DIETARY VITAMINS C AND D AFFECTS SEA BASS LARVAL MORPHOGENESIS

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An overdose produce growth delay and induce cephalic malformations and one vertebra less (Gisbert et al., 2005; Villeneuve et al., 2005, 2006; Mazurais et al., 2009)

Larvae need more VM than juveniles. A small amount of VM induce malformations (Mazurais et al., 2008)
### Vitamin D (VD)
- ✔ Calcium and phosphate homeostasis
- ✔ Protection of **skeletal integrity**

### Vitamin C (VC)
- ✔ Co-substrate for hydroxylase and oxygenase enzymes involved in the biosynthesis of **pro-collagen**
- ✔ Antioxidant
- ✔ Pro-oxidant

### Functions

### State of the Art

#### Vitamin D (VD)
- Hilton & Ferguson, 1982
- Graff et al., 2002
- Haga et al., 2004
- No studies at larval stage

#### Vitamin C (VC)
- Halver, 1957, 1989
- Andrews & Murai, 1975
- Lim & Lovell, 1978
- Chávez de Martínez et al., 1990
- Soliman et al., 1986
- Studies at larval stage
- Dabrowski, 1990
- Gapasin et al., 1998
**Vitamin D (1,25-dihydroxy VD, VD₃)**

- **Poor mineralization**
  - 11.2 IU VD₃/g diet
  - Pugheadness
  - Deformities of the caudal fin
  - Vertebral deformities (Kyphosis, scoliosis)
  - Branchiostegal rays deformities

- **Mineralization delay**
  - 42-120 IU VD₃/g diet
  - Vertebral deformities (kyphosis, scoliosis)
  - Branchiostegal rays deformities

**Vitamin C (L-ascorbic acid phosphate)**

- **Poor mineralization**
  - 0-30 mg VC/kg diet
  - Cartilage damage
  - Pugheadness
  - Haemal arch not formed
  - One vertebrae lost
  - Epurals, uroneural, specialized neural arch

- **Supernumerary vertebrae**
  - 8 times the requirements of juvenile fish (NRC, 1993)

- **Mineralization delay**
  - 400 mg VC/kg diet
  - Epurals, uroneural, specialized neural arch
  - Deformities of dentary
  - Deformities of the dorsal and anal fin
  - Supernumerary vertebrae
  - Vertebral deformities (kyphosis, scoliosis)
  - Branchiostegal rays deformities

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**Graphs and Images:**

- Bone density analysis
- Frequency and occurrence of deformities
- Comparison of vertebral formations between different vitamin levels.
MOLECULAR PATHWAYS INVOLVED IN MINERALIZATION

VD₃ → Vitamin D Receptor (VDR) → VC

VDR → INTESTIN
Calcium absorption

TRPV-6
SVCT-1

INTESTIN
Malformations ?

BONE
Regulation of osteoblasts differentiation, proliferation and mineralization

TRPV-6
SVCT-1

BMP-4/PPAR γ
IGF-1, RARγ
Osteocalcin

Regulation of bone mineralization
CONTROL OF INTESTINAL ABSORPTION

Vitamin D (VD)

TRPV-6

Vitamin C (VC)

Ca$^{2+}$ absorption

Expression of TRPV-6

<table>
<thead>
<tr>
<th>Day 11</th>
<th>Day 22</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>VD-0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD-19.2</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>VD-38.4</td>
<td>b</td>
<td>bc</td>
</tr>
<tr>
<td>VD-140</td>
<td></td>
<td></td>
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</table>

Expression of TRPV-6

<table>
<thead>
<tr>
<th>Day 11</th>
<th>Day 25</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC-0</td>
<td></td>
<td></td>
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<tr>
<td>VC-5</td>
<td></td>
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<tr>
<td>VC-15</td>
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<tr>
<td>VC-30</td>
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<tr>
<td>VC-50</td>
<td></td>
<td></td>
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<tr>
<td>VC-400</td>
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</tbody>
</table>

INDIRECT EFFECT ON BONE MINERALIZATION AND DEVELOPMENT
CONTROL OF INTESTINAL ABSORPTION

**SVCT-1**

**Vitamin C (VC)**

VC absorption

Day 45

- **Total pixel number / larvae**
  - Strong blue
  - Light Blue

- **Expression of SVCT-1**

Day 11, Day 15, Day 25, Day 45

**Legend**
- VC-0
- VC-5
- VC-15
- VC-30
- VC-50
- VC-400

**Significance**
- a
- b
- ab
CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD)  IGF-1  Vitamin C (VC)

Osteoblasts proliferation and stimulation of its function

Expression of IGF-1

Day 11  Day 22  Day 45

Expression of IGF-1

Day 11  Day 25  Day 45

Legend:
- VD-0
- VD-19.2
- VD-38.4
- VD-140

Legend:
- VC-0
- VC-5
- VC-15
- VC-30
- VC-50
- VC-400

Bars with different letters indicate significant differences.
CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD)  Osteocalcin  Vitamin C (VC)

Osteoblasts mineralization

Expression of osteocalcin

Day 11  Day 22  Day 45

Expression of osteocalcin

Day 11  Day 25  Day 45
CONTROL OF BONY TISSUE DEVELOPMENT

**Vitamin D (VD)**

- Stimulation of TRPV-6 and osteocalcin expression

**VDR**

**Vitamin C (VC)**

- Expression of VDRβ at Day 11, Day 22, Day 45
- Expression of VDRβ at Day 11, Day 25, Day 45

- VC-0, VC-15, VC-30, VC-50, VC-400
CONTROL OF BONY TISSUE DEVELOPMENT

Vitamin D (VD)  
BMP-4  
Vitamin C (VC)  

Osteoblasts differentiation

Expression of BMP-4

Day 11  Day 22  Day 45

Day 11  Day 15  Day 25  Day 45
CONTROL OF CARTILAGE TISSUE DEVELOPMENT

Vitamin D (VD)

RARγ

Cartilage formation

Vitamin C (VC)

PPARγ

Adipocyte differentiation

Expression of PPARγ

Expression of RARγ

Day 11  Day 22  Day 45

Day 11  Day 25  Day 45

VC-0  VC-5  VC-15  VC-30  VC-50  VC-400
Before day 22 …

After day 22

Skeletal elements that developed during this period were sensitive to low VD3 levels and also those that developed later on were sensitive to low VD3 levels. Skeletal elements that developed during this period were less sensitive to high VD3 levels and those that developed later on were more sensitive to high VD3 levels.

Before day 22, pugheadness and caudal fin deformities (5 mm TL, 11 dph) and branchiostegal rays (8 mm TL, 25 dph) were observed. After day 22, vertebral column and branchiostegal rays deformities were observed.

VITAMIN D

OSTEOBLASTS

OST

ENTEROCYTES

TRPV-6

Ca^{2+}

Multipotent cells

BMP-4

VDR

IGF-1

RARγ

ACCELERATION

OSSIFICATION

DELAY

MALFORMATIONS

49%

59%

20%

~14 IU g^{-1}

~16 IU g^{-1}

11.2 IU g^{-1}

27.6 IU g^{-1}

42-120 IU g^{-1}

11.2 IU g^{-1}
Skeletal elements that developed early were sensitive to low VC levels and also those that developed later on.

Epurals & specialized neural arch

Skeletal elements that developed early were less sensitive to high VC levels.

Epurals, specialized neural arch, dentary, dorsal & anal fins, supernumerary vertebrae

At day 45...

Pugheadness & haemal arches

At day 22...

ADIPOCYTES

OSTEOBLASTS

Multipotent cells

BMP-4

IGF-1

RARγ

OST

OPTIMAL OSSIFICATION

VC

ENTEROCYTES

TRPV-6

SVCT-1

VITAMIN C

VDR

Ca^{+2}

Malformations

OAD

MALFORMATIONS

> 30 mg Kg^{-1}

50 mg kg^{-1}

400 mg kg^{-1}
CONCLUSIONS

**Vitamin D (VD)**

- Maturation of the intestinal functions delayed
  - => effect on larval development
- Disruption of intestinal Ca\(^{2+}\) absorption (TRPV-6)
- Disruption of the expression of genes involved in skeletogenesis (BMP-4, IGF-1, RARγ) and bone mineralization (VDR, osteocalcin)

Skeletal elements that developed in early and later stages were equally sensitive to low VD\(_3\) levels

Skeletal elements that developed in early stages were more resistant to high VD\(_3\) than those that developed later on

Optimal VD\(_3\) is very restricted

27.6 IU VD\(_3\)/g diet (11.5 x dose of juveniles; NRC, 1993)

**Vitamin C (VC)**

- No evident effect on larval development
- Disruption of intestinal Ca\(^{2+}\) & VC absorption (TRPV-6, SVCT-1)
- Disruption of the expression of genes involved in skeletogenesis (IGF-1, RARγ) and bone mineralization (VDR, osteocalcin) in favor of adipocytic tissue formation (PPARγ)

Skeletal elements that developed in early and later stages were equally sensitive to low VC levels

Skeletal elements that developed in early stages were more resistant to high VC than those that developed later on

Optimal VC is restricted

50 mg VC/kg diet (0.5 x dose of juveniles; NRC, 1993)

MALFORMATIONS ~30%
Perspectives

**Vitamin D (1,25-dihydroxy VD, VD₃)**
- 11.2 IU VD₃/g diet
  - Pugheadness ↓ osteocalcin
  - Deformities of the caudal fin ↓ RARg
  - Vertebral deformities ↓ osteocalcin
  - Branchiostegal rays deformities ↓ osteocalcin

- 42-120 IU VD₃/g diet
  - Vertebral deformities ↑↑ osteocalcin
  - Branchiostegal rays deformities ↑↑ osteocalcin

**Vitamin C (L-ascorbic acid phosphate)**
- 0-30 mg VC/kg diet
  - Cartilage damage
  - Pugheadness
  - Cartilaginous vertebrae
  - Haemal arch not formed
  - One vertebrae lost
  - Epurals, specialized neural arch

- 400 mg VC/kg diet
  - Epurals, specialized neural arch
  - Deformities of dentary
  - Deformities of the dorsal and anal fin
  - ↓ osteocalcin
  - ↑ PPARg

≠ DISRUPTIONS
DIFFERENT FUNCTION
DIFFERENT MODE OF ACTION
DIFFERENT ACTION ON THE RATE OF BONE MINERALIZATION
DIFFERENT TYPE OF BONE MINERALIZATION
CHONDRALE INTRAMEMBRANOUS

STUDY OF THE SPECIFIC MOLECULAR MARKERS OF EACH TYPE OF OSSIFICATION
ADAPTATION OF THE AMOUNT OF VITAMINS TO THE DEVELOPMENTAL STAGE
ACKNOWLEDGEMENTS

Lab of Adaptation Reproduction & Nutrition of marine fish

Centre de Brest
France

Unit of Fish Biology & Quality in Aquaculture

Biology Department
University of Patras
Greece

Thank You for Your Attention!
Before day 22 ...

Skeletal elements that developed during this period were very sensitive to low VD3 levels. And also those that developed later on were sensitive to low VD3 levels. Skeletal elements that developed during this period were less sensitive to high VD3 levels. Skeletal elements that developed later on were more sensitive to high VD3 levels.

After day 22

59%

pugheadness & caudal fin deformities (5 mm TL, 11 dph) branchiostegal rays (8 mm TL, 25 dph)

After day 22 Vertebral column & branchiostegal rays deformities

BMP-4

OSTEOBLASTS

OSTEOCYTES

VITAMIN D

ENTEROCYTES

TRPV-6

Ca++

~14 IU g⁻¹

11.2 IU g⁻¹

~16 IU g⁻¹

27.6 IU g⁻¹

42-120 IU g⁻¹

VDR

SCL

OST

OSTEOCYTES

OPTIMAL OSSIFICATION

ACCELERATION OSSIFICATION

MALFORMATION

ACCELERATION

DELAY

IGF-1

RARγ

OSTEOBLASTS

VITAMIN D

MUTIPOTENT CELLS

BMP-4

VDR

SCL

OST

OSTEOCYTES

OPTIMAL OSSIFICATION

ACCELERATION OSSIFICATION

MALFORMATIONS

59%

49%

20%