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Physiological Function Disturbances and Adaptive Responses in Nile Tilapia (*Oreochromis niloticus*) Under Different Salinity Stresses

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Abstract: The physiological functions of aquatic organisms are closely linked to changes in environmental salinity. High-salinity environments can disrupt energy metabolism, induce inflammation, and negatively impact normal growth and development. However, aquatic organisms possess self-regulatory mechanisms that can mitigate these impacts to some extent. This study aimed to investigate the adaptive regulatory processes in Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) exposed to high-salinity environments by evaluating metabolic enzyme activities and levels of inflammatory markers. The increased levels of IL-1β and elevated ACP activity suggested that high-salinity conditions (15 and 30 ppt) induced intestinal inflammation. Concurrently, the elevated activities of SOD and GSH, along with decreased SDH activity, pointed to heightened oxidative stress in the brain and a reduced mitochondrial energy supply. Additionally, the adaptive features of intestinal energy metabolism under high-salinity conditions were evident, with adjustments in HK and PK activities mitigating the effects of suppressed PFK activity. Moreover, elevated lipase (LPS) activity in muscle tissue under salinity stress indicated that fat is mobilized to supply energy for muscle activity without affecting muscle protein. In conclusion, salinity stress triggered inflammatory and oxidative stress responses in Nile tilapia, yet the fish exhibited self-regulatory processes in energy metabolism. This study provides a theoretical basis for understanding the adaptive mechanisms of aquatic organisms in stressful environments.

Keywords: oxidative stress; energy metabolism; immunity; adaptation

Key Contribution: This study provides scientific evidence for elucidating the physiological regulatory mechanisms and adaptive strategies of fish in response to salinity changes, offering valuable insights into the optimization and practical applications of aquaculture management.

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1. Introduction

Salinity is an important environmental factor affecting the survival and adaptability of aquatic organisms. With the rapid changes in global climate, fluctuations in water salinity within marine and coastal ecosystems worldwide are occurring, leading to widespread and irreversible impacts [1,2]. For fish, these salinity changes may affect physiological functions and metabolic regulation [3–5]. Generally speaking, fish can regulate osmotic pressure to maintain homeostasis when environmental salinity changes [6]. However, osmotic pressure regulation is itself an energy-consuming process [7], and fluctuations in environmental salinity can disrupt energy allocation, potentially leading to imbalances in osmoregulation [8]. For example, the energy cost required to regulate ion concentrations in the tissues of Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) increases with increasing salinity [9]. In addition, an increase in plasma triglyceride levels after transferring Gilthead seabream (*Sparus aurata*, Linnaeus, 1758) from low- to high-salinity waters may be related to the reallocation of energy resources [10]. In addition to osmoregulation, energy

metabolism is also linked to the growth and development of fish. Changes in salinity alter food conversion rates [11]; an interaction analysis has shown that high-salinity waters decreased starch digestibility in Atlantic salmon (*Salmo salar*, Linnaeus, 1758) [12], ultimately affecting fish growth. Therefore, it is particularly important to investigate the intrinsic relationship between salinity changes and energy metabolism.

When salinity exceeds the regulatory capacity of the fish, it can lead to internal environmental disturbances, which may trigger further adverse reactions such as inflammation and stress [13–15]. For example, prolonged exposure to a low-salinity environment (<4 PSU) can cause physiological stress in Blackhead seabream (*Acanthopagrus schlegelii*, Bleeker, 1854), including oxidative stress (OS) and endoplasmic reticulum stress (ERS) [16]. Additionally, a high-salinity environment can induce acute stress responses and inflammation in the Clown knifefish (*Chitala chitala*, Hamilton, 1822), leading to cell death and impaired histological function [17]. Studies have also shown that prolonged exposure to a hyperosmotic environment can lead to excessive immune responses in Striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878), resulting in histological damage and disrupting physiological homeostasis [18]. Therefore, studying the adaptive mechanisms of euryhaline fish in response to salinity stress in their environment is of significant importance.

Nile tilapia (*Oreochromis niloticus*) is a freshwater fish species with a certain tolerance to salinity and can survive in low-salinity water environments. This study evaluated the effects of different salinity conditions on the activities of metabolic enzymes, inflammatory responses, and the expression of genes related to energy metabolism and inflammation in the intestines, brain, and muscle. This research also explored potential adaptive regulatory mechanisms, providing a theoretical basis for understanding the physiological response mechanisms and adaptive strategies of fish in response to salinity changes and offering insights for practical applications in aquaculture.

2. Materials and Methods

2.1. Animal Acquisition and Maintenance

Three-month-old Nile tilapia were purchased from a fish farm in Yantai, China, with an average body length of 9.25 ± 0.65 cm and an average weight of 10.51 ± 0.76 g. Healthy and uniformly sized Nile tilapia were randomly selected and placed into nine 120 L glass tanks for a 28-day period. The tanks were maintained under controlled conditions, with a light cycle of 14 h of light and 10 h of darkness, a water temperature of 28 ± 1 °C, pH 7.9 ± 0.2, and a salinity of 0 parts per thousand (ppt). The salinity of the water was measured using a salinometer (HSA-35, Shanghai Precision Instruments Co., Ltd., Shanghai, China). The dissolved oxygen content was kept above 8.0 mg/L, and half of the water was replaced daily. The fish were fed a commercial feed (Jiakang Fodder Co., Ltd., Xiamen, China; crude protein content of ≥30%) twice daily (5% of body weight) at 09:00 and 16:00, and feces were promptly removed to maintain water quality.

2.2. Experimental Design and Sample Collection

Exposure to different salinities began after the initial 28-day acclimation period. Based on previous studies [19], we established the following three treatment groups: freshwater, moderate salinity, and high salinity (0 ppt, 15 ppt, and 30 ppt). Each treatment consisted of three parallel experiments, resulting in a total of nine 120 L glass tanks. Each tank was stocked with 30 Nile tilapia. According to previous studies [19], the water salinity in the experimental group tanks (15 and 30 ppt) was gradually increased by 3 ppt per day until the target salinities were reached, while the control group remained at 0 ppt. Salinity levels were monitored regularly to ensure stability. Once the target salinities were achieved, the exposure experiments commenced, lasting a total of 21 days. The purpose of this study was to monitor the physiological changes in Nile tilapia under different salinity conditions. Samples were collected after 7, 14, and 21 days following anesthetization by 0.002% MS-222. The Nile tilapia were handled according to ARRIVE 2.0 standards [20]. All experiments were carried out in line with the requirements of the Chinese Association for

Laboratory Animal Sciences. This study was approved by the animal ethics committee of Shandong University.

2.3. Biochemical Analysis

In this study, biochemical assay kits purchased from the Nanjing Jiancheng Bioengineering Institute were used to measure the biochemical parameters in the intestine, brain, and muscle tissues (each indicator included three biological replicates for each group). Commercial kits were used for the detection of intestinal biochemical indicators, which included Phosphofructokinase (PFK, A129-1-1), Pyruvate Kinase (PK, A076-1-1), Hexokinase (HK, A077-3-1), Acid Phosphatase (ACP, A060-2-1), and Interleukin-1 beta (IL-1β). These indicators were determined using ELISA kits (CD-JK90018, Chundubio Technology Co., Ltd., Wuhan, China). Brain tissue biochemicals were assayed using Superoxide Dismutase (SOD, A001-1-1), Malondialdehyde (MDA, A003-1-2), Glutathione (GSH, A006-2-1), Succinate Dehydrogenase (SDH, A022-1-1), Malate Dehydrogenase (MDH, A021-2-1), and Na^+/K^+-ATPase (NKA) enzymes, which were tested as described by Bouskill et al. [21]. The commercial kit for the muscle biochemical index was Lipase (LPS, A054-1-1). We used Bradford methods [22] to evaluate muscle protein content.

2.4. Quantitative Real-Time PCR (qPCR) Analysis

To further investigate the effects of salinity stress on tissue metabolic regulation, immune responses, and oxidative stress, this study measured the expression levels of the following: *hk1a*, *pfk*, *il-1β*, and *G6PD* in the intestine; *sod*, *cat*, *gst*, and *nkaα1a* in the brain; and *myog* and *PPAR-α* in the muscle at 21 days (with each sample synthesized from the tissues of three fish). The AG RNAex Pro reagent (Accurate Biotechnology Co., Ltd., Hunan, China) was used to extract the total RNA, and the extraction method was followed according to the merchant's instructions. An Evo M-MLV RT Mix Kit (Accurate Biotechnology Co., Ltd., Hunan, China) was used to perform reverse transcription to synthesize first-strand cDNA for the qRT-PCR. Quantitative real-time fluorescence quantitative PCR was performed using a LightCycler 96 Instrument(Roche, Mannheim, Germany). *β-actin* was used as an internal reference gene, and mRNA relative expression levels were calculated using $2^{-\Delta\Delta Ct}$. We referenced previous papers for specific procedures [23]. The primer sequences used for this study are all in Table 1.

Table 1. Primer sequences of RT-PCR involved in this study.

Gene Name	Sequences of Primers (5′–3′)	Reference
hk1a F *hk1a* R	TGCCACTGCTACACTGAAGATGC TCCTCGGGCGTGTCGTAGATTT	[24]
pfk F *pfk* R	CATCCCAGCGTTCATTCCTG TGCCGTCTCCACCGATTACACA	[25]
G6PD F *G6PD* R	TGCTCCTGTTTCTCTCTCCG CATCCCAGCGTTCATTCCTG	[26]
il-1β F *il-1β* R	TCAGTTCACCAGCAGGGATG GACAGATAGAGGTTTGTGCC	[23]
myog F *myog* R	CCACAATGGAGGTCAAGG AGAGTGTCGTCGTCAAGC	[27]
PPAR-α F *PPAR-α* R	TACGGTGTTTACGAAGCCCT AGGAAGGTGTCATCTGGGTG	[28]
sod F *sod* R	GGTGCCCTGGAGCCCTA ATGCGAAGTCTTCCACTGTC	[29]

Table 1. *Cont.*

Gene Name	Sequences of Primers (5′–3′)	Reference
cat F	TAATGGGAGAGGGAAGATGG	[29]
cat R	ATCTTAGATGAGGCGGTGATG	
gst F	TAATGGGAGAGGGAAGATGG	[30]
gst R	CTCTGCGATGTAATTCAGGA	
nkaα1a F	AACTGATTTGGTCCCTGCAA	[19]
nkaα1a R	ATGCATTTCTGGGCTGTCTC	
β-actin F	TGGTGGGTATGGGTCAGAAAG	[19]
β-actin R	CTGTTGGCTTTGGGGTTCA	

2.5. Integrated Biomarker Response (IBR)

In this study, ten indicators, including PFK, PK, HK, ACP, IL-1β, SOD, MDA, GSH, MDH, and NKA, were selected for the calculation of IBR, which was used to assess the stress of salinity exposure to Nile tilapia.

The respective mean (m) and standard deviation(s) of biomarker X under different treatment conditions were first calculated. Subsequently, the biomarker data (Xi) were standardized to obtain Yi according to the formula, where Yi = (Xi − m)/s. Zi was obtained by assigning a value to Yi; Zi = Yi if the biomarker was induced under environmental conditions, and Zi = −Yi if it was inhibited. The final biomarker score (Si) was calculated for all treatment groups and plotted on a radar plot as Si = Zi + $|Z_{min}|$, where Z_{min} takes the value of the absolute value of the smallest value of Zi in all treatment groups. The IBR value for each treatment group was calculated from the sum of the areas of triangles Ai enclosed by consecutive neighboring biomarkers, where Ai = Si * Si + 1 * sin(2π/k)/2 and k is the number of biomarkers (k = 10).

The IBR calculation formula is as follows:

$$\mathrm{IBR} = \sum_{i=1}^{k} \mathrm{Ai}$$

2.6. Statistical Analysis

The data in this study conform to a normal distribution and homogeneity of variance. Data were expressed as mean values ± standard error of the mean (SE) and were normally distributed. ANOVA was conducted to analyze the experimental data using IBM SPSS Statistics 27, New York, NY, USA with $p < 0.05$ considered statistically significant. Multiple comparisons were performed using Tukey's post-hoc test. Pearson correlation coefficients were calculated using Origin 2024 for the correlation analysis.

3. Results

3.1. Evaluation of Intestinal Energy Metabolism and Immune Indicators

Two inflammation-related factors, IL-1β and ACP, were measured in this study. In the 15 ppt group, IL-1β levels were higher than the control at 14 and 21 days. In the 30 ppt group, the IL-1β level at 21 days was significantly higher than that of the control (Figure 1A). Additionally, the ACP activity in the salinity groups increased significantly, except at 7 days. Notably, at 7 days, the ACP activity in the 15 ppt group was the lowest across all treatments (Figure 1B).

The intestinal samples from Nile tilapia revealed that PFK activity in both the 15 and 30 ppt groups was significantly lower at 7, 14, and 21 days compared to the control (Figure 1C). Additionally, the PK activity decreased in the 15 ppt group across all time points compared to the control. In the 30 ppt group, the activity was higher than in the control group (Figure 1D). At the high salinity of 30 ppt, HK activity was significantly lower than the control at all time points, a pattern not observed in the control or the 15 ppt group (Figure 1E). Two-way ANOVA analysis (Table 2) showed that ACP, PFK, PK, and HK

activities, as well as IL-1β levels, were affected by time, salinity, and interactions. However, the combined effect of salinity and time on HK activity was not significant.

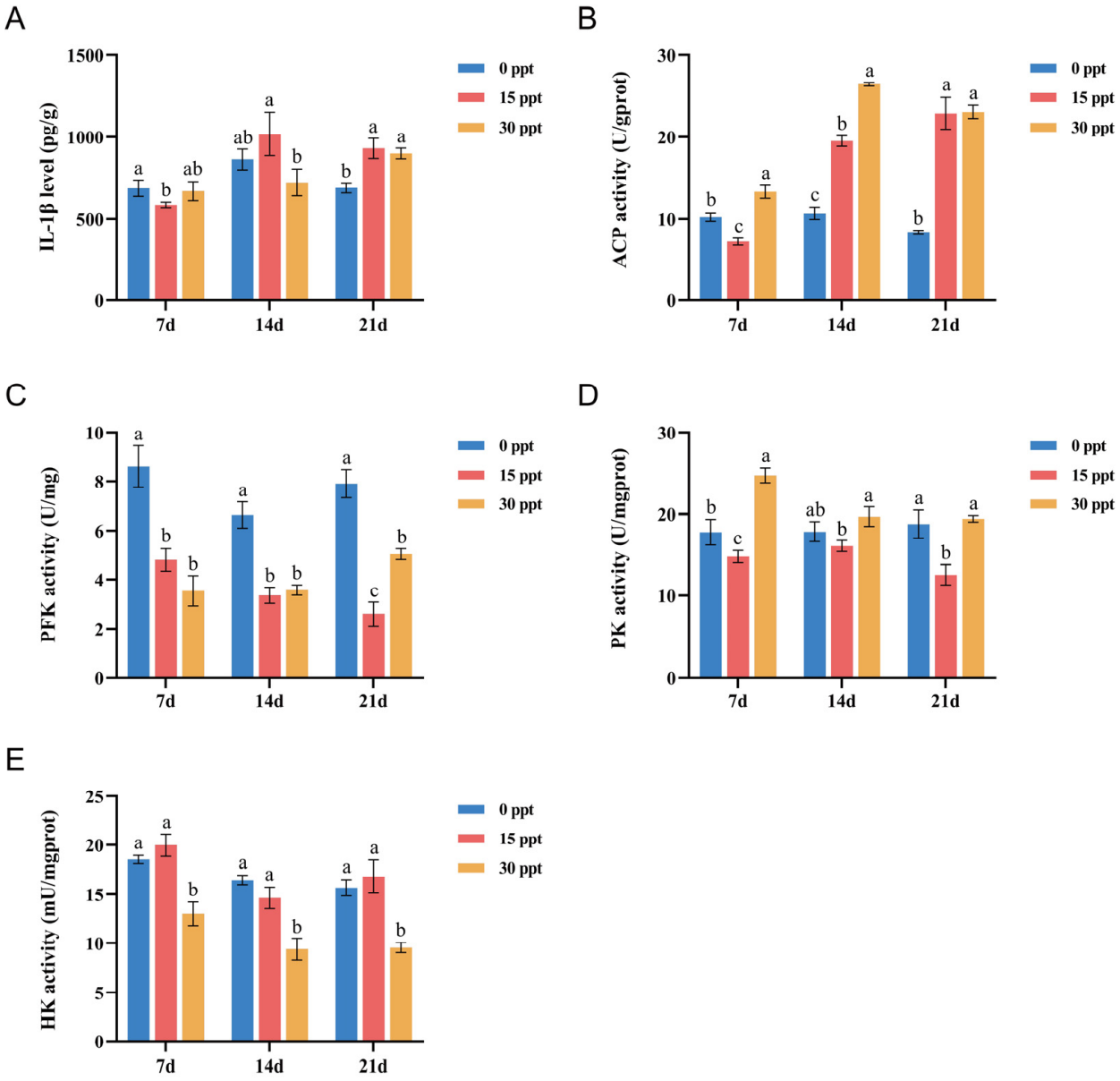

Figure 1. Values (mean ± SD) of intestinal biochemical indices of Nile tilapia (0 ppt is the control, the rest are experimental groups, the same below) for 7, 14, and 21 days. (**A**) IL-1β level; (**B**) ACP activity; (**C**) PFK activity; (**D**) PK activity; and (**E**) HK activity. Different letters indicate significant differences over different treatment groups (One-way ANOVA, $p < 0.05$).

Table 2. Interaction between intestinal biochemical markers, time, and salinity in Nile tilapia using two-way ANOVA.

Factors/Interactions	IL-β			ACP		PFK		PK		HK	
	DF	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Time	2	28.911	0.000	289.271	0.000	11.120	0.000	8.261	0.003	33.963	0.000
Salinity	2	5.535	0.013	398.737	0.000	175.659	0.000	79.464	0.000	116.685	0.002
Time ∗ Salinity	4	11.296	0.000	100.448	0.000	11.611	0.000	10.183	0.000	2.425	0.086

3.2. Assessment of Brain Energy Metabolism and Antioxidant Indices

In this study, NKA activity showed no significant difference between the control and high-salinity treatments at both 14 and 21 days (Figure 2A). However, SDH activity differed significantly among all treatments at 14 and 21 days, following a trend of control > 15 ppt > 30 ppt (Figure 2B). Additionally, MDH activity in the 15 and 30 ppt groups was significantly lower than the control at 7 days. At 14 and 21 days, MDH activity was only observed to be higher in the 15 ppt group compared to the control (Figure 2C).

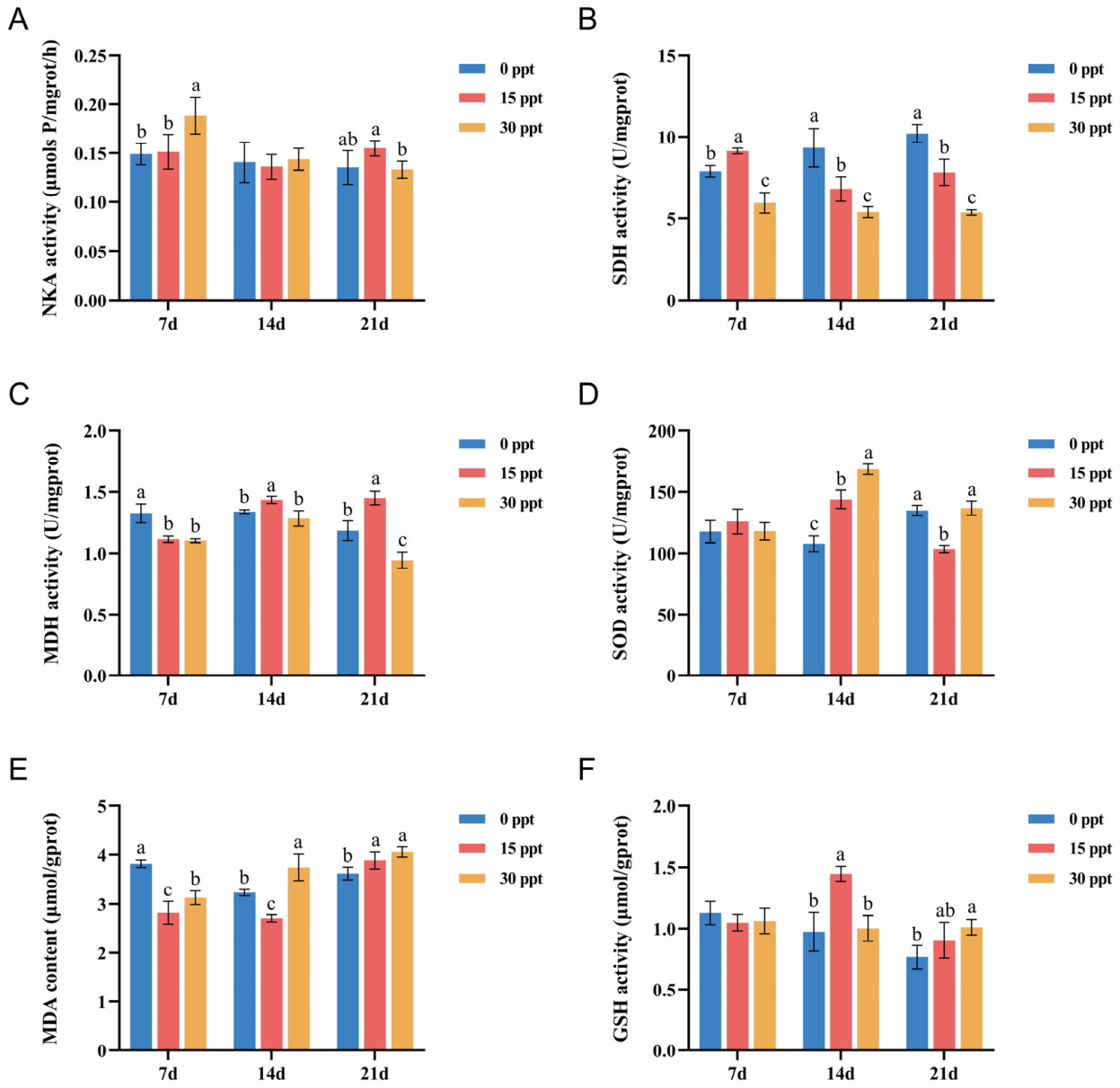

Figure 2. Brain energy metabolism and oxidative stress markers in Nile tilapia at 7, 14, and 21 days in different salinity environments. (**A**) NKA activity; (**B**) SDH activity; (**C**) MDH activity; (**D**) SOD activity; (**E**) MDA activity; and (**F**) GSH activity. Different letters represent significant differences between treatments (One-way ANOVA, $p < 0.05$).

The results of oxidative stress index measurements are presented in Figure 2D–F. There was no significant difference in the SOD activity among the treatment groups at 7 days. However, by 14 days, the SOD activity had increased in the 15 and 30 ppt groups. This trend reversed at 21 days, with the SOD activity in the 15 ppt group decreasing significantly, making it lower than in the other two groups (Figure 2D). The MDA activity

was significantly lower in the 15 ppt group at 7 and 14 days, but by 21 days, it had increased significantly, surpassing the levels observed in the control group. For GSH, no significant trend was observed at 7 and 14 days. It was only after 21 days that an increase in GSH levels in the brain was observed with increasing exposure to salinity (Figure 2F).

Two-way ANOVA was performed on time, salinity, and each of the indicators (Table 3), and it was observed that SDH, MDH, NKA, SOD, MDA, and GSH activities were significantly affected by both time and salinity together.

Table 3. Two-way ANOVA analysis of brain oxidative stress and energy metabolism markers in interaction with exposure time and salinity.

Factors/Interactions		SDH		MDH		NKA		SOD		MDA		GSH	
	DF	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Time	2	2.433	0.116	28.390	0.000	9.241	0.000	20.892	0.000	62.475	0.000	13.508	0.000
Salinity	2	74.303	0.000	42.494	0.000	2.533	0.098	24.026	0.000	36.462	0.000	6.543	0.007
Time ∗ Salinity	4	9.414	0.000	23.143	0.000	4.766	0.005	30.534	0.000	29.843	0.000	8.609	0.000

3.3. Assessment of Basic Muscle Indicators

By examining Nile tilapia tail muscle homogenates, it was found that at 7 days, the LPS activity in the control group was significantly lower compared to the other two groups, at all three time points (Figure 3A). The LPS activity was highest in the 15 ppt group, significantly surpassing that of the 30 ppt group at 14 and 21 days (Figure 3A). However, the results showed no significant difference in muscle protein content across the groups (Figure 3B).

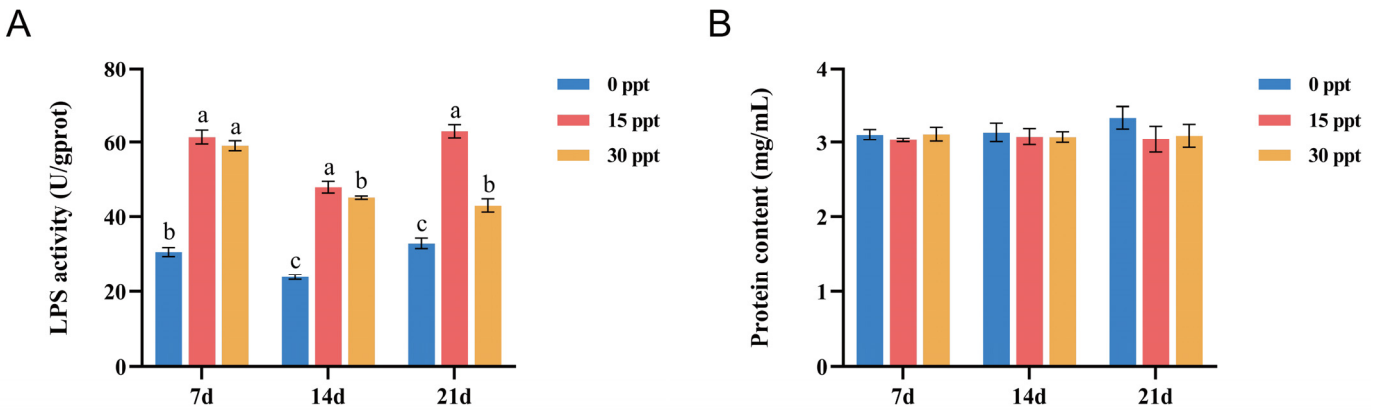

Figure 3. Effects of different salinity conditions on muscle-related indices of Nile tilapia at 7, 14, and 21 days (mean ± SD). (**A**) LPS activity and (**B**) Protein content. Different letters denote statistically significant differences between treatments, ($p < 0.05$).

3.4. Related Gene Expression Assessments

In order to determine the mechanism of changes in Nile tilapia-related genes under changing salinity conditions, this study determined the relative expression levels of genes at 21 days. Compared with the control, the 15 ppt group somewhat promoted the relative expression levels of *cat*, *gst*, and *il-1β*, whereas it produced a strong inhibitory effect on *pfk* and *G6PD*. In the 30 ppt group, the expression of *hk1a and G6PD* were suppressed, while *il-1β and myog* were promoted (Figure 4A).

Considering the possible interactions between the genes, we used the Pearson correlation coefficient to calculate the correlation links between them. The analysis showed that there was a significant positive correlation between *PPAR-α* and *G6PD* in the 15 ppt group ($p < 0.05$) (Figure 4B), and a significant positive correlation was also observed in the 30 ppt

group in the three groups of genes, *G6PD* and *hk1a*, *gst* and *pfk*, as well as *sod* and *myog* ($p < 0.05$) (Figure 4B).

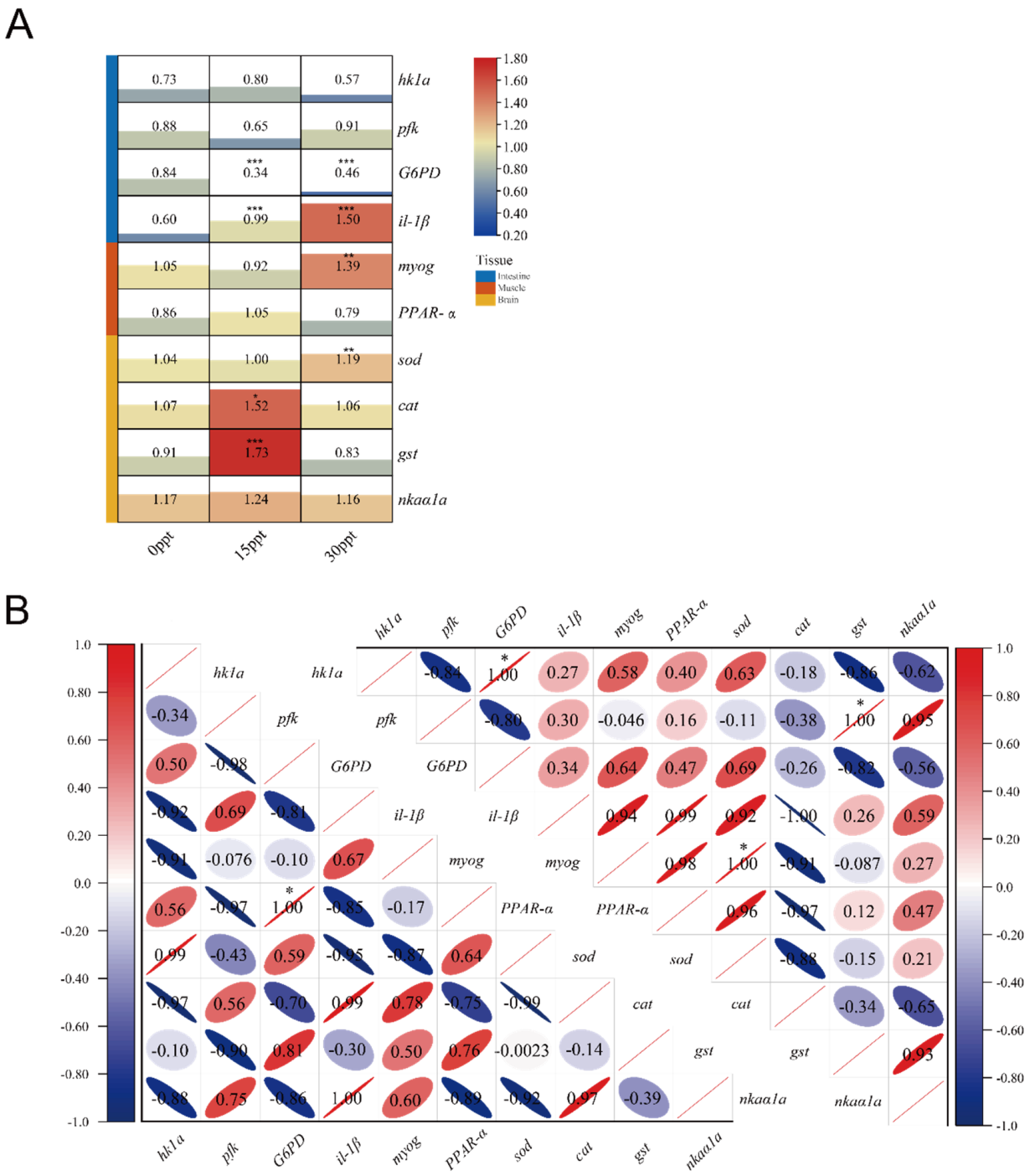

Figure 4. (**A**) Heat map of the relative mRNA expression levels in the intestine, brain, and muscle of Nile tilapia exposed to different salinities for 21 days. The numbers indicate the gene expression levels (* means $p < 0.05$, ** means $p < 0.005$, *** means $p < 0.001$). (**B**) Pearson correlation analysis of intestine, brain, and muscle genes in Nile tilapia at 21 days of exposure (left part is the 15 ppt group, right part is the 30 ppt group, ∗ indicates statistical significance).

3.5. Integrated Biomarker Response (IBR) Analysis

The IBR star plots are shown in Figure 5A–C. At 7 and 14 days, the 15 ppt group had the largest area of star plots and the IBR values were higher in the 15 ppt group in the histograms.

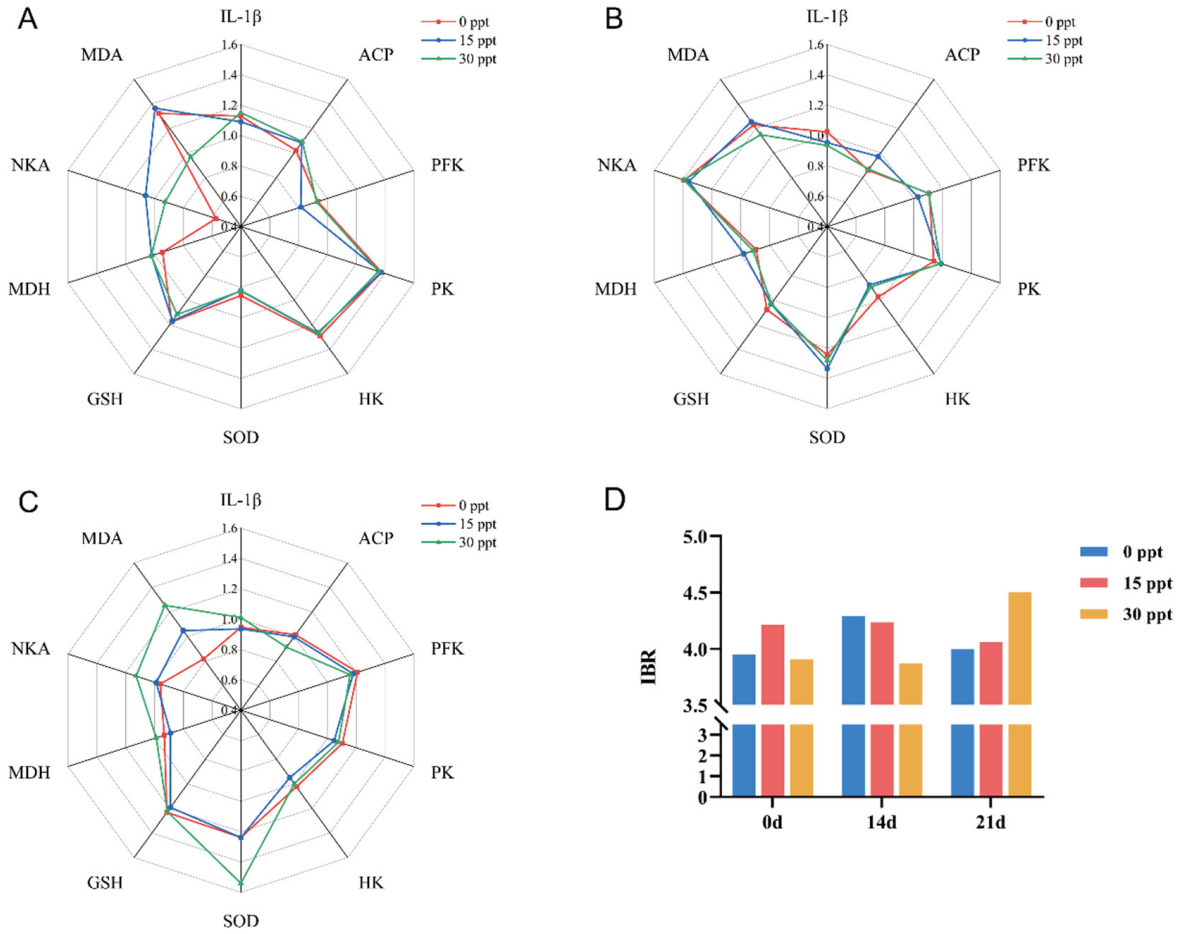

Figure 5. Star plots of Integrated Biomarker Response (IBR) scores of Nile tilapia in different salinity environments. (**A**) Star plot of IBR at 7 days; (**B**) star plot of IBR at 14 days; (**C**) star plot of IBR at 14 days; and (**D**) histogram of IBR values.

4. Discussion

Metabolic processes, as well as intermediate metabolic pathways, are altered to meet the energy requirements for self-regulation of fish growth and development due to changes in environmental salinity [31]. The results of this study showed that exposure to salinity stress induced inflammation and oxidative stress. In addition, the energy metabolic processes in Nile tilapia were disturbed during the 21-day exposure period, and this disturbance may have been staged to mitigate the negative effects of salinity change.

However, in reality, the effects of salinity stress on different tissues are not uniform over time and salinity but are determined by physiological functions [32]. According to existing studies, the impact of salinity on the gut can be phase-dependent [33]. IL-1β and ACP are important antimicrobial factors in the immune system [34,35]. The results showed that the immune response was not obvious at 7 days. ACP played a dominant role at 14 and 21 days, and IL-1β began to participate in the immune response as time increased. In addition, as the levels of IL-1β increased, the expression of *il-1β* also gradually rose. This suggests that salinity stress stimulates immune function in Nile tilapia, similar to the study about black seabream [16]. This immunization effect changes over time. HK, PFK, and

PK are key rate-limiting enzymes in glycolysis for the conversion of exogenous glucose to pyruvate [36]. Under stress conditions, fish can control energy metabolism processes by regulating their enzyme activities in sugar metabolism pathways [37]. In this study, salinity stress (15 and 30 ppt) inhibited PFK activity, whereas HK and PK were differently expressed. It has been shown that PFK has a higher priority in glycolysis [38], and the phenomenon of increased HK activity at 15 ppt and PK activity at 30 ppt in this study may be an adaptive regulation by Nile tilapia to compensate for the risk of energy metabolism due to reduced PFK activity. This compensatory regulation also occurs in white shrimp (*Litopenaeus vannamei*) to minimize damage from salinity stress [39]. However, this trend is not evident in the expression of the corresponding mRNAs, such as *hk1a* and *pfk*, which may be related to post-translational modifications of the proteins.

Changes in energy metabolism can affect the redox status of the cell. ROS are a class of molecules associated with organ and tissue senescence and damage, with mitochondrial respiration being its most important source [40,41]. Stress responses due to salinity stress are often linked to increased ROS [13,42]. SOD is involved in the defense against oxidative stress [43], and its activity can reflect the degree of tissue oxidative stress. GSH is a self-protective factor with the ability to scavenge oxygen radicals [44], and in general, GSH activity is proportional to the oxidative stress to which organisms are exposed [45]. MDA is a lipid peroxidation product, and high levels may lead to cellular damage [46]. In this study, under three different salinity exposures, there were no differences in SOD activity and GSH activity in brain tissue in the short term (7 days), which may be due to the oxidative stress induced by increased salinity remaining within a controllable range in the short term [43]. At 14 days, the increase in SOD activity and GSH activity at 15 ppt salinity indicates an increased risk of oxidative stress in the brain, where a large amount of ROS generated at this point is converted to H_2O_2 by SOD [47]. This suggests that medium- and low-salinity stimulation enhances SOD and GSH levels to protect the body from damage caused by excessive ROS [48]. Notably, the decrease in MDA levels at this time may suggest a reduction in ROS levels, which weakens lipid peroxidation. In contrast, at 30 ppt salinity, GSH activity decreased while MDA levels increased, indicating an exacerbation of lipid peroxidation in brain tissue [49]. By 21 days, SOD activity decreased at 15 ppt, and RT-PCR results did not show an excessive expression level of the *sod* gene. Meanwhile, GSH activity increased to eliminate the lipid peroxidation products induced by salinity, which also explains the elevated expression of *gst* at 21 days, promoting GSH activity. At 30 ppt salinity, both SOD and GSH activities increased, along with MDA levels, indicating a severe degree of oxidative stress in brain tissue at this salinity at 21 days.

As the control center, the physiological state of the brain also changes under different salinity environments. NKA in the brain can play a role in altering intra- and extracellular ion concentrations and regulating osmotic pressure, as well as being used for nerve impulse conduction [50], and Nile tilapia is capable of adapting to a certain range of salinity changes in the water column [19]. Therefore, in the present study, brain NKA activity did not show large changes, and the RT-PCR results indicated no significant change in the expression of *nkaα1a* at 21 days, which is consistent with the adaptation of Nile tilapia. The present study also showed that under salinity stress, the mitochondrial energy supply in the brain appeared to be affected, which was mainly reflected in the reduction of SDH activity [51,52]. The effect was more intense with the increase of salinity, and it is worth noting that the perturbation of the mitochondrial energy supply may lead to apoptosis or even tissue necrosis in the brain [53,54]. MDH activity in the brain showed a weak increase under salinity stress, mainly in the 15 ppt group, which could be attributed to the increased H_2O_2 content in the brain [47], and transient exposure of cells to H_2O_2 leads to an increase in MDH activity [55]. Additional results showed a decrease in MDH activity at 7 days, which, in combination with the oxidative stress results above, suggests that brain MDH activity is inhibited for a short period of time, interfering with the catalytic reaction of malate and inhibiting the generation of brain adipose tissue [56–58].

When fish are exposed to unfavorable environmental conditions, stored energy substances (fat, glycogen) can be used for energy expenditure [59]. In this study, the activity of LPS in the muscle was higher than that of the control group under all salinity conditions, indicating that increased salinity leads to the breakdown of muscle fat, contributing to the energy supply in the muscle [60], a phenomenon similar to that seen in Atlantic cod (*Gadus morhua*, Linnaeus, 1758), which may be triggered by an insufficient hepatic glycogen supply [59]. This may be attributed to cellular regulatory mechanisms, as there were no significant changes in the expression of LPS-related genes under high-salinity conditions. Proteins are used as energy substances for physiological activities only when lipids and glycogen are depleted [61], so there was no significant change in muscle protein content in this study. In terms of aquaculture, the indicators measured in this study are insufficient to determine whether the muscle growth of Nile tilapia is affected by salinity; further research is needed, such as measuring fat content and the RNA/DNA ratio in muscle tissue [62].

5. Conclusions

In conclusion, these findings suggest that fluctuations in environmental salinity disrupt the normal physiological functions of Nile tilapia. High-salinity exposure triggers an intestinal inflammatory response and affects metabolic enzyme activity, with the degree of impact influenced by the duration of exposure. Additionally, high salinity induces oxidative stress in the brain, impairing mitochondrial function, which may disrupt energy supply processes. Changes in muscle lipase activity under salinity stress further indicate that muscle energy metabolism could be compromised. This study provides a theoretical basis for understanding the physiological responses and adaptive strategies of fish to salinity fluctuations while also offering valuable insights into the aquaculture of Nile tilapia.

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Institutional Review Board Statement: All the procedures and animal handling complied with the guidelines approved by the Chinese Society of Laboratory Animal Science. This research was approved by the Animal Ethics Committee of Shandong University.

Data Availability Statement: Data are available on request due to restrictions.

Conflicts of Interest: The authors declare no conflicts of interest.

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