The long term effects of DHA supplementation in live feed for first feeding pike perch larvae on development of neural tissue and behavioral responses

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ghent university, belgium, 2-5 september 2013
Importance of DHA for first feeding pike perch (Sander Lucioperca) larvae – Influence on behavioural responses

Larvi – Ghent
2-6 Sept. 2013

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Challenges in production of pike perch fry

- Cannibalism
- Quality of fry, and survival rate
- High stress sensitivity

Causes
- Lack of breeding programme
- Environmental and nutritional issues

- Optimal nutritional requirements not known (lipids, fatty acids, amino acids, vitamins etc.)
Influence of DHA on stress and neural development in pike perch larvae

The developing brain may be vulnerable to DHA deficiency. Neurologic processing, Synapse formation, Neurite outgrowth, Myelination, Neurotransmitter secretion Neurological function.

Low brain DHA levels during development can be restored to normal by subsequent n-3 fatty acid supplementation, but will physiological functions be affected?
Experimental set up:

OO: refined olive oil (0.79 kg 18:1 kg oil-1)
DHA oil: INCROMEGA, CRODA, (0.51 kg DHA Kg oil-1)
Phospholipid oil (PL): fish oil rich in phospholipids (0.44 kg phospholipid kg oil-1)
**Experimental set up:**

- Behaviour of larvae (28-30 dph) in presence and absence of a simulated predator
- Fast-escape response (avoidance behaviour) of larvae (28 dph) and juveniles (+120 dph) to a mechanosensory stimulus
- Long terms effects on learning ability and stress responsiveness by maze tests and cortisol analyses

**Physiological implications of early larvae FA nutrition:**
Short & long term consequences on wellfare, cognitive behaviour; stress
Experimental set up:

Behaviour of larvae in absence and presence of a simulated predator
Fast/escape (avoidance) test for larvae & juveniles
Learning ability – maze test
### Larvae FA composition 27 dph:

<table>
<thead>
<tr>
<th>Dietary inclusion:</th>
<th>A: 890 g OO 50 g DHA oil</th>
<th>B: 840 g OO 500 g DHA oil</th>
<th>C: 390 g OO 500 g DHA oil</th>
<th>D: 390 g OO 500 g PL.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae FA comp (% total) (27 dph):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>16.9±0.4</td>
<td>17.0±0.3</td>
<td>15.9±1.0</td>
<td>15.9±0.4</td>
</tr>
<tr>
<td>18:0</td>
<td>8.3±0.3</td>
<td>8.6±0.3</td>
<td>8.9±0.3</td>
<td>7.8±0.3</td>
</tr>
<tr>
<td>SFA</td>
<td>26.6</td>
<td>27.2</td>
<td>26.6</td>
<td>25.5</td>
</tr>
<tr>
<td>16:1 n-7</td>
<td>1.6±0.1</td>
<td>1.5±0.1</td>
<td>1.3±0.1</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>34.1±1.4</td>
<td>31.9±1.5</td>
<td>24.0±1.1</td>
<td>28.1±1.0</td>
</tr>
<tr>
<td>MUFA</td>
<td>38.6</td>
<td>36.4</td>
<td>29.6</td>
<td>34.1</td>
</tr>
<tr>
<td>18:3 n-6</td>
<td>0.4±0.0</td>
<td>0.4±0.0</td>
<td>0.3±0.0</td>
<td>0.3±0.0</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>1.5±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>7.9</td>
<td>7.5</td>
<td>6.7</td>
<td>6.4</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>18.6±0.3</td>
<td>18.9±0.5</td>
<td>19.2±0.7</td>
<td>17.2±0.3</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>2.6±0.2</td>
<td>3.3±0.3</td>
<td>5.4±0.3</td>
<td>6.4±0.4</td>
</tr>
<tr>
<td>22:6 n-3</td>
<td>3.5±0.6</td>
<td>4.6±0.1</td>
<td>10.3±1.3</td>
<td>8.5±1.4</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>36.2</td>
<td>36.2</td>
<td>36.2</td>
<td>33.3</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>1.4</td>
<td>1.4</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>n-3 /n-6</td>
<td>3.3</td>
<td>3.7</td>
<td>5.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>
Larvae growth until 27 Dph

<table>
<thead>
<tr>
<th>Dietary Code</th>
<th>Larvae Weight (mg dw⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: OO</td>
<td></td>
</tr>
<tr>
<td>B: +5% DHA</td>
<td></td>
</tr>
<tr>
<td>C: +50% DHA</td>
<td></td>
</tr>
<tr>
<td>D: +50% PL</td>
<td></td>
</tr>
</tbody>
</table>

The graph shows the comparison of larval weight at different dietary codes.
Larvae behaviour in absence and presence of a simulated predator

**Time at edge**
- Rest
- Predator stressor

**Time spent in lower half**
- Stress vs no stress
  - Stress
  - No stress
  - Significant interaction between Diet and Stress. (P = 0.029)

**Routine swimming speed**
- BL sec\(^{-1}\)
  - A
  - B
  - C
  - D

**Time in rheotaxis**
- Stress vs no stress
  - Stress
  - No stress
  - Significant interaction between Diet and Stress. (P = 0.029)
Larvae (avoidance) escape response

Annova, P=0.1192

Annova, P=0.399

Annova, P=0.656

Annova, P=0.001
Juvenile (avoidance) escape response

**Escape latency (ms)**

- A: 25
- B: 20
- C: 15
- D: 20

**Distance covered (bl in 80 ms)**

- A: 0.3
- B: 0.3
- C: 0.3
- D: 0.3

**Peak velocity (bl s⁻¹)**

- A: 10
- B: 10
- C: 10
- D: 10

**Peak acceleration m s⁻²**

- A: 1000
- B: 1000
- C: 1000
- D: 1000

Anova, P=0.093

Anova, P=0.106

Anova, P=0.107

Anova, P=0.04
Learning ability and stress responsiveness in maze tests

Two way repeated ANOVA
Learning session:P<0.001
Fatty acid:P<0.05
Fatty acid x Trail:0.56

Two way repeated ANOVA
Learning session:P<0.001
Fatty acid:P<0.20
Fatty acid x Trail:0.55
Cortisol levels in juveniles after test in maze

Significance: (P=0.25).
Conclusions

- Dietary DHA influences on larvae tissue composition and affects larvae stress responsiveness and behavior during rest or to an acute stressor.
- No apparent influence on growth or visual acuity.
- Peak acceleration for larvae and juveniles were significantly decreased by low dietary DHA concentrations.
- n should be increased in such studies as individual variation is high.
- Juvenile learning ability in a maze was similar between groups, but “initial freezing time” higher in groups fed low levels of DHA as larvae.
- future studies

- How is stress and behavior linked to neurophysiology? A cross-disciplinary approach including brain signaling systems as well as behavior and metabolic physiology.

- From an applied point of view, gene expression or protein levels of BDNF (i.e., neutrophic factor associated with learning) may be utilized as biological markers for stress coping ability and mental robustness in fish.
Aknowledgements

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