

UPLC-MS/MS Detection

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Abstract

To comprehensively investigate the effects of different diets on fish muscle quality and metabolic alterations, a large-scale metabolomics study was performed.

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Protocol

Sample Collection

Step 1.

White muscle and gonadal tissues were taken from 250 tails per experimental group (AF/GF).

Sample Grouping

Step 2.

The sex of the grass carp was determined by the contour of the gonad and further results of the gonad tissue slice. Based on the results of the sex determination, metabolomic analysis of muscle samples were divided into four test groups (n = 10): female fish of the grass feeding group (FGF), male fish of the grass feeding group (MGF), female fish of the artificial diet group (FAF), as well as male fish of the artificial diet group (MAF).

Sample Preparation for LC-MS

Step 3.

Each replicate from each experimental group was taken from 5 individuals (approximately 25 mg per sample). Samples of 5 tails were placed in an EP tube and mixed with 800 μ L of an ice-cold mixture of methanol and water (1:1 ratio), with two steel balls added to each tube. The tissues were then broken at 60 Hz for 5 minutes by the TissueLyser, then 300 μ L of supernatant from each tube was collected after a 10-minute centrifugation at 25000 g at 4 °C and then injected into the LC-MS system.

Chromatography

Step 4.

Chromatographic separations were performed using ultra performance liquid chromatography Ultimate 2777C (UPLC) system (Waters, UK). An ACQUITY UPLC BEH C18 column (100 mm* 2.1mm, 1.7 μ m, Waters, UK) was used for the reversed phase separation. The column oven was maintained at 50°C. The injection volume for each sample was 10 μ L, and the flow rate was 0.4 ml per minute. Additionally, the mobile phase consisted of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid). Gradient elution conditions were set as follows: 02 min, 100% phase A; 211 min, 0% to 100% B; 1113 min, 100% B; and 1315 min, 0% to 100% A.

Mass spectrometry

Step 5.

The eluents were introduced into a high-resolution tandem mass spectrometer SYNAPT G2-XS QTOF (Waters, UK) by electrospray ionization with capillary voltages set in the positive and negative modes

to 2.0 kV and 1.0 kV, respectively. The cone voltages of both modes were 40V. The mass spectrometry data were acquired in Centroid MSE mode. The TOF mass scan range of both simultaneous low- and high-energy mass scan functions was from 50 m/z to 1200 m/z with a scan time 0.2 seconds. For the MS/MS detection, all precursors were fragmented using 20 - 40 eV. During the acquisition, the mass spectrometry signal was acquired every 3 seconds to calibrate the mass accuracy.

Data transformation

Step 6.

The peak intensity tables of detected features were inputted into the MetaboAnalyst 4.0 (<http://www.metaboanalyst.ca/>) "Statistical Analysis" module for univariate and multivariate data analysis [27]. The input data were normalized by a pooled sample (quality control, QC) from the two experimental groups. Meanwhile, the log transformation and autoscaling were also used in data normalization procedures.

Metabolite identifications

Step 7.

For qualitative and quantitative metabolomics, raw data were processed using Progenesis Q1 software (Nonlinear Dynamics, 2017, version: 2.2, Waters, MA, US). First, data were cropped to remove external standards. Masses were detected, and the chromatogram for each mass was built using the Centroid mass detector and Chromatogram builder, respectively. Smoothed data were then deconvoluted using a noise amplitude algorithm and deisotoped. The conditions for chromatographic alignment were 0.01 m/z tolerance and 0.1 min RT-tolerance. Finally, sodium and ammonium adducts search was performed prior to exporting the data to Excel for post-processing. The compound identification list, which contained the molecular weight, compound name, statistical scores, and other information to show the result of the identifications was exported as an excel file (.csv).

Double-check with Identified Metabolites

Step 8.

To verify and confirm compound identifications, the METLIN batch Metabolite Search Database (<http://metlin.scripps.edu/>), Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg/>), Human Metabolite Database (<http://www.hmdb.ca/>) and ChemSpider (<http://www.chemspider.com/>) databases were used by comparing molecular weights and MOL files. The molecular and structural formulas of the candidate compounds were retrieved by the comparison and then confirmed by MS/MS scans for the characteristic ions and fragmentation patterns of the metabolites.