

Bioaccumulation, Oxidative Stress, Immune Responses in *Channa Argus* Exposure to Waterborne Hexavalent Chromium

Jia-hua du

Heilongjiang Bayi Agricultural University

Zhe Yu

Heilongjiang Bayi Agricultural University

Jun-liang Zhao

Heilongjiang Bayi Agricultural University

Zhi-hui Bai

Heilongjiang Bayi Agricultural University

Zehao Guo

Heilongjiang Bayi Agricultural University

De-hui Li

Heilongjiang Bayi Agricultural University

Gui-qin Wang

Jilin Agricultural University

Mu-yang Li

Heilongjiang Bayi Agricultural University

Lei Zhao (✉ zlhlijbyau@126.com)

Heilongjiang Bayi Agricultural University <https://orcid.org/0000-0002-3768-9812>

Research Article

Keywords: Cr (VI), Bioaccumulation, Oxidative stress, Immune responses

Posted Date: June 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-633529/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Hexavalent chromium (Cr (VI)) is a very common and harmful heavy metal pollutant in the world. However, the responses of Cr (VI) to aquatic environmental toxicants have not been well described. In this study, we evaluated the waterborne Cr (VI) [0 mg/L (C1), 0.5 mg/L (C2), 1 mg/L (C3), 2 mg/L (C4)] exposed chronically to *Channa argus*. After 14 and 28 days, we measured biochemical parameters, Cr (VI) accumulation, antioxidant activity and immune response in the serum, liver and gill, respectively. Our results show that Cr (VI) accumulation will occur in certain tissues as the time of exposure in water prolongs, and the biochemical parameters in the serum will increase. Antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) gradually decreased with the increasing time of Cr (VI) concentration, while the malondialdehyde concentration increases. In addition, in the immune response, cortisol, aminotransferase (ALT), aspartate aminotransferases (AST), alkaline phosphatase (AKP), immunoglobulin M increase, but lysozyme decrease. These results indicated that exposure to waterborne Cr (VI) can exert adverse effects in *C. argus* by inducing Cr (VI) accumulation, immune responses and oxidative stress.

1. Introduction

Due to human activities, the content of heavy metals in the environment has increased gradually, and the deterioration of environmental quality has seriously affected human life and health. At present, environmental heavy metals have become a worldwide environmental problem. Heavy metal pollution is mainly manifested in water pollution. Because of the importance of fish in the aquatic ecosystem, fish are typically used for environmental monitoring. Chromium is a key micronutrient for maintaining physiological homeostasis. There are two main forms of Cr in the aquatic environment: trivalent chromium (Cr (III)) and hexavalent chromium (Cr (VI)). Cr (III) is an important trace element. Cr (VI) is an important environmental pollutant released from domestic and industrial wastewater, which harms animal health and causes malformations, allergic reactions, and damages the liver and kidneys as well as animal cancer [1]. Cr (VI) is more active on living cells than Cr (III), and it easily penetrates cell membranes. Therefore Cr (VI) causes greater damage to cells [2]. Exposure to Cr (VI) may lead to metabolic conversion of normal growth and development to detoxified energy chromium toxicity and inhibit the growth of fish [3]. It is well known that Cr (VI) and other toxic heavy metals will accumulate in the specific tissues of a variety of fish through exposure to heavy metals in water and in the diet, and continue to exert toxic effects leading to sub-health and even death of fish [4–5].

Aquatic animal blood factors have been widely used as indicators of physiological and pathological changes in toxicology and environmental research, aiming to assess the impact of toxin exposure [6]. Because fish blood has a close relationship with the external environment through the circulatory system [7]. Therefore, fish blood parameters are a good indicator to reflect water environmental toxicity. Shaheen et al. (2012) also reported that since blood is highly sensitive to environmental changes and therefore can be used as a reliable parameter to measure environmental toxicity, and Cr (VI) has negative effects on aquatic animal blood and biochemical factors [8].

Cr exposure in water can lead to accumulation of specific tissues in aquatilia, influence the normal metabolic enzyme activity, cause the reactive oxygen species (ROS) and tissues precipitation, peroxidation of lipid and protein, and later cause oxidant stress. Aquatic lives contain an active anti-oxidation system with various protective enzymes preventing or limiting tissue damaged by inhibiting the generation of ROS [9]. The anti-oxidation system of fish is an important element in the decomposition of H₂O₂, including antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (Gsh-Px), which are recognized as antioxidant damage and response. An indicator of irritating response [10]. The production of lipid peroxide and malondialdehyde (MDA) can be also reduced by Gsh-Px [11]. Therefore, the effects of chromium on fish exposure can be studied by the use of antioxidant enzymes. Besides, it has been proposed that selenium exposure can influence oxidant stress along with immune function [12]. Trace elements in water can affect the immune indices of fish [13]. Lysozyme (LZM) which is important is a natural immune defense molecule. In a non-specific immune response, not only does LZM dissolve bacteria, but also activates the complement system and is important in preventing microbial invasion [14]. Immunoglobulin M (IgM) is a large molecule involved in a specific immune response [15]. Researches have revealed that the levels of LZM and IgM are convenient parameters to monitoring the possible effects of environmental impacts on fish immune responses [16–17]. In addition, serum cortisol (COR) is a key parameter to measure the physiological state of exposure to pollutants. Therefore, they can be used as indicators of stress response.

Channa argus is a carnivorous freshwater fish, commonly known as black fish and mullet [13]. Because of its high nutritional value, strong environmental adaptability and fast growth, it is one of the main fish species cultured in freshwater ponds in China, North Korea, Japan, India and Russia widely [16]. It's an expensive edible fish, but few knows about the Cr (VI) effects. Thus, the purpose of this study is to make an investigation of the Cr (VI) accumulation in *C. argus* tissue and its adverse effects on Cr (VI) antioxidant activity, oxidant stress, serum biochemistry and tissue accumulation.

2. Materials And Methods

2.1. Experimental Fish and Design

C. argus (13.42 ± 0.35 g) were collected from a commercial fish farm. During the acclimation period, *C. argus* were cultured in 100 L polyethylene tanks with aerated water and fed commercial carp diet twice daily at 1–2% BW for 14 days.

After acclimation, 240 *C. argus* were randomly assigned to four groups (5 tanks in each group, 12 fish in each tank). The Cr (VI) concentrations in blue plastic boxes were 0, 0.5, 1.0, and 2.0mg/L, respectively. Cr (VI) solution was prepared from potassium dichromate solution (CAS:7778-50-9, MDL: MFCD00011367, Macklin Biochemical Co., Ltd, Shanghai, China). Commercial feed (**Table 1**) of *C. argus* (Dry matter: crude protein 48.1%, crude lipid 11.3%, ash 12.3%, carbohydrate 20.1%, gross energy, 19.3 kJ/g and protein: energy ratio, 26.0 mg/kJ, Alpha Feed Co., Ltd Shenzhen, China) was used as experimental feed. The tanks were placed under experimental conditions (temperature: $22.3 \pm 1.2^\circ\text{C}$; pH: 7.6 ± 0.2 ; ammonia: less

than 0.3 mg/L; nitrites: less than 0.04 mg/L and dissolved oxygen: 5.82 ± 0.22 mg/L) and in photoperiod (14 h light/10 h dark photoperiod). Feed the fish twice a day (08:00 and 16:00) at 3% body weight for 28 days. A siphon was used to remove debris from the bottom of the tank every day and half of the water in each tank was changed every two days. The fish for research were performed according to the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee, Heilongjiang Bayi Agricultural University. The concentration of Cr (VI) were approximately equal to or higher than that present in the environment.

2.2. Sample Collection

The fish were put on a 24-hour fast before the samples were collected. On days 14 and 28, fish were anesthetized in buffered Methane-Sulfonate-222 (MS-222). Blood samples were taken from the tail vein, and centrifugation was performed at 3000 RPM for 10 min and serum was collected, which was stored at -20°C for future research. Tissue samples were taken from liver, intestine, gill. All tissues were rapidly frozen in liquid nitrogen and stored at -80°C for future research.

2.3. Chromium Analysis

On days 14 and 28, in order to observe Cr concentration, 3 tissues (liver, intestine, gill) were taken from each group. All tissues were dried in an oven at 120°C before analysis and the dried samples were cold-digested with 20 mL of concentrated nitric acid (65% HNO_3). The digested samples were collected and centrifuged at 14,000 $\times g$ for 20 min at 4°C and the purified liquid was used for determining Cr by atomic absorption spectrometer AA-6300 (Shimadzu, Japan).

2.4. Serum Immunological Parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferases (AST), alkaline phosphatase (AKP), cortisol (COR), lysozyme (LZM), immunoglobulin M (IgM) and lactate dehydrogenase (LDH) concentration were measured at 14 and 28 days using commercial assay kit as described by Gou et al. (2018) [18], and it is in accordance with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.5. Antioxidant Parameters

Antioxidant indices in the liver, intestine and gills were analyzed on the 14 and 28 days. Catalase (CAT), glutathione peroxidase (Gsh-Px), superoxide dismutase, (SOD) and malondialdehyde (MDA) content were determined using business kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) as described by Yu et al. (2020) [19].

2.6. Statistical Analysis

The results of the research are expressed as mean \pm S.D. One-way analysis of variance (ANOVA) was used to determine the significant differences. In order to compare the mean values ($P < 0.05$), Duncan's multiple range test was used, which had significant differences. SPSS statistics 19.0 software (IBM, USA) was used for analysis.

3. Result

3.1. Chromium Accumulation

As Fig. 1 shown, after 14 and 28 days of Cr (VI) exposure, the accumulation levels in each tissue of *C. argus* was liver > gill > intestine. Compared with the 0 mg/L group, the accumulation of Cr (VI) in gill, intestine and liver were significantly increased ($P < 0.05$) in all groups exposed to Cr (VI).

3.2. Antioxidant Parameters in Liver and Gill

The liver and gill antioxidant coefficients of *C. argus* in response to Cr (VI) are shown in Fig. 2. As shown in the above results, compared with 0 mg/L, the activity of CAT, Gsh-Px and SOD in liver and gill significantly reduced ($P < 0.05$) when exposed to 2 mg/L Cr (VI) at the 28 day. On the contrary, MDA content in liver dramatically increased ($P < 0.05$) when exposed to 2 mg/L Cr (VI), and MDA content in gill approached the peak level at 2 mg/L on the 28 day.

3.3. Serum Immunological Parameters

Changes in serum immunological parameters after 28 days of *C. argus* exposure to Cr (VI) are shown in Fig. 3 and Fig. 4. Compared with 0 mg/L group, LZM activity was obviously decreased ($P < 0.05$) when it was exposed to 0.5-2mg/L Cr (VI) in the 14 day and the 28 day, and was lower than the other groups when it was exposed to 2mg/L Cr (VI) in the 28 day. However, the activity of AST, AKP, LDH and COR content were different. In dose-dependent effect, it significantly increased ($P < 0.05$) when it was exposed to 1-2mg/L Cr (VI) on the days 14 and 28. On day 28 after Cr (VI) exposure, IgM concentration and ALT activity were significantly higher than others when they were exposed to 2mg/L Cr (VI).

4. Discussion

Heavy metals are known to be a toxic pollutant that has always been existed, which may cause harmful effects on fish in aquatic ecosystems. The exposure of heavy metal toxic substances will have toxic effects on aquatic animals, but such toxic effects will vary with the biological conditions of the animals, the physical and chemical properties of the water, and the types, types and exposure time of toxic substances [20]. We also know that the type of fish, age and metabolic activity, the concentration of metals in food and sediments, as well as the temperature and salinity of environmental factors, may all affect the degree of heavy metal accumulation in their tissues [21]. Fish are usually at the top of the aquatic food chain, and exposure to heavy metals from bioaccumulation in fish can be considered as an assessment of metal pollution in the aquatic environment [22]. In addition, the accumulation of Cr (VI) in fish may lead to potential health risks for those who consume them [23]. Therefore, it is necessary to explore the accumulation and purification of water. Cr (VI) in fish tissues, to evaluate the toxic effects of Cr (VI) as antioxidants in fish systems and relative eating risks. And the route of ingestion affects accumulation in aquatic animals, whether through respiration or through digestive organs.

In this study, through exposure to heavy metals in water, the highest accumulation of Cr (VI) in the tissue was observed in the liver of *C. argus*. In general, the accumulation of fish in the liver is exposed to a much greater amounts of tested metals than other organs. This is attributed to the higher tendency of the elements to be related to the sulfur Al-sulfhydryl groups in carboxylate, amino, nitrogen and metallothionein. Vutukuru et al. (2007) reported that there is a large accumulation of chromium in the liver of major carp in India, and *Labeo rohita* is exposed to Cr (VI), leading to metabolic processes and detoxification [24]. Intestines and gills are important in ingestion. The content of heavy metals and other toxic substances in fish [25]. Intestinal tract is very crucial for the nutrients digestion and absorption in fish, and the integrity of its organizational structure is crucial for the growth and development of fish. In general, the ingestion of metals in the intestine is mainly largely diet-related, because it will ingest metals in the case of water transmission. The high accumulation of metals in intestines may be related to enterohepatic circulation that bile could excrete metals into the intestines [24]. This research showed that the bioaccumulation of Cr (VI) aqueous exposed to different tissues is as follows: liver > gills > intestine. Because of the interpenetration between fish blood and the external environment, blood parameters have proven to be sensitive and reliable indexes of toxic metal exposure for aquatic animals [26–29].

The SOD, CAT, Gsh-Px and MDA evaluation are generally known as the important indicators to detect the antioxidant capacity of aquatilia [30]. Antioxidant enzymes such as SOD, CAT, and Gsh-Px are important in the cellular defense against xenobiotic exposure [31]. It is known that SOD can catalyze the search for superoxide anion radicals and can quickly turn oxygen-free radicals into hydrogen peroxide molecules, so as to balance the metabolism of free radicals and protect the cells [32–33]. Both CAT and Gsh-Px have the ability to eliminate H₂O₂ and transform it into H₂O and O₂, thereby helping to remove H₂O₂ and reduce tissue damage [34]. All of the enzymes mentioned can defense against the stress of exposure to toxins in the organism. Their proper function and activity are essential to prevent any cell damage or death [35]. The secondary product MDA is produced by lipids peroxidation, indicating that oxidant cell damage caused by binding to free protein amino acids and then crosslinking within and between protein molecules [36–37]. So, an increased MDA level is the main mechanism for oxidative damage of cells. Therefore, MDA accumulation is the sign of the risk of oxidant cell damages [38]. The results above indicated, compared with 0 mg/L, the CAT activity, Gsh-Px and the SOD levels in liver and gill markedly decreased ($P < 0.05$) when they were exposed to 2 mg/L Cr (VI) on the 28 day. Exposure of Cr (VI) in water can cause massive generation of free radicals and reduce the antioxidant capacity of aquatilia. Opposite trend, in liver and gill, the level of MDA increased significantly ($P < 0.05$) when exposed to 2 mg/L Cr (VI) compared with 0 mg/L, in the 28 day. The level of MDA which exposed to 2 mg/L Cr (VI) is the highest, especially in the liver at 28 day.

Because of the interpenetration between fish blood and the external environment, blood parameters have proven to be sensitive and reliable indexes of toxic metal exposure for aquatic animals [39–41]. In order to determine the degree of liver and gill toxicity caused by Cr (VI) exposure, the levels of several enzymes were estimated, including ALT, AST and LDH. ALT and AST are important transaminases in fish liver, and AST is an indicator of liver tissue. The serum biochemical parameters of injury are therefore considered

to be important indicators for the diagnosis of liver diseases in aquatic animals [42–43]. Compared with the control, they were greatly improved at 28 day. This increase may be due to damage to liver cells and the leakage of these enzymes from damaged cells [44]. COR is synthesized by the adrenal cortex and is for regulating glucose metabolism and stress response. Meanwhile, the serum COR level is related to the stress process [45]. Serious metal pollution can cause abnormal COR metabolism of fish [46]. Previous studies have shown that the accumulation of Cr (VI) tends to lead to immunosuppression, thus increasing sensitivity of fish to diseases [47]. AKP is an important indicator of liver disease diagnosis. When the liver is damaged, liver cells overproduce AKP. LZM is an essential protective immune factor, hydrolyzing the peptidoglycan structure to remove all kinds of bacteria [48]. IgM is a large molecule involved in the specific immune response and is an important index to measure immune response [49]. This study shows that as the concentration of Cr (VI) increases, the level of LZM in the serum decreases significantly. It is suggested that immunosuppression may be caused because of inflammation by heavy metal stress [49]. At the same time, with the increase of lead concentration, the content of COR and AKP in this research increased significantly, which indicated that the lead accumulation caused an increase in the degree of stress. This could be due to the damage of the liver tissue structure caused by the accumulation of Cr (VI), and the COR and AKP in the serum also increase. However, IgM shows an upward trend with the prolonged exposure time in water. Some reports indicate that exposure to heavy metals and other toxic substances can cause changes in the body's immune system parameters [17]. Besides, higher levels of LZM and IgM have been observed in diseased fish [50]. Some studies have revealed that the environmental pressure has a negative impact on the immunity system, leading to increased sensitivity to diseases [51]. As the Cr (VI) content of water increases, the IgM level of *C. argus* also increases.

5. Conclusion

This research indicated that the waterborne Cr (VI) exposure to *C. argus* leads to the significant Cr (VI) accumulation in organs. Besides, Cr (VI) exposure in water changed antioxidant parameters (MDA, CAT, SOD and Gsh-Px) and immune coefficients (LZM, IgM, ALT, AST, AKP, COR, LDH) expression. These results suggest that the Cr (VI) exposure has a negative effect *C. argus*.

6. Declarations

Acknowledgment

The research was supported by the Personnel Foundation of Heilongjiang Bayi Agricultural University (NO.XYB202015).

Author Contributions Section

Jia-Hua Du and Zhe Yu✉writing-original draft preparation &designed study

Jun-Liang Zhao, Zhi-Hui Bai✉formal analysis

Ze-Hao Guo and De-Hui Li **Investigation**

Gui-Qin Wang, Mu-Yang Li and Lei Zhao: writing-review and editing

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.

Conflict of interest

The authors declare that they have no conflict of interest.

7. References

1. K.C. Tagliari, V.M.F. Vargas, K. Zimiani, R. Cecchini, Oxidative stress damage in the liver of fish and rats receiving an intraperitoneal injection of hexavalent chromium as evaluated by chemiluminescence, *Environ. Toxicol. Pharmacol.* 17 (2004) 149–157.
2. H. Oliveira, T.M. Santos, J. Ramalho-Santos, M.D. Pereira, Histopathological effects of hexavalent chromium in mouse kidney, *Bull. Environ. Contam. Toxicol.* 76 (2006) 977–983.
3. A.P. Roberts, J.T. Oris, Multiple biomarker response in rainbow trout during exposure to hexavalent chromium, *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* 138 (2004) 221–228.
4. S. Squadrone, M. Prearo, P. Brizio, et al., Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. *Chemosphere*, 90 (2013) 358-365.
5. Z. Yu, Z.Y. Dai, G.X. Qin, M.Y. Li, L.F. Wu, Alleviative effects of dietary microbial floc on copper-induced inflammation, oxidative stress, intestinal apoptosis and barrier dysfunction in *Rhynchocypris lagowski* Dybowski, *Fish & Shellfish Immunology*. 106 (2020) 120-132.
6. Z. Yu, X.Q. Wu, L.Q. Zheng, Z.Y. Dai, L.F. Wu, Effect of acute exposure to ammonia and BFT alterations on *Rhynchocypris lagowski*: Digestive enzyme, inflammation response, oxidative stress and immunological parameters. *Environmental Toxicology and Pharmacology*. 78 (2020) 103380.
7. R. Vinodhini, M. Narayanan, The impact of toxic heavy metal on the hematological parameters in common carp (*Cyprinus carpio L.*). *Iran. J. Environ. Health Sci. Eng.* 6 (2009) 23–28.
8. T. Shaheen, T. Akhtar, Assessment of chromium toxicity in *Cyprinus carpio* through hematological and biochemical blood markers. *Turk. J. Zool.* 36 (2012) 682–690.
9. L. Wang, B. Zheng, W.R. Meng, Molecular biomarkers in aquatic organisms in relation to the oxidative stress imposed by environmental pollutants. *Acta Ecol Sin.* 98 (2007) 78–91
10. Z. Yu, L. Li, R. Zhu, M. Li, L.F. Wu, Effects of bioflocs with different C/N ratios on growth, immunological parameters, antioxidants and culture water quality in *Opsariichthys kaopingensis* Dybowski. *Aquaculture Research*. 51 (2020) 805-815.
11. Z. Yu, Z.Y. Dai, L. Li, G.X. Qin, L.F. Wu, Dietary supplementation with biofloc promotes growth, improves immune and antioxidant status, and upregulates NF-κB/Nrf2 signalling molecules and stress resistance in *Rhynchocypris lagowskii* Dybowski. *Aquaculture Nutrition*. 27 (2021) 225-239.

12. Fairbrother A, Fowles J (1990) Subchronic effects of sodium selenite and selenomethionine on several immune-functions in mallards. *Arch Environ Contam Toxicol* 19(38):836–844
13. L. Zhao, Y.G. Zheng, Y.H. Feng, et al. Toxic effects of waterborne lead (Pb) on bioaccumulation, serum biochemistry, oxidative stress and heat shock protein-related genes expression in *Channa argus*. *Chemosphere*. 261 (2020) 127714.
14. Z. Yu, Y.G. Zheng, H.L. Du, H.J. Li, L.F. Wu, Influence of differential protein levels of feed on growth, copper-induced immune response and oxidative stress of *Rhynchocypris lagowski* in a biofloc-based system. *Aquaculture Nutrition*. 26 (2020) 2211-2224.
15. L. Zhao, M. Li, K. Sun, et al. *Hippophae rhamnoides* polysaccharides protect IPEC-J2 cells from LPS-induced inflammation, apoptosis and barrier dysfunction in vitro via inhibiting TLR4/NF-κB signaling pathway. *International Journal of Biological Macromolecules*. 155 (2020) 1202-1215.
16. M.Y. Li, C.S. Gao, X.Y. Du, et al. Effect of sub-chronic exposure to selenium and astaxanthin on *Channa argus*: Bioaccumulation, oxidative stress and inflammatory response. *Chemosphere*. 244 (2019) 125546.
17. Y. Yin, P. Zhang, X. Yue, et al, Effect of sub-chronic exposure to lead (Pb) and *Bacillus subtilis* on *Carassius auratus gibelio*: bioaccumulation, antioxidant responses and immune responses. *Ecotoxicol Environ Saf*. 161 (2018) 755–762.
18. C. Gou, J. Wang, Y. Wang, et al. *Hericium caput-medusae* (Bull.:Fr.) Pers. polysaccharide enhance innate immune response, immune-related genes expression and disease resistance against *Aeromonas hydrophila* in grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology* 72 (2019) 604-605
19. Z. Yu, L. Li, R. Zhu, M. Li, J. Duan, J.Y. Wang, Y.H. Liu, L.F. Wu, Monitoring of growth, digestive enzyme activity, immune response and water quality parameters of Golden crucian carp (*Carassius auratus*) in zero water exchange tanks of biofloc systems. *Aquaculture Reports*. 16 (2020) 100283.
20. S.S. Vutukuru, Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major Carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health* 2 (2005) 456–462.
21. S.M, Allen-Gil, V.G. Martynov, Heavy metal burdens in nine species of freshwater and anadromous fish from the Pechora River, northern Russia. *Science of the Total Environment*. 160–161 (1995) 653-659.
22. P. Tawari-Fufeyin, S.A. Ekaye, Fish species diversity as indicator of pollution in Ikpoba river, Benin City, Nigeria. *Rev. Fish Biol. Fish*. 17 (2007) 21–30.
23. J. Yin, L. Wang, Q. Liu, et al. Metal concentrations in fish from nine lakes of Anhui Province and the health risk assessment. *Environmental Science and Pollution Research*, 34(2020) 1-8.
24. S.S. Vutukuru, N.A. Prabhath, M. Raghavender, A. Yerramilli, Effect of arsenic and chromium on the serum amino-transferases activity in Indian Major Carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health* 4 (2007) 224–227.

25. Y. Xu, W. Wang, Exposure and potential food chain transfer factor of Cd, Se and Zn in marine fish *Lutjanus argentimaculatus*. Mar Ecol Prog. 238 (2002) 173–186
26. M.H. Kraal, M.H.S. Kraak, C.J. Degroot, C. Davids, Uptake and tissue distribution of dietary and aqueous cadmium by carp (*Cyprinus carpio*). Ecotoxicol. Environ. Saf. 31 (1995) 179–183.
27. J. Cazenave, D.A. Wunderlin, A.C. Hued, M. Bistoni, Haematological parameters in a neotropical fifish, *Corydoras paleatus*, captured from pristine and polluted water. Hydrobiologia. 537 (2005) 25-33.
28. Z. Yu, Y.N. Quan, Z.Q. Huang, H.H. Wang, L.F. Wu, Monitoring oxidative stress, immune response, Nrf2/NF-κB signaling molecules of *Rhynchocypris lagowski* living in BFT system and exposed to waterborne ammonia. Ecotoxicology and Environmental Safety. 205 (2020) 111161.
29. C.B.R. Martinez, M.Y. Nagae, C.T.B.V. Zaia, D.A.M. Zaia, Acute morphological and physiological effects of lead in the neotropical fifish *Prochiloduslineatus*. Braz. J. Biol. 64 (2004) 797-807.
30. M.A.O. Dawood, S. Koshio, M. Ishikawa, et al., Dietary supplementation of selenium nanoparticles modulated systemic and mucosal immune status and stress resistance of red sea bream (*Pagrus major*). Fish Physiol. Biochem. 45 (2019) 219–230.
31. M. Sevcikova, H. Modra, A. Slaninova, Z. Svobodova, Metals as a cause of oxidative stress in fish: a review. Vet Med-Czech 56 (2011) 537–546.
32. Z. Yu, L. Li, M. Li, L.F. Wu, Dietary supplementation of microbial floc heightens growth and improves digestive, immune, antioxidant enzymes activity and ammonia resistance in *Opsariichthys kaopingensis*. Aquaculture Research. 51 (2020) 4054-4064.
33. L. Cao, W. Huang, J.H. Liu, X.B. Yin, S.Z. Dou, Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. Comp Biochem Physiol Part C. 151 (2010) 386–392.
34. C.L. Yao, Z.Y. Wang, J.H. Xiang, Crustacean haemocytes and their function in immune responses. Zool Res. 27 (2006) 549–557.
35. S. Pandey, S. Parvezb, A.R. Ahamed, et al., Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chem-Biol Interact. 174 (2008) 183–192.
36. E. Papadimitriou, N.S. Loumbourdis, Exposure of the frog *Rana ridibunda* to copper impact on two biomarkers, lipid peroxidation, and glutathione. Bull Environ Contam Toxicol. 69 (2002) 885–891.
37. V.L.D. Badisa, L.M. Latinwo, C.O. Odewumi, et al., Mechanism of DNA damage by cadmium and interplay of anti-oxidant enzymes and agents. Environ Toxicol. 22 (2007) 144–215.
38. Y. Zhou, W. Jing, H.U. Dahms, J.S. Hwang, L. Wang, Oxidative damage, ultrastructural alterations and gene expressions of hemocytes in the freshwater crab *Sinopotamon henanense* exposed to cadmium. Ecotoxicol Environ Saf. 138 (2017) 130–138.
39. M.Y. Li, X.Y. Liu, C.G. Xia, et al. Astaxanthin enhances hematology, antioxidant and immunological parameters, immune-related gene expression, and disease resistance against in *Channa argus*. Aquaculture International 27 (2019) 735–746

40. J.Y. Ko, E.A. Kim, J.H., Lee, et al. Protective effect of aquacultured flounder-derived peptide against oxidative stress in zebrafish. *Fish Shellfish Immunol.* 36 (2014) 320-323.
41. C.B.R. Martinez, M.Y. Nagae, C.T.B.V. Zaia, D.A.M. Zaia, Acute morphological and physiological effects of lead in the neotropical fish *Prochiloduslineatus*. *Braz. J. Biol.* 64 (2004) 797-807.
42. G.I. Hemre, R. Waagbø, B. Hjeltnes, A. Aksnes, Effect of gelatinized wheat and maize in diets for large Atlantic salmon (*Salmo salar L.*) on glycogen retention, plasma glucose and fish health. *Aquac. Nutr.* 2 (2010) 33-39.
43. N. Sheikhzadeh, A.K. Oushani, M.H.N. Enferadi, Effects of *Haematococcus pluvialis* supplementation on antioxidant system and metabolism in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* 38 (2011) 413-419.
44. M. Oener, G. Atli, M. Canli, Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. *Environmental Toxicology & Chemistry* 27 (2010) 360-366.
45. C.B.R. Martinez, M.Y. Nagae, C.T.B.V. Zaia, D.A.M. Zaia, Acute morphological and physiological effects of lead in the neotropical fish *Prochiloduslineatus*. *Braz. J. Biol.* 64 (2004) 797-807.
46. S. Garcia-Santos, A. Fontainhas-Fernandes, S.M. Monteiro, J.M. Wilson, Effects of exposure to cadmium on some endocrine parameters in tilapia, *Oreochromis niloticus*. *Bull. Environ. Contam. Toxicol.* 90 (2013) 55-59.
47. L. Zhao, B.D. Yuan, J.L. Zhao, et al. Amelioration of hexavalent chromium-induced bioaccumulation, oxidative stress, tight junction proteins and immune-related signaling factors by *Allium mongolicum Regel* flavonoids in *Ctenopharyngodon idella*. *Fish and Shellfish Immunology*, 106 (2020) 993-1003.
48. H.Y. Yildiz, Plasma lysozyme levels and secondary stress response in rainbow trout, *Oncorhynchus mykiss* (Walbaum) after exposure to Leteux-Meyer mixture. *Turk. J. Vet. Anim. Sci.* 30 (2006) 265-269.
49. Z. Yu, Y.G. Zheng, H.L. Du, et al. Bioflocs protects copper-induced inflammatory response and oxidative stress in *Rhynchocypris lagowski Dybowski* through inhibiting NF- κ B and Nrf2 signaling pathways. *Fish Shellfish Immunol.* 98 (2020) 466-476.
50. M. Studnicka, A. Siwicki, The nonspecific immunological response in carp (*Cyprinus carpio L.*) during natural infection with *Eimeria subepithelialis*. *Bamidgeh*. 42 (1990) 18-21.
51. J.H. Kim, J.C. Kang, Oxidative stress, neurotoxicity, and non-specific immune responses in juvenile red sea bream, *Pagrus major*, exposed to different waterborne selenium concentrations. *Chemosphere*. 135 (2015) 46-52.

8. Tables

Table 1

Formulation and nutrient content of the basal diet

| Ingredients (%) | | Nutrient content (% or kJ/g) | |
|-----------------------|------|------------------------------|------|
| Fish meal | 38.7 | Crude protein | 48.1 |
| Soybean meal | 4 | Crude lipid | 11.3 |
| Poultry meal | 12.9 | Ash | 12.3 |
| Gluten | 5 | Carbohydrate | 20.1 |
| Spraying blood meal | 5 | Gross energy | 19.3 |
| Peanut meal | 3 | | |
| Flour | 17.5 | | |
| Fish oil | 5.9 | | |
| Squid offal | 1 | | |
| Monocalcium phosphate | 1.5 | | |
| Vitamin premix | 1.5 | | |
| Mineral mixture | 1 | | |
| Zeolite | 3 | | |

Figures

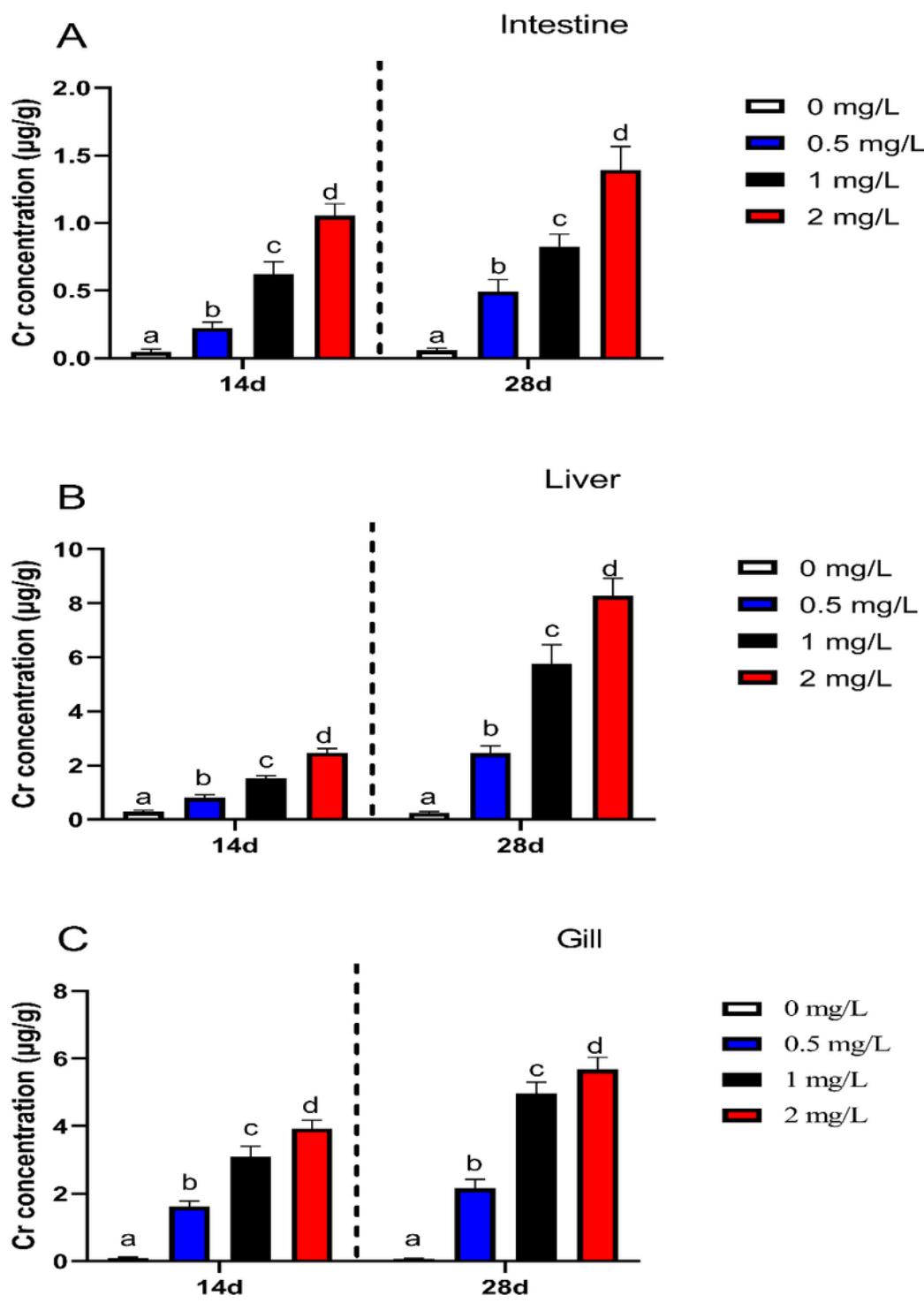


Figure 1

Cr accumulation (Intestine (A), Liver (B) and Gill (C)) of *Channaargus* ($n = 5$) after exposure to Cr. Data are expressed as the mean \pm S.D. Bar with different letters are significantly ($P < 0.05$) different by Tukey test on the same sampling interval.

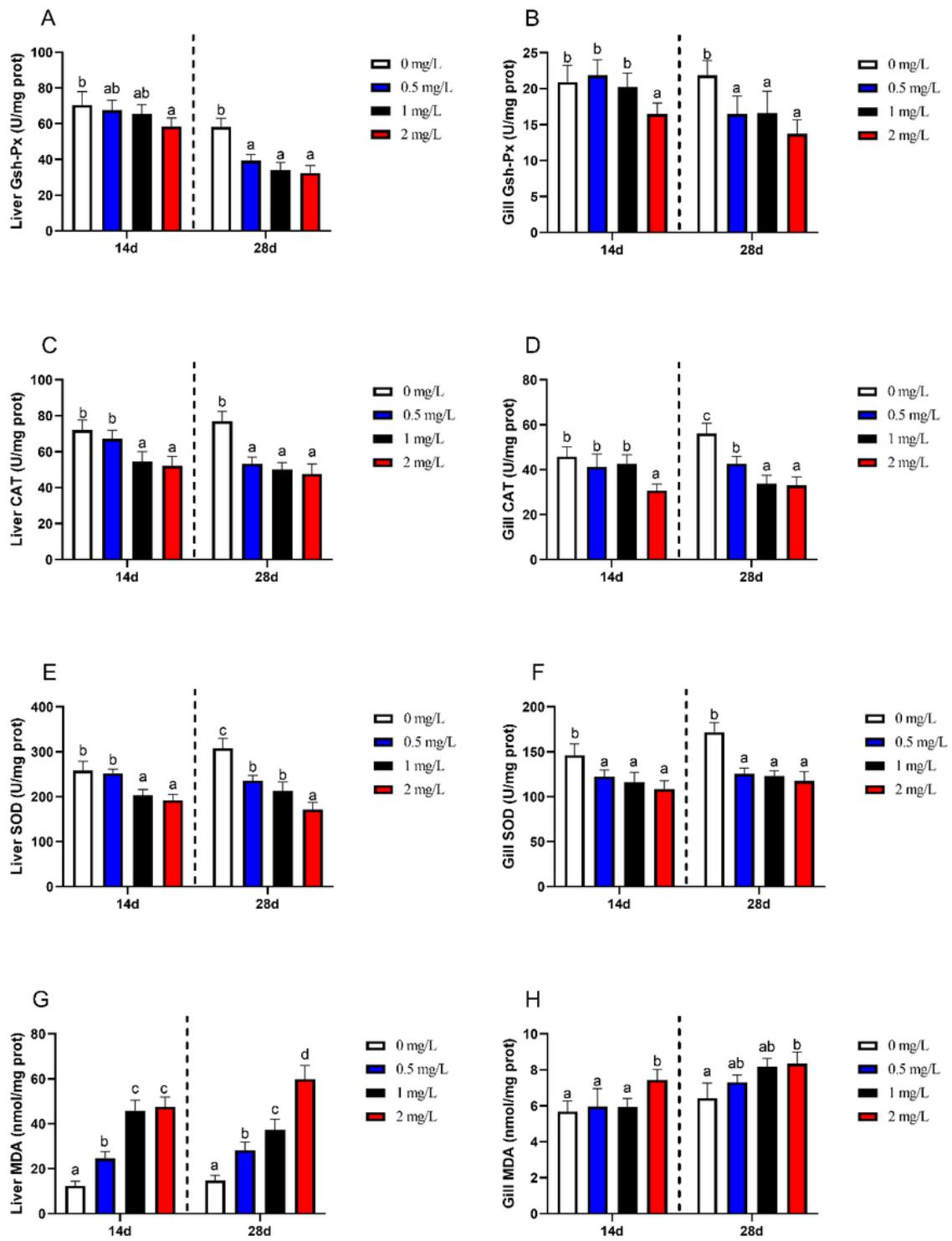


Figure 2

Oxidative stress parameters in the liver and gill (GPx activity (A, B), CAT activity (C, D), SOD activity (E, F) and MDA (G, H)) of *Channa argus* ($n = 5$) after exposure Cr. Data are expressed as the mean \pm S.D. Bar with different letters are significantly ($P < 0.05$) different by Tukey test on the same sampling interval.

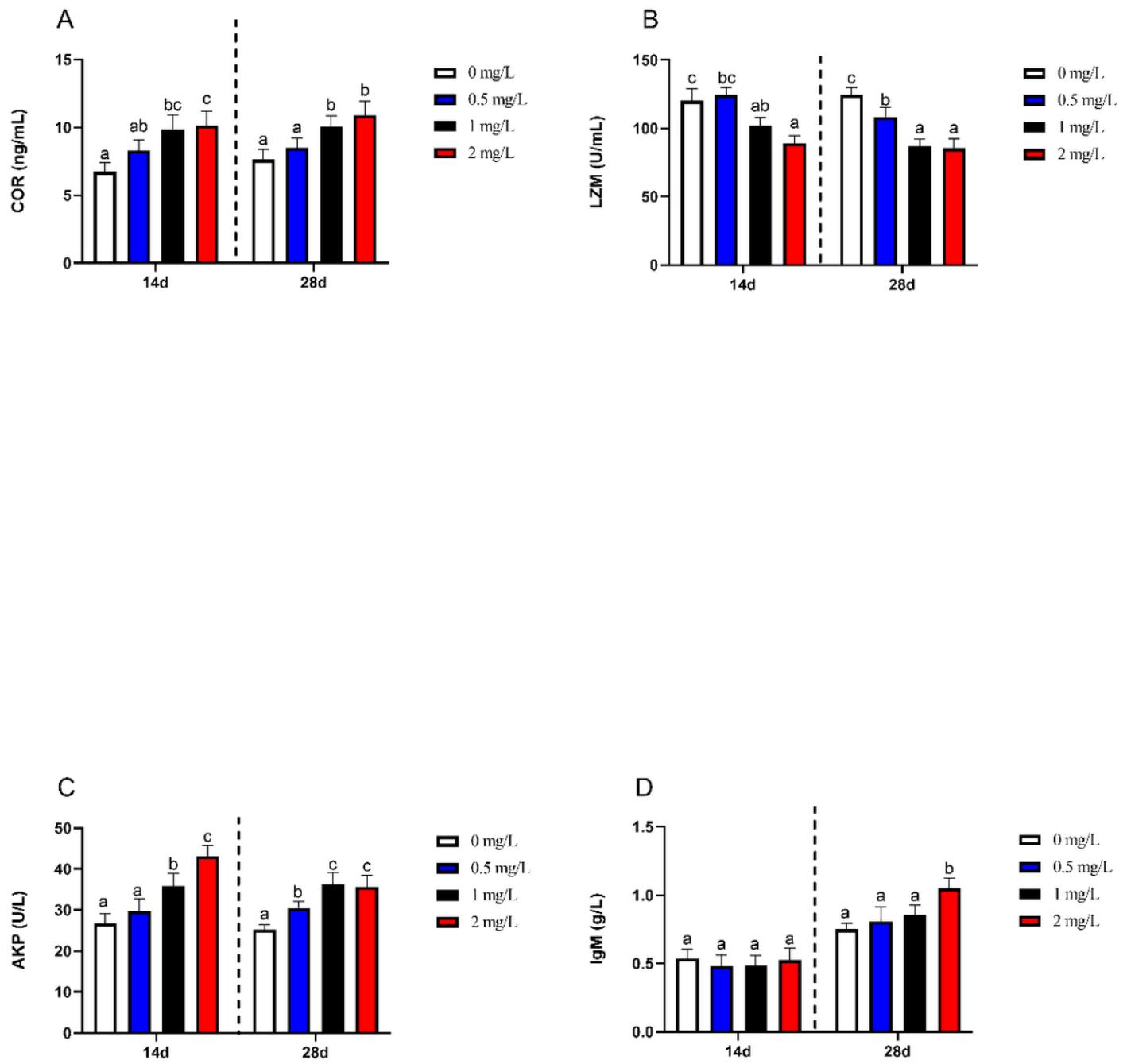


Figure 3

The serum immunological parameters(COR activity(A),LZM activity(B),AKP activity(C) and IgM(D)) of Channa argus ($n = 5$) after exposure Cr. Data are expressed as the mean \pm S.D. Bar with different letters are significantly ($P < 0.05$) different by Tukey test on the same sampling interval.

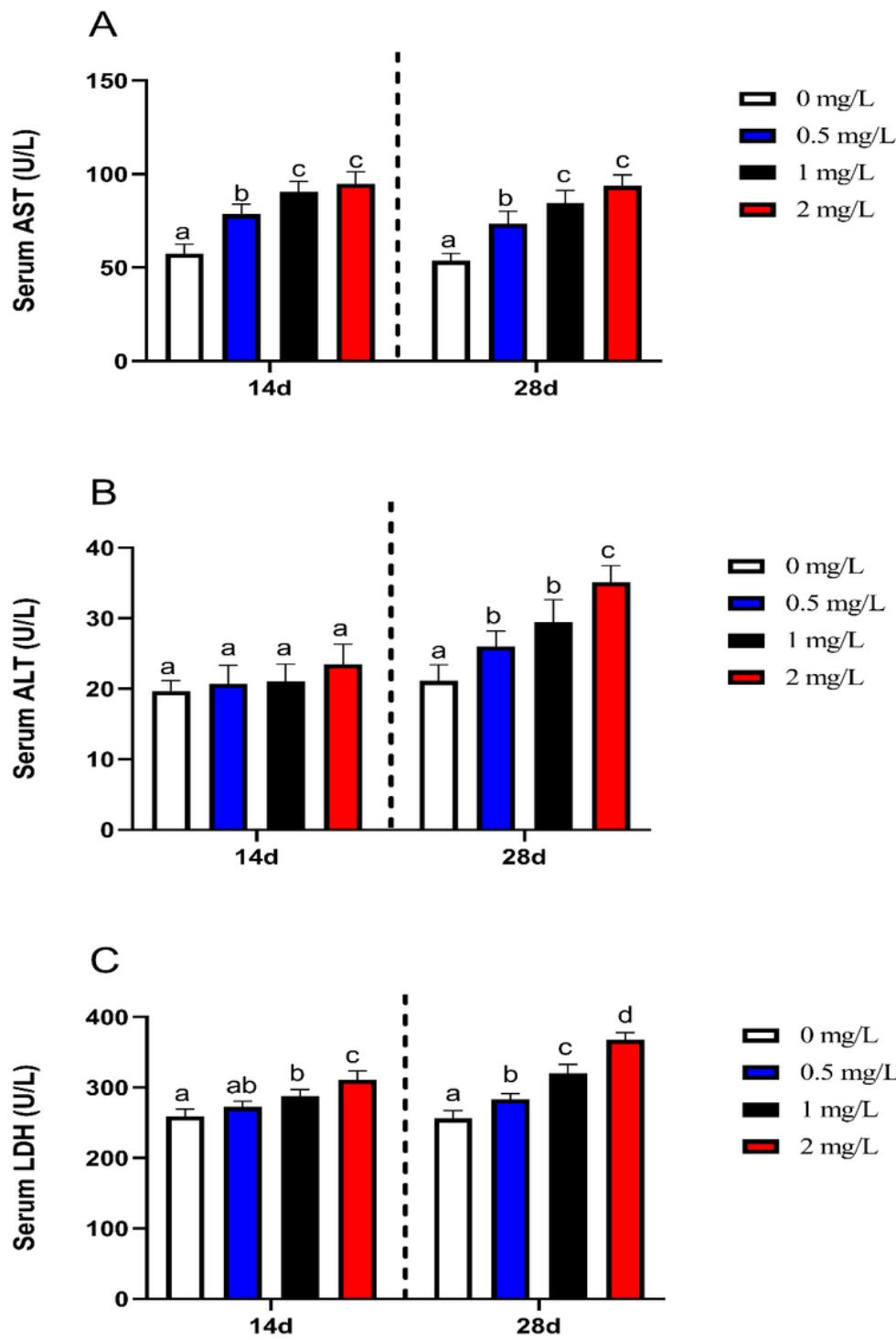


Figure 4

The serum immunological parameters(AST activity(A),ALT activity(B) and LDH activity(C)) of Channa argus ($n = 5$) after exposure Cr. Data are expressed as the mean \pm S.D. Bar with different letters are significantly ($P < 0.05$) different by Tukey test on the same sampling interval.