EFFECTS OF T3 & CORTISOL ON DIGESTIVE ENZYMES GENE EXPRESSION IN DEVELOPING SEABASS (LATES CALCARIFER) LARVAE

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At first-feeding, larval gut is relatively simple and lacks a functional stomach

- No acid secretion
- No acid protease secretion (pepsin)
- Unable to digest protein efficiently
Days post hatching

0 5 10 15 20 25 30 35

Relative abundance of mRNA

0 1 2 3 4 5 6 7 8

Pepsinogen

First feeding
(Rotifers)

Artemia feeding

Days post hatching
Exogenous thyroid hormone and cortisol treatment has been shown to accelerate

- Yolk absorption
- Morphogenesis
- Growth and survival
- Metamorphosis
Thyroid hormone and cortisol treatment has also been shown to accelerate

- Differentiation of stomach
- Formation of gastric glands
- Appearance of pepsinogen
Objective of the present study

To evaluate the effects of T3 and cortisol on proteolytic digestive enzymes gene expression at two critical stages of larval development

- First feeding
- Metamorphosis
Materials and methods

Experiment I – First feeding

Newly hatched seabass larvae were distributed into 15, 20 l conical glass tanks.

The larvae were reared in seawater alone or in seawater containing T3 (5 nM and 10 nM) or cortisol (100 nM and 200 nM) with each treatment in triplicate.

The treatments were administered on day 1 post hatching (1 dph) and the media were not replaced until 3 dph.

The larvae were fed with rotifers at a density of 15 ml⁻¹ from 3 dph onwards.

The larval samples were collected on 1, 3, and 5 dph for RNA extraction.
Total RNA from the larval samples was extracted using TRI Reagent and 1 mg was reverse transcribed in a total volume of 10 ml.

PCR amplification was performed on 0.5 ml cDNA using trypsinogen, aminopeptidase N, and pepsinogen specific intron-flanking oligonucleotide primers.

PCR products were run on ethidium bromide stained agarose gel and the band volume measured using a Gel-Doc2000 system and Quality one software (Biorad).
Aminopeptidase N

Days post hatching (dph)

- 1 dph
- 3 dph
- 5 dph

Relative abundance of mRNA

- Control
- F-100
- F-200
- T3-5
- T3-10

Days post hatching (dph)
Trypsinogen

Days post hatching (dph)

Relative abundance of mRNA

Control
F-100
F-200
T3-5
T3-10

b  b
a

b  b
a  a

b  b
a  a

1 dph
3 dph
5 dph

Days post hatching (dph)
Days post-hatching (dph)

Relative abundance of mRNA

Control
F-100
F-200
T3-5
T3-10

1 dph
3 dph
5 dph

Pepsinogen
Experiment II – Metamorphosis

Fifteen-day-old seabass larvae were stocked in 20 l tanks at a density of 15 ml\(^{-1}\) and reared in the same treatments as in Experiment I.

Each treatment was triplicated and the media were changed and replaced with fresh media daily.

The larvae were fed with *Artemia* nauplii ad libitum daily.

The larva samples for RNA were collected on 15, 17, 21, 23, 26, and 28 dph.
Days post hatching

Relative abundance of mRNA

Control

Start of hormone treatment

100 nM F

200 nM F
Pepsinogen

Days post hatching

15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Relative abundance of mRNA

0 1 2

Start of hormone treatment

Control

5 nM T3

10 nM T3
Aminopeptidase N

Days post hatching (dph)

Relative abundance of mRNA

Start of hormone treatment

Control
F-100 nM
F-200 nM

Days post hatching (dph)
Aminopeptidase N

Days post hatching (dph)

Relative abundance of mRNA

Control
T3-5 nM
T3-10 nM

Start of hormone treatment

Relative abundance of mRNA vs. Days post hatching (dph) for Aminopeptidase N with different hormone treatments (Control, T3-5 nM, T3-10 nM).
Trypsinogen

Days post hatching (dph) 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Relative abundance of mRNA

Control  F-100 nM  F-200 nM

Start of hormone treatment

Relative abundance of mRNA

Days post hatching (dph)
Trypsinogen

Days post hatching (dph)

Start of hormone treatment

Relative abundance of mRNA

Control  T3-5 nM  T3-10 nM
Days post hatching

Relative abundance of mRNA

Pepsinogen

First feeding (Rotifers)

Artemia feeding

Days post hatching
Pepsinogen

Relative abundance of mRNA

Days post hatching

Start of hormone treatment

Control

5 nM T3

10 nM T3

Relative abundance of mRNA vs. Days post hatching
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