The importance of arachidonic acid, as a modulator of stress resistance through the HPI axis, in gilthead seabream larvae (*Sparus aurata*)

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Essential Fatty Acids

- ArA (20:4n-6)
  - not essential in membranes
  - precursor for prostaglandins
  - cellular regulation

- DHA (22:6n-3) and EPA (20:5n-3)
  - essential function in cellular membranes
  - required for good growth
  - retinal function and improved vision
**Selected fatty acids of the enrichment phospholipid and biomeal preparations from Martek Biosciences and the commercial product AlgaMac 2000**

<table>
<thead>
<tr>
<th>Components</th>
<th>DHA-PL +</th>
<th>ARA-PL +</th>
<th>AlgaMac 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algal meal</td>
<td>Algal meal</td>
<td></td>
</tr>
<tr>
<td><strong>Selected Fatty acids (% of TFA)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoic acid (DHA)</td>
<td>35.9</td>
<td>2.4</td>
<td>24</td>
</tr>
<tr>
<td>Arachidonic Acid (ArA)</td>
<td>0.0</td>
<td>52.5</td>
<td>0</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA)</td>
<td>0</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Docosapentaenoic acid (DPA)</td>
<td>0</td>
<td>0</td>
<td>12.9</td>
</tr>
</tbody>
</table>
The values (mg g$^{-1}$ ± SEM) of selected fatty acids and fatty acid ratios of the rotifers fed the enrichment treatments

<table>
<thead>
<tr>
<th></th>
<th>AlgaMac</th>
<th>0% ArA</th>
<th>12.5% ArA</th>
<th>25% ArA</th>
<th>50% ArA</th>
</tr>
</thead>
<tbody>
<tr>
<td>22:6n-3 (DHA)</td>
<td>7.7 ± 2.0</td>
<td>6.5 ± 1.4</td>
<td>5.7 ± 0.1</td>
<td>6.2 ± 2.2</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>20:5n-3 (EPA)</td>
<td>3.4 ± 0.8</td>
<td>2.3 ± 0.4</td>
<td>3.0 ± 1.0</td>
<td>2.2 ± 0.8</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td>22:5n-6 (DPA)</td>
<td>2.2 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>20:4n-6 (ArA)</td>
<td>1.5 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>3.0 ± 1.0</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>2.3 ± 0.6</td>
<td>3.0 ± 0.6</td>
<td>2.4 ± 0.7</td>
<td>2.8 ± 0.1</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>DHA/ArA</td>
<td>5.2 ± 1.4</td>
<td>9.1 ± 1.6</td>
<td>3.4 ± 0.4</td>
<td>2.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>EPA/ArA</td>
<td>2.2 ± 0.0</td>
<td>3.4 ± 0.9</td>
<td>1.7 ± 0.3</td>
<td>0.7 ± 0.0</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>
Transfer from tanks to aquaria characterized handling stress

400 L V-tanks
40,000 eggs/tank
rotifer feeding

27 L aquaria
200-300 larvae/aquarium
Artemia feeding
Effect of ArA during rotifer feeding

400 L tanks

rotifers

Handling stress

Artemia

30 L aquaria

Alga ArA

+ ArA

++ ArA

+++ ArA

++++ ArA

Alga mac

Alga mac

Alga mac

Alga mac

Alga mac
Accumulated larval mortality in ArA treatments following transfer to aquaria

Accumulated larval mortality in Accumulated larval mortality in ArA treatments following transfer to aquaria

Days after transfer

Accumulated larval mortality treatment$^{-1}$

- Alga (1.5 mg/g DW)
- 5.3 mg/g DW
- 0.8 mg/g DW
- 3.0 mg/g DW
- 1.7 mg/g DW
Effect of ArA in rotifers and Artemia

400 L tanks

Rotifers

Alga 1.3
ArA 0.8
ArA 1.7

Handling stress

30 L aquaria

Artemia
Accumulated mortality, as a result of Artemia treatment, in larvae fed the (a) 1.7mg ArA and (b) 0.8mg ArA rotifers.

Graph showing daily cumulative mortality treatment for larvae fed different concentrations of Artemia.
Accumulated mortality in seabream larvae as a result of ArA levels in rotifers fed prior to handling stress.

Accumulated mortality over days after handling stress for different ArA levels:
- 0.8 mg ArA/g DW
- 1.3 mg ArA/g DW
- 2.7 mg ArA/g DW
Conclusions from these studies

- Dietary ArA improved resistance to handling stress

- Dietary ArA fed prior to stress more effective than when fed after stress.
Does the effect of ArA vary with larval development and/or stress type?

From M. Jobling, 1995
Experimental setup

**400 L tanks**
- **AGE GROUPS**
  - **SALINITY CHANGE**
    - Alga 1.3
    - ArA 0.8
    - ArA 1.7
  - **NO SALINITY CHANGE**
    - Alga 1.9
    - ArA 0.9
    - ArA 4.6

- **30 L aquaria**

**Handling stress**
- 20 day old larvae: pre-metamorphosis
- 30 day old larvae: metamorphosis
The effect of dietary ArAA on survival in larvae exposed to chronic salinity change or no salinity change.

**pre-metamorphosis**

*20-30 days*

**metamorphosis**

*30-40 days*
Why does dietary ArA correlate with improved larval survival in the no salinity change group but shows a negative correlation in the salinity change group?
The suggested role of Arachidonic acid (ArA) in the HPI axis during the stress response

- Prostaglandins (PG)
  - EPA
  - ArA
  - PGE2-ArA

- Hypothalamus
  - Hydromineral balance
  - gill blood flow
  - oxygen uptake and transfer
  - immune functions
  - mobilizes energy reserves

- Pituitary
  - 1
  - 2

- Head kidney
  - Interrenal cells
  - Chromaffin cells

- Cortisol
  - Hydromineral balance
  - immune functions
  - mobilizes energy reserves

- Catecholamines
  - Hydromineral balance
  - gill blood flow
  - oxygen uptake and transfer
Cortisol basal levels 10 days after transfer of 30 day old larvae to aquaria

![Graph showing cortisol levels with salinity change and no salinity change for different ArA (mg/g DW) concentrations (0.9, 3.8, 8.4). The graph indicates significant changes with asterisks (*) and letters a, b, and c for statistical comparisons.]
These studies showed ArA effect on basal cortisol levels after acclimation to salinity and/or handling stress.

In a following study, larvae were fed for 10 days on ArA poor or ArA rich naupliii. The salinity was then reduced from 40-25 ppt over 1 hour and the stress response was followed over time.
Cortisol response to salinity change
(40-25 ppt within 1h)

0.9mg ArA/g DW
6.9mg ArA/g DW

Cortisol (pg/g DW)

time (h)
Na\textsuperscript{+}/K\textsuperscript{+}-ATPase response to salinity change
(41-25 ppt within 1h)

Time (h)

µmol Pi/h/mg protein

- 0.9mg ArA/g DW
- 6.9mg ArA/g DW
Whole body sodium levels in response to salinity change (41-25 ppt within 1h)

- 0.9 mg ArA/g DW
- 6.9 mg ArA/g DW

![Graph showing sodium levels over time](image-url)
Whole body potassium levels
(41-25 ppt within 1h)

K (µmol/g DW)

Time (h)

0 0.3 0.6 1 2 24

0.9 mg ArA/g DW
6.9 mg/g DW
Current conclusions

- ArA improves survival following an acute stress event if fed prior to the event.
- ArA may be most effective during metamorphosis.
- ArA, as a precursor to PGE$_2$, is involved in regulation of basal cortisol levels and cortisol levels during the stress response.
- Improves stress resistance by regulating osmoregulation.
- High levels of ArA can be detrimental during chronic stress.
Special Thanks

Benny Ron
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Kutsal Gamsiz
Marc Lacuisse
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Body osmolarity of Sparus aurata larvae after abrupt transfer from 40 to 25 or 15 ppt