the food samples using an atomic absorption spectrometer (AA-6300, Shimadzu, Japan) (Zhai et al., 2017).

Table 1. The experimental design of the present study

Exposure period (days 46–66)		Pre-exposure period (days 0–45)		Groups
Lead	Probiotic	Lead	Probiotic	
–	–	–	–	Negative control
+	–	–	–	Positive control
+	+	–	+	Probiotic
+	+	–	+	Encapsulated probiotic

Fish maintenance

Fingerling rainbow trout were obtained from a local farm (Dezful, Khuzestan, Iran) and transferred to the laboratory. Fish were acclimatized for 14 days prior to commencement of the experiment, and then a total of 300 rainbow trout with an

average weight of 16 ± 4 g were randomly divided into twelve round fiberglass tanks (300 l) with three replicate tanks per treatment at final density of 25 fish per tank (75 fish per treatment). Fish were fed with the assigned diets based upon 2.5% of their body weight twice per day. After feeding, the remaining feed was siphoned out and then dried to accurately calculate the amount of feed consumed. During the adaptation and experimental periods, the tanks were supplied with dechlorinated and aerated tap water, and physicochemical parameters of the water were monitored during the whole experiment and maintained within a normal range as follows: dissolved oxygen, 7.5–8 ppm; temperature, $14 \pm 1^\circ\text{C}$; pH, 7 ± 0.5 ; $\text{NO}_2\text{-N}$, $<0.05 \text{ mg l}^{-1}$; $\text{NH}_3\text{-N}$, $<0.02 \text{ mg l}^{-1}$.

Growth performance

After being fasted for 24 h, the fish from each group were weighed at days 0 and 45. The growth parameters were subsequently calculated using the following formula:

$$\text{Weight gain percentage (WGP): } [(FW - IW) / IW] \times 100;$$

where: *FW* and *IW* are final weight (g) and initial weight (g), respectively.

Condition factor (CF): $(FW \times 100) / \text{standard length}^3$ (cm).

Specific growth rate (SGR): $[(\ln FW - \ln IW) \times 100] / \text{experimental period (d)}$.

Feed conversion ratio (FCR): $\text{feed consumed (g)} / (FW - IW)$.

Protein efficiency ratio (PER): $(FW - IW) / \text{protein intake (g)}$.

Daily weight gain (DWG): $(FW - IW) / \text{experimental period (d)}$.

Feed efficiency ratio (FER): $[(FW - IW) \times 100] / \text{feed consumed (g)}$.

Sample collection

Blood, liver and gut samples were taken at days 0, 45, 52, 59 and 66 (3 fish per replicate, 9 fish per treatment). Prior to sampling, all fish were starved for 24 h and then anesthetized in water containing 0.3 ml l^{-1} of 2-phenoxyethanol (Shanghai Reagent, China) (Velišek and Svobodová, 2004). Blood samples were collected from the caudal vein using a 3 ml syringe (AVA Luer lock, Tehran, Iran). The blood collected was transferred to Eppendorf tubes, allowed to clot at 4°C for 60 min and then centrifuged ($3,000 \text{ g}$ for 10 min at 4°C) to separate serum for assessment of the biochemical and oxidative stress parameters. The sera were stored at -80°C for further analysis. Thereafter, fish were aseptically dissected and the specimens of liver and foregut were removed to measure the activities of hepatic and intestinal enzymes, respectively (Mohammadian et al., 2021).

Intestinal enzymes activity

The intestinal samples were initially homogenized in a cold homogenization buffer containing 100 mM Tris-HCl, 0.1 mM EDTA and 0.1% Triton X-100 (pH=7.8, 1:10 v:w), and then the homogenates were centrifuged at $13,500 \text{ g}$ for 30 minutes at 4°C . The obtained supernatants were collected and placed at -80°C for further tests. Total protein content of the supernatants was measured by the Bradford's method

using bovine serum albumin as standard. The activities of trypsin, α -amylase, lipase and ALP were measured according to Serrano et al. (2018) and Hahor et al. (2019). Trypsin activity was determined using BAEE (N α -benzoyl-L-arginine ethyl ester) as the substrate. α -amylase activity was measured using soluble starch as a substrate hydrolysable to maltose. Lipase activity was calculated based on the measurement of fatty acids resulting from enzymatic hydrolysis of triglycerides present in olive oil emulsion. The activity of intestinal ALP was quantified using p-nitrophenyl phosphate (PNPP) as substrate by a commercially colorimetric kit (Pars Azmoon Co., Tehran, Iran).

Serum biochemical parameters and hepatic enzymes activity

The concentrations of glucose, total protein, albumin, calcium, phosphorus, magnesium, triglyceride and cholesterol in serum were measured spectrophotometrically (Unico, UV-2802S; Shanghai, China) using commercially available kits according to the manufacturer's guidelines (Pars Azmoon Co., Tehran, Iran). The levels of total protein, albumin, glucose, calcium, triglyceride and cholesterol were determined using the standard methods as described previously (Vazirzadeh et al., 2020). Evaluation of the levels of magnesium and phosphorus in serum was performed by the xylidyl blue and molybdate UV methods, respectively (Chromý et al., 1973; Berti et al., 1988).

The activities of liver enzymes including lactate dehydrogenase (LDH), ALT, AST and ALP were kinetically measured by commercial kits according to the manufacturer's guidelines (Pars Azmoon Co., Tehran, Iran). The activities of serum AST and ALT were determined following the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) recommendations. Serum ALP and LDH were measured according to the standard method suggested by DGKC (Deutsche Gesellschaft für Klinische Chemie) (Safari et al., 2016).

Oxidative stress markers

Serum CAT activity was measured by the method described by Kurhaluk et al. (2017). CAT activity was estimated by the decrease of hydrogen peroxide in the reaction mixture following the development of the yellow complex resulting from the reaction between ammonium molybdate and hydrogen peroxide at 410 nm. One unit of CAT activity is defined as the amount of enzyme required for catalytic decomposition of one μ mol hydrogen peroxide per min per mg of protein. The activity of serum total SOD (CuZnSOD and MnSOD) was determined by the hydroxylamine method as suggested by Mittal et al. (2008). The rate of the reduction of nitro blue tetrazolium (NBT), which is mediated by hydroxylamine hydrochloride by production of a blue color, was measured at 560 nm. One unit of SOD is defined as the amount of enzyme which leads to 50% inhibition of NBT reduction. Ellman's assay was used for quantification of the thiol groups present in GSH according to the reaction between the thiol groups and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB, Sigma, Germany) at slightly alkaline condition (Tipple and Rogers, 2012). The absorbance of the resultant TNB (5-nitro-2-thiobenzoic acid) was subsequently measured by a spectrophotometer at 412 nm. The concentration of free thiols in the samples was

determined using a calibration curve generated from the absorbance values of the standards versus cysteine concentrations. The levels of MDA in serum samples were assayed by the thiobarbituric acid reactive substances (TBARS) assay (Naderi et al., 2019). The concentration of MDA was calculated using the molar extinction coefficient of MDA ($156,000 \text{ M}^{-1} \text{ cm}^{-1}$).

Data analysis

The results were analyzed by SPSS software version 22 and expressed as mean $\pm$ standard deviation (SD). All data were checked for normal distribution using the Shapiro-Wilk test. The results obtained from the measurement of growth performance, digestive enzymes activity, hepatic enzymes activity, oxidative stress markers and serum biochemical parameters were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine the differences of the mean values of tested parameters. Differences were considered significant at $P < 0.05$.

Results

Growth performance

As summarized in Table 2, dietary supplementation with microencapsulated or non-encapsulated probiotic significantly promoted WGP, SGR, FCR, PER and FER in the probiotic groups compared to the NC group ($P < 0.05$). However, PER and FER were significantly higher in the PR group than the EN-PR group ($P < 0.05$). Moreover, improved levels of CF and DWG were observed after feeding of fish with diets supplemented with microencapsulated or non-encapsulated *L. delbrueckii* subsp. *bulgaricus*. However, significant differences of these parameters were observed only in the PR group relative to the NC group ($P < 0.05$).

Table 2. Growth performance of rainbow trout fed with or without dietary *L. delbrueckii* subsp. *bulgaricus* for 45 days

Parameter	Negative control	Probiotic	Encapsulated probiotic
WGP (%)	104.22 $\pm$ 8.29 b	201.04 $\pm$ 2.08 a	193.93 $\pm$ 8.89 a
CF (%)	1.02 $\pm$ 0.08 b	1.31 $\pm$ 0.01 a	1.18 $\pm$ 0.12 ab
SGR (%)	1.60 $\pm$ 0.16 b	2.46 $\pm$ 0.23 a	2.42 $\pm$ 0.25 a
FCR (g g ⁻¹)	1.66 $\pm$ 0.21 a	0.59 $\pm$ 0.05 b	0.77 $\pm$ 0.07 b
PER (g g ⁻¹)	1.72 $\pm$ 0.24 c	4.22 $\pm$ 0.36 a	3.37 $\pm$ 0.28 b
DWG (g d ⁻¹)	0.51 $\pm$ 0.19 b	0.92 $\pm$ 0.15 a	0.86 $\pm$ 0.06 ab
FER (%)	69.15 $\pm$ 7.47 c	168.92 $\pm$ 14.54 a	134.73 $\pm$ 10.58 b

Data indicate mean $\pm$ SD of three tanks per treatment (n = 3). Various lowercase letters denote significant differences between the values in each row ($P < 0.05$). WGP (weight gain percentage); CF (condition factor); SGR (specific growth rate); FCR (feed conversion ratio); PER (protein efficiency ratio); DWG (daily weight gain); FER (feed efficiency ratio).

Digestive enzymes activity

As shown in Figure 1, fish fed with probiotic (non-encapsulated or microencapsulated) for 45 days had significantly higher activities of trypsin, lipase, ALP and α -amylase compared to those receiving the basal diet ($P < 0.05$). The activities of trypsin, lipase and ALP were reduced in the probiotic groups after exposure to lead (days 59 and 66) compared to pre-exposure time (day 45), whereas the activities of lipase at day 52 and ALP at days 52 and 59 were significantly higher in the probiotic groups than the PC group ($P < 0.05$). The levels of α -amylase activity were significantly higher in the probiotic treatments relative to the PC group after administration of lead ($P < 0.05$). Moreover, significantly higher activities of α -amylase and lipase (days 59 and 66) were recorded in the EN-PR group compared to the PR group ($P < 0.05$). No significant changes in the levels of trypsin and ALP were observed between the NC and PC groups ($P > 0.05$). However, significantly higher levels of α -amylase (days 52 and 59) and lower activities of lipase (days 52 and 66) were observed in the PC group compared to the NC group ($P < 0.05$).

Figure 1. Intestinal enzymes activity (unit mg pro⁻¹) in rainbow trout fed with or without dietary *L. delbrueckii* subsp. *bulgaricus* before and after dietary exposure to lead. Data represent the mean $\pm$ SD ($n = 9$). Different capital letters represent the significant differences between each of the treatments at various sampling times ($P < 0.05$). Different lowercase letters denote significant differences between the treatments at a specified time ($P < 0.05$)

Liver enzymes activity

After 45 days feeding with the experimental diets (Table 3), significantly lower levels of ALP, LDH and ALT were observed in both probiotic groups (PR and EN-PR) compared to the control group ($P < 0.05$). No significant change in the level of liver AST was detected between the experimental groups at day 45 ($P > 0.05$). Following dietary exposure to lead, the levels of ALP (day 66) and AST (days 52, 59

and 66) were significantly higher in the PC group than the NC group ($P<0.05$). On the other hand, reduced values of ALP and AST (days 52, 59 and 66) were recorded in the probiotic groups compared to the PC group ($P<0.05$). The lowest activities of hepatic ALP (days 59 and 66) and AST (days 52 and 66) were detected in fish fed with microencapsulated probiotic ($P<0.05$). A significant increase in the ratio of AST/ALT was observed in the EN-PR group relative to the NC and PR groups before administration of lead ($P<0.05$). After incorporation of lead to diet, the highest values in the ratio of AST/ALT were recorded in the PC group (days 52, 59 and 66), whereas the lowest levels of this ratio were observed in the EN-PR group (day 66) ($P<0.05$).

Serum biochemical parameters

The results of assessment of serum biochemical parameters are presented in Table 4. Serum total protein and albumin were significantly ($P<0.05$) higher in fish supplemented with dietary probiotics than those receiving the basal diet prior to and after administration of lead at all intervals (except for total protein in the EN-PR group at day 45). The level of total protein was decreased in the PC group compared to the NC group, although this reduction was only significant at day 59 ($P<0.05$). Moreover, administration of lead resulted in the significant reduction of serum total protein and albumin in the PC group at day 66 relative to day 52 ($P<0.05$). Lower levels of serum cholesterol were noticed in the EN-PR group compared to the other groups at day 45 ($P<0.05$). Significantly reduced levels of serum cholesterol were observed in the PC group than the control group at all intervals after exposure to lead ($P<0.05$). Moreover, serum cholesterol was significantly higher in the probiotic groups than the PC group at day 66 ($P<0.05$). No remarkable alteration of serum glucose was observed between the treatments after feeding with the experimental diets for 45 days. However, significantly higher levels of glucose were detected in the probiotic groups relative to the control groups (day 66) after dietary exposure to lead ($P<0.05$). Moreover, glucose contents were significantly lower in the PC group than the NC group at day 59 ($P<0.05$). Serum triglycerides were significantly increased in the NC and EN-PR groups at day 45 relative to day 0 ($P<0.05$). However, the levels of serum triglycerides were significantly lower in the probiotic groups than the NC group at day 45 ($P<0.05$). Moreover, the values of serum triglycerides were significantly higher in the NC (days 59 and 66), PR (day 59) and EN-PR (days 59 and 66) groups relative to the PC group after dietary exposure to lead ($P<0.05$). As can be seen in Table 4, the levels of serum phosphorus and magnesium were elevated in the probiotic groups after 45 days, significantly higher than those in the NC group ($P<0.05$). Fish exposed to lead (PC and probiotic groups) had higher levels of serum phosphorus compared to non-exposed fish at days 52 and 66 ($P<0.05$). However, the content of serum phosphorus was significantly higher in the probiotic groups compared to the PC group after administration of lead (day 66) ($P<0.05$). The concentrations of serum calcium were significantly higher in fish fed with microencapsulated probiotic than those receiving the basal diet for 45 days ($P<0.05$). Although, no remarkable alterations in serum calcium and magnesium were observed between the treatments after exposure to lead.

Table 3. Liver enzymes activity in rainbow trout fed with or without dietary *L. delbrueckii* subsp. *bulgaricus* before and after dietary exposure to lead

Parameter	Treatments	Day 0	Day 45	Day 52	Day 59	Day 66
ALP (U l ⁻¹)	Negative control	275.94±5.63 Aa	282.33±13.43 Aa	281.03±11.01 Aa	292.84±17.25 Aa	279.17±7.77 Ab
	Positive control	ND	ND	277.71±17.79 Ba	223.68±9.01 Cb	389.83±13.44 Aa
	Probiotic	276.01±5.54 Aa	192.99±11.12 Cb	234.52±16.02 Bb	161.74±10.58 Dc	157.71±2.53 Dc
	Encapsulated probiotic	278.27±5.06 Aa	199.12±13.65 Cb	227.52±10.44 Bb	116.46±9.62 Dd	88.04±7.38 Ed
LDH (U l ⁻¹)	Negative control	19.32±1.58 ABa	18.04±2.22 Ba	17.84±1.30 Ba	21.90±2.90 Aa	17.81±2.52 Ba
	Positive control	ND	ND	13.54±1.34 Cb	20.61±1.76 Aa	16.24±1.50 Ba
	Probiotic	17.80±1.64 Aa	10.87±1.01 Bc	11.75±1.10 Bb	11.59±0.87 Bb	12.82±1.28 Bb
	Encapsulated probiotic	17.82±1.29 Aa	14.25±1.14 Bb	11.75±1.60 Cb	10.72±0.95 Cb	12.82±1.68 BCb
AST (U l ⁻¹)	Negative control	0.85±0.11 Ca	1.14±0.15 BCa	1.18±0.13 Bc	1.88±0.06 Ab	1.87±0.27 Ab
	Positive control	ND	ND	2.53±0.27 Ba	2.83±0.28 Aa	3.05±0.18 Aa
	Probiotic	0.88±0.13 Da	1.16±0.07 Ca	1.83±0.07 Ab	1.71±0.16 Ab	1.38±0.08 Bc
	Encapsulated probiotic	0.88±0.14 CDa	1.31±0.17 Ba	1.10±0.13 BCc	1.58±0.13 Ab	0.82±0.08 Dd
ALT (U l ⁻¹)	Negative control	1.67±0.06 Aa	1.68±0.09 Aa	1.72±0.35 Aa	1.49±0.10 Ab	1.68±0.08 Aa
	Positive control	ND	ND	1.74±0.14 Aa	1.66±0.08 ABa	1.58±0.11 Ba
	Probiotic	1.62±0.08 Aa	1.54±0.10 Ab	1.58±0.21 Aab	1.42±0.13 Ab	1.62±0.10 Aa
	Encapsulated probiotic	1.60±0.08 Aa	1.45±0.09 BCb	1.32±0.05 CDb	1.21±0.22 Dc	1.59±0.07 ABa
AST/ALT ratio	Negative control	0.53±0.08 Ba	0.69±0.02 Bb	0.71±0.09 Bc	1.20±0.03 Ab	1.12±0.16 Ab
	Positive control	ND	ND	1.44±0.15 Ba	1.72±0.18 Aa	1.91±0.19 Aa
	Probiotic	0.54±0.07 Ca	0.75±0.10 Bb	1.15±0.14 Aab	1.21±0.14 Ab	0.86±0.08 Bc
	Encapsulated probiotic	0.53±0.08 Ca	0.90±0.10 Ba	0.85±0.08 Bbc	1.29±0.14 Ab	0.52±0.04 Cd

Data were expressed as mean ± SD (n = 9). ND: not determined. Different capital letters represent significant differences between the values within each row (p<0.05). Different lowercase letters indicate significant differences between the values in each column (P<0.05). ALP (alkaline phosphatase); LDH (lactate dehydrogenase); AST (aspartate aminotransferase); ALT (alanine transaminase).

Table 4. Serum biochemical parameters in rainbow trout fed with or without dietary *L. delbrueckii* subsp. *bulgaricus* before and after dietary exposure to lead

Parameter	Treatments	Day 0	Day 45	Day 52	Day 59	Day 66
1	2	3	4	5	6	7
Total Protein (g dl ⁻¹)	Negative control	5.19±0.13 Aa	5.12±0.09 Ab	5.20±0.10 Ab	5.14±0.11 Ab	5.19±0.10 Ab
	Positive control	ND	ND	5.32±0.22 Ab	4.29±0.39 Bc	4.18±0.97 Bb
	Probiotic	5.18±0.14 Da	5.80±0.34 Ca	6.01±0.13 BCa	6.96±0.30 Aa	6.32±0.42 Ba
	Encapsulated probiotic	5.13 ±0.14 Ca	4.78±0.32 Cb	6.03±0.41 Ba	6.82±0.17 Aa	6.42±0.32 ABa
Albumin (g dl ⁻¹)	Negative control	1.95±0.11 Aa	1.96±0.16 Ab	1.96±0.16 Ab	1.93±0.11 Ab	2.02±0.07 Ac
	Positive control	ND	ND	2.02±0.13 Ab	1.75±0.08 ABb	1.58±0.38 Bc
	Probiotic	1.96±0.16 Da	2.55±0.20 Ca	3.03±0.28 BCa	3.95±0.25 Aa	3.29±0.50 Bb
	Encapsulated probiotic	1.96±0.15 Ca	2.79±0.34 Ba	2.92±0.18 Ba	3.76±0.13 Aa	4.05±0.19 Aa
Cholesterol (mg dl ⁻¹)	Negative control	162.60±5.28 Ca	219.89±10.88 Aa	219.88±10.88 Aa	182.61±17.40 Ba	221.87±10.89 Aa
	Positive control	ND	ND	140.30±6.38 Ab	144.33±5.87 Ab	139.60±5.02 Ab
	Probiotic	159.83±11.39 Ca	196.62±16.26 Bb	133.22±5.94 Dbc	144.39±13.39 CDb	231.80±14.49 Aa
	Encapsulated probiotic	156.82±8.42 Ba	140.30±16.12 BCc	118.81±8.87 Cc	140.30±16.12 BCb	244.22±18.03 Aa
Glucose (mg dl ⁻¹)	Negative control	55.46±7.53 Aa	47.23±2.22 Ab	47.23±2.22 Ab	55.46±7.52 Aa	46.35±1.25 Ab
	Positive control	ND	ND	45.00±5.35 Ab	42.91±5.82 Ab	40.39±7.34 Ab
	Probiotic	61.61±6.60 ABa	54.90±5.89 BCab	44.53±4.28 Db	45.42±3.38 CDab	69.52±6.36 Aa
	Encapsulated probiotic	60.90±7.40 BCa	59.17±8.73 BCa	88.34±8.86 Aa	54.50±7.30 Ca	70.42±7.38 Ba
Triglycerides (mg dl ⁻¹)	Negative control	178.40±15.07 Ba	253.25±10.63 Aa	253.25±36.44 Aab	235.50±12.75 Aa	243.72±12.45 Aa
	Positive control	ND	ND	217.01±19.79 Ab	178.39±12.75 Bb	172.79±13.48 Bb
	Probiotic	177.06±14.53 Ba	156.76±10.09 Bc	247.87±12.45 Aab	223.59±21.08 Aa	183.89±16.15 Bb
	Encapsulated probiotic	171.84±15.17 Ca	206.69±5.58 Bb	268.22±18.91 Aa	173.00±20.00 Cb	219.34±19.27 Ba

Table 4 – contd.

1	2	3	4	5	6	7
Phosphorus (mg dl ⁻¹)	Negative control	4.57±0.61 Ba	6.09±0.35 Ac	6.09±0.35 Ac	4.57±0.68 Bb	6.09±0.35 Ac
	Positive control	ND	ND	11.39±1.22 Ab	10.31±1.11 Aab	10.51±1.30 Ab
	Probiotic	4.65±0.63 Ca	11.89±1.06 Ba	16.14±1.26 Aa	10.63±0.64 Ba	15.04±2.02 Aa
	Encapsulated probiotic	4.89±0.49 Ca	10.37±0.85 Bb	12.64±1.19 Ab	8.77±1.16 Bb	14.10±0.96 Aa
Calcium (mg dl ⁻¹)	Negative control	9.28±0.54 Ba	8.44±0.88 Bb	8.46±0.75 Bb	12.66±1.42 Aa	8.44±0.96 Bb
	Positive control	ND	ND	10.96±1.02 Aa	10.59±0.77 Aa	10.68±1.28 Aa
	Probiotic	8.95±0.80 Ba	9.10±0.94 Bb	10.02±1.40 Bab	12.23±1.08 Aa	9.17±1.45 Bab
	Encapsulated probiotic	9.33±0.71 Ba	12.29±0.82 Aa	10.48±0.58 ABa	11.70±1.49 Aa	10.19±1.25 ABab
Magnesium (mg dl ⁻¹)	Negative control	1.88±0.18 Aa	2.07±0.18 Ab	2.07±0.18 Ab	1.49±0.14 Bb	2.07±0.18 Aa
	Positive control	ND	ND	1.91±0.12 Ab	1.96±0.12 Aa	1.88±0.22 Aa
	Probiotic	2.05±0.14 BCa	2.31±0.10 ABa	2.57±0.13 Aa	2.03±0.17 BCa	1.83±0.19 Ca
	Encapsulated probiotic	1.89±0.12 BCa	2.29±0.11 Aa	2.09±0.16 ABb	2.09±0.19 ABa	1.77±0.17 Ca

Data were expressed as mean ± SD (n = 9). ND: not determined. Different capital letters show significant differences between the values within each row (P<0.05). Various lowercase letters denote significant differences between the values in each column (P<0.05).

Oxidative stress markers

Figure 2 represents the data related to the measurement of oxidative stress responses. The activities of serum SOD were significantly increased following the administration of dietary probiotic (non-encapsulated and microencapsulated) before and after administration of lead ($P < 0.05$). However, the highest SOD activities were observed in fish fed with microencapsulated probiotic at all intervals (days 52, 56 and 66) after dietary exposure to lead ($P < 0.05$). Serum CAT activities had no significant differences between the experimental groups after 45 days feeding ($P > 0.05$). On the other hand, serum CAT activities were significantly higher in the probiotic groups (days 59 and 66) relative to the PC group after administration of dietary lead ($P < 0.05$). Fish exposed to dietary lead (PC group) for 3 weeks (day 66) had significantly ($P < 0.05$) lower levels of serum CAT and SOD compared to those exposed for 1 week (day 52). Serum GSH levels were significantly higher in the EN-PR group compared to the PR and NC groups at day 45 ($P < 0.05$). Moreover, serum GSH content was significantly higher in the probiotic treatments compared to the PC group at days 52, 59 and 66 ($P < 0.05$). Feed supplementation with microencapsulated *L. delbrueckii* subsp. *bulgaricus* led to the highest GSH content at day 59 ($P < 0.05$). The concentration of serum MDA showed no significant alteration between the experimental groups at day 45. After dietary exposure to lead, the highest level of serum MDA was recorded in the PC group, whereas the lowest level was observed in the EN-PR group at days 52 and 59 ($P < 0.05$).

Figure 2. Antioxidant activities and lipid peroxidation ($\mu\text{mol mg pro}^{-1}$) in rainbow trout fed with or without dietary *L. delbrueckii* subsp. *bulgaricus* before and after dietary exposure to lead. Data represent the mean $\pm$ SD ($n = 9$). Different capital letters represent the significant differences between each of the treatments at various sampling times ($P < 0.05$). Different lowercase letters denote significant differences between the treatments at a specified time ($P < 0.05$). SOD (superoxide dismutase); CAT (catalase); GSH (glutathione); MDA (malondialdehyde)

Discussion

It is well established that use of dietary probiotics can positively affect the growth of fish through stimulation of appetite, elevation of digestive enzymes activity, regulation of the population of the gut microflora, modification of the intestinal morphology, promotion of feed utilization and provision of micronutrients (Salinas et al., 2008; Suzer et al., 2008; Pinpimai et al., 2015; Tarkhani et al., 2020). In this study, feeding of fingerling trout with diets containing non-encapsulated or microencapsulated *L. delbrueckii* subsp. *bulgaricus* for 45 days improved the growth parameters. Similarly, significant improvement or promotion of growth parameters have been reported in Nile tilapia (*Oreochromis niloticus*) and rainbow trout fed with diets containing free or encapsulated *Saccharomyces cerevisiae* and *L. rhamnosus*, respectively (Pinpimai et al., 2015; Hooshyar et al., 2020). Moreover, improvement of FCR was recorded in rainbow trout supplemented with encapsulated *L. fermentum* at 1×10^7 CFU g⁻¹ feed (Madreseh et al., 2019). The improvement of PER, FER and FCR in the probiotic groups reveals that inoculation of the tested probiotic into diet could improve feed utilization and reduce the amount of feed consumed for growth (El-Haroun et al., 2006). According to the results, fish fed with both forms of *L. delbrueckii* subsp. *bulgaricus* for 45 days had significantly higher activities of intestinal trypsin, α -amylase, lipase and ALP. Therefore, the enhancement of digestive enzymes activity was apparently one of the main reasons for growth-stimulatory effects of the probiont used. The elevated activities of digestive enzymes have been reported to enhance the digestion of macromolecules and therefore facilitate the absorption of nutrients within the gut lumen (Jang et al., 2019).

Following dietary exposure to lead, the activities of lipase and α -amylase were respectively decreased and increased in the PC group relative to the NC group, although the activities of trypsin and ALP had no significant alterations in these groups. Similar patterns were also reported in Nile tilapia subjected to lead (Álvarez-González et al., 2020). In contrast to our results, reduced activities of α -amylase, lipase and trypsin were observed in digestive-associated tissues of Nile tilapia after dietary exposure to lead (Dai et al., 2009). *In vitro* studies have revealed that low and high concentration of lead can respectively increase and decrease the activities of digestive enzymes (α -amylase and trypsin). At high concentration, lead could interact with functional groups present in digestive enzymes and change their secondary structure via binding to active sites and displacement of ions available in the metal-binding sites, thus resulting in inhibition of the activities of lead-associated enzymes (Hong, 2003; Yang et al., 2005). However, to our knowledge, there is no clear mechanism implying the higher activity of digestive enzymes under lead treatment *in vitro*. In the study conducted by Álvarez-González et al. (2020), the activity of intestinal α -amylase was increased in Nile tilapia fry exposed to lead for 30 days for better use of the carbohydrates within the intestinal lumen. Therefore, the enhancement of α -amylase in lead-exposed fish may suggest that higher energy was required to cope with stress induced by lead toxicity. On the other hand, long-term exposure to lead can result in higher accumulation of this metal in the digestive tract, which subsequently may inhibit the activity of digestive enzymes such as α -amylase

(Dai et al., 2009). However, further *in vivo* studies are required to investigate the effects of dietary lead on digestive enzymes activity in fish models. The discrepancies related to the effects of heavy metals on digestive enzymes may be associated with metal type, metal concentration, exposure time, exposure dose and fish age (Dai et al., 2009; Álvarez-González et al., 2020). In the present research, higher levels of α -amylase (days 52, 59 and 66), lipase (day 52) and ALP (days 52 and 59) were recorded in the probiotic groups than the PC group. Consistently with our findings, dietary *L. plantarum* and *L. reuteri* were respectively able to promote the production of digestive enzymes in Nile tilapia and common carp (*Cyprinus carpio*) after exposure to waterborne lead (Zhai et al., 2017; Giri et al., 2018). These protective effects of *Lactobacillus* spp. can be related to their capabilities for reduction of metal absorption from the gut (ion exchange, precipitation, complexation) and protection of the intestinal barrier against metal-induced inflammation (Monachese et al., 2012; Mrvčić et al., 2012; Bhattacharya, 2020). Therefore, administration of dietary *Lactobacillus* spp. can be effective against digestive enzymes disturbance caused by heavy metals in fish gut.

Enzymes such as ALT, AST, LDH and ALP are useful biomarkers that may reflect tissue injuries resulting from heavy metal toxicity in fish blood (Khan et al., 2020). Reduction or no alteration of the activities of ALT, AST, ALP and LDH following the incorporation of dietary supplements to fish diet, as observed in the present study, may suggest that they have no deleterious effects on fish tissues (Abdel-Tawwab et al., 2010; Ghanei-Motlagh et al., 2020). With respect to their higher metabolic activities, liver and kidney are known as the pivotal organs involved in detoxification and bioaccumulation of heavy metals (Kim and Kang, 2017; Mustafa et al., 2020). In this regard, dietary exposure to lead was associated with a significant accumulation of lead in liver and kidney of Korean rockfish (*Sebastes schlegelii*), rainbow trout and Nile tilapia (Alves et al., 2006; Dai et al., 2009; Kim and Kang, 2017). Moreover, histopathological alterations of liver and kidney have been previously reported in fish exposed to lead (Dai et al., 2009; Ahmed et al., 2014). In this study, significantly higher levels of liver ALP (day 66) and AST (days 52, 59 and 66) were recorded in the PC group compared to the NC group. In agreement with our results, higher enzymatic activities of ALP and AST have been reported in serum or tissues of fish exposed to lead (Firat et al., 2011; Mirmazloomi et al., 2015). Based on the results, the highest AST/ALT ratios were noticed in the PC group after exposure to lead at all intervals. AST/ALT ratio is a biochemical indicator that is used to predict the degree of hepatic damage (Li et al., 2017). Higher AST/ALT is associated with more severe liver cell injury, which may indicate the possible damage induced by dietary lead in this study. On the other hand, incorporation of microencapsulated *L. delbrueckii* subsp. *bulgaricus* into diet resulted in the lowest levels of hepatic enzymes and AST/ALT upon lead exposure. This may suggest that encapsulated probiotic cells had higher viability and tolerance in the gastrointestinal tract and could more efficiently prevent the lead-induced liver injury. Similarly, the positive effects of *Lactobacillus* spp. were demonstrated against metabolic enzymes dysfunction caused by heavy metal toxicity in fish models (Zhai et al., 2016; Giri et al., 2018).

According to the results, serum total protein (days 59 and 66) and albumin (day 66) were significantly decreased in the PC group than the NC group after administration of lead. Regarding the fact that albumin is synthesized by the liver, a concomitant decline in the content of serum total protein and albumin may be associated with dysfunctions induced by lead in the liver of fish exposed (Firat et al., 2011). Diminished levels of total protein have been also reported in other fish species after exposure to sub-lethal concentrations of lead (Martinez et al., 2004; Firat et al., 2011). Albumin is the predominant protein present in the blood with well-known antioxidant properties and regulatory effects on plasma homeostasis (Roche et al., 2008; Belinskaia et al., 2020). Higher content of total protein in the PR group and higher levels of total protein and albumin in the PR and EN-PR groups were detected after 45 days. Inoculation of probiotics to diet has been reported to improve the utilization of protein in the gut, thus resulting in a higher synthesis of protein in the liver and a higher content of protein in serum (Dai et al., 2019; Vazirzadeh et al., 2020). Fish in the PR and EN-PR groups had higher levels of total protein and albumin compared to fish in the PC group after exposure to lead at all intervals, which may be attributed to the protective effects of the probiont used against lead-induced liver damage. Consistently with our results, incorporation of *L. reuteri* to diet of common carp could enhance the levels of total protein prior to and after exposure to lead (Giri et al., 2018).

In this study, serum cholesterol was reduced in the PC and the probiotic groups compared to the control group after 7 and 14 days exposure to lead in diet. However, the levels of cholesterol were recovered in the probiotic groups relative to the PC group after administration of dietary lead for 21 days. A similar hypocholesterolemia has been also reported in rosy barb (*Pethia conchonius*) and streaked prochilod (*Prochilodus lineatus*) exposed to lead (Tewari et al., 1987; Martinez et al., 2004). The observed hypocholesterolemia may be related to the inhibition of cholesterol biosynthesis in the hepatic cells which typically produce the greatest amounts of cholesterol in the body (Firat and Kargin, 2010). It can be also correlated to the increased utilization of cholesterol in the pathway of corticosteroid synthesis in the interrenal tissue of fish (Tewari et al., 1987). On the contrary, increased values of serum cholesterol were observed in common carp exposed to lead (Giri et al., 2018). The elevated levels of cholesterol following the exposure to heavy metals have been attributed to disturbance of lipid metabolism in liver, degeneration of cell membranes and enhancement of the catabolism of total lipids (Javed et al., 2017; Kim and Kang, 2017).

Serum triglycerides were significantly lower in the probiotic groups than the NC group at day 45. Similarly, a hypotriglyceridemic response was observed in red sea bream (*Pagrus major*) fed with *Lactobacillus* spp. (Dawood et al., 2016). However, plasma triglycerides and glucose had no significant changes in rainbow trout fed with diets containing encapsulated or non-encapsulated *L. rhamnosus* (Hooshyar et al., 2020). In this study, lower concentrations of serum triglycerides were found in the PC group than the NC group at days 59 and 66. On the other hand, serum triglycerides were higher in the PR (day 59) and EN-PR (days 52 and 66) groups than the PC group post-exposure to lead. In this regard, a reduction in serum triglyceride was recorded in Nile tilapia exposed to cadmium. Moreover, administration of dietary *L. plantarum* could recover the reduced levels of serum triglycerides in this species

(Zhai et al., 2015). Depressed level of serum triglycerides after exposure of fish to heavy metals may be attributed to reduction of food intake, enhancement of the rate of membrane biogenesis, and impairment of gut and liver functions (Javed et al., 2017).

Significantly lower concentration of glucose was detected in the PC group than the NC group (day 59). It has been reported that exposure to heavy metals can stimulate the stress responses in fish, resulting in a temporary elevation in blood glucose to fulfill the energy required for detoxification of toxic metals and re-establish the homeostatic conditions (Fantin et al., 1998; Martinez et al., 2004). Prolonged exposure to heavy metals, however, will result in the depletion of the body energy reserves and reduction of fish survival (El-Shafei, 2017). In this regard, enhanced serum glucose was recorded in Nile tilapia exposed to lead for 4 days. Thereafter, the levels of glucose were reached to the basal level after 21-day exposure of the fish to lead (Firat et al., 2011). Moreover, a 30-day exposure of rainbow trout to waterborne lead led to the reduction of glucose content. However, serum glucose reached a normal level following the maintenance of fish in lead-free water for 49 days (Haux and Larsson, 1982). In the present study, therefore, an increase in serum glucose might have occurred during the initial days after dietary exposure to lead. On the other hand, the levels of serum glucose were higher in fish fed with probiotic (day 66) than those fed with basal diet after administration of lead. This may indicate that the tested *L. delbrueckii* subsp. *bulgaricus*, particularly in its microencapsulated form, could mitigate the metabolic stress induced by lead in the probiont-fed fish. The increments of serum glucose, cholesterol and triglycerides have been also observed in tilapia fed with *L. plantarum* compared to those nourished by basal diet after exposure to aluminum (Yu et al., 2017). However, further *in vivo* studies should be conducted to examine the effects of probiotic supplementation on carbohydrate metabolism after exposure to lead.

In this study, higher levels of serum minerals (calcium, magnesium and phosphorus) were recorded in fish treated with probiotics for 45 days. In this context, the elevated levels of serum calcium have been also reported in African catfish (*Clarias gariepinus*) fed with diets containing *L. acidophilus* for 21 days (Al-Dohail et al., 2011). No remarkable alterations of serum calcium and magnesium were observed in lead-exposed fish compared to non-exposed fish. Similar results have been reported in rainbow trout subjected to lead (Haux and Larsson, 1982). In this study, a significant elevation in serum phosphorus was observed in fish exposed relative to non-exposed fish. Moreover, significantly higher level of serum phosphorus was observed in the PR group compared to PC group (days 52 and 66). In this regard, serum phosphorus showed a significant increase in Mozambique tilapia exposed to waterborne lead for 7 days (Kaya et al., 2015). On the other hand, reduced levels of serum calcium, magnesium and phosphorus have been recorded in rainbow trout and stinging catfish (*Heteropneustes fossilis*) after exposure to lead (Srivastav et al., 2013). Moreover, acute toxicity of lead in rainbow trout resulted in the elevated and reduced levels of plasma calcium and magnesium, respectively (Rogers et al., 2003). The disturbed regulation of blood ions after lead toxicity in freshwater fish has been attributed to the impairment of kidney or gill function, the reduction of absorption

of ions at intestine and the potential of lead for interaction with blood proteins or calcium in bone tissue (Fantin et al., 1988; Rogers et al., 2003; Alves et al., 2006; Srivastav et al., 2013).

Previous studies have revealed that there are insufficient levels of endogenous antioxidants in cultured fish to cope with external stressors, and improvement of antioxidant defense capacities of fish is highly important (Ghanei-Motlagh et al., 2017). On the other hand, *Lactobacillus* spp. have direct scavenging activities against active oxidants by production of enzymes or metabolites with potent antioxidant abilities such as SOD, GSH, folate and butyrate (Wang et al., 2017). Likewise, administration of *Lactobacillus* spp. can positively alter the antioxidant defense system of fish through regulation of antioxidant-dependent signaling pathways (Hoseinifar et al., 2020; Kong et al., 2020). Prior to exposure to lead, the enhancement of activities of serum SOD was observed in fish fed with dietary non-encapsulated and microencapsulated *L. delbrueckii* subsp. *bulgaricus*. Moreover, no significant alterations of serum CAT, GSH and MDA were recorded before exposure to lead. Similarly, feed supplementation of *L. rhamnosus* (non-encapsulated or microencapsulated) led to the augmented levels of SOD (although the values of CAT had no alteration) in the liver of rainbow trout (Hooshyar et al., 2020). It has been demonstrated that heavy metals have high potentials to induce oxidative stress in fish tissues (Javed et al., 2017). SOD, CAT, GSH and MDA are known as reliable markers associated with heavy-metal induced oxidative stress (Hoseini et al., 2019; Lee et al., 2019). SOD and CAT are involved in the disproportionation of superoxide anion radical, and the degradation of hydrogen peroxide, respectively (Yousefi et al., 2019; Ghanei-Motlagh et al., 2021). GSH is a tripeptide non-enzymatic antioxidant which plays an important role in the balance of intracellular redox reactions (Haddad and Harb, 2005). MDA is a secondary product of lipid peroxidation reflecting the cell membrane injury mediated by free radicals (Grotto et al., 2009). After dietary exposure to lead, significantly higher levels of serum MDA were noticed in the PC group compared to the control and probiotic groups. Moreover, a time-dependent reduction in the serum levels of SOD and CAT was observed in lead-exposed fish. Similar patterns associated with oxidative stress have been also reported in northern snakehead (*Channa argus*), African catfish and Nile tilapia exposed to lead (Awoyemi et al., 2014; Zhao et al., 2020). In this study, higher activities of serum SOD, CAT and GSH and lower levels of MDA were observed in the probiotic groups and in particular in the microencapsulated group after exposure to lead. These protective effects of probiotics have been shown to be associated with their regulatory functions on the NF- κ B- and Nrf2-mediated signaling pathways (Mu et al., 2019; Yang et al., 2020; Yu et al., 2020). In agreement with our results, the protective effects of encapsulated or non-encapsulated *Lactobacillus* strains have been reported previously against oxidative stress caused by heavy metals in aquatic and terrestrial animal models (Balakrishnan et al., 2013; Yu et al., 2017; Zhai et al., 2017; Giri et al., 2018; Muhammad et al., 2018; Banwo et al., 2021).

Conclusion

In summary, the results of the present study revealed the potentially protective effects of dietary non-encapsulated and microencapsulated *L. delbrueckii* subsp. *bul-*

garicus against chronic lead toxicity (via diet) in fingerling rainbow trout. Dietary supplementation with this probiotic, particularly in the microencapsulated form, could improve growth parameters, reverse the negative effects of lead on hepatic enzymes, prevent the lead-induced dysfunctions of digestive enzymes, modulate serum biochemical parameters and potentiate antioxidant defense system in trout fingerlings. Therefore, *L. delbrueckii* subsp. *bulgaricus* can be applied as a potential dietary supplement for positive modulation of physiological responses of rainbow trout against dietborne lead toxicity. However, further investigations using molecular and pathological approaches are required to explore the exact mechanisms of action of encapsulated probiotics against chronic lead toxicity in fish.

Acknowledgments

The present study was funded by a grant (Grant No.: 98/3/02/89903) from Research Council and Excellence Center of Warm Water Fish Health of Shahid Chamran University of Ahvaz.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

The authors of this research followed the instructions approved by the Animal Ethics Committee of Shahid Chamran University of Ahvaz.

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Received: 15 I 2021

Accepted: 15 IV 2021