Metabonomics Study on the Mechanism of the Effect of Low Salt on the Liver of Qinghai Lake Naked Carp

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Abstract: In order to explore the metabonomics study of the mechanism of low salt on the liver of Qinghai Lake naked carp, this study investigated the liver enzyme activity, tissue structure and related immune genes during the salinity change of Qinghai Lake naked carp. The role of naked carp in immunity can provide a theoretical basis for the research on the adaptability of Qinghai Lake naked carp to changes in salinity. Two experimental groups of Qinghai Lake naked carp with different salinities were established in the Emergency Center (JH) and Qinghai Lake (QH). The rescue center is Freshwater, and the salinity of Qinghai Lake is 1.24‰. This experiment uses ultra-high performance liquid chromatography non-targeted metabonomics technology, and the differential metabolites were screened according to the variable weight value (VIP) and independent sample T test, and the KEGG pathway enrichment and annotation analysis were performed. The results showed that compared with the JH group, in this study, 221 metabolites among the 1,525 differential metabolites identified received 90 KEGG annotations. We performed KEGG enrichment analysis on the 65 differential proteins obtained, and the results showed that the differentially expressed proteins mainly come from primary bile acid biosynthesis: M (Primary bile acid biosynthesis: M), Parkinson's disease: (Parkinson disease: HD), Linoleic acid metabolism: M (Linoleic acid metabolism: M), protein digestion and absorption: OS (Protein digestion and absorption: OS), cancer choline metabolism: HD (Choline metabolism in cancer: HD) , This study clarified the metabonomics of the liver metabolism mechanism of Qinghai Lake naked carp with different salinities, and the study of Qinghai Lake naked carp liver function has a good guiding significance for molecular biology.

1 INTRODUCTION

Qinghai Lake naked carps belong to the genus Cypriniformes, Cyprinidae, Schizoma subfamily, and naked carps, commonly known as Huangyu. It is the only commercial fish in Qinghai Lake and occupies a very important position in the Qinghai Lake ecosystem. (CAO, WU, SHAO, 2010). Due to its historical and natural reasons, Qinghai Lake naked carp resources were once greatly destroyed. In order to accelerate its resource recovery in Qinghai Lake, the Qinghai Lake Naked Carp Rescue Center has carried out artificial breeding and breeding of Qinghai Lake naked carp resources in the past 20 years, especially in recent years through the establishment of a factory circulating water breeding system to develop Qinghai Lake naked carp Great progress has been made in breeding. Qinghai Lake naked carp has the habit of reproductive migration, has a strong adaptability to changes in salinity, and can live in fresh water, brackish water, and alkaline water (Walker, Dunn, Edwards, Petr, Yang, 1995). Wang Ping et al. (WANG, LAI, YAO, 2015) Metabonomics studies and studies on the mechanism of the intestine of Qinghai Lake naked carp under different salinity environments have shown that there are gene expression in many tissues such as Qinghai Lake naked carp intestine and liver.

Salinity is one of the important factors affecting the survival and growth of fish. Especially for

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euryhalinic fish, the salinity changes in the water environment in which they live will cause the adaptation of enzyme activities, tissue structure and gene expression in the fish. Sexual changes (Yong Zhong); (Wang, 2018); (Zhang, Wen, Zhang, 2018), studying the growth, survival, metabolism and other physiological activities of fish under low-salt conditions (Lian, 2012) can help understand the anti-low-salt mechanism of fish and guide the healthy breeding of fish. Although predecessors performed correlation analyses on liver tissues at different salinities to explain the differences in gene transcription level expression of Qinghai Lake naked carp, but due to the regulation of gene expression and translation level, Qinghai Lake naked carp cannot perform liver transcriptomics research without saline environment. Fully explain the types of products involved in the synthesis of liver metabolites.

Non-targeted metabolomics can perform qualitative and relative quantitative analysis of small molecular metabolites in biological systems for specific physiological conditions, and reflect the total metabolite information to the greatest extent (Wen, 2019); (Yang, 2020). Non-targeted metabolomics can provide the maximum amount of information about the metabolism of the central carbon cycle, reflecting the body's metabolism after being subjected to environmental stress through biological processes such as gene expression, transcription, post-transcriptional regulation, protein translation and modification, etc. Changes in the final product. In this part of the research, we used the naked carp of Qinghai Lake (JH) and Qinghai Lake (QH) as the research object, and used non-targeted metabolomics to study the metabolic changes of Qinghai Lake naked carp in response to low-salt stress, hoping to reveal Qinghai Lake naked carp. Carp provides metabolite data and basic information on the regulation mechanism of low-salt stress.

2 MATERIALS AND METHODS

2.1 Materials

The test materials were naked carps in the rescue center (JH) and Qinghai Lake (QH) in September 2020. All naked carps were fresh and live fish species identified by the staff of Qinghai Lake Naked Carp Rescue Center. 10 samples of each were collected. The collected naked carp was washed and deplaned, and the liver was collected, and immediately subjected to biological reaction inactivation treatment (liquid nitrogen freezing), and stored in a refrigerator at -80°C.

2.2 Sample Pretreatment

The pretreatment method of the liver tissue sample was prepared according to the method of He et al. (He, An, Huang, 2019). Accurately weigh 50 mg of the liver tissue sample and transfer it to a 1.5 mL EP tube; add 400 μl of extraction solution (methanol: water=4:1) to the sample at low temperature , High-throughput tissue disrupter (-20°C, 50HZ, 6min); vortex (30s) to mix, then low-temperature ultrasonic extraction for 30min (5°C, 40KHz); place the sample at -20°C, 30min; centrifuge Centrifuge the sample at 13000 rpm and 4°C for 15 minutes, aspirate the supernatant, and transfer it to an LC-MS vial for analysis. The quality control sample (QC) is prepared by mixing equal volumes of all sample extracts, each The QC volume is the same as the sample. All extraction reagents are pre-cooled at -20 °C before use.

2.3 LC-MS Detection

The instrument platform for this LC-MS analysis is the UPLC-TripleTOF system of AB SCIEX. The chromatographic conditions are: The chromatographic column is a BEH C18 column (100 mm × 2.1 mm id, 1.7 μm; Waters, Milford, USA); the mobile phase A is water (containing 0.1% formic acid), and the mobile phase B is acetonitrile/isopropanol (1/1) (containing 0.1% formic acid); the mobile phase elution gradient program is as follows: 0 min, 5% B; 3 min, 20% B; 9 min, 95% B; 13.0 min, 95% B; 13.1 min, 5% B; 16min, 5%B. The flow rate is 0.40mL/min, the injection volume is 10μL, and the column temperature is 40°C.

The sample mass spectrum signal acquisition adopts positive and negative ion scanning mode and ion spray voltage. The mass spectrometry parameters are as follows: spray gas 50V; auxiliary heating gas 50V; curtain gas 30V; ion source heating temperature 500℃; ionization voltage (positive) 5000V, ionization voltage (negative) -4000V; interface heating on; declustering voltage 80V, The collision energy is 20-60(rolling)V.

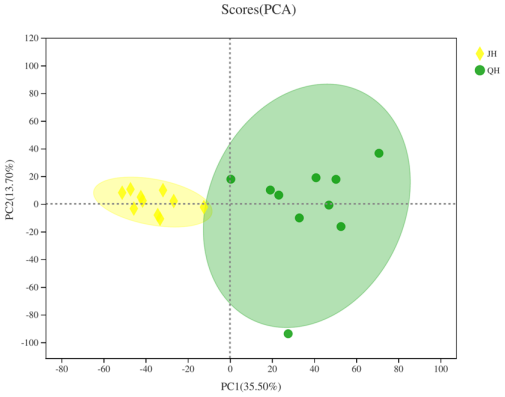
2.4 Quality Control

Quality control samples (QC) are prepared by mixing the extracts of all samples in equal volumes. The volume of each QC is the same as that of the sample. It is processed and tested in the same way as the analysis sample. In the process of instrument analysis, every 10 analysis Insert a QC sample into the sample to examine the repeatability of the entire analysis process.

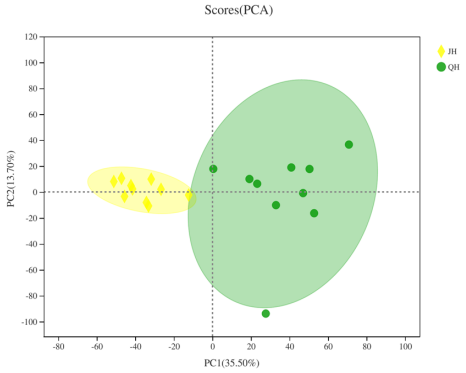
3 RESULTS AND ANALYSIS

3.1 Principal Component Analysis

Principal component analysis is used for pattern recognition analysis of multivariate variables. Principal component analysis (PCA) is a statistic that converts a set of observed possibly related data into linear uncorrelated data (ie principal components) through orthogonal transformationmethod. As an unsupervised analysis method, it can automatically analyze the income without default grouping arrangement. Model construction of sample metabolism data, using a small amount of principal components to reduce data dimensions, represent data information, and reveal The internal structure of the data. Among them, 35.5% of the data in Figure 1aPCA participated in the model construction; in the P-LCA model established in Figure 1b, 35.3% of the data participated in the model construction. The closer R2Y and Q2 are to 1, the more stable and reliable the model is. Generally, the model with Q2 greater than 0.5 is stable and reliable. As shown in the table, the tested model is reliable regardless of the cation or anion mode. The metabolites identified on this basis are Believed to be reliable.



a.PCAScore graph



b.PLS-DAScore graph

Figure 1: Principal component analysis diagram.

3.2 Screening of Differential Metabolites

Obtain more accurate labeled compounds, and further conduct biomarker mining and functional analysis of compounds with complete secondary information detected in the positive and negative ion mode. The differential metabolite identification standard used in this study was P<0.05 by paired t-test, and it also satisfies log 2 Fold change<-1 or log 2 Fold change>1. See the volcano map for the screening results of differential metabolites (Figure 2). Each point in the volcano map represents a metabolite, the abscissa represents the fold change of each compound compared to the Qinghai Lake naked carp and the rescue center control (take the logarithm to the base 2), and the ordinate represents the P value of the paired t test (Take the negative logarithm to the base 10). The scattered colors represent the final screening results. There are a total of 901 differential metabolisms, of which 124 are up-regulated and 97 are down-regulated.

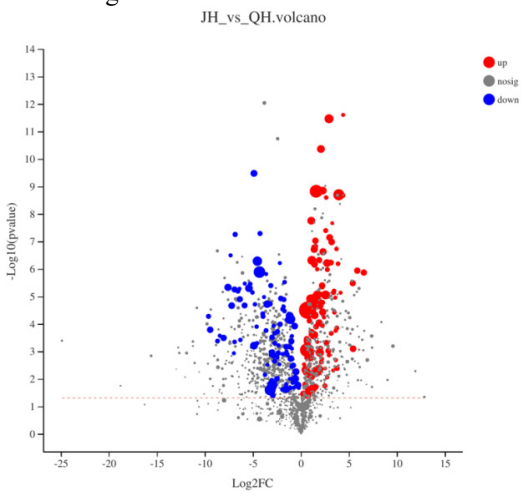
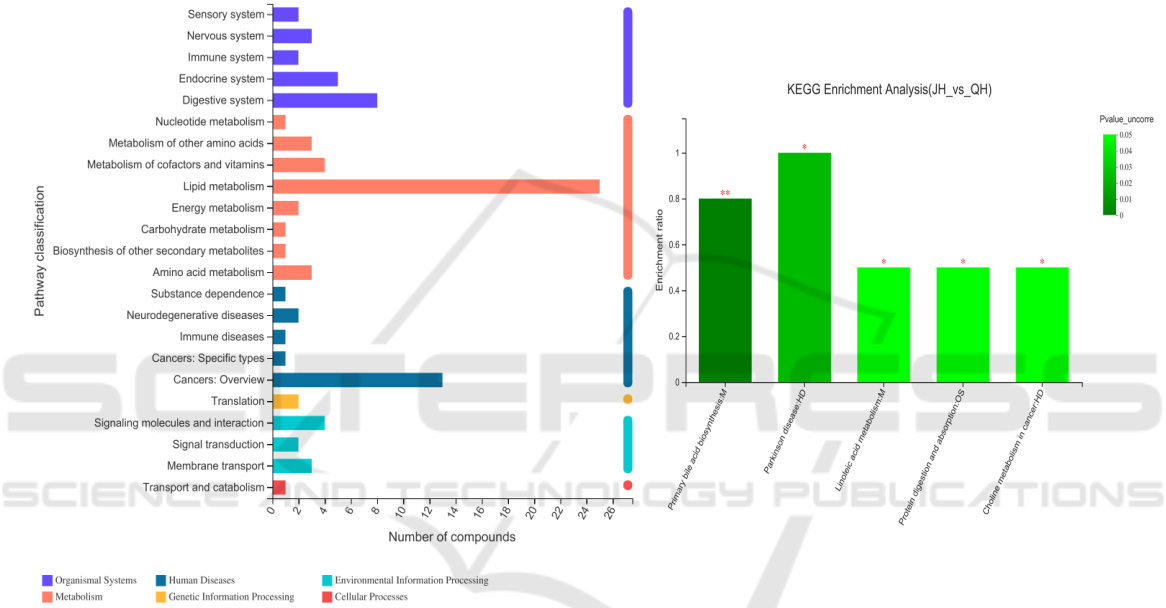


Figure 2: Volcano map of differential metabolites.

3.3 KEGG Pathway Annotation and Enrichment Analysis

In this study, out of the 1,525 differential metabolites identified, 221 metabolites received 90 KEGG annotations. In order to further reveal the overall pathway enrichment characteristics of all differential metabolites, we performed an enrichment analysis on the KEGG annotation results of the differential metabolites. The KEGG annotation analysis of the differential proteins is shown in Figure 3a. The results showed that under high altitude stress conditions, the liver metabolites of Qinghai Lake naked carp were significantly enriched in the sensory system, Nervous system, Immune system, Endocrine system, and digestive system. Digestive system, Nucleotide metabolism, Metabolism of other amino acids, Metabolism of cofactors and vitamins, Lipid metabolism, Energy metabolism, carbohydrate metabolism (Biosynthesis of other secondary metabolism), amino acid metabolism (substance dependence), neurodegenerative diseases, cancer: specific types, cancer: overview, transport (Translation), signaling molecules and interactions (Signaling molecules and interaction), signal transduction, membrane transport and catabolism.



a.KEGG annotation analysis of differentialproteins *b.*KEGG enrichment analysis of differential proteins

Figure 3: Annotation diagram of KEGG pathway enrichment of differential proteins.

We performed KEGG enrichment analysis on the 65 differential proteins obtained. The analysis results are shown in Figure 3band table 1. The results indicate that the differentially expressed proteins mainly come from Primary bile acid biosynthesis: M, Parkinson disease: HD, Linoleic acid metabolism: M, Protein digestion and absorption: OS, cancer choline metabolism: HD.

Table 1: Enrichment analysis of KEGG pathway in the liver of Qinghai Lake naked carp.

Metabolite	Metab ID	Formula	VIP_pred_OPLS-DA	FC(JH/QH)	P_value	mode
Taurine	metab_7226	C2H7NO3S	2.727	2.869	<0.001	neg
3a,7a,12a-Trihydroxy-5b-cholestan-26-al	metab_106	C27H46O4	7.024	0.127	0.0165	pos
Taurocholic acid	metab_610	C26H45NO7S	1.290	0.013	<0.001	pos
5b-Cyprinol sulfate	metab_2325	C27H48O8S	7.908	0.110	0.025	pos

9,10-DiHOME	metab_6109	C18H34O4	1.628	10.651	<0.001	neg
PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:2(9Z,12Z))	metab_9591	C48H80NO8P	5.434	7.685	<0.001	neg
PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	metab_9633	C52H80NO8P	1.479	1.704	0.008	neg
9-OxoODE	metab_2646	C18H30O3	2.090	0.108	<0.001	pos
L-Isoleucine	metab_5783	C6H13NO2	4.023	0.447	<0.001	pos
Piperidine	metab_1437	C5H11N	1.843	0.422	<0.001	pos
L-Tyrosine	metab_1454	C9H11NO3	1.047	0.528	<0.001	pos

4 DISCUSSION

Salinity is one of the important environmental factors that affect the survival of fish. Changes in salinity will cause changes in enzyme activities, tissue structure and gene expression levels in fish. The liver is an important metabolism-based organ in the fish body. It plays a vital role in the synthesis and catabolism of carbohydrates, fats and proteins. Therefore, the strength of the liver can be used as the ability of fish to resist external environmental stress. An important indicator of size. We mainly focus on Primary bile acid biosynthesis: M, Linoleic acid metabolism: M, Protein digestion and absorption: OS, several major The differential metabolism of the KEGG pathway was analyzed. The liver occupies an important position in the metabolism of bile acids, which is closely related to the synthesis, secretion, and conversion of bile acids. The changes of bile acids are also known as metabonomic markers of liver injury (Liu, Jiang, Shen, 2016), the primary bile acid is directly synthesized by hepatocytes using cholesterol as a raw material. It is secreted from the liver and enters the intestinal lumen, where it becomes a secondary bile acid under the metabolism of some enzymes and bacteria (MA, XIE, WANG, 2019). Li Xiulong et al. (LI, HU, LI, 2020) found that the acute liver injury induced by acetaminophen in rats may be related to glycerophospholipid metabolism, sphingolipid metabolism, and primary bile acid biosynthesis and other metabolic pathways. related. Jin Wenjie (Jin, Li, Ran, 2021) et al. conducted a transcriptome analysis of copper toxicology in naked carp, and the results showed that several genes involved in oxidative stress in the gill and liver were up-regulated. Up-regulation of these genes indicates that copper treatment causes oxidative stress, which may cause ribosome damage. The metabolism of linoleic acid is the main component of the cell membrane. The increase and decrease of its content may be related to the necrosis and apoptosis of hematopoietic stem cells. Liu Teng et al. (LIU, XU, LU, 2020) studied the intervention effect of Astragalus injection on leukopenia model mice based on LC-MS metabonomics and found that Astragalus injection can increase the white blood cells, lymphocytes and neutral of leukopenia model mice. Granulocyte and monocyte count; its effect of increasing white blood cells may be related to the metabolism of linoleic acid, the biosynthesis of phenylalanine, tyrosine and tryptophan, and the metabolism of phenylalanine. Protein digestion and absorption: OSis a component of the animal body and participates in various life activities of the Qinghai Lake naked carp (Deng, 2020), Li et al. (Li, Wang, Xu, 2015) used transcriptomics to study hens before and after laying eggs In liver tissue, a large number of differential genes were found to participate in amino acid metabolism pathways.

5 CONCLUSION

In this paper, a non-targeted metabolomics study was conducted on 30 cases of naked carp liver tissue samples using LC-MS analysis method. The main conclusions can be summarized as follows: (1) Obtain the metabolite list and data matrix, combine the T test and VIP(OPLS-DA) to screen out the different metabolites, (2) Among the 1,525 differential metabolites identified, there are 221 90 KEGG annotations were obtained for metabolites (3) The 65 differentially expressed proteins identified were mainly derived from primary bile acid biosynthesis: M, linoleic acid metabolism: M, protein digestion and absorption: OS. In future work, Progenesis QI (Waters Corporation, Milford, USA) software is used for metabolite annotation, data preprocessing, etc., to carry out metabonomics research on the mechanism of low-salt impact on the

liver of Qinghai Lake naked carp, and to improve the use of pathway analysis, Advanced analysis, such as association analysis and cluster analysis, mine the biological information of differential metabolism.

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