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Supplementary Materials

LC-MS Based Metabolomic Profiling of Largehead Hairtail (*Trichiurus japonicus*) Ovary Reveals Metabolic Signatures of Ovarian Developmental Process (II–IV)

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1. Sample preparation

50mg ovary were accurately weighed and extracted using a 400 μL methanol: water (4:1, v/v) solution. The mixture was allowed to settle at −20 °C and treated by High throughput tissue crusher Wonbio-96c (Shanghai wan bo biotechnology Co.,Ltd., Shanghai, China) at 50 Hz for 6 min, then followed by vortex for the 30s and ultrasound at 40 kHz for 30 min at 5 °C. The samples were placed at −20 °C for 30min to precipitate proteins. After centrifugation at 13,000g at 4 °C for 15min, the supernatant was carefully transferred for LC-MS analysis.

2. LC-MS analysis conditions

2.1. Chromatographic conditions：

2μL of sample was separated by HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm) and then entered into mass spectrometry detection.The mobile phases consisted of 0.1% formic acid in water:acetonitrile (95:5, v/v) (solvent A) and 0.1% formic acid in acetonitrile:isopropanol:water (47.5:47.5:5, v/v)(solvent B). The solvent gradient changed according to the following conditions: from 0 to 0.1 min, 0% B to 5% B; from 0.1 to 2 min, 5% B to 25% B; from 2 to 9 min, 25% B to 100% B; from 9 to 13 min, 100% B to 100% B; from 13 to 13.1 min, 100% B to 0% B; from 13.1 to 16 min, 0% B to 0% B for equilibrating the systems. The sample injection volume was 2 μL and the flow rate was set to 0.4 mL/min. The column temperature was maintained at 40 °C. During the period of analysis, all these samples were stored at 4 °C.

2.2. MS conditions:

The mass spectrometric data was collected using a Thermo UHPLC-Q Exactive Mass Spectrometer equipped with an electrospray ionization (ESI) source operating in either positive or negative ion mode. The optimal conditions were set as followed: heater temperature, 400 °C; Capillary temperature, 320 °C; sheath gas flow rate, 40 arb; Aux gas flow rate, 10 arb; ion-spray voltage floating (ISVF), −2800V in negative mode and 3500V in positive mode, respectively; Normalized collision energy, 20-40-60V rolling for MS/MS. Full MS resolution was 70,000, and MS/MS resolution was 17,500. Data acquisition was performed with the Data Dependent Acquisition (DDA) mode. The detection was carried out over a mass range of 70–1050 m/z.