



Full length article

Effect of *Bacillus cereus* against cadmium induced hematological disturbances and immunosuppression in *Carassius auratus gibelio*Nan Wang^{a,b}, Chunshan Gao^c, Peijun Zhang^d, Lili Guan^{b,e}, Yunxiang Wang^{a,b}, Yue Qin^{a,b}, Yuehong Li^{a,b,*}^a College of Animal Science and Technology, Jilin Agricultural University, Changchun, 130118, China^b Ministry of Education Laboratory of Animal Production and Quality Security, Jilin Agricultural University, Changchun, 130118, China^c Freshwater Fisheries Research Institute of Jilin Province, Changchun, 130000, China^d Health Monitoring and Inspection Center of Jilin Province, Changchun, 130062, China^e College of Life Sciences, Jilin Agricultural University, Changchun, 130118, China

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ABSTRACT

Cadmium (Cd) is the most common heavy metal and is easily detected in aquatic environments worldwide. The genus *Bacillus* was one of dominant probiotics, which was commonly used in aquaculture. The present study was undertaken to explore the effects of *Bacillus cereus* (*B. cereus*) supplementation on hematological parameters and the immune response of *Carassius auratus gibelio* (*C. gibelio*) following Cd exposure. Fish were exposed to waterborne Cd (0, 1 and 2 mg/L) and/or treated with dietary *B. cereus* at 10⁸ cfu/g for four weeks. The hematological disturbances observed after exposure of waterborne Cd included significant decreases in red blood cell (RBC) count, hemoglobin (Hb) concentration and hematocrit (HCT). While significant elevation ($P < 0.05$) of RBC count, HCT and Hb levels in the 1 and 2 mg/L Cd-*B. cereus* administration group at 4 weeks, compared with the Cd-only group. Among serum enzymatic activities, aspartate aminotransferase (AST) and alanine transaminase (ALT) activities by Cd exposure were significantly higher than controls, but this increase was effectively inhibited in Cd-*B. cereus* administration groups. In the Cd-*B. cereus* administration group, significant down-regulation of Hsp70, Hsp90, IL-1 β , IL-6, IL-10 and TNF- α in conjunction with the up-regulation of IgM and LZM in the spleen indicated that *B. cereus* alleviated the Cd-induced damage to the immune system to some degree. The results of this study suggested that *B. cereus* has the potential to countermeasure Cd-induced hematological disturbances and immunosuppression in *C. gibelio*.

1. Introduction

Heavy metal contamination appears as one of the major global threats not only from the survival of human and other organisms, particularly aquatic animals but also from natural environmental viewpoint because of its nonbiodegradable, hazardous and toxic properties. The heavy metal cadmium (Cd), is one of the 10 most toxic metals in the Priority List of Hazardous Substances (ATSDR 2007) for causing a grossly biological impact by bioconcentration, bioaccumulation and biomagnification phenomena [1].

Fish culturing in contaminated effluents may lead to heavy metal bioaccumulation in tissue system [2,3]. Cd accumulation may negatively affect fish hematology, metabolism, reproduction, development; and immunodeficiency [4]. The hematopoietic system is one of the most sensitive system to assess the toxicity of environmental toxins and

drugs in humans and animals, and changes in levels of hematological parameters were observed in rats after Cd exposure reveals the hematological disturbances, including significantly decreased in RBCs, Hb, HCT, PLTs and lymphocyte count [5]. It has been shown that Cd exposure may result in the accumulation of non-essential toxic metals in the kidneys, spleen and liver, thereby inhibiting the activity of these hematopoietic tissues and causing anemia [6,7]. In addition, longer term exposure to Cd gradually reduces the immune response as indicated by decrease in IgM levels, and the up-regulation of the cytokine genes (TNF- α , IL-1 β , IL-6, IL-10, and IFN- γ), and alters the expression of immune-related and HSP genes [4,5,8]. In addition, significant alterations of immune parameters demonstrated the immunotoxic effects induced by Cd, including serum IgM levels, lysozyme and serum glutamic-pyruvic transaminase activities [4]. Thus, there is an urgent need to explore more effective methods for removing cadmium

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contamination from the environment.

Probiotics are living microorganisms that confer beneficial effects on the host when ingested in sufficient quantities [9]. In aquaculture, probiotics may be administration via dietary supplementation or as a water additive [10]. Ingested probiotics break down toxic and non-nutritious dietary components, facilitating host digestion. Probiotics also provide antimicrobial compounds, preventing pathogenic colonization of the gut by excluding pathogens from nutrient supplies and mucosal spaces [11]. Recently, Zhai et al. showed that the dietary supplementation of *L. plantarum* CCFM8661 provided significant protective effects against waterborne Pb exposure in Nile tilapia [12]. It was also reported that the addition of the *Lactobacillus acidophilus* improved the survival rate of the *Oreochromis niloticus*, decreasing the harmful effects of heavy metal pollution by forming complexes with Cd, these complexes are not easily absorbed by the host [13]. Several studies have shown that *Bacillus* strains are also efficient probiotics, improving growth, immunity, and disease resistance in aquatic organisms [14–16]. Yin et al. demonstrated that *Bacillus subtilis* has the potential to alleviate the effects of lead toxicity in *C. gibelio* [17].

Although, *C. gibelio* is a commonly farmed species in China, few studies have investigated Cd toxicity in this species. Furthermore, it is apparent that no attempt has been made so far to investigate the effects of *B. cereus* against Cd toxicity in *C. gibelio*. The objective of this study was conducted to research the effect of Cd exposure and *B. cereus* administration on hematological parameters and immune response in *C. gibelio*.

2. Materials and methods

2.1. *Bacillus cereus*

The *B. cereus* strain used in this study was isolated from the gut of *C. gibelio* obtained from Jilin Agricultural University, Jilin Province, China. This bacterial isolation was performed following previous studies [18]. Prior to use, the organism was cultured in Luria-Bertani (LB) medium at 30 °C for 18 h using an orbital shaker at 150 rpm under aerobic conditions.

2.2. Diet preparation

Commercial feed (crude protein 37.7%, crude lipid 7.4%, and ash 10.8%) obtained from Jinyanhong Aquarium Products Co., Hangzhou, China was used as basal diet. Three experimental diets were prepared by supplementing the basal diet with *B. cereus* (at a final dose of 10^8 cfu/g diet) and/or Cd (1 mg/L and 2 mg/L). The commercial feed was made of powder and the powder was sifted through 120 µm mesh. *B. cereus* or/and Cd at specified concentration was mixed thoroughly in cooled conditions and then pellets were made by a hand pelletizer. The concentration of *B. cereus* in the feed was determined using the spread plate technique (nutrient agar incubated at 30 °C for 24 h). The control group was fed a basal diet with an equivalent volume of sterile saline, and all diets were stored at 4 °C until use.

2.3. Experimental diets

C. gibelio (60.84 ± 0.48 g; 14.2 ± 0.2 cm) were obtained from a specialized aquatic fry farm (Jinlin province, China). Fish were allowed to acclimate to laboratory conditions for two weeks before the exposure trial. Fish were granted with the Commercial feed (crude protein 37.3%, crude lipid 7.45, and ash 10.8%; Jinyanhong Aquarium Products Co., Hangzhou, China) at a rate of 1–2% of body weight twice daily. Laboratory conditions were kept consistent throughout (dissolved oxygen: 6.59 ± 0.07 mg/L; pH: 7.2 ± 0.3 ; ammonia: ≤ 0.5 mg/L; nitrites: ≤ 0.05 mg/L). Water temperature was adjusted to 25 ± 2 °C and a natural light were used for all experimental illumination with a photoperiod of 13 h light and 11 h dark circularly during the period of

Table 1

Nominal and actual Cd concentration in water (mg/L).

Groups	Nominal concentration	Actual Cd concentration in the experiment weeks	
		2 weeks	4weeks
CK	0	0.004 ± 0.001	0.003 ± 0.001
CB	0	0.002 ± 0.002	0.002 ± 0.001
LC	1	0.995 ± 0.001	0.990 ± 0.001
LCB	1	0.976 ± 0.003	0.975 ± 0.001
HC	2	1.994 ± 0.005	2.006 ± 0.003
HCB	2	1.978 ± 0.002	1.976 ± 0.005

adaptation. Experiments were performed according to the research protocols approved by the Institutional Animal Care and Use Committee, Jilin Agricultural University.

2.4. Diets and experimental design

360 fish were randomly divided into six groups (with three replicates per group), which resided in 80 L plastic tanks. Each group was treated with waterborne Cd and/or dietary *B. cereus*. The six groups were divided as follows: CK group (control group), CB group (*B. cereus* 10^8 cfu/g), LC group (Cd 1 mg/L), LCB group (Cd 1 mg/L plus *B. cereus* 10^8 cfu/g), HC group (Cd 2 mg/L), HCB group (Cd 2 mg/L plus *B. cereus* 10^8 cfu/g). The fish were fed twice daily (at 9:00 a.m. and at 3:00 p.m.) for four weeks. Light and water quality parameters during the experimental period were identical to those during the acclimation period. 20 mL water samples were collected from each of the tanks, and Cd concentration was analyzed using the same method as tissue samples. The actual concentration of water-borne Cd is shown in Table 1. Cadmium chloride (CdCl_2) was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Cd was added based on the experimental concentration. Half of the water in each tank was replaced daily.

2.5. Sample collection

Fish were fasted for 24 h before collecting samples. Six fish were randomly selected from each tank for 2 and 4 weeks to collect tissue samples and blood samples. Fish were euthanized with 300 mg/L of Methane-Sulfonate-222 (MS-222). Tissue samples were subsequently collected as spleen and gut. Blood samples were collected using heparin-treated syringes, and then immediately separated using centrifugation at 4000g for 5 min at 4 °C. The red blood cell (RBC) was analyzed by counting using microscope. All samples were frozen in liquid nitrogen and stored at -80 °C until the experimental assays.

2.6. Hematological assays

The obtained blood was assayed for concentration of cadmium using Atomic Absorption spectrometer AA-6300 (Shimadzu, Japan). Hb and HCT levels were assayed using a clinical kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.7. Biochemical analysis

The concentrations of Cd, magnesium (Mg), glucose (Glu), total cholesterol (TC) and total protein with Aspartate aminotransferase (AST), Alanine transaminase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), immunoglobulin M (IgM) and Lysozyme (LZM) levels in the blood, as well as levels of SIgA levels in the gut were conducted using commercial kits (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China).

Table 2
Primers used in this study.

Genes	Sequences(5'-3')	Accession no.
β-actin	Forward	TGAAGATCTGACCGAGCGT
	Reverse	GGAAGAAAGGCGAGCGGTTTC
HSP70	Forward	ATTGAGACCGCAGGTGGAGT
	Reverse	GGCTGGTTGTCGGAGTAGGT
IgM	Forward	AGCTCAACCATCTGCACCAA
	Reverse	ATGTAAGCGAGTCCGCAGGT
HSP90	Forward	GTATGGAGCAGCAAGACCGAGAC
	Reverse	CAACCTCAGCCTCATCTTCAGTGG
IL-1β	Forward	CAGTAAGACCAGCCTGACCTTGC
	Reverse	GCACCTCAGCGTCACAGCCTTC
IL-6	Forward	CTGCCTGTCTCAGAGATCACAAAGC
	Reverse	GCCGACAGTATGCCGAAGAAG
TNF-α	Forward	CGCGACTGACACTGAAGACC
	Reverse	GCAGGAGTTCTGTGGTGGTG
IL-10	Forward	TTCAGGAAGTCAAGCGGGATAT
	Reverse	GCTGTGACTTCAAAGGATTTT
LZM	Forward	TGTGTCTGATGTGGCTGTGC
	Reverse	TGCACACATAGTTGCCAAGTGA

2.8. Reverse-transcriptase real-time PCR

The spleen tissues were used to analyze the immune-related gene expression at the end of the exposure trial. Total RNA was extracted from spleen using a Trizol kit (Takara, Dalian, China). RNA concentration and quality was analyzed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and purified RNA with an OD260/OD280 absorption ratio of 1.8–2.0 was then used to synthesize cDNA using RT-PCR cDNA kit (Takara, Dalian, China). Primer 5.0 program was used for the design of all primers and was submitted to Shanghai Invitrogen Biotech Co., Ltd for synthesis. Eight immune-related gene expression including IL-1, IL-6, IL-10, LZM, IgM, Hsp70 and Hsp90 were used for qRT-PCR reaction and the housekeeping gene *β-actin* was used as reference [4,17,19]. The correlation sequences of specific primers are shown in Table 2. The RT-PCR reaction was performed in a total volume of 20 μL, containing 1 μL cDNA, 2 μL each primer, 7 μL DEPC-treated water and 10 μL SYBR Premix Ex Taq Master Mix. The thermal reaction included 5 min at 94 °C, followed by 30 cycles at 94 °C for 5 s, 60 °C for 30 s, and 72 °C for 30 s. The reaction was repeated 3 times for each sample. Data were converted into Ct values after the end of the reaction. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative gene expression levels.

2.9. Statistical analysis

Results are presented as means ± standard deviation (SD). Statistical analyses were performed in SPSS 20.0 (SPSS, Chicago, IL, USA). Data were analyzed with two-way ANOVA, followed by Tukey's multiple comparison tests. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Hematological parameters

Cd accumulation, hematocrit, hemoglobin concentration and RBC count of *C. gibelio* following exposure to waterborne Cd and/or *B. cereus* administration are present in Fig. 1.

Significant elevation of RBC counts in the 1 and 2 mg/L Cd-*B. cereus* administration group at 4 weeks, compared with the Cd-only administration ($P < 0.05$) (Fig. 1 A). Compared with the Cd-only administration group, Cd accumulation in the blood significantly decreased ($P < 0.05$) in the 1 mg/L Cd-*B. cereus* administration group at 2 and 4 weeks and only decreased significantly in the 2 mg/L Cd-*B. cereus* administration group at 4 weeks (Fig. 1 B). At 4 weeks, significant

increase ($P < 0.05$) was observed on hemoglobin concentration in the 1 and 2 mg/L Cd-*B. cereus* administration group compared with the Cd-only administration group (Fig. 1 C). At 4 weeks, compared with the Cd-only administration group, hematocrit in the blood was significantly increased ($P < 0.05$) in the 0, 1 and 2 mg/L Cd-*B. cereus* administration group (Fig. 1 D).

3.2. Blood biochemistry

Ca and Mg concentration, Glu and TC level, also ALP and ALT activity in the blood of *C. gibelio* following exposed to dietary and/or *B. cereus* are shown in Fig. 2.

Compared with the Cd-only administration group, Ca and Mg concentration in the blood significantly increased ($P < 0.05$) in the 1 and 2 mg/L Cd-*B. cereus* administration group at 4 weeks (Fig. 2 A and B). In addition, there is significant decrease ($P < 0.05$) in Glu were found in the 0, 1 and 2 mg/L Cd-*B. cereus* administration group at 4 weeks, compared with the Cd-only administration group (Fig. 2 C). Compared with the Cd-only administration group, significantly reduced level of TC was observed in the 0, 1 mg/L Cd-*B. cereus* administration group at 2 and 4 weeks and only decreased significantly in the 2 mg/L Cd-*B. cereus* administration group at 4 weeks ($P < 0.05$) (Fig. 2 D). At 4 weeks, Cd-*B. cereus* administration group showed a significant increase ($P < 0.05$) of ALP activities in 0, 1 and 2 mg/L when compared with the Cd-only administration group (Fig. 2 E). Significant decrease in ALT activity was found in the 1 mg/L Cd-*B. cereus* administration group at 2 and 4 weeks and only decreased significantly in the 2 mg/L Cd-*B. cereus* administration group at 4 weeks ($P < 0.05$), compared with the Cd-only administration group (Fig. 2 F).

AST, LDH and LZM activity, total protein level, and IgM concentration in the blood also SIgA concentration in the gut of *C. gibelio* following exposed to dietary and/or *B. cereus* are shown in Fig. 3.

There is significant decrease in AST activity were found in the 0, 2 mg/L Cd-*B. cereus* administration group at 2 and 4 weeks and only decreased significantly in the 1 mg/L Cd-*B. cereus* administration group at 4 weeks, compared with the Cd-only administration group ($P < 0.05$) (Fig. 3 A). However, significant increase ($P < 0.05$) in total protein merely were found in the 0 mg/L Cd-*B. cereus* administration group at 2 and 4 weeks, compared with the Cd-only administration group (Fig. 3 B). In addition, significantly decrease on LDH activities between Cd-only and Cd-*B. cereus* group at all levels was observed in Fig. 3 C ($P < 0.05$). At 4 weeks, IgM concentration and LZM activities in the blood also SIgA concentration in the gut significantly increased ($P < 0.05$) in Cd-*B. cereus* administration group at all levels, compared with the Cd-only administration group (Fig. 3 D, E and F).

3.3. Immune-related gene expression

Fig. 4 showed changes in the levels of immune-related gene expression in spleen after Cd and *B. cereus* administration for 4 weeks. The expression of Hsp70 and Hsp90 were significantly decreased ($P < 0.05$) in the 1 and 2 mg/L Cd-*B. cereus* administration group, compared with the Cd-only group (Fig. 4 A and B). There is significant increase ($P < 0.05$) in IgM expression were found in the 0, 2 mg/L Cd-*B. cereus* administration group, compared with the Cd-only administration group (Fig. 4 C). Compared with the Cd-only administration group, IL-1β, 6, and 10 also TNF-α in the spleen significantly decreased ($P < 0.05$) in the 1 and 2 mg/L Cd-*B. cereus* administration group at 4 weeks (Fig. 4 D, E, F and H). In addition, Cd-*B. cereus* administration group showed a significant increase ($P < 0.05$) of LZM expression in 0 and 2 mg/L when compared with the Cd-only administration group (Fig. 4 G).

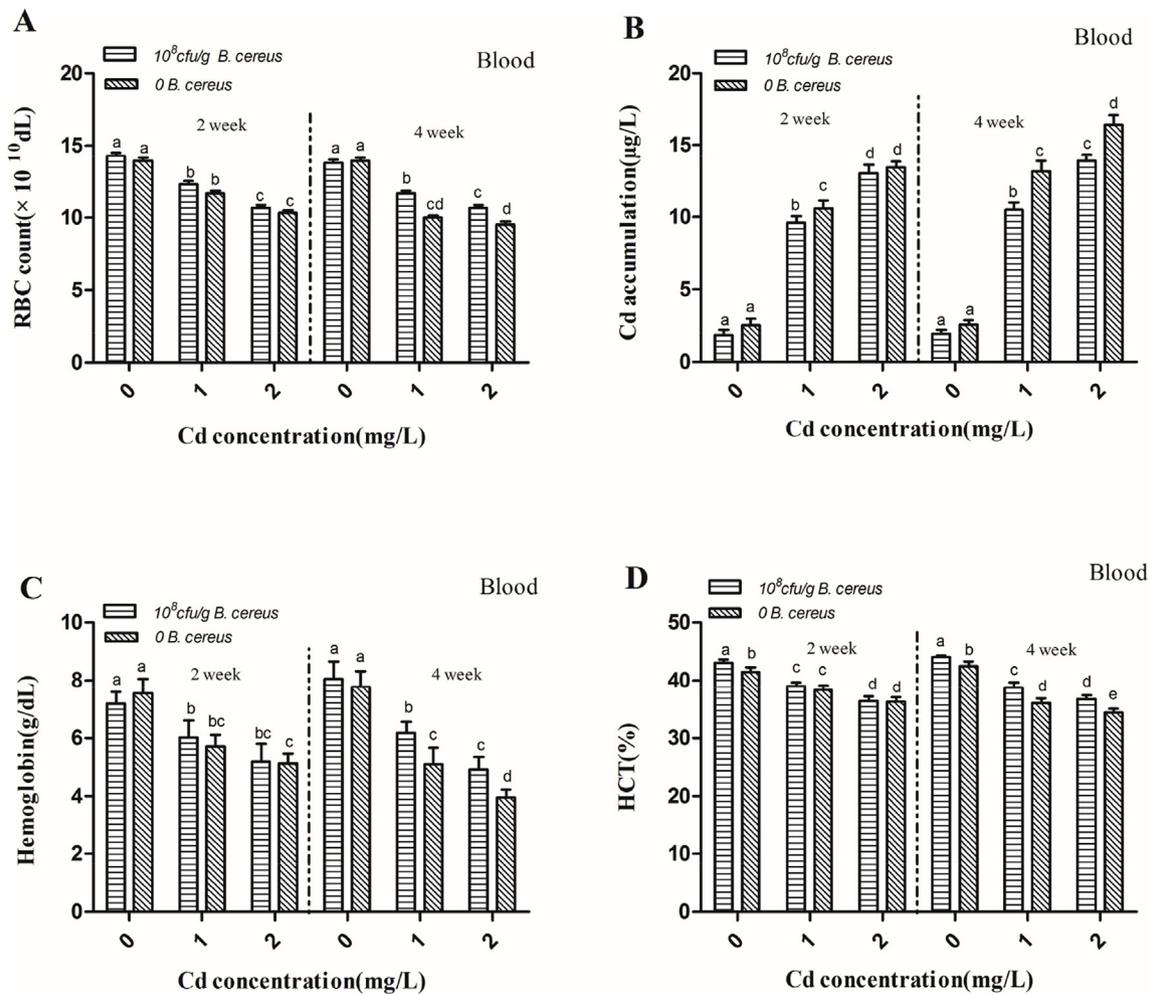


Fig. 1. RBC count, Cd accumulation, hemoglobin concentration and hematocrit of *C. gibelio* ($n = 6$) after exposure to waterborne Cd and/or *B. cereus* administration. Data are expressed as the mean \pm S.D. Bar with different letters are significantly ($P < 0.05$) different by Turkey test on the same sampling interval.

4. Discussion

Hematopoietic system is highly sensitive to environmental toxins, and is thus often used to assess the toxicity of drugs and contaminants in humans and animals [20]. In this study, significantly enhanced concentration of Cd was observed in *C. gibelio* with Cd exposure ($P < 0.05$). Our results indicated that *B. cereus* supplementation significantly reduced the accumulation of Cd in the blood. Our results are consistent with previous studies, which showed that exposure to heavy metals led to a significant reduction in various hematological parameters, including RBC, HCT and Hb [21]. Heavy metal exposure generally induces erythrocyte lysis in aquatic animals, resulting in the depletion of HCT and Hb [22]. Cd exposure may cause anemia by a complex mechanism involving interference with the effective use of Fe in Hb synthesis [23]. In this study, Cd accumulation in the blood significantly decrease ($P < 0.05$), also significant elevation ($P < 0.05$) of RBC count, HCT and Hb levels in the 1 and 2 mg/L Cd-*B. cereus* administration group at 4 weeks, compared with the Cd-only administration group. Similar results obtained by previous research where they observed a same trend in recovering hematological parameters of *Cyprinus carpio*. *Bacillus spp.* have high adsorption capacities due to the high content of peptidoglycan and teichoic acid in the cell walls. De et al. showed that *Bacillus cereus* BW-03 can be used as a suitable candidate strain and also a substitute strain for inorganic mercury remediation [24,25]. Here, *B. cereus* may have adsorbed Cd, reducing the amount of cadmium concentration in the serum of *C. gibelio* and somewhat alleviating the damage to the hematopoietic system.

Transaminases like ALT and AST play important roles in amino acid and protein metabolism and may be released into plasma following tissue injury and dysfunction. Ha et al. suggested that changes in serum enzyme activity levels might signal organismal damages caused by heavy metals [26]. Indeed, 20 days of continuous exposure to sub-lethal concentrations of Cd (0.05 mg/L) resulted in significantly elevated AST and ALT activity levels in *Oreochromis niloticus* [27]. Consistent with this, serum ALT and AST activities in the Cd-only group were significantly increased compared with the corresponding control group. Significant decrease in AST and ALT activity were found in the Cd-*B. cereus* group compared with the Cd-only group. Zhai et al. suggested that dietary administration of *L. plantarum* might rescue ALT and AST activities in Nile tilapia [12,28]. In addition, supplementation with the probiotic *L. reuteri* P16 restored serum AST and ALT levels after Cd-induced damage to the liver and heart of *C. carpio* [29]. Similarly, changes in ALP activity and blood glucose levels, which can be sensitive indicators of environment stress in fish, were restored in the Cd-*B. cereus* group.

Studies have shown that Cd has immunosuppressive effects [5,8,30]. The activity of LZM and other humoral components may affect the innate immune responses of aquatic organisms such as fish [4,31,32]. As IgM neutralizes foreign objects such as bacteria and viruses, low IgM levels in fish may indicate immune system dysfunction [4,33]. In this study, both LZM and IgM activity decreased gradually as Cd exposure duration increased. Our results are consistent with previous studies, which demonstrated that arsenic induced lymphocyte apoptosis in fish through oxidative stress, causing decreased Ig

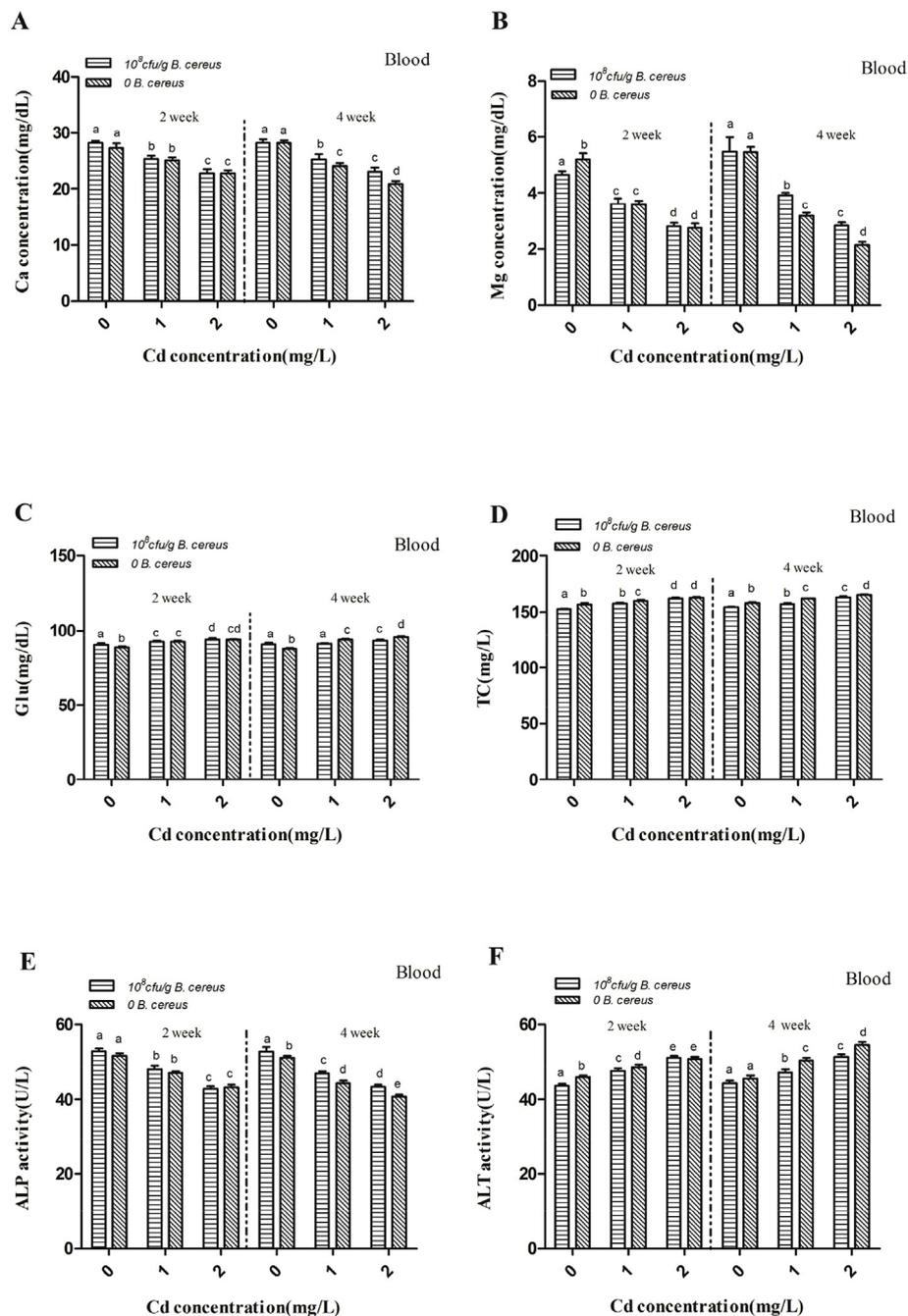


Fig. 2. Ca and Mg concentration, Glucose (Glu) and total cholesterol (TC) level, also alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activity in the blood of *C. gibeli* (n = 6) after exposure to waterborne Cd and/or *B. cereus* administration. Data are expressed as the mean ± S.D. Bar with different letters are significantly ($P < 0.05$) different by Turkey test on the same sampling interval.

production [34,35]. However, serum IgM concentration, LZM activity, and SIgA concentration in the gut were significantly greater in the Cd-*B. cereus* group in all levels at 4 weeks, compared with the Cd-only group. It was shown that LZM levels increased and disease resistance was enhanced in the common carp after *Bacillus Licheniformis* due to the secondary metabolites produced by this bacterium [36]. Therefore, *B. cereus* supplementation may reduce the oxidative stress associated with Cd accumulation in the tissues and blood and may improve innate immune function.

Cytokines, including interleukin, tumor necrosis factor and chemokines, play an important role in innate immunity [37]. And it is worth noting that TNF- α is the cytokine most cytokine associated with immune modulation, inflammation, tumorigenesis, cell proliferation

and apoptosis in the Cd²⁺-regulated gene network [38]. In the present study, our results showed that expression of IL-1 β and TNF- α were up-regulated after Cd exposure. This was consistent with a previous study, which showed that exposure to 0.005 mg/L Cd up-regulation of IL-1 β and TNF- α in zebrafish embryos [39]. However, an additional study found that IL-1 β and TNF- α transcription decreased after fish were exposed to 0.65 mg/L Cd [4]. It has been suggested that the secondary metabolites generated by probiotics upregulated IL-10 and TNF- α expression in the host [40]. In contrast, we found that the genes encoding the pro-inflammatory cytokines IL-10 and TNF- α were upregulated. The gene encoding LZM was significantly upregulated after treatment with *B. cereus*.

Heat shock proteins (HSPs) may be synthesized when aquatic

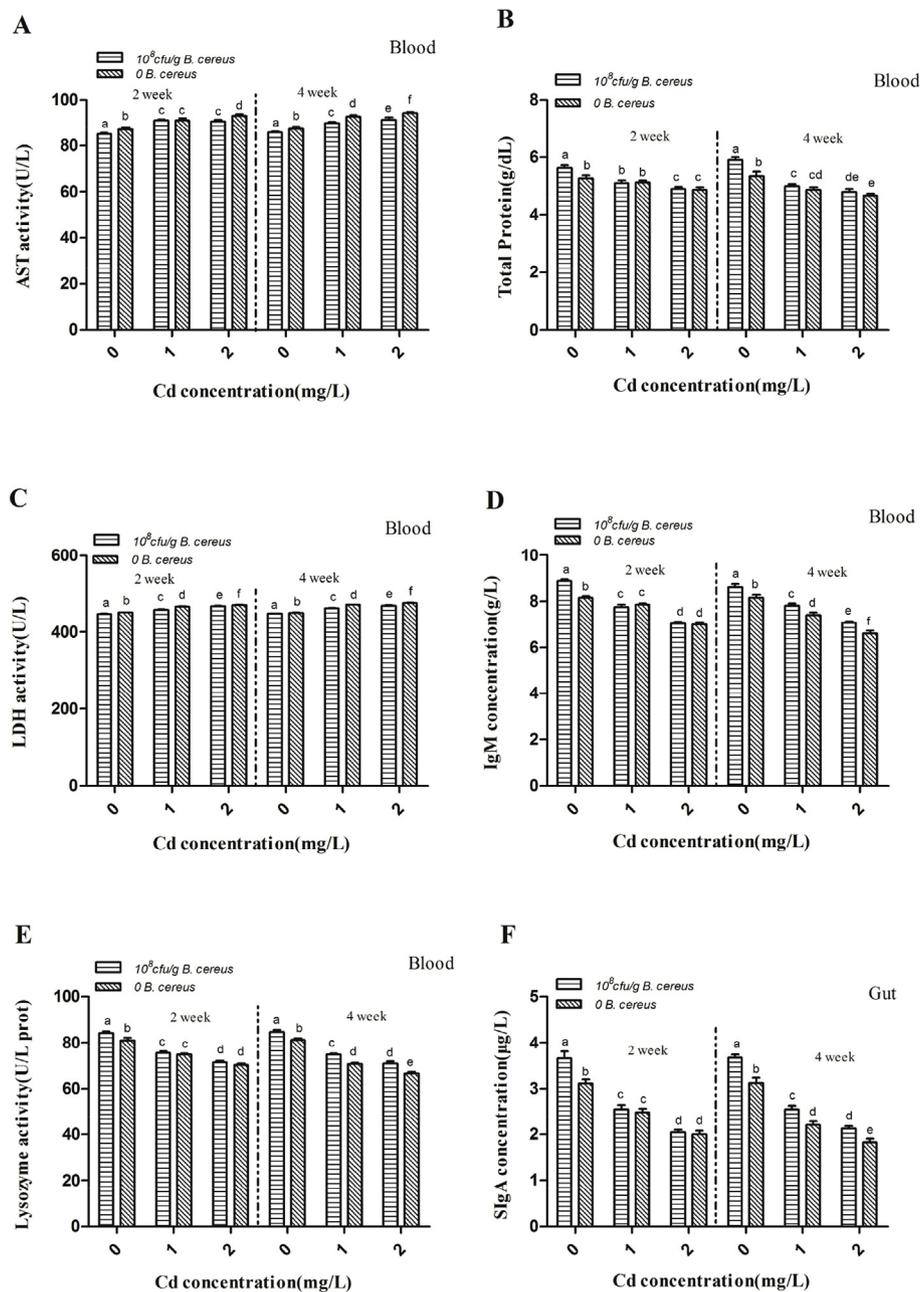


Fig. 3. Aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and lysozyme (LZM) activity, total protein level, and IgM concentration in the blood also SIgA concentration in the gut of *C. gibelii* (n = 6) after exposure to waterborne Cd and/or *B. cereus* administration. Data are expressed as the mean ± S.D. Bar with different letters are significantly ($P < 0.05$) different by Turkey test on the same sampling interval.

organisms are stimulated by physical, chemical, biological, mental and other stimuli in the environment to induce stress reactions, so as to play a protective role in the stress process, and therefore are considered as good biomarkers of environmental stress [41,42]. Several studies have shown that Hsp70 expression increased following exposure to heavy metals, and decreased after probiotic administration [43,44]. Mohapatra et al. showed that dietary supplementation with a probiotic mixture consisting of *Bacillus subtilis*, *Lactococcus lactis*, and *Saccharomyces cerevisiae* decreased the expression of Hsp70 in *L. rohita* [45]. This corroborates current data that Cd exposure significantly enhanced the expression of Hsp70 and Hsp90, and *B. cereus* administration was effective in reducing transcription level. The stress-reducing factors produced by the administered probiotic might have reduced the HSP protein expression, decreased Cd-induced stress, and improved growth and immunity in fish. Interestingly, some studies have not observed

alterations in HSP expression levels in response to Cd exposure [46]. However, it is perhaps not surprising that the dose, duration, mode, and source of heavy metal exposure are crucial to immune regulation in mammals and fish [47]. These results suggested that *B. cereus* might have a positive regulatory effect on the immune damage caused by Cd exposure.

5. Conclusion

Taken together, our results suggest that *B. cereus* can reduce serum Cd accumulation in blood, regulate hematological parameters (RBC count, hemoglobin concentration and hematocrit value), biochemical parameters (including blood calcium, magnesium and glucose, total protein, TC, ALP, ALT, AST, LDH and LZM activity, IgM and SIgA levels) and regulate immune-related gene expression (including Hsp70, Hsp90,

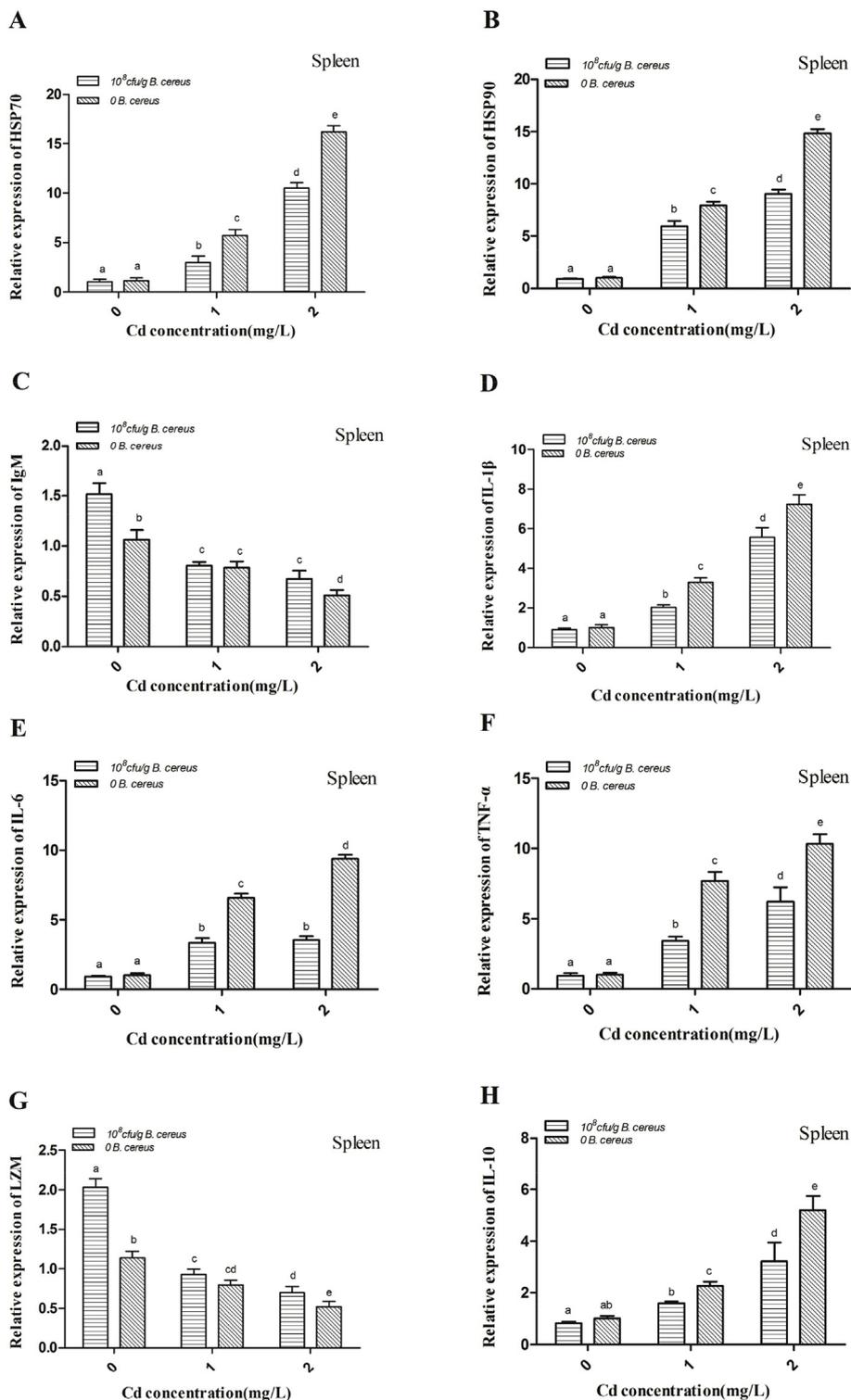


Fig. 4. Immune-related gene expression (Hsp70, Hsp90, IgM, IL-1 β , IL-6, TNF- α , LZM and IL-10) in the spleen of *C. gibelio* (n = 6) after exposure to waterborne Cd and/or *B. cereus* administration. Data are expressed as the mean \pm S.D. Bar with different letters are significantly ($P < 0.05$) different by Turkey test on the same sampling interval.

IgM, IL-1 β , IL-6, IL-10, LZM and TNF- α expression) following Cd exposure in *C. gibelio*. These results demonstrate that *B. cereus* might be useful for the mitigation of cadmium toxicity in aquaculture.

Conflicts of interest

The authors declare that they have no conflict of interest.

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