

Effects of stocking density on the antioxidant status, nutrient composition and metabolic function of *Cyprinus carpio* in Integrated Rice-Fish farming systems



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Introduction

Integrated rice-fish farming is widely recognized as an effective mechanism for increasing agricultural biodiversity and developing agroecology, which can provide grain and meat to humans while reducing the risk of environmental pollution. In recent years, owing to the good ecological and economic benefits of integrated rice-fish farming, it has expanded rapidly worldwide. In China, it has become one of the main modes of food production. However, the lack of appropriate stocking densities has led to some constraints on its extensive practice.

Therefore, the purpose of this study was aim to evaluate the effects of stocking density on the redox status, nutrient composition and metabolic function of *Cyprinus carpio* in an integrated rice-fish farming system.

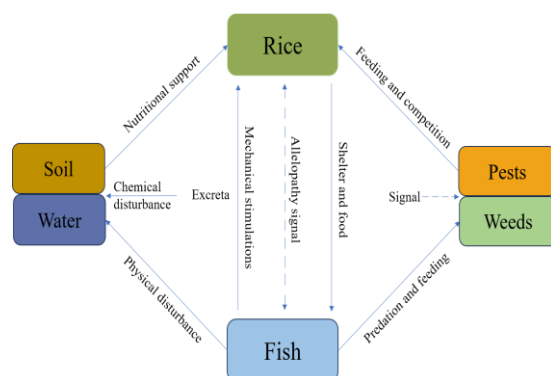


Figure 1. Chart of energy flow in rice-fish co-culture system

Methods

In the rice-fish co-culture system, three stocking densities were set: low stocking density (LD, 52.9 g/m²), middle stocking density (MD, 105.8g/m²) and high stocking density (HD, 158.7g/m²), and each density contained three replicates. The common carp used in the experiment was with an average initial weight of 105.83±2.52 g and a commercial diet was fed at 2-3% of the total body weight of the fish twice a day.

After 60 days of rearing in paddy fields, the muscles of *C. carpio* were collected to detect the oxidative stress indices, amino acid and fat composition. In addition, muscle tissue was used for transcriptome sequences to investigate the effects of high stocking densities on the molecular function of the system.

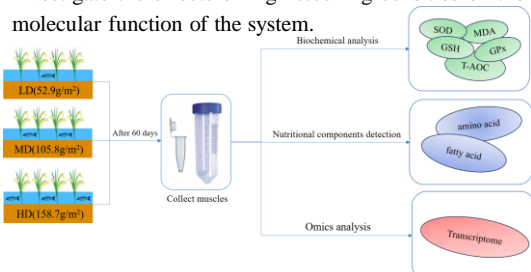


Figure 2. Flowchart of the entire research process

Results

1. Changes in Oxidative Stress Parameters

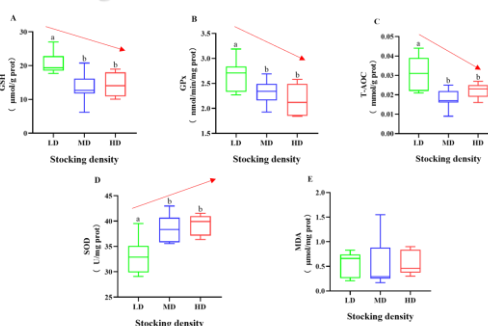


Figure 3. Antioxidative parameters in muscles of *C. carpio* under different densities in an integrated rice-fish farming system at the end of the experiment.

At the end of the experiment, the SOD levels of the MD and HD groups increased markedly compared with the LD group, while the activities of T-AOC, GSH and GPx in the MD and HD groups was significantly lower than those in the LD group. In addition, the MDA content at different densities was not notably affected.

2. Changes in nutrient composition

2.1 Amino acid composition in muscles

Items	LD	HD
EAA	5.90±0.26	5.60±0.19
HEAA	1.47±0.07	1.43±0.05
NEAA	6.85±0.32	6.36±0.22
TAA	14.22±0.65	13.33±0.46

Table 1. Amino acid content and composition (g/100g wet weight) in muscle of *C. carpio* at different stocking densities.

No matter at high or low density, most of the amino acid contents in the muscle of *C. carpio* were similar, and there was no statistical significance.

2.2 Fatty acid composition in muscles

Items	LD	HD
SFA	143.2±4.13	185.2±7.59*
MUFA	135.1±6.38	194.1±14.43*
PUFA	189.2±7.25	236.6±7.52
n-3 PUFA	78.9±2.86	96.4±5.83*
n-6 PUFA	106.2±5.57	135.2±5.39
n-3/n-6	0.75±0.04	0.72±0.06

Table 2. Fatty acid content and composition (mg/100g wet weight) in muscle of *C. carpio* at different stocking densities.

Compared with the LD group, SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA increased significantly in the muscle of *C. carpio* in the HD group, and there was no significant difference in the ratio of n-3/n-6 PUFA.

3. Changes in transcriptome sequencing

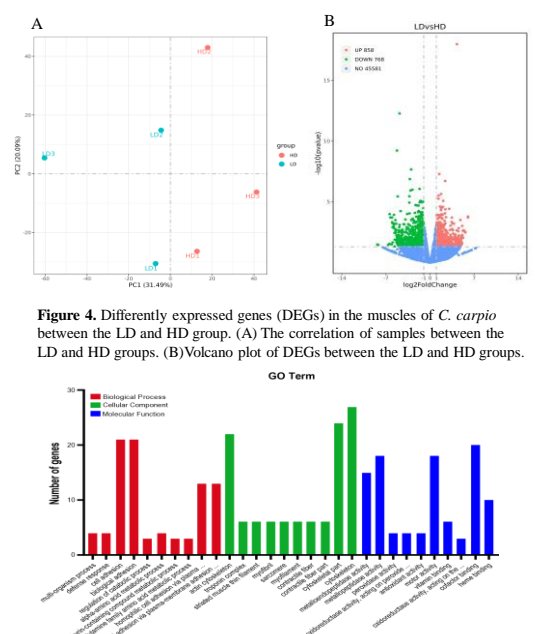


Figure 4. Differently expressed genes (DEGs) in the muscles of *C. carpio* between the LD and HD group. (A) The correlation of samples between the LD and HD groups. (B) Volcano plot of DEGs between the LD and HD groups.

Figure 5. GO enrichment terms of DEGs in three ontologies.

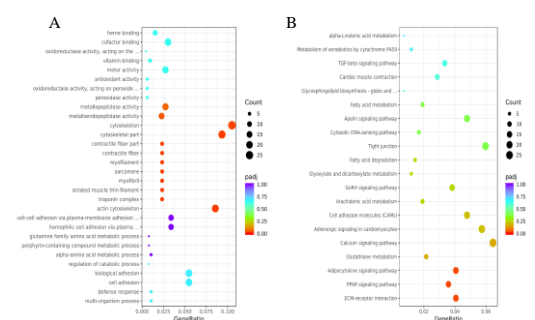


Figure 6. (A) Top 10 GO terms in the biological process, cellular component and molecular function. (B) Top 20 enriched KEGG pathways.

Further transcriptome analysis of the muscles of *C. carpio* showed that there were 1626 differentially expressed genes between the LD group and the HD group, including 858 upregulated genes and 768 downregulated genes, were annotated to 141 KEGG pathways. Moreover, the pathways related to ECM-receptor interaction, PPAR signaling pathway and Adipocytokine signaling pathway were significantly influenced by stocking density.

Conclusion

- The antioxidant defense system of *C. carpio* was inhibited at high stocking density.
- The fatty acid content in the muscle of *C. carpio* increased significantly at high stocking density, while the amino acid content was not statistically significant.
- In the HD group, two key pathways related to lipid metabolism were inhibited, including the PPAR and Adipocytokine signaling pathways, resulting in abnormal muscle metabolism in *C. carpio* in an integrated rice-fish farming system.