BEHAVIORAL RESPONSES AND PHYSIOLOGICAL MECHANISMS OF GRASS CARP IN HYPOXIC CONDITIONS

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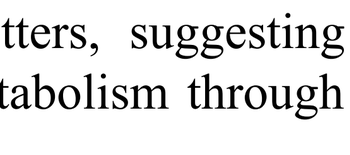
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Abstract

In this study, juvenile grass carp were exposed to three dissolved oxygen (DO) levels (7, 5, and 3 mg/L) for 60 days to investigate the effects of chronic hypoxia on growth and behavior, as well as to reveal the mechanisms of behavioral response. Chronic hypoxia significantly reduced body length and body weight, and markedly inhibited feeding and locomotor activities. Hypoxia exposure impaired energy metabolism that may contribute to behavioral suppression. In addition, chronic hypoxia induced intestinal damage and gut microbiota dysbiosis, characterized by reduced abundances of beneficial bacteria, including *Phocaeicola_A* and *Akkermansia*.These microbial alterations were accompanied by decreased concentrations of short-chain fatty acids (SCFAs) as well as significant changes in neurotransmitter levels in both the intestine and brain, including increased NE and 5-HT levels. Correlation analysis revealed significant associations among gut microbiota, SCFAs, and neurotransmitters, suggesting that hypoxia-induced intestinal dysfunction and microbial imbalance may impact neurotransmitter metabolism through the gut–brain axis, ultimately leading to impaired swimming and feeding behaviors in grass carp.

Introduction

- Dissolved oxygen is one of the main environmental factors that affect the growth and metabolism of fish and induce various fish diseases.insufficient dissolved oxygen may inhibit fish growth, affecting tissue metabolism, function and morphological structure.
- Grass carp (*Ctenopharyngodon idella*), the most widely farmed freshwater fish in China, also faces the challenge of hypoxia.



Methods

Juvenile grass carp (4.50 ± 2.23 g) were exposed to three dissolved oxygen (DO) levels (7, 5, and 3 mg/L) in a recirculating aquaculture system for 60 days. Each treatment was conducted in triplicate.

Behavioral tests were performed after 60 days of exposure. Locomotor activity, feeding behavior were recorded using video analysis.

Neurotransmitters and short-chain fatty acids (SCFAs) were quantified using LC-MS/MS and triple quadrupole mass spectrometry, respectively. Intestinal microbiota composition was analyzed by 16S rRNA sequencing.

Results

- Compared with the normoxia group (7 mg/L), body length and body weight significantly decreased in the hypoxia groups (5 and 3 mg/L).
- Chronic hypoxia (3 mg/L DO) significantly decreased feed intake and feeding frequency in juvenile grass carp.

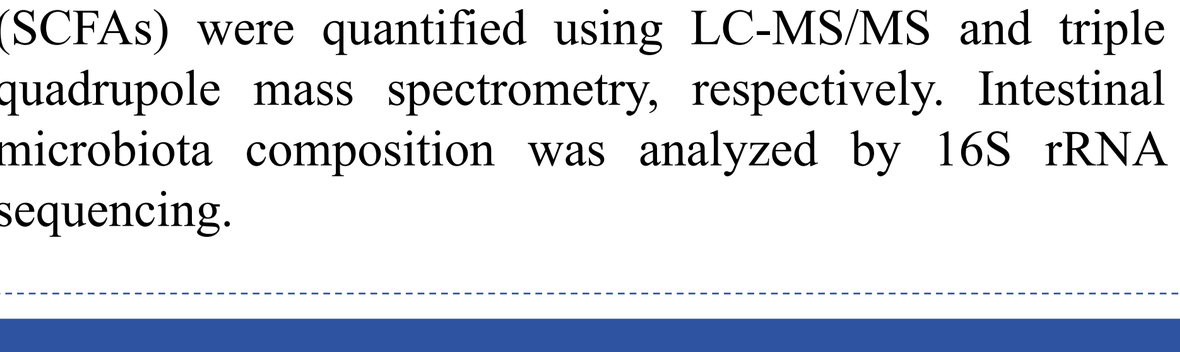


Fig.1. Effects of 60 day hypoxia stress on growth, development and feeding behaviorof juvenile grass carp.

- Chronic hypoxia significantly affected oxidative stress and energy metabolism in grass carp.
- Chronic hypoxia induced intestinal damage of grass garp, characterised by observed reduction in villus height and muscle layer thickness, as well as decreased goblet cell density.

Results

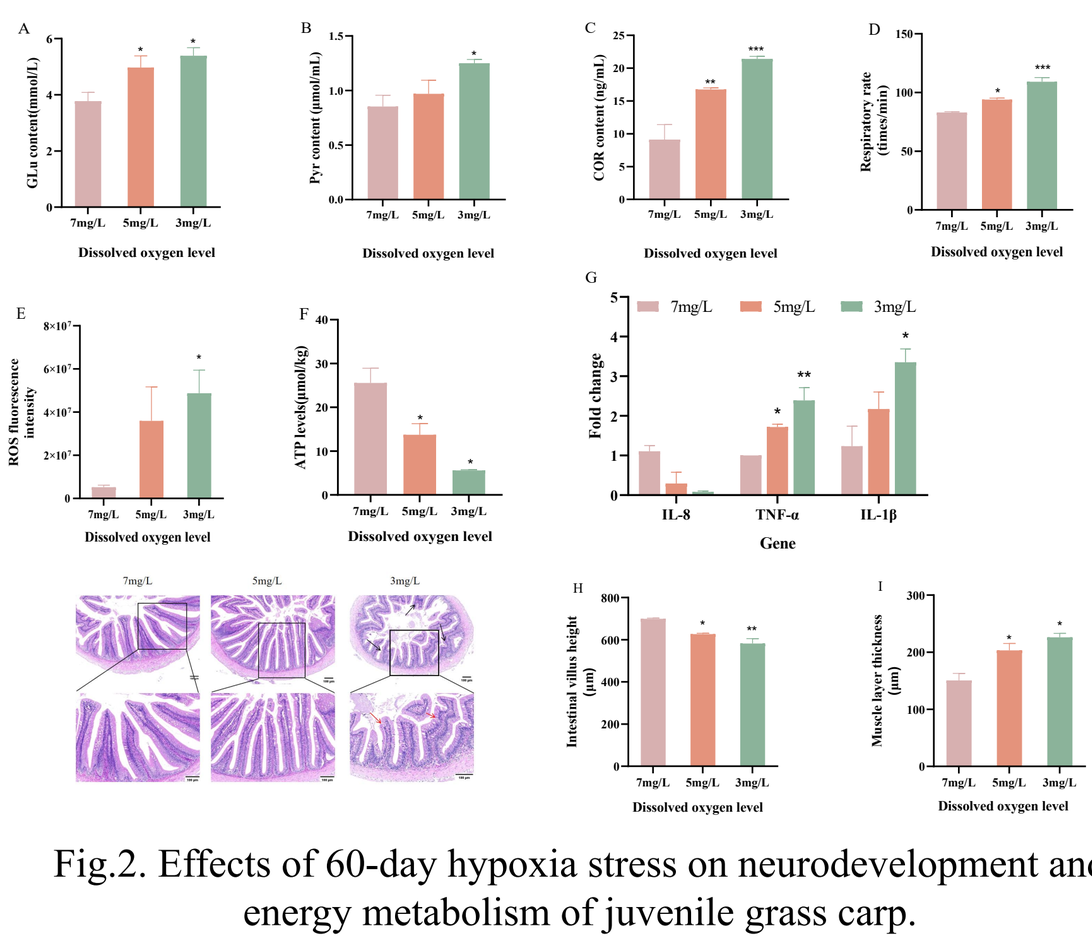


Fig.2. Effects of 60-day hypoxia stress on neurodevelopment and energy metabolism of juvenile grass carp.

- Chronic hypoxia causes gut microbiota dysbiosis and significant changes of neurotransmitters in the gut and brain.These microbial changes are significantly correlated with neurotransmitter alterations.

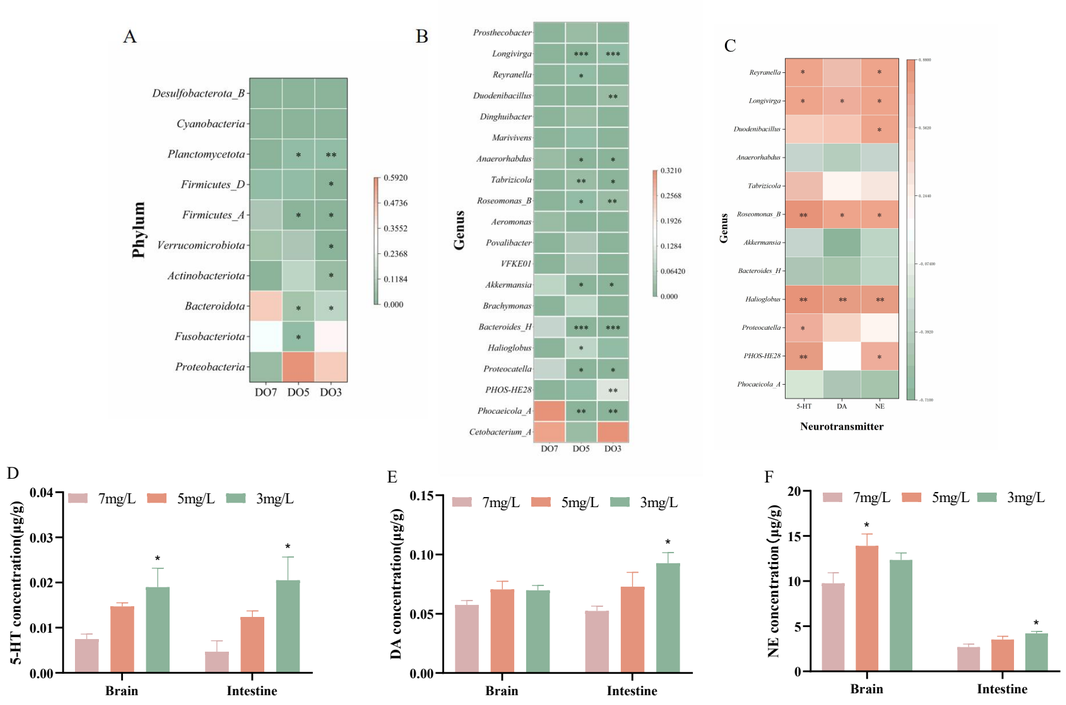


Fig.3. Effects of 60-day hypoxia stress on gut microbiota and neurotransmitter, and the microbiota-neurotransmitter correlation, in juvenile grass carp.

Conclusion

Chronic hypoxia significantly inhibited growth, feeding, and locomotor behaviors in juvenile grass carp, while inducing oxidative stress and energy metabolism disorders. Hypoxia also caused intestinal damage, gut microbiota dysbiosis, and significant changes in SCFAs and neurotransmitter levels in the gut and brain, particularly increased 5-HT and NE levels. Correlation analysis suggested that hypoxia-induced microbial imbalance may regulate neurophysiological responses through the gut–brain axis, ultimately contributing to impaired feeding and swimming behaviors in grass carp.