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Research Article

Effects of Vitamin E on Immune Response, Antioxidant Capacity, and Liver Tissue Structure of Crucian Carp under Acute Cold Stress

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The purpose of this study was to investigate the effects of vitamin E addition to the water column on immune response, antioxidant capacity, and liver tissue structure of crucian carp (*Carassius auratus*) under acute cold stress. Crucian carp was placed in the aqueous solution of control group (E1), negative control group (E2), and 100 mg/L vitamin E (E3) and cooled from 20 to 5°C by cold acclimation intelligent cooling device. Samples were taken at three temperature points of 20, 10, and 5°C, respectively, and the samples were detected and analyzed. The results showed that the content of each index increased under acute cold stress. The contents of white blood cell, red blood cell, hemoglobin, platelet, lysozyme, and glutathione peroxidase in E3 treatment group were significantly lower than those in E1 and E2 treatment groups ($P<0.05$). The contents of cholinesterase, alkaline phosphatase, acid phosphatase, hydrogen peroxide, superoxide anion, superoxide dismutase, peroxidase, and malondialdehyde in E3 treatment group were significantly lower than those in E1 and E2 treatment groups ($P<0.01$). Under acute cold stress, the liver tissue structure of crucian carp also changed, such as hepatocyte vacuolization, and the damage degree of E3 treatment group was lower than that of E1 and E2 treatment groups. In summary, the addition of vitamin E in water to treat crucian carp can reduce the harm caused by acute cold stress to its body and provide a theoretical reference for the application of vitamin E in water to alleviate fish stress.

1. Introduction

Low-temperature waterless transportation is a new type of green and safe living circulation mode, which has the advantages of low cost, high survival rate, high carrying capacity, and small quality change [1]. Low-temperature anhydrous live transportation is a method of inducing a dormant state in fish through low temperatures, packaging them anhydrically, and placing them in a specific low-temperature environment for live storage and transportation [2]. Temperature is an important environmental condition that affects the growth, development, and reproduction of fish. In the process of low-temperature-induced fish dormancy, a sudden drop in temperature will adversely affect the normal life activities of fish, and in serious cases, it may even cause the death of fish [3, 4]. Studies have shown that fish can cope with the oxidative damage caused by low-temperature stress by activating the regulation of physiological processes such as antioxidant enzyme activity, releasing neurotransmitters and hormones [5]. For example, superoxide dismutase, catalase, and glutathione peroxidase, as important enzymes with antioxidant defense function, can effectively scavenge reactive oxygen species (ROS) and maintain intracellular ROS concentration at normal physiological levels [6]. In addition,

the physiological and biochemical reactions of cells and tissues caused by low-temperature environment may lead to changes in tissue structure and function [7]. Therefore, it is of great significance to study the methods to alleviate the stress response induced by low temperature during fish dormancy and to maintain the quality of fish without water.

Vitamin E is one of the most important fat-soluble vitamins, which can improve the fat metabolism and antioxidant capacity of aquatic products, enhance the body's immunity, resistance to disease, and other important physiological functions [8–10]. Vitamin E is an important free radical scavenger in the body. It is beneficial to the metabolism of substances by inhibiting lipid peroxidation in the body and protecting biofilm from damage. Therefore, to a certain extent, alleviate the body's stress response caused by low-temperature environment. The suitable survival temperature of crucian carp is 15–25°C. When the temperature is lower than the suitable survival temperature range of crucian carp, it will cause the stress response of the body. Previous studies have shown that the addition of vitamin E to the water body can alleviate the stress response of the body caused by cooling stress [11]. As a vitamin, vitamin E plays an important role in the health of the body within the allowable dose range, whether in the field of food or external use. Adding vitamin E to water reduces the cost of making other bait materials. At the same time, due to the significantly shortened time of vitamin E action on fish and the improved survival ability of waterless survival, the loss is reduced, and the actual sales volume is largely maintained. Therefore, it is necessary to add vitamin E to water. However, so far, there is still a lack of research on the effects of vitamin E supplementation in water on the immunity, antioxidant enzyme activity, and histological structure of fish during stress. In this study, crucian carp was used as the research object to study the effects of vitamin E on the immune, antioxidant level and histological structure of crucian carp under acute cold stress. The purpose of this study was to provide a theoretical reference for the application of vitamin E in water to alleviate fish stress and improve the circulation efficiency of live fish.

2. Materials and Methods

2.1. Materials. Crucian carp was purchased from the seafood market in Jinan and transported to the National Engineering Research Center for Agricultural Products Logistic. Adult fish with initial body weight (0.3 ± 0.1 kg), body length (15.7 ± 1.1 cm), healthy body quality, no damage to the body surface, intact scales, and uniform size were selected and temporarily reared in the temperature-controlled recirculating water filtration system of aquatic products of the National Engineering Research Center for Agricultural Products Logistic for use. The temporary culture conditions were as follows: they were transported to the Research Center for temporary breeding for 2 weeks, continuously inflated, and fed with commercial compound feed once in the morning and once in the evening every day and treated with sewage and water exchange. During this period, fish that died or were in poor health were removed. Feeding was prohibited 24 hr before the start of the experiment. Dissolved oxygen ≥6 mg/L, pH 7–8, and water temperature 20 ± 1°C.

2.2. Instruments and Equipment. BK-280 automatic biochemical analyzer, Shandong Boke Biological Industry Co., Ltd., China; XT-1800IV Sysmex se-9500 blood cell analyzer, Shenzhen Mindray Biomedical Electronics Co., Ltd., Shenzhen, China; T9S ultraviolet spectrophotometer, Beijing Purkinje General Instrument Co., Ltd., China; the intelligent cold acclimation/wake-up box for fresh aquatic products was independently developed by the Key Laboratory of Agricultural Products Storage and Transportation Technology in Shandong Province (patent no. ZL201310447777.8); Artificial climate chamber QHX-300BSH-III, Shanghai Xinmiao Medical Device Manufacturing Co., Ltd., China; aquaculture and circulating water treatment system, Qingdao Zhongke Seawater Treatment Equipment Engineering Co., Ltd., China; jPB-607A Portable Dissolved Oxygen Analyzer, Shanghai LeiMag Electric Scientific Instruments Co., Ltd., China.

2.3. Test Method

2.3.1. Vitamin E Treatment. In the experimental group, vitamin E was dissolved in anhydrous ethanol at a ratio of vitamin E : anhydrous ethanol = 1 : 5 to make a reserve liquid. The stock solution was added with water to prepare vitamin E aqueous solution with a mass concentration of 100 mg/L, which was set as E3. Crucian carp without vitamin E (0 mg/L) treatment was used as the control group E1. A negative control group, E2, was set up, and E2 was a solution configured with the same level of ethanol as "the amount of ethanol required to dissolve vitamin E in E3." The transiently reared crucian carp was transferred to the cold domestication intelligent cooling device ($200 \times 120 \times 130$ cm^3) with aqueous solutions (160 L) of E1, E2, and E3 treatment groups for drug baths. The time was 2 hr, which was the duration of the exposure. The total number of experimental crucian carp is 54, with 18 for E1, E2, and E3, respectively. The experiment was repeated three times with six fish per experiment.

2.3.2. Cold Acclimation Treatment. The cold acclimation of crucian carp was completed by a self-designed cold acclimation intelligent cooling device [12]. The device continues to run the circulatory system during the cooling period. First, the water temperature is reduced from 20 to 10°C at a cooling rate of 10°C/hr, and then the water temperature is reduced from 10 to 5°C at a cooling rate of 5°C/hr, which takes 2 hr. During the cold acclimation, the feeding of crucian carp was prohibited, and the temperature was recorded in real time with a precision temperature detector. Six crucian carps were randomly sampled at 20, 10, and 5°C, respectively.

2.3.3. Determination of Indicators. All animal experiments were performed in accordance with the British Animals (Scientific Procedures) Act 1986 and the National Institutes of Health Laboratory Animal Care and Use Guidelines (NIH publication No. 8023, revised in 1978). After the crucian carp was taken out from the cold acclimation intelligent cooling device, more than 5 mL of whole blood was taken from the

tail vein with a 5-mL disposable syringe. The blood was taken and placed in a refrigerator at 4°C for 2 hr. After centrifugation at 4,000 rpm for 20 min at 4°C, the supernatant was taken and stored in a refrigerator at −80°C. After having taken the blood, six crucian carps were placed on a tray containing ice for dissection, and its liver tissue was taken. After liquid nitrogen treatment, it was placed in a refrigerator at −80°C for storage. According to the method of Liu et al. [13], an appropriate amount of liver tissue from two fishes was randomly selected and separately infiltrated in 4% formaldehyde fixative in order to prepare paraffin sections.

(1) Determination of Blood Physiological Indexes. White blood cell (WBC) ($4–10 \times 10^9$/L), red blood cell (RBC) (120–160 g/L), hemoglobin (HGB) (101–200 g/L), and platelet (PLT) ($100–300 \times 10^9$/L) were measured by XT-1800IV Sysmex se-9500 blood cell analyzer and a kit (Shenzhen Mindray Biomedical Electronics Co., Ltd., Shenzhen, China).

(2) Determination of Serum Biochemical Indicators. Cholinesterase (CHE) (0.5–150 U/mL) and alkaline phosphatase (ALP) (0.05–50.0 Kim's units/100 mL) in serum were determined using an automatic biochemical analyzer (BK-280). Acid phosphatase (ACP) (0.4–32.0 Kim's units/100 mL), lysozyme (LZM) (5–1,000 U/mL), hydrogen peroxide (H_2O_2) (0.0097–1.5 μmol/mL), and superoxide anion (O_2^-) (0.5–250 U/L) were determined by using the kit from Nanjing Jianjian Bioengineering Institute.

(3) Determination of Antioxidant Index. Superoxide dismutase (SOD) (5.0–122.1 U/mL), glutathione peroxidase (GSH-Px) (20–330 U), catalase (CAT) (0.2–24.8 U/mL), and malondialdehyde (MDA) (0–113.0 nmol/mL) contents in liver were determined by using a kit from Nanjing Jianjian Bioengineering Institute. Protein content in liver was determined by total protein assay kit (BCA, 20–2,000 μg/mL).

(4) Observation of Organizational Structure. The liver tissue of E1 group at 20°C was used as the blank control group, and the liver tissue structure of E1, E2, and E3 groups at 10 and 5°C was observed. We carefully cut the liver into 5–6 μm thick slices with scalpel. Liver tissues in 4% formaldehyde fixative were dehydrated with ethanol and anhydrous ethanol in volume fractions of 50%, 70%, 85%, and 95% for 2 hr each; after paraffin embedding, the histological features were observed under a light microscope after the steps of sectioning, hematoxylin and eosin (H&E) staining, and sealing with neutral gum.

2.4. Statistical Analysis. All data are expressed as mean ± standard deviation. Statistical analysis of the data was performed in SPSS 22.0 software (version 22, IBM Corp., Armonk, NY, USA). Based on single-factor analysis of variance, Duncan's multiple comparison method was used for the analysis. In all cases, the minimum level of significance was determined at both $P<0.05$ and $P<0.01$. The results were plotted using Origin 2019 software.

3. Results

3.1. Effects of Vitamin E on Blood Physiological Indexes of Crucian Carp under Acute Cold Stress. With the decrease of temperature, the WBC content of E1, E2, and E3 treatment groups increased, while the WBC content of E1 and E3 treatment groups showed an upward trend, while the WBC content of E2 treatment group increased first and then decreased. At 10°C, the WBC content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.05$) (Figure 1(a)). During the cooling process, the RBC content of E1 and E2 treatment groups increased with the decrease of temperature, while the RBC content of E3 treatment group decreased first and then increased. At 5°C, the RBC content in E3 treatment group was significantly lower than that in E1 and E2 treatment groups ($P<0.05$) (Figure 1(b)). During the cooling process, the HGB content of the E1 treatment group increased with the decrease of temperature, while the E2 and E3 treatment groups increased first and then decreased. At 5°C, the HGB content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.05$) (Figure 1(c)). During the cooling process, the PLT content of the E1 and E2 treatment groups increased first and then decreased with the decrease of temperature, while the PLT content of the E3 treatment group decreased with the decrease of temperature. At 10°C, the PLT content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.05$) (Figure 1(d)).

3.2. Effects of Vitamin E on Serum Biochemical Indexes of Crucian Carp under Acute Cold Stress. As shown in Figure 2(a), the ALP concentration of E1 and E2 treatment groups increased first and then decreased with the decrease of temperature, while the ALP concentration of E3 treatment group decreased first and then increased. At 10°C, the ALP concentration of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.01$). During the cooling process, the ACP concentration of E1 and E2 treatment groups increased with the decrease of temperature, while the ACP concentration of E3 treatment group decreased with the decrease of temperature. At 5°C, the ACP content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.01$) (Figure 2(b)). During the cooling process, the LZM concentration of the E1 and E2 treatment groups increased first and then decreased with the decrease of temperature, while the E3 treatment group decreased first and then increased. At 10°C, the LZM concentration in the E3 treatment group was significantly lower than that in the E1 and E2 treatment groups ($P<0.05$) (Figure 2(c)).

As shown in Figure 3(a), the content of CHE in E1, E2, and E3 treatment groups increased with the decrease of temperature. When the temperature decreased to 5°C, the content of CHE in E3 treatment group was significantly lower than that in E1 and E2 treatment groups ($P<0.01$). As shown in Figure 3(b), the content of H_2O_2 in E1 and E2 treatment groups increased first and then decreased with the decrease of temperature, while the content of H_2O_2 in E3 treatment group decreased with the decrease of temperature. At 5°C, the H_2O_2 content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.01$). As shown in Figure 3(c), the O_2^- content of the E2 treatment group increased with the decrease of temperature,

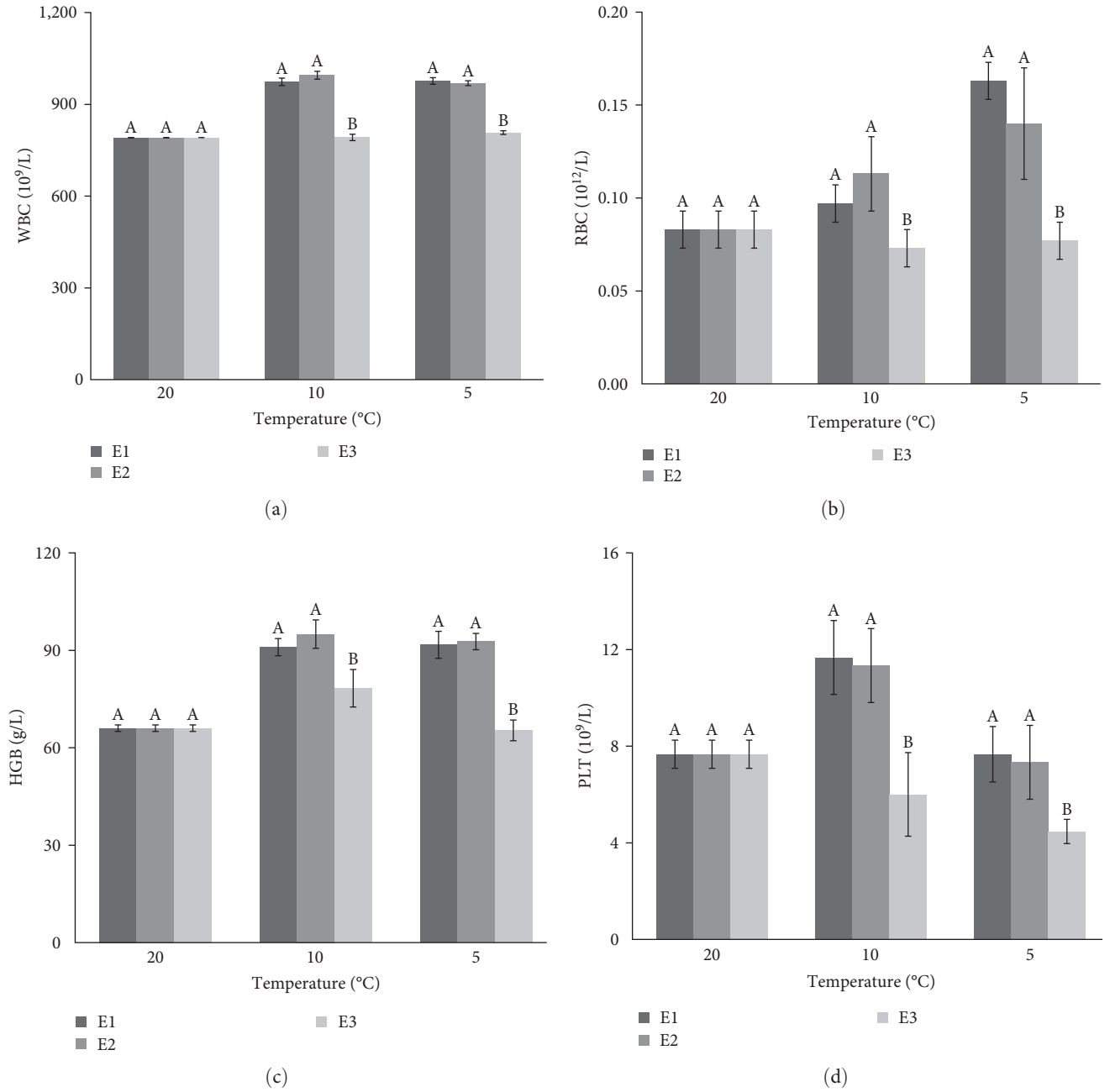

FIGURE 1: Effects of vitamin E on blood physiological indexes of crucian carp under acute cold stress: (a) white blood cell (WBC), (b) red blood cell (RBC), (c) hemoglobin (HGB), and (d) platelet (PLT). Different uppercase letters above columns indicate significant differences, and the same uppercase letters indicate no significant differences.

and the O_2^- content of the E1 and E3 treatment groups increased first and then decreased with the decrease of temperature. At 5°C, the O_2^- content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.01$).

3.3. Effects of Vitamin E on MDA, CAT, SOD, and GSH-Px in Liver of Crucian Carp under Acute Cold Stress. With the decrease of temperature, the MDA content of E1 and E2 treatment groups increased first and then decreased, while the MDA content of E3 treatment group decreased with the decrease of temperature. At 5°C, the MDA content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.01$) (Figure 4(a)). As shown in Figure 4(b), during the cooling process, the CAT content of the E1 and E2 treatment groups increased with the decrease of temperature, while the CAT content of the E3 treatment group decreased first and then increased. At 10°C, the CAT content of E3 treatment group was significantly lower than that of E1 and E2 treatment groups ($P<0.01$). During the cooling process, the SOD content of E1, E2, and E3 treatment groups increased with the decrease of temperature, and when the temperature decreased to 5°C, the SOD content of E3 treatment group was significantly lower than

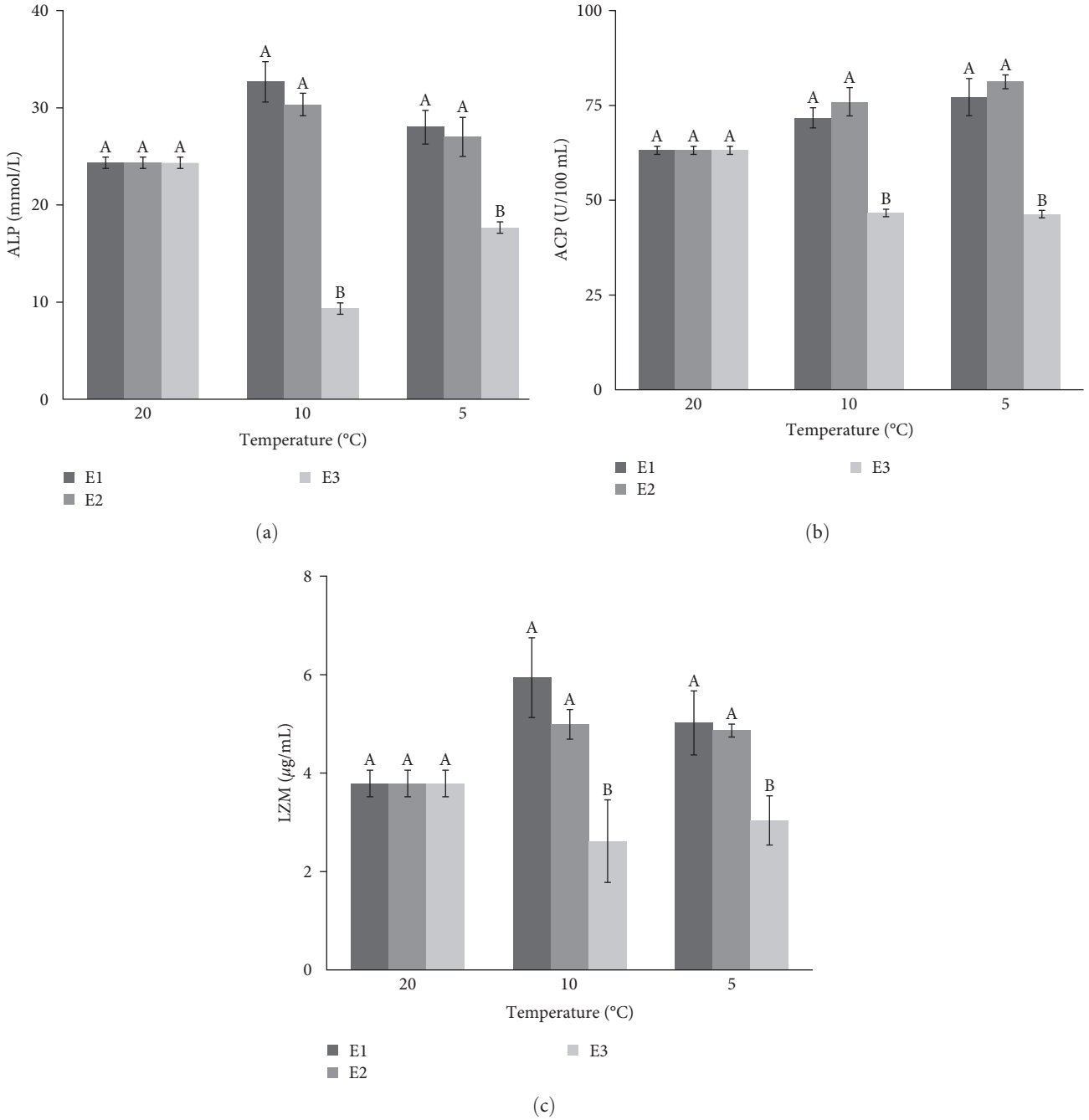

Figure 2: Effects of vitamin E on serum ALP, ACP, and LZM of crucian carp under acute cold stress: (a) alkaline phosphatase (ALP), (b) acid phosphatase (ACP), and (c) lysozyme (LZM). Different uppercase letters above columns indicate significant differences, and the same uppercase letters indicate no significant differences.

that of E1 and E2 treatment groups ($P<0.01$) (Figure 4(c)). During the acute cold process, the GSH-Px content of E1, E2, and E3 treatment groups increased first and then decreased with the decrease of temperature. At 5°C, the content of GSH-Px in E3 treatment group was significantly lower than that in E1 and E2 treatment groups ($P<0.05$) (Figure 4(d)).

3.4. Effects of Vitamin E on Liver Tissue Morphology of Crucian Carp under Acute Cold Stress. Under the light microscope, the hepatocytes in the liver tissue of the blank control group were closely arranged, and a small amount of vacuoles were distributed in the cytoplasm (Figure 5(a)). At 10°C, there was no obvious abnormality in the basic structure of the liver in the E1 treatment group, and the amount of vacuoles increased relatively (Figure 5(b)). The basic structure of the liver in the E2 treatment group was relatively complete at 10°C, but the amount of vacuoles was relatively increased (Figure 5(c)). At 10°C, the hepatocytes in the liver tissue of the E3 treatment group were closely arranged and the cytoplasm was relatively slightly increased (Figure 5(d)).

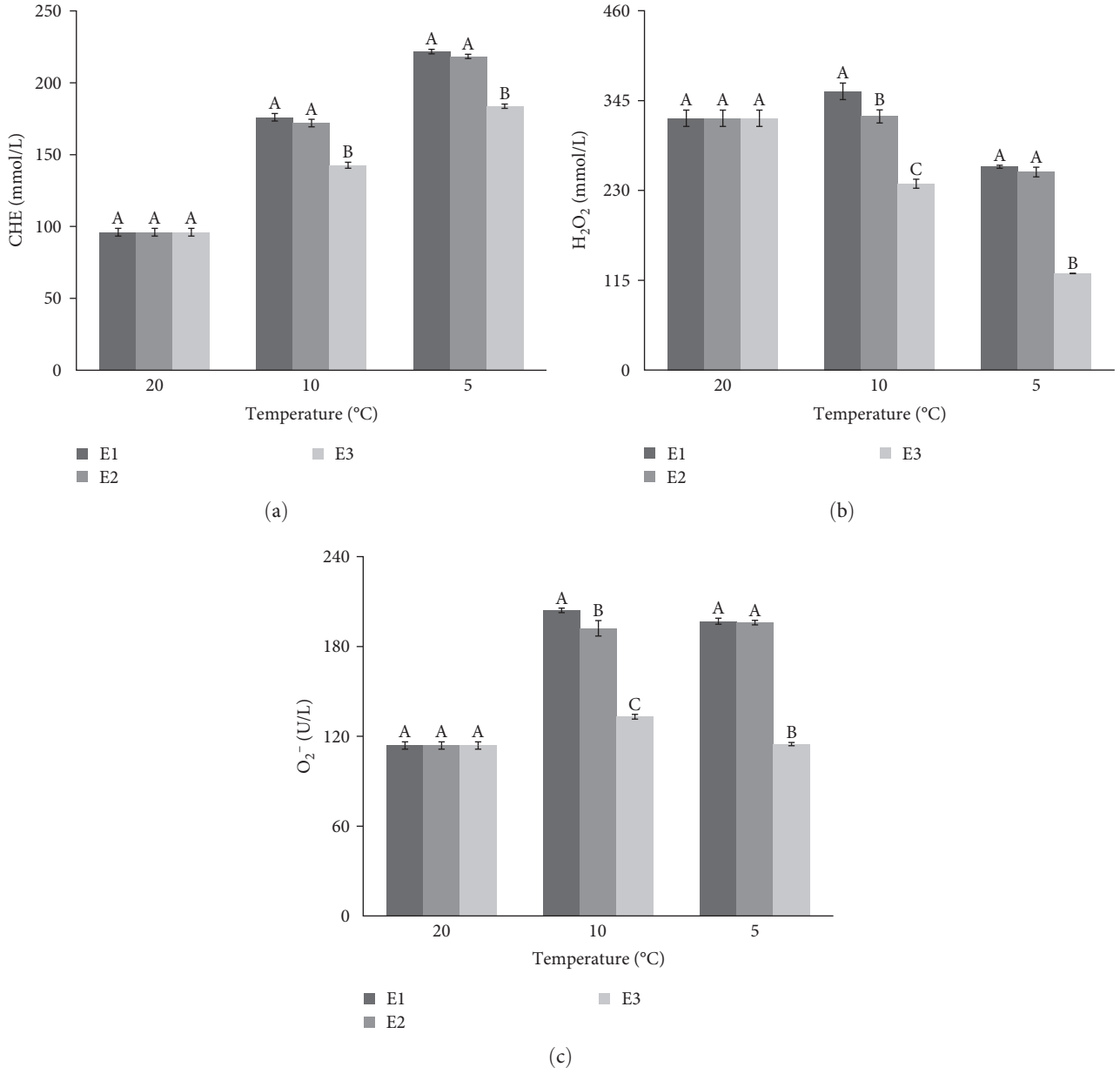

Figure 3: Effects of vitamin E on serum CHE, H_2O_2, and O_2^- of *Carassius auratus* under acute cold stress: (a) cholinesterase (CHE), (b) hydrogen peroxide (H_2O_2), and (c) superoxide anion (O_2^-). Different uppercase letters above columns indicate significant differences, and the same uppercase letters indicate no significant differences.

As shown in Figure 6, the liver tissue structure of the blank control group was complete, and a small amount of vacuoles appeared in the cytoplasm (Figure 6(a)). At 5°C, the hepatocytes in the E1 treatment group were closely arranged and the vacuoles were relatively increased (Figure 6(b)). The structure of the liver tissue in the E2 treatment group was relatively complete at 5°C, and the vacuoles were relatively increased (Figure 6(c)). At 5°C, the basic structure of the liver in the E3 treatment group was not significantly abnormal, but the cytoplasm was relatively increased (Figure 6(d)).

4. Discussion

4.1. Effects of Vitamin E on Blood Physiological and Biochemical Indexes of Crucian Carp under Acute Cold Stress. The WBC is involved in the regulation of immune work in various organisms, and related studies have shown that stress treatment can lead to an increase in WBC content in organisms [14–16]. In this study, the increase of WBC content in E1, E2, and E3 treatment groups after cold stress treatment was the result of the body's immune system responding to cold stress. However, the WBC content in

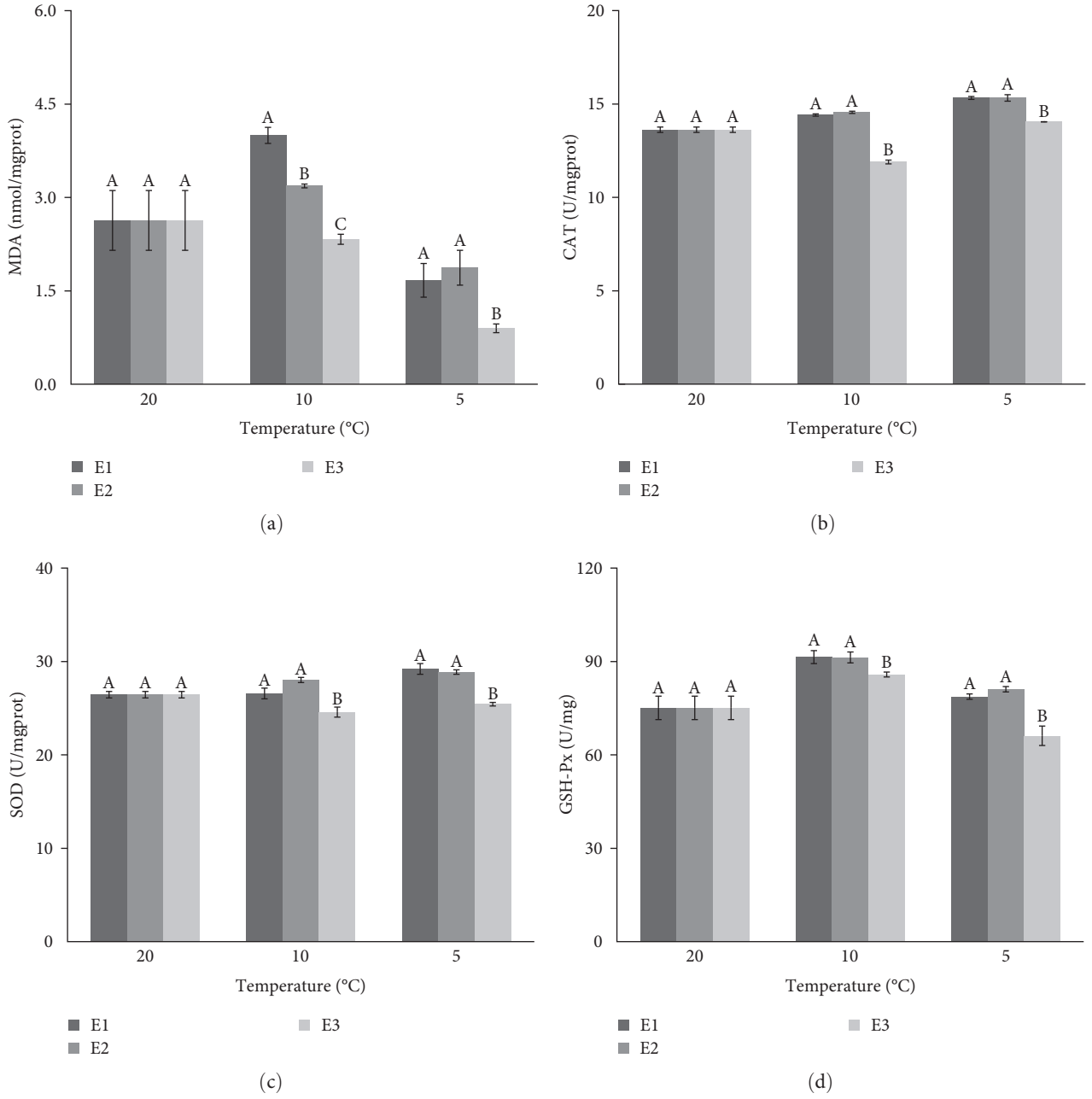

Figure 4: Effects of vitamin E on antioxidant indexes of crucian carp under acute cold stress: (a) malondialdehyde (MDA), (b) catalase (CAT), (c) superoxide dismutase (SOD), and (d) glutathione peroxidase (GSH-Px). Different uppercase letters above columns indicate significant differences, and the same uppercase letters indicate no significant differences.

the E3 treatment group was significantly lower than that in the E1 and E2 treatment groups. This indicates that vitamin E treatment alleviates fish stress, and the body's immune system responds to cold stress to a corresponding decrease. The RBC is the most important component in the blood, which mainly carries and transports oxygen. Under stress, the physiological mechanism of the body is activated, thus promoting the production of more RBCs to meet the needs of physiological activities and exercise [17, 18]. The HGB is the direct carrier of oxygen in the blood. Studies have shown that with the increase of stress, hemoglobin tends to increase, which may be to enhance the oxygen carrying capacity of the blood to cope with the increase of energy demand under stress [19–21]. In this study, under the cooling stress, in order to cope with the stress, the crucian carp increased the content of RBC and HGB, so as to enhance the oxygen carrying capacity of the blood and provide energy for the body. However, the RBC and HGB contents in the E3 treatment group were significantly lower than those in the E1 and E2 treatment groups. This indicated that the effect of low-temperature stress on crucian carp treated with vitamin E was smaller than that of E1 and E2 treatment groups, and the body consumed less energy to resist cold than that of E1 and E2 treatment groups. Therefore, the effect of oxygen carrying

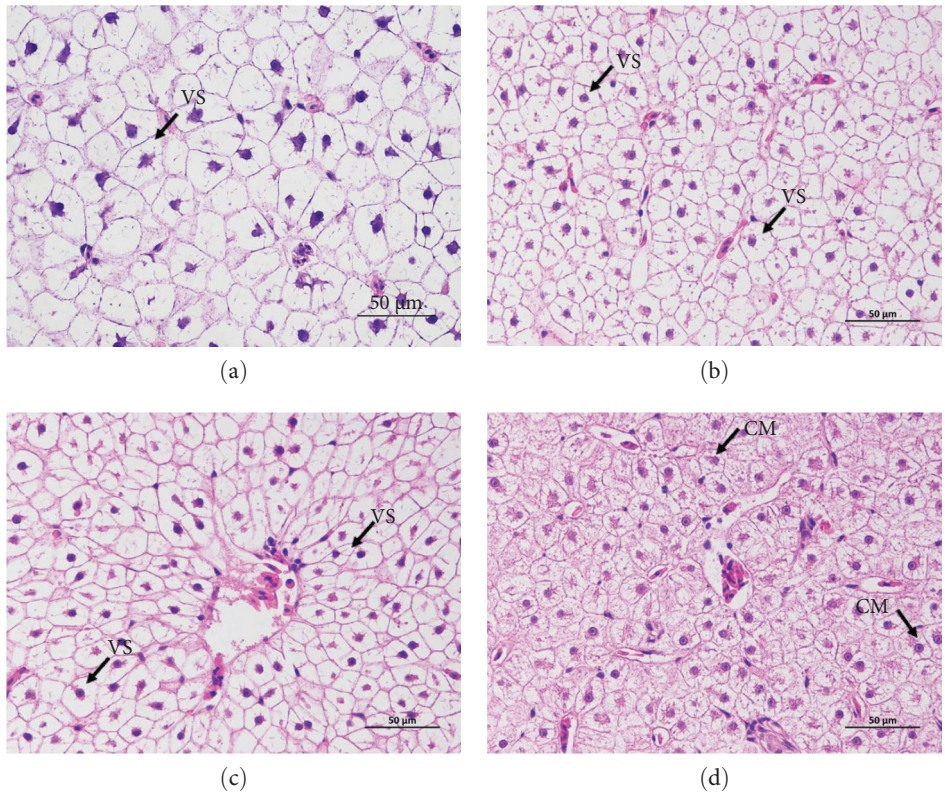

Figure 5: Effects of vitamin E on liver tissue structure of *Carassius auratus* under acute cold stress at 10°C: (a) blank control group; (b) E1 group; (c) E2 group; (d) E3 group. VS, vacuole structure; CM, cytoplasm.

and energy supply was weaker than that of E1 and E2 treatment groups, and the RBC and HGB contents of E3 treatment group were also reduced accordingly. The role of PLT in the blood of fish is to coagulate and stop bleeding and repair damaged blood vessels. Under stress, the PLT content of the body shows an increase [22, 23]. In the study of juvenile Chinese sea bass (*Lateolabrax maculatus*), the PLT content increased significantly under hypoxia stress [24]. In this study, the PLT content in the blood of crucian carp increased under cooling stress, but the PLT content in the E3 treatment group was significantly lower than that in the E1 and E2 treatment groups, indicating that vitamin E treatment could alleviate the negative effects of stress. Compared with the nonvitamin E treatment, the PLT of crucian carp treated with vitamin E had less effect on its body.

The LZM, ALP, and ACP play an important role in the immune response of fish. The LZM is one of the important nonspecific immune factors in organisms, and its level and activity are directly related to the immune ability and health of fish. Related studies have found that after the body undergoes stress, the activity of LZM will increase, thereby activating the body 's protective mechanism to maintain the body's own balance [25–27]. In this study, the content of LZM in fish increased under cooling stress, but the content of LZM in E3 treatment group was significantly lower than that in E1 and E2 treatment groups, indicating that vitamin E treatment could alleviate stress and reduce immune response. The ALP is produced by intrahepatic bile duct cells and is an important marker of liver dysfunction [28–30]. In the study of rohu (*Labeo rohita*), it was found that the ALP activity of rohu was significantly increased under DEP toxicity stress [31]. In this study, the ALP activity of crucian carp increased under acute cold stress, but the ALP activity of E3 treatment group was significantly lower than that of E1 and E2 treatment groups, indicating that vitamin E treatment could alleviate the cooling stress of fish and reduce the ALP activity, which was similar to the results of Sarma et al. [32]. The ACP is one of the important indicators to measure the body's immune function and health. The fish body regulates the metabolism of the body through phosphatase catalysis. After being stressed, the ACP activity in the fish body increases, and the body responds to environmental changes by dephosphorylating the stress response [33, 34]. In this study, under acute cold stress, the ACP activity of E1 and E2 treatment groups increased, and the ACP activity of E3 treatment group was significantly lower than that of E1 and E2 treatment groups, indicating that vitamin E-treated crucian carp can alleviate stress, enhance immunity, and dephosphorylation stress response is weaker than that of crucian carp without vitamin E treatment.

The CHE is mainly derived from liver cholinesterase (CHE), which can reflect the function of liver protein synthesis [35]. In the study of medaka, it was found that the enzyme activity of CHE increased and induced oxidative stress under sewage stress [36]. In this study, after the three groups of crucian carp were subjected to acute cold stress, the activity of CHE enzyme increased, but the activity of CHE enzyme in serum of crucian carp treated with vitamin E was

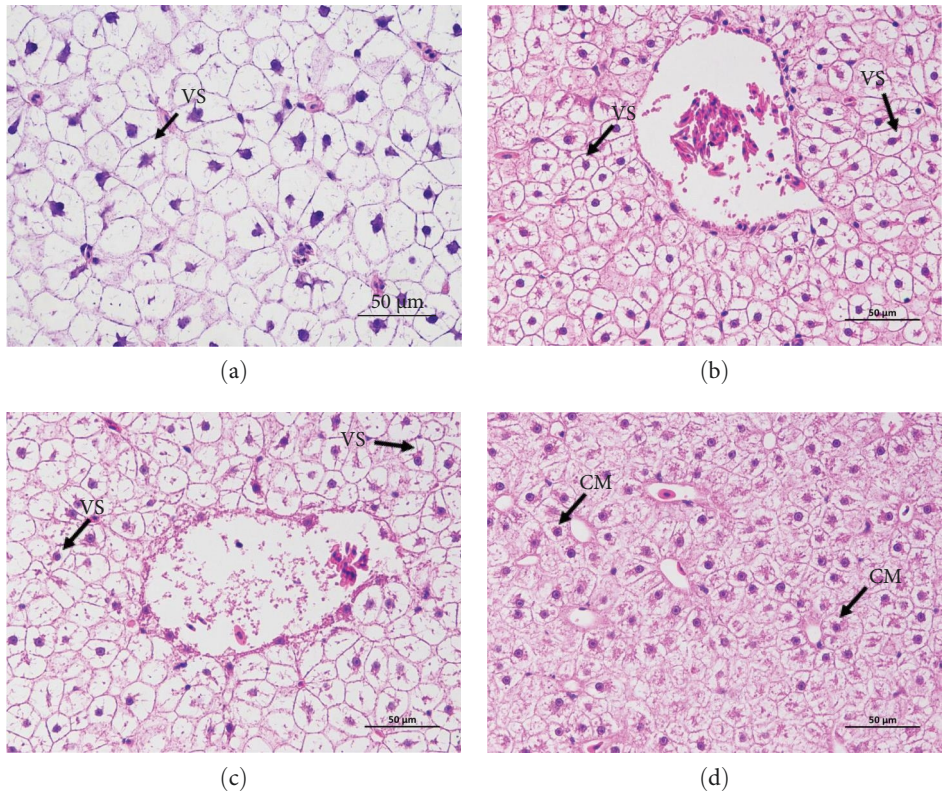

FIGURE 6: Effects of vitamin E on liver tissue structure of *Carassius auratus* under acute cold stress at 5°C: (a) blank control group; (b) E1 group; (c) E2 group; (d) E3 group. VS, vacuole structure; CM, cytoplasm.

significantly lower than that without vitamin E treatment, indicating that vitamin E treatment could reduce the negative effects of stress on the body. When fish is under stress, a large amount of ROS will be accumulated in the body, such as H_2O_2 and O_2^-. If it is not cleaned up in time and effectively, it will react with biological macromolecules in the body, resulting in lipid peroxidation, which will affect the normal physiological activities of fish [37–39]. In this study, after cooling stress treatment, the content of H_2O_2 and O_2^- increased, and the body began to accumulate a large amount of ROS. If it is not cleaned up, it will cause oxidative damage to the body. The accumulation of ROS in the vitamin E group was significantly lower than that in the nonvitamin treatment group, which slowed down the degree of oxidative damage to the body.

4.2. Effects of Vitamin E on Antioxidant Indexes of Crucian Carp under Acute Cold Stress. The MDA is the most abundant active aldehyde in the secondary products of lipid peroxidation. It can affect the structure and function of proteins in the body and cause damage to cells by damaging mitochondrial respiratory function and related dehydrogenases. It is one of the important markers of lipid peroxidation [40–42]. Studies have found that under stress treatment, the content of MDA in the blood and tissues of fish will increase significantly [43]. In this study, the MDA content in the liver of crucian carp increased after exposure to cooling stress, while the MDA content in the liver of crucian carp treated with vitamin E was significantly lower than that in the untreated group, indicating that vitamin E-treated crucian carp can alleviate the stress and the body induced the degree of oxidative stress is low. When fish are stressed, the body will initiate an antioxidant defense system to remove ROS such as H_2O_2 and O_2^- and reduce oxidative damage [44]. The enzymatic system is composed of antioxidant enzymes such as CAT, SOD, and GSH-Px. It is one of the antioxidant defense systems, which can effectively clean up H_2O_2 and O_2^-. The change of its activity can also indirectly reflect the degree of oxidative damage in the body [45, 46]. Studies have found that the activities of CAT and SOD in liver tissue of fish increased significantly under stress [47]. Studies have shown that the activities of GSH-Px, CAT, and SOD in fish liver increased significantly under high temperature and hypoxia stress [48]. In this study, the activities of CAT, SOD, and GSH-Px in the liver of crucian carp were increased under cooling stress, but the activities of CAT, SOD, and GSH-Px in the liver of crucian carp treated with vitamin E were significantly lower than those in the untreated group, indicating that vitamin E can reduce the oxidative damage of the body and alleviate the adverse effects of stress.

4.3. Effects of Vitamin E on Histopathological Changes of Crucian Carp under Acute Cold Stress. The liver bears important physiological functions such as metabolism, excretion, and detoxification. Its state in the body can best reflect the nutritional physiology and pathological state of the body [49–51]. Many studies have shown that various environmental stresses can cause changes in liver structure and even

affect its function. For example, persistent heat stress causes liver damage in barracuda, including cytoplasmic vacuolar degeneration [52]. In the study of catfish, heat stress can lead to some pathological changes in the liver, including vacuolization [53]. In this study, under cooling stress, the liver tissue of E1 and E2 treatment groups caused liver damage, while the liver tissue damage of E3 treatment group was smaller than that of E1 and E2 treatment groups, indicating that vitamin E treatment can alleviate liver damage.

5. Conclusion

This study shows that acute cold stress treatment can increase the content of WBC, RBC, HGB, PLT, CHE, ALP, ACP, LZM, H_2O_2, O_2^-, MDA, CAT, SOD, and GSH-Px. Changes in tissue structure were observed in the liver, which showed adverse effects on crucian carp under cooling stress, and the liver tissue was seriously damaged. However, vitamin E treatment can reduce the content of physiological and biochemical indexes, alleviate liver injury, and improve histological changes. In summary, this study revealed the physiological response level of vitamin E in water on crucian carp under acute cold stress. This will undoubtedly help to provide a theoretical reference for the study of the effects of vitamin E treatment on fish under low-temperature stress.

Data Availability

The data are available from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Data management, J.T., L.J., and D.C.; formal analysis, J.T.; funding acquisition, B.H., C.Z., and P.J.; methodology, B.H. and C.Z.; resources, P.J. and D.C.; writing-original draft, J.T.; writing-review and editing, J.T. All authors have read and agreed to the published version of the manuscript.

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References

[1] W. Wang, Y. Zhang, Y. Liu, Nóra Adányi, and X. Zhang, "Effects of waterless live transportation on survivability, physiological responses and flesh quality in Chinese farmed sturgeon (Acipenser schrenckii)," *Aquaculture*, vol. 518, Article ID 734834, 2020.

[2] P. Zeng, T. Chen, and J. Shen, "Effects of cold acclimation and storage temperature on crucian carp (Carassius auratus gibelio) in a waterless preservation," *Fish Physiology and Biochemistry*, vol. 40, no. 3, pp. 973–982, 2014.

[3] R. J. Schepers and M. Ringkamp, "Thermoreceptors and thermosensitive afferents," *Neuroscience & Biobehavioral Reviews*, vol. 33, no. 3, pp. 205–212, 2009.

[4] X. W. Zhang, Y. J. Zhang, W. Li, W. Q. Zhong, and L. B. Chen, "Cloning and function exploration comparison of Grik1 in Antarctic crocodile icefish (Chionodmco hamatus) and Nile tilapia (Oreochromis niloticus) exposed to cold stress," *Journal of Dalian Ocean University*, vol. 38, no. 1, pp. 68–75, 2023.

[5] S. Yang, T. Yan, L. Zhao et al., "Effects of temperature on activities of antioxidant enzymes and Na+/K+-ATPase, and hormone levels in Schizothorax prenanti," *Journal of Thermal Biology*, vol. 72, pp. 155–160, 2018.

[6] L. Cao, W. Huang, X. Shan, Z. Ye, and S. Dou, "Tissue-specific accumulation of cadmium and its effects on antioxidative responses in Japanese flounder juveniles," *Environmental Toxicology and Pharmacology*, vol. 33, no. 1, pp. 16–25, 2012.

[7] H. Elabd, H.-P. Wang, A. Shaheen, H. Yao, and A. Abbass, "Anti-oxidative effects of some dietary supplements on Yellow perch (Perca flavescens) exposed to different physical stressors," *Aquaculture Reports*, vol. 8, pp. 21–30, 2017.

[8] D. Montero, M. Marrero, M. S. Izquierdo, L. Robaina, J. M. Vergara, and L. Tort, "Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (Sparus aurata) juveniles subjected to crowding stress," *Aquaculture*, vol. 171, no. 3-4, pp. 269–278, 1999.

[9] R. Guzmán-Guillén, A. I. Prieto Ortega, A. Martín-Caméan, and A. M. Cameán, "Beneficial effects of vitamin E supplementation against the oxidative stress on Cylindrospermopsin-exposed tilapia (Oreochromis niloticus)," *Toxicon*, vol. 104, pp. 34–42, 2015.

[10] L. Wang, B. Ma, D. Chen et al., "Effect of dietary level of vitamin E on growth performance, antioxidant ability, and resistance to Vibrio alginolyticus challenge in yellow drum Nibea albiflora," *Aquaculture*, vol. 507, pp. 119–125, 2019.

[11] J. M. Tang, L. X. Jiang, C. F. Zhang et al., "Effect of vitamin E on physiology and biochemistry, muscle texture, and flavor of crucian carp (Carassius auratus) and its effect on mitigation of cooling stress," *Food and Fermentation Industries*, vol. 49, no. 19, pp. 226–234, 2023.

[12] L. Li, C. F. Zhang, J. J. Wu, Q. Liang, Y. J. Liu, and B. S. Huang, "Effect of high pressure on the water-free survival of crucian carp," *Science and Technology of Food Industry*, vol. 41, pp. 261–265, 2020.

[13] E. Liu, X. Zhao, C. Li et al., "Effects of acute heat stress on liver damage, apoptosis and inflammation of pikeperch (Sander lucioperca)," *Journal of Thermal Biology*, vol. 106, Article ID 103251, 2022.

[14] A.-W. A. Abdel-Warith, E.-S. M. I. Younis, N. A. Al-Asgah, A. M. Rady, and H. Y. Allam, "Bioaccumulation of lead nitrate in tissues and its effects on hematological and biochemical parameters of Clarias gariepinus," *Saudi Journal of Biological Sciences*, vol. 27, no. 3, pp. 840–845, 2020.

[15] P. Bujjamma and P. Padmavathi, "Effect of cadmium on Haematological changes in a freshwater catfish, Heteropneustes fossilis," *International Journal of Zoology Studies*, vol. 3, pp. 132–141, 2018.

[16] S. P. Trivedi, S. Singh, A. Trivedi, and M. Kumar, "Mercuric chloride-induced oxidative stress, genotoxicity, haematological

changes and histopathological alterations in fish *Channa punctatus* (B loch, 1793)," *Journal of Fish Biology*, vol. 100, no. 4, pp. 868–883, 2022.

[17] V. Parlak, B. Ozgeris, A. Ucar et al., "Hematotoxic, oxidative and genotoxic damage in rainbow trout (Oncorhynchus mykiss) after exposure to 3-benzoylpyridine," *Toxicology Mechanisms and Methods*, vol. 32, no. 7, pp. 501–509, 2022.

[18] A. Roesner, S. A. Mitz, T. Hankeln, and T. Burmester, "Globins and hypoxia adaptation in the goldfish, Carassius auratus," *The FEBS Journal*, vol. 275, no. 14, pp. 3633–3643, 2008.

[19] C. F. Mazur and G. K. Iwama, "Effect of handling and stocking density on hematocrit, plasma cortisol, and survival in wild and hatchery-reared chinook salmon (Oncorhynchus tshawytscha)," *Aquaculture*, vol. 112, no. 4, pp. 291–299, 1993.

[20] A. K. Srivastava and I. Sahai, "Effects of loading density on carbohydrate metabolism and hematology in the Indian freshwater catfish, Heteropneustes fossilis," *Aquaculture*, vol. 66, no. 3-4, pp. 275–286, 1987.

[21] C. E. Trenzado, A. E. Morales, and M. de la Higuera, "Physiological effects of crowding in rainbow trout, Oncorhynchus mykiss, selected for low and high stress responsiveness," *Aquaculture*, vol. 258, no. 1–4, pp. 583–593, 2006.

[22] H. T. Li, W. Xu, Z. G. Zhao et al., "Effects of alkalinity acclimation on blood physiology and biochemistry and liver antioxidant system of juvenile luciobarbus capito," *Progress in Fishery Sciences*, vol. 43, pp. 12–21, 2022.

[23] L. T. Wang, C. X. Ge, C. S. Gao et al., "Effect of different concentrations of saline-alkali stress on the chronic injury of carassius auratus gibelio," *Chinese Journal of Veterinary Medicine*, vol. 55, pp. 84–87, 2019.

[24] Z. C. Chang, H. S. Wen, M. Z. Zhang et al., *Effects of Dissolved Oxygen Levels on Oxidative Stress Response and Energy Utilization of Juvenile Chinese Sea Bass(Lateolabrax Maculatus) and Associate Physiological Mechanisms*, vol. 48, pp. 20–28, Periodical of Ocean University of China, 2018.

[25] J. Chen, N. Liu, B. Li, H. Zhang, Y. Zhao, and X. Cao, "The effects of fipronil exposure on oxidative stress, non-specific immunity, autophagy, and apoptosis in the common carp," *Environmental Science and Pollution Research*, vol. 28, no. 22, pp. 27799–27810, 2021.

[26] P. Sun, B. Tang, and F. Yin, "Physiological responses and non-specific immune parameters in *Epinephelus moara* exposed to repeated physical stress," *Indian Journal of Animal Research*, vol. 53, no. 5, pp. 578–582, 2017.

[27] F. Yin, H. Gong, Q. Ke, and A. Li, "Stress, antioxidant defence and mucosal immune responses of the large yellow croaker Pseudosciaena crocea challenged with Cryptocaryon irritans," *Fish & Shellfish Immunology*, vol. 47, no. 1, pp. 344–351, 2015.

[28] Z.-H. Li, V. Zlabek, J. Velisek et al., "Acute toxicity of carbamazepine to juvenile rainbow trout (Oncorhynchus mykiss): effects on antioxidant responses, hematological parameters and hepatic EROD," *Ecotoxicology and Environmental Safety*, vol. 74, no. 3, pp. 319–327, 2011.

[29] Nde M. Lovatto, F. R. Goulart, S. T. de Freitas et al., "Nutritional evaluation of phosphorylated pumpkin seed (Cucurbita moschata) protein concentrate in silver catfish Rhamdia quelen (Quoy and Gaimard, 1824)," *Fish Physiology and Biochemistry*, vol. 41, no. 6, pp. 1557–1567, 2015.

[30] A. Ademola zaid, "Growth response, nutrient utilization, biochemical and hematological parameters of Juvenile African catfish (clarias gariepinus) fed diet supplemented with gongronema latifolia (benth) extract," *Egyptian Journal of Aquatic Biology and Fisheries*, vol. 22, no. 4, pp. 167–179, 2018.

[31] M. Latif, M. Faheem, and Asmatullah, "Study of oxidative stress and histo-biochemical biomarkers of diethyl phthalate induced toxicity in a cultureable fish, labeo rohita," *Pakistan Veterinary Journal*, vol. 40, pp. 202–208, 2020.

[32] K. Sarma, K. Prabakaran, P. Krishnan, G. Grinson, and A. Anand Kumar, "Response of a freshwater air-breathing fish, Clarias batrachus to salinity stress: an experimental case for their farming in brackishwater areas in Andaman, India," *Aquaculture International*, vol. 21, no. 1, pp. 183–196, 2013.

[33] R. K. Pipe, "Hydrolytic enzymes associated with the granular haemocytes of the marine mussel Mytilus edulis," *The Histochemical Journal*, vol. 22, no. 11, pp. 595–603, 1990.

[34] B. Wang, Y. Wang, T. Jia et al., "Changes in physiological responses and immunity of blunt snout bream Megalobrama amblycephala from transport stress," *Fish Physiology and Biochemistry*, vol. 48, no. 5, pp. 1183–1192, 2022.

[35] Y. X. Hu, *The Physiological Adaptation of Stocking Silver Carp and Bighead Carp to Water Environmental Change in the Three Gorges Reservoir Water Ranch*, Southwest University, 2014.

[36] S. Hamidi, M. Banaee, H. R. Pourkhabbaz, A. Sureda, S. Khodadoust, and A. R. Pourkhabbaz, "Effect of petroleum wastewater treated with gravity separation and magnetite nanoparticles adsorption methods on the blood biochemical response of mrigal fish (*Cirrhinus cirrhosus*)," *Environmental Science and Pollution Research*, vol. 29, no. 3, pp. 3718–3732, 2022.

[37] L. Cao, W. Huang, J. Liu, X. Yin, and S. Dou, "Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure," *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, vol. 151, no. 3, pp. 386–392, 2010.

[38] A. Hallare, K. Nagel, H.-R. Köhler, and R. Triebskorn, "Comparative embryotoxicity and proteotoxicity of three carrier solvents to zebrafish (Danio rerio) embryos," *Ecotoxicology and Environmental Safety*, vol. 63, no. 3, pp. 378–388, 2006.

[39] E. López, C. Arce, M. J. Oset-Gasque, S. Cañadas, and M. P. González, "Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture," *Free Radical Biology and Medicine*, vol. 40, no. 6, pp. 940–951, 2006.

[40] T. Ishii, S. Kumazawa, T. Sakurai, T. Nakayama, and K. Uchida, "Mass spectroscopic characterization of protein modification by malondialdehyde," *Chemical Research in Toxicology*, vol. 19, no. 1, pp. 122–129, 2006.

[41] T. U. Krohne, E. Kaemmerer, F. G. Holz, and J. Kopitz, "Lipid peroxidation products reduce lysosomal protease activities in human retinal pigment epithelial cells via two different mechanisms of action," *Experimental Eye Research*, vol. 90, no. 2, pp. 261–266, 2010.

[42] X. X. Lin, Y. T. Ye, C. F. Cai et al., "Malondialdehyde causes glutathione/glutathione transferase pathway oxidative stress in intestine and hepatopancreas of grass carp (Ctenopharyngodon idellus)," *Chinese Journal of Animal Nutrition*, vol. 27, pp. 3604–3612, 2015.

[43] S. Prince, C. Pooja, and K. Mandeep, "Organic flame retard ant induced oxidative stress in various organs of freshwater fish channa punctatus," *Pollution Research*, vol. 37, pp. 1008–1013, 2018.
[44] M. Qi, Y. L. Hou, T. Liu et al., "The effects of acute hypoxia stress and re-oxygenation on oxidative stress and energy metabolism of juvenile Cyprinus carpio var qingtianensis," *Freshwater Fisheries*, vol. 50, pp. 92–98, 2020.
[45] E. Farombi, O. Adelowo, and Y. Ajimoko, "Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River," *International Journal of Environmental Research and Public Health*, vol. 4, no. 2, pp. 158–165, 2007.
[46] A. F. Welker, É. G. Campos, L. A. Cardoso, and M. Hermes-Lima, "Role of catalase on the hypoxia/reoxygenation stress in the hypoxia-tolerant Nile tilapia," *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, vol. 302, no. 9, pp. R1111–R1118, 2012.
[47] A. Ratn, Y. Awasthi, M. Kumar, S. K. Singh, R. Tripathi, and S. P. Trivedi, "Phorate induced oxidative stress, DNA damage and differential expression of p53, apaf-1 and cat genes in fish, Channa punctatus (Bloch, 1793)," *Chemosphere*, vol. 182, pp. 382–391, 2017.
[48] J.-L. Sun, L.-L. Zhao, L. Liao et al., "Interactive effect of thermal and hypoxia on largemouth bass (Micropterus salmoides) gill and liver: aggravation of oxidative stress, inhibition of immunity and promotion of cell apoptosis," *Fish & Shellfish Immunology*, vol. 98, pp. 923–936, 2020.
[49] A. Khieokhajonkhet, S. Muichanta, N. Aeksiri, K. Ruttarattanamongkol, J. Rojtinnakorn, and G. Kaneko, "Evaluation of sacha inchi meal as a novel alternative plant protein ingredient for red hybrid tilapia (Oreochromis niloticus× O. mossambicus): growth performance, feed utilization, blood biochemistry, and histological changes," *Animal Feed Science and Technology*, vol. 278, Article ID 115004, 2021.
[50] B. Liu, P. Xu, P. B. Brown et al., "The effect of hyperthermia on liver histology, oxidative stress and disease resistance of the Wuchang bream, Megalobrama amblycephala," *Fish & Shellfish Immunology*, vol. 52, pp. 317–324, 2016.
[51] S. Sun, Y. Wu, H. Yu et al., "Serum biochemistry, liver histology and transcriptome profiling of bighead carp Aristichthys nobilis following different dietary protein levels," *Fish & Shellfish Immunology*, vol. 86, pp. 832–839, 2019.
[52] Y. Wang, C. Li, C. Pan, E. Liu, X. Zhao, and Q. Ling, "Alterations to transcriptomic profile, histopathology, and oxidative stress in liver of pikeperch (Sander lucioperca) under heat stress," *Fish & Shellfish Immunology*, vol. 95, pp. 659–669, 2019.
[53] A. Khieokhajonkhet, S. Sangphrom, N. Aeksiri, P. Tatsapong, K. Wuthijaree, and G. Kaneko, "Effects of long-term exposure to high temperature on growth performance, chemical composition, hematological and histological changes, and physiological responses in hybrid catfish [♂Clarias gariepinus (Burchell, 1822) ×♀C. macrocephalus (Günther, 1864)]," *Journal of Thermal Biology*, vol. 105, Article ID 103226, 2022.