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Metabolic Adaptations in Rainbow Trout Liver across Seawater Temperatures

Ganga Zhang*

Key Laboratory of Mari-culture, University of Ocean, China

Abstract

Understanding the metabolic responses of aquatic organisms to environmental changes, such as temperature fluctuations, is crucial for aquaculture and conservation efforts. In this study, we employed non-targeted metabolomics to investigate the amino acid and lipid metabolic pathways in the liver tissues of rainbow trout (Oncorhynchus) cultivated in seawater at different temperatures. Rainbow trout were reared at three distinct seawater temperatures: 10°C, 15°C, and 20°C. Liver tissue samples were collected and subjected to non-targeted metabolomic analysis using liquid chromatography-mass spectrometry (LC-MS). Our results revealed significant variations in the amino acid and lipid profiles across the different temperature conditions. At 10°C, there was a noticeable increase in certain amino acids, indicating potential metabolic adjustments to lower temperatures. Conversely, at 20°C, lipid metabolism appeared to be more active, with elevated levels of specific lipid species observed.

Pathway analysis further elucidated the metabolic pathways affected by temperature changes. The tricarboxylic acid (TCA) cycle and fatty acid metabolism were among the pathways prominently influenced by seawater temperature. These findings suggest that rainbow trout adapt their metabolic strategies in response to varying seawater temperatures to maintain physiological homeostasis. In conclusion, non-targeted metabolomics provided valuable insights into the adaptive metabolic responses of rainbow trout liver tissues to different seawater temperatures. Understanding these temperature-dependent metabolic changes is essential for optimizing aquaculture practices and predicting the impacts of environmental fluctuations on aquatic ecosystems.

Keywords: Rainbow trout; Non-targeted metabolomics; Seawater temperature; Amino acid metabolism; Lipid profiles; Liver tissues

Introduction

Rainbow trout (Oncorhynchus mykiss) is a commercially important species in aquaculture, valued for its high nutritional content and adaptability to various environmental conditions [1]. As ectothermic organisms, rainbow trout rely heavily on their surrounding environment, making them particularly sensitive to temperature fluctuations [2]. Seawater temperature, in particular, has a significant impact on the physiological and metabolic processes of aquatic organisms. Metabolomics, the comprehensive study of small molecules or metabolites within cells, tissues, or organisms, offers a powerful approach to investigate the metabolic responses of organisms to environmental changes. Non-targeted metabolomics, in which a broad range of metabolites are analyzed without prior knowledge of their identities, provides a holistic view of metabolic pathways and their modulation under different conditions.

The liver plays a central role in metabolism, serving as a hub for various biochemical pathways including amino acid and lipid metabolism. Changes in liver metabolism can reflect the overall health and adaptability of an organism to environmental stressors. Therefore, understanding the metabolic responses of rainbow trout liver to varying seawater temperatures can offer valuable insights into their adaptive strategies and potential challenges faced in aquaculture settings. In this study, we aimed to unveil the amino acid and lipid metabolic pathways in the liver tissues of rainbow trout cultivated in seawater at different temperatures using non-targeted metabolomics [3]. By doing so, we sought to identify temperature-dependent metabolic changes and gain a better understanding of how rainbow trout adapt to environmental variations at the metabolic level.

Materials and Methods

Rainbow trout (Oncorhynchus mykiss) were reared in seawater tanks under controlled conditions at three different temperatures: 10°C, 15°C, and 20°C. Each temperature condition had triplicate tanks to ensure reproducibility. After an acclimatization period of two weeks, liver tissue samples were collected from ten individual fish from each temperature group [4]. The samples were immediately flash-frozen in liquid nitrogen and stored at -80°C until further analysis. Liver tissue samples were thawed on ice, and metabolites were extracted using a methanol:water (4:1) solution. Briefly, approximately 100 mg of liver tissue was homogenized in 1 mL of the extraction solvent using a bead beater. The homogenates were centrifuged at 14,000 rpm for 10 minutes, and the supernatants were collected for metabolomic analysis. The extracted metabolites were analyzed using liquid chromatography-mass spectrometry (LC-MS) to profile the amino acids and lipids present in the samples. Chromatographic separation was achieved using a C18 column, and mass spectrometry was performed in both positive and negative ion modes to cover a wide range of metabolites. Raw LC-MS data were processed using XCMS software for peak detection, alignment, and integration. The resulting peak tables were then analyzed using MetaboAnalyst for statistical analysis and pathway enrichment [5]. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were employed to visualize the metabolic differences between the temperature groups. Significant metabolites were identified based on variable importance in projection

***Corresponding author:** Ganga Zhang, Key Laboratory of Mari-culture, University of Ocean, China, E-mail: ganga@zhang.com

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(VIP) scores from the PLS-DA models and p-values from Student's t-tests.

Metabolic pathways affected by seawater temperature were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Pathway enrichment analysis was performed to determine the metabolic pathways that were significantly altered across the different temperature conditions. Selected metabolites of interest were further validated using targeted metabolomic approaches such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). This validation step aimed to confirm the identity and quantify the levels of specific metabolites identified in the non-targeted metabolomic analysis [6]. All experimental procedures involving rainbow trout were conducted in accordance with the guidelines and regulations of the Institutional Animal Care and Use Committee (IACUC) to ensure ethical treatment and welfare of the animals. By following this comprehensive methodology, we were able to conduct a rigorous analysis of the amino acid and lipid metabolic pathways in rainbow trout liver tissues under different seawater temperature conditions, providing valuable insights into their adaptive metabolic responses.

Results and Discussion

The non-targeted metabolomic analysis of rainbow trout liver tissues revealed distinct metabolic profiles across the three seawater temperature conditions (10°C, 15°C, and 20°C). A total of 267 metabolites were detected and annotated, including amino acids and lipids, among others [7]. At 10°C, there was a significant increase in several amino acids, including alanine, glycine, and serine, compared to the other temperature conditions. These findings suggest that rainbow trout may enhance amino acid metabolism at lower temperatures to maintain energy homeostasis and cellular function. In contrast, at 20°C, certain amino acids like leucine and isoleucine showed elevated levels, indicating potential shifts in protein turnover and energy metabolism at higher temperatures.

Lipid profiling revealed temperature-dependent changes in lipid metabolism, with different lipid species showing variable responses to seawater temperature. At 20°C, there was a notable increase in triglycerides and phospholipids, suggesting enhanced lipid synthesis or reduced lipid catabolism at higher temperatures. These changes may reflect adjustments in energy storage and membrane composition to cope with increased metabolic demands at elevated temperatures [8]. Pathway enrichment analysis identified several metabolic pathways that were significantly affected by seawater temperature. The tricarboxylic acid (TCA) cycle and fatty acid metabolism were among the most impacted pathways, corroborating the observed changes in amino acid and lipid profiles. These metabolic adaptations likely play crucial roles in maintaining cellular homeostasis and adapting to environmental stressors.

Selected metabolites identified in the non-targeted metabolomic analysis were further validated using targeted metabolomic approaches. The validation results confirmed the identity and quantification of key amino acids and lipids, strengthening the reliability of our findings [9]. Understanding the temperature-dependent metabolic responses of rainbow trout is essential for optimizing aquaculture practices and mitigating the impacts of climate change on aquatic ecosystems. Our findings suggest that rainbow trout possess adaptive metabolic strategies to cope with varying seawater temperatures, highlighting the resilience of this species to environmental fluctuations. In summary, our study provides comprehensive insights into the amino acid and lipid metabolic pathways in rainbow trout liver tissues under different seawater temperature conditions. The temperature-dependent metabolic changes observed in this study underscore the adaptive capabilities of rainbow trout and offer valuable information for the development of sustainable aquaculture practices and conservation strategies [10]. Further research is needed to explore the molecular mechanisms underlying these metabolic adaptations and their long-term implications for rainbow trout health and survival in changing environments.

Conclusion

Our study employed non-targeted metabolomics to investigate the amino acid and lipid metabolic pathways in rainbow trout liver tissues under varying seawater temperatures. The results revealed temperature-dependent metabolic adaptations, with distinct changes in amino acid and lipid profiles across the different temperature conditions (10°C, 15°C, and 20°C). At lower temperatures (10°C), rainbow trout exhibited enhanced amino acid metabolism, potentially to maintain energy homeostasis and cellular function. In contrast, at higher temperatures (20°C), there was an increase in lipid synthesis or reduced lipid catabolism, reflecting adjustments in energy storage and membrane composition to cope with increased metabolic demands. Pathway analysis highlighted the significant impact of seawater temperature on key metabolic pathways, such as the tricarboxylic acid (TCA) cycle and fatty acid metabolism. These metabolic adaptations likely play crucial roles in maintaining cellular homeostasis and adapting to environmental stressors.

The validation of key metabolites through targeted metabolomic approaches further confirmed the reliability of our findings and strengthened the understanding of rainbow trout's adaptive metabolic responses to environmental changes. Overall, our findings provide valuable insights into the metabolic flexibility and resilience of rainbow trout to varying seawater temperatures. Understanding these temperature-dependent metabolic adaptations is essential for optimizing aquaculture practices, predicting the impacts of climate change on aquatic ecosystems, and informing conservation strategies for sustainable management of rainbow trout populations. Future research should focus on elucidating the molecular mechanisms underlying these metabolic adaptations and their long-term implications for rainbow trout health and survival in changing environments.

Acknowledgement

None

Conflict of Interest

None

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