Specific activity of the main digestive enzymes in grouper *Epinephelus coioides* larvae

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Artificial larval feed

Newly-hatched larvae

copepods

artemia

rotifers

Artificial larval feed

60 day-old larvae

macronutrients
Objectives

- To determine the protein content of crude enzyme extract from grouper larvae at different stages of development for the computation of specific enzyme activity.

- To determine the specific activity of alkaline and acid type proteases, α-amylase and lipase during larval development of grouper.
Materials and Methods
Quantitation of digestive enzymes in the crude extract

Larvae
Day 0, 2, 4, 8, 12, 16, 20, 25, 30, 35, 40, 45, 50, 55, and 60.

Freeze-dried larvae

Representative sample (70 mg)

2.5 ml 50 mM Tris-HCl buffer pH 7.5, 4°C

Homogenate

Filter

Sephadex Gel 25M column 2.5 ml sample volume 3.5 ml eluent

Supernatant 2.5 ml

Centrifuge

13,500 rpm at 4°C for 30 min

Crude enzyme extract in 3.5 ml 50 mM Tris-HCl buffer (store in -80°C)
Crude Enzyme Extract in 3.5 ml 50 mM Tris-HCl buffer

- Total protein
  - Micro Lowry Method
  - Bovine serum albumin
  - Wavelength = 630 nm (Microplate)

- Enzyme Activity
  - Alkaline and Acid type (Pepsin)
  - Proteases
  - α-Amylase
  - Lipase
Total Protein Assay by Micro Lowry Method (Microplate Reader)
Spectrophotometric Assay

Alkaline type protease
Acid type protease
α-Amylase

Spectrophotometric Assay
Potentiometric Assay - Lipase
Results
Total protein concentration in the crude enzyme extract from grouper larvae.
Protease Activity

- **Alkaline Type Protease**
- **Acid Type Protease X 1000**

**mU/larva**

**Age of larvae**

- metamorphosis
- stomach formation
- mouth and anus
Alpha-amylase activity of the crude enzyme extract from grouper larvae

- **U/larva**
- **Age of larvae (day)**
- **metamorphosis**
- **mouth and anus**
Lipase activity of the crude enzyme extract from grouper larvae

- Age of larvae (day)
- U/larva X 10^5

Key:
- Mouth and anus
- Pyloric caeca prominent
- Metamorphosis
Conclusions
A gradual increase in the total protein concentration was observed from day 12 to day 50. The highest concentration was obtained at day 60.

All enzyme activities were detected early and during the whole larval stage.

Alkaline type protease activity decreased from day 50 to day 60 while acid type protease activity increased.
- Acid type protease activity could be related to the stomach formation at day 12 in grouper larvae.

- A progressive increase in alpha-amylase activity was observed from day 0 to day 60.

- Maximum lipase activity was related to the development of pyloric caeca and intestine and to metamorphosis.
Thank You !!