Effects of Microcystin-LR Exposure on Intestinal Injury and Hepatic Lipid Metabolism in GIFT Tilapia

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1. Introduction

The frequent occurrence of cyanobacterial blooms due to water eutrophication poses a significant concern, with the cyanotoxin MC-LR being particularly severe. MC-LR, renowned for its stability and high toxicity, has exceeded permissible limits in numerous water bodies globally, posing a threat to aquatic organisms and human health. This toxin primarily targets the liver, causing inflammation, apoptosis, and disrupting lipid metabolism, though the underlying mechanisms remain elusive. Furthermore, MC-LR notably alters the gut microbiota composition of its hosts, compromising gut health and metabolic functions, with consequential impacts on overall wellbeing. As a pivotal species in aquaculture, GIFT Oreochromis niloticus (GIFT tilapia) faces challenges related to fat accumulation. This study focuses on the effects of MC-LR on GIFT tilapia, particularly regarding its gut microbiota and hepatic lipid metabolism. By investigating the changes in gut microbiota composition and the potential influence of their metabolites on hepatic lipid metabolism under MC-LR exposure, we aim to provide a novel perspective for fat control in aquaculture while highlighting the profound impacts of environmental pollutants on aquatic organism health.

2. Methods

Experimental design

After being temporarily raised for two weeks at the Wuxi Fisheries College of Nanjing Agricultural University, 160 experimental fish were divided into a control group and a treatment group, with four replicates in each group. They were subjected to intraperitoneal injection at a dose of 200 μg/kg. The control group received an equal volume of saline solution. Blood, liver, and intestinal samples were collected from each group at 0, 12, 24, 48, and 96 hours for subsequent detection.

Indicator testing

- **Biochemical indicators testing**

Biochemical indicators, including GOT, GPT, TG, TC, TP, T-AOC, CAT, SOD, MDA, and TP, were measured using Nanjing Jiancheng Bioengineering Institute kits. The levels of LYZ and C3 were detected using ELISA kits.

- **16S rDNA assay for gut microorganisms**

The process involved DNA extraction from intestinal samples, followed by PCR amplification, product purification, library quantification, and sequencing.

Sequencing Platform: The NovaSeq 6000 sequencer was utilized for paired-end sequencing.

Subsequently, α- and β-diversity indices, relative abundance at the phylum and genus levels, and differential microbial indicators were calculated.

- **Targeted metabolomic assays**

Sample Processing and Analysis: The process of intestinal sample handling involves thawing, weighing, extraction, derivatization treatment, and detection analysis using LC-MS/MS.

Chromatography-Mass Spectrometry Conditions: These conditions encompass the type of chromatographic column used, temperature settings, injection volume, and the composition of the mobile phase, among others.

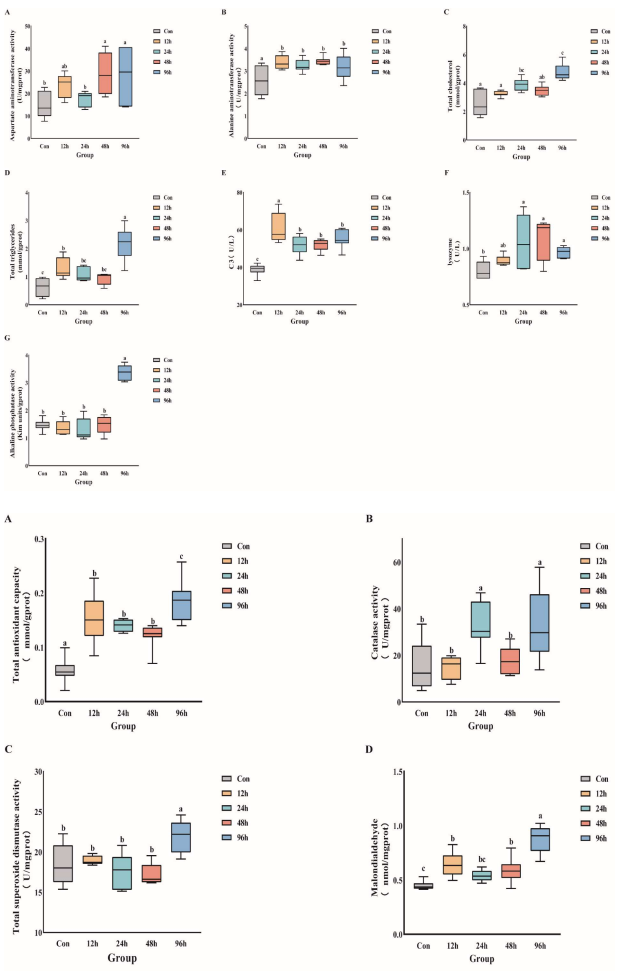
- **data processing**

Based on the normality of the data and homogeneity of variance, appropriate statistical methods (ANOVA, Duncan's test, or Kruskal-Wallis test) are selected to conduct the differential analysis, with a significant difference level set at $P < 0.05$.

3. Results

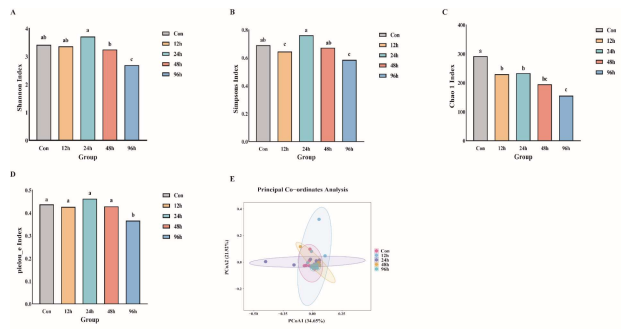
biochemical index

- **Physiological and biochemical indicators**

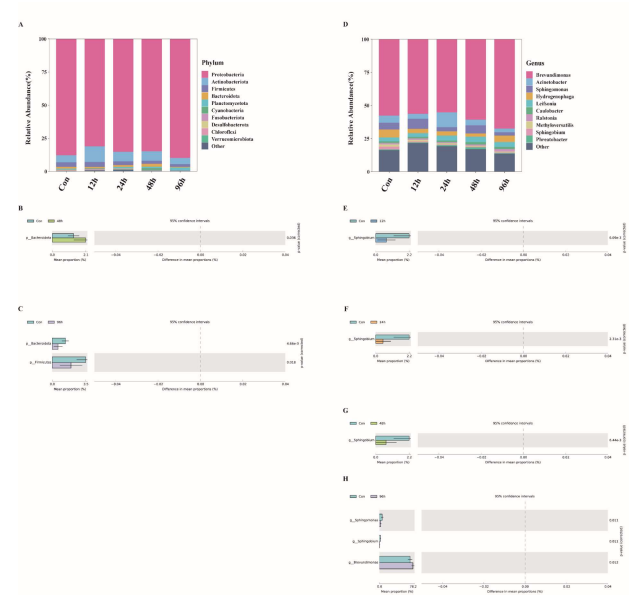


16S rDNA sequencing

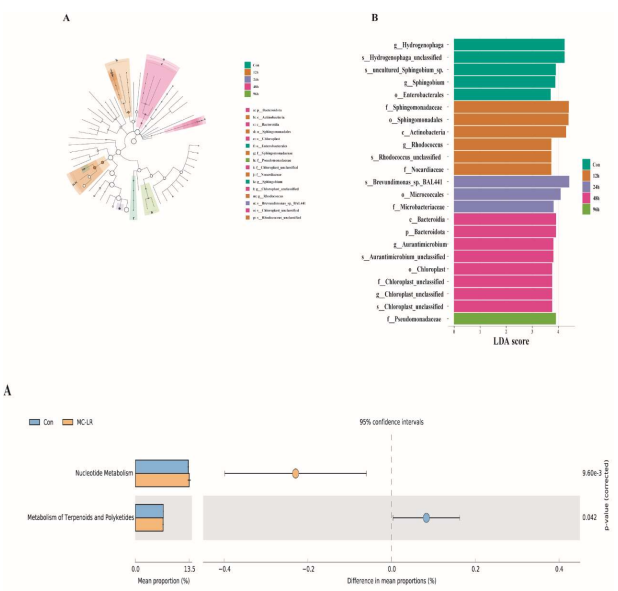
- **diversity index**



- **Relative abundance and differential microbial**

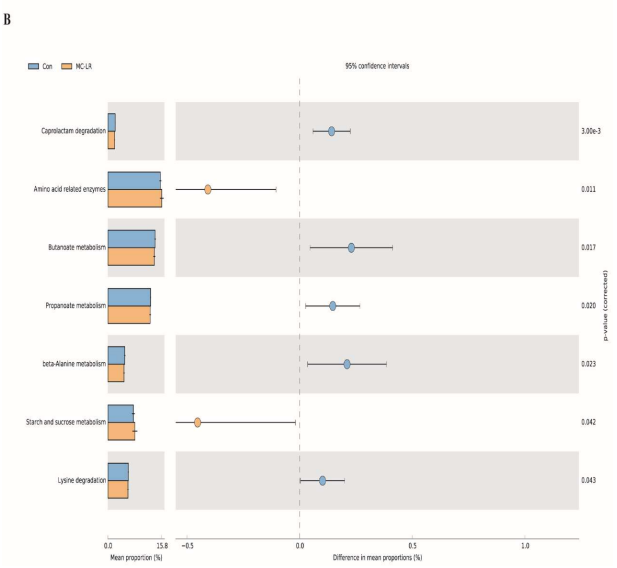


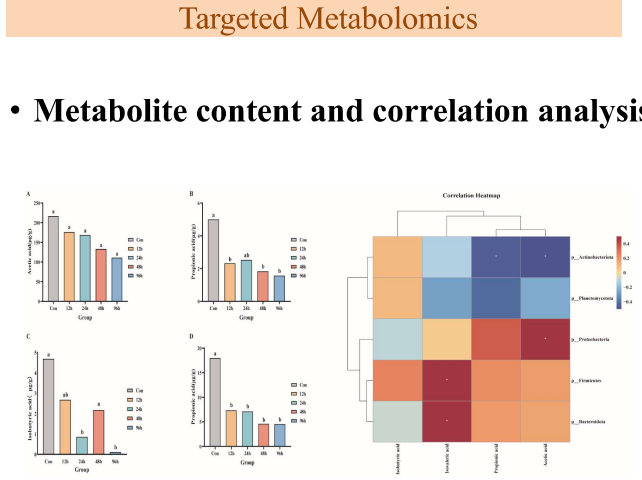
- **Biomarkers and function prediction**



Targeted Metabolomics

- **Metabolite content and correlation analysis**





4. Conclusion

This study demonstrates that MC-LR exposure disrupts and activates oxidative stress in GIFT tilapia, impairs immune function, and alters the composition of gut microbiota, leading to intestinal damage and changes in metabolites, which subsequently affect hepatic lipid metabolism. Our findings provide a theoretical reference for elucidating the toxic mechanisms of MCs and contribute to the advancement of healthy aquaculture practices for GIFT tilapia. Additionally, we hypothesize a correlation between gut microbiota, particularly short-chain fatty acids (SCFAs) as metabolites, and lipid metabolism in GIFT tilapia; however, the specific mechanisms underlying this relationship require further investigation.

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