Histological Observation of Tissues

Honghao Zhao,Jasmine Chong,Rong Tang,Li Li,Jianguo Xia,Dapeng Li

Abstract

The different kinds of stained histological sections are widely used to detect and observe physiological and pathological changes in various tissues.

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Before start

Bouin's fixative was formulated by saturated solution of picric acid (75 ml), 40% aqueous formaldehyde (25 ml), and glacial acetic acid (5 ml), kept at room temperature.

Protocol

Blood Samples Collection

Step 1.
Both white muscle and gonad tissues were taken from 250 tails per experimental group (AF/GF).

Storage of muscle samples used for H&E staining

Step 2.
White muscle samples were taken from each fish in GF and AF experimental groups, and temporarily stored in tissue fixative..

Storage of gonad samples used for H&E staining

Step 3.
500 gonad samples were also collected from every experimental fish and kept in Bouin's fixative in 2 ml plastic tubes at 4 ℃.

Storage of samples used for Oil red O staining

Step 4.
The back and abdominal muscle samples were immediately harvested and frozen in liquid nitrogen, then were transferred and preserved at -80 ℃ until Oil red O staining.

Preparation for Samples Staining

Step 5.
Serial transverse 10 μm-thick sections of muscles and gonads were stained routinely with hematoxylin & eosin (H&E). To determine the presence of fat in the muscles, frozen muscle tissues were stained with Oil red O solution, which would color any fat contained in the muscle.

Sex Determination

Step 6.
The sex of each grass carp was determined by the contour of the gonad and further results of the gonad tissue slice (Jensen and Shelton, 1983; Ke, 1975).

Sample Grouping

Step 7.
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).

The observation of Muscular Characteristics

Step 8.
A total of 200 - 400 fibers of per experimental group fish were studied using a Leica MZ 6 microscope for their cross sectional area. A size limit for identifying fibers was set at fiber diameters ≥ 10 μm as the optical resolution below this limit did not allow for sufficient identification and accuracy in the analyses.

Warnings

Picric acid does not cause any direct allergies, but it would be converted into an allergenic chemical after contacting with tissue.