Dietary neutral lipid level and source affect food intake, nutrient absorption, gut structure, enzymatic activity and growth in marine fish larvae

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**Background**

*Fish larvae*

Extremely high growth rates \(\rightarrow\) high demands for energy and structural components

*Lipids*

- Important source of metabolic energy
- Components of biological membranes
- Precursors of essential metabolites

The requirements for essential fatty acids (EFA) have been extensively studied in marine fish larvae.

Few studies have examined the effect of *total lipid levels* in larval diets.
Meet larval requirements using poor sources of EFA (predominantly neutral lipids):

- excessive lipid content
- imbalanced lipid class composition

High dietary neutral lipid levels ➔ several effects can be speculated:

- **Digestion** (decrease in efficiency or activity of digestive enzymes)
- **Absorption** (reduction in absorption efficiency)
- **Ingestion** (regulation of food intake according to dietary energy ?)
Dietary Triacylglycerol Source and Level Affects Performance and Lipase Expression in Larval Seabass (*Dicentrarchus labrax*)

## Experimental Diet Formulation and Proximate Composition

<table>
<thead>
<tr>
<th>Diet Ingredients (g/kg dry diet)</th>
<th>F7.5</th>
<th>T7.5</th>
<th>C7.5</th>
<th>F15</th>
<th>T15</th>
<th>C15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constant ingredients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>540</td>
<td>540</td>
<td>540</td>
<td>540</td>
<td>540</td>
<td>540</td>
</tr>
<tr>
<td>Hydrolyzed fish meal</td>
<td>130</td>
<td>130</td>
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<tr>
<td>Soybean lecithin</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Betaine</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Variable ingredients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish oil (cod liver oil)</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Triolein (purified)</td>
<td>0</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td>Starch</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Proximate Composition

<table>
<thead>
<tr>
<th></th>
<th>F7.5</th>
<th>T7.5</th>
<th>C7.5</th>
<th>F15</th>
<th>T15</th>
<th>C15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM, %)</td>
<td>97.8±0.0</td>
<td>97.6±0.0</td>
<td>97.6±0.1</td>
<td>97.1±0.0</td>
<td>97.4±0.2</td>
<td>97.5±0.0</td>
</tr>
<tr>
<td>Protein (N x 6.25) (% DM)</td>
<td>58.3±0.2</td>
<td>58.7±0.4</td>
<td>58.8±0.2</td>
<td>58.5±0.0</td>
<td>57.7±0.0</td>
<td>58.4±0.4</td>
</tr>
<tr>
<td>Lipid (% DM)</td>
<td>17.0±0.6</td>
<td>16.9±0.7</td>
<td>16.0±0.1</td>
<td>24.2±0.5</td>
<td>24.4±0.6</td>
<td>23.9±0.7</td>
</tr>
<tr>
<td>Ash (% DM)</td>
<td>12.0±0.1</td>
<td>12.0±0.0</td>
<td>12.2±0.1</td>
<td>12.0±0.0</td>
<td>13.5±0.1</td>
<td>12.0±0.0</td>
</tr>
<tr>
<td>Protein energy / Lipid energy(^{1/1})</td>
<td>1.5</td>
<td>1.5</td>
<td>1.6</td>
<td>1.1</td>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Lipase specific activity

- ✓ Source of dietary lipid
- ✗ Level of dietary lipid

Fish oil: age-dependent effect
Growth

Not related to lipase specific activity

Lipase activity not limiting for growth?
Conclusions

The results suggest the existence of a regulatory mechanism of neutral lipolytic activity and secretion according to the FA composition of the diet.

The understanding of the underlying mechanisms controlling such and adaptation is still scarce – several hypothesis:

- Specificity of lipase towards FA differing in chain length and degree of saturation;
- Endocrine factors; chemical nature of FA (particularly chain length) affects CCK secretion in humans and other mammals;
Conclusions

However... growth was not related to lipase activity ➔ the two factors might be independent (possibly a secretion in excess to dietary needs)

Physiological consequence of such a regulation?

For fish oil, the effect was age-dependent ➔ not only nutritional requirements but also digestive function may show important changes between early larvae and later larvae/juvenile stages
Growth, gut histology and absorption of fatty acids and amino acids in response to dietary neutral lipid level in Senegalese sole (*Solea senegalensis* Kaup 1858) larvae

*Morais et al. (2005) British Journal of Nutrition 93, 813-820*

*Morais et al. (2005) Aquaculture 246, 347-357*
Diets:
- Non-enriched *Artemia* (NEA);
- *Artemia* enriched on a soybean oil emulsion (EA).
Non-enriched *Artemia* (NEA): better larval growth, compared to *Artemia* enriched on a soybean oil emulsion (EA), a diet containing a higher neutral lipid level.
Artemia enriched on soybean oil (EA) induced a much higher accumulation of lipid droplets within the gut enterocytes.

Can this accumulation function as a physical barrier to efficient lipid absorption?
**Lipid absorption**

**Tube feeding** (32 DAH):

- $^{14}$C-Triolein (TRI), a triacylglycerol
- $^{14}$C- Phosphatidylcholine 1,2 di-oleoyl (PC), a phospholipid
- $^{14}$C- Oleic acid (OA), a free fatty acid

Significantly lower label absorption in larvae fed EA

*Lipid accumulation ➔ barrier for FA absorption?*
Lipid absorption

Tube feeding: Free FA (32 DAH)
- $^{14}$C- Oleic acid (OA), 18:1(n-9)
- $^{14}$C- Stearic acid (SA), 18:0
- $^{14}$C- Docosahexaenoic acid (DHA), 22:6(n-3)

Absorption of lipids differing in FA composition may not be equally affected by lipid droplet accumulation in the enterocytes
Protein and amino acid absorption

Larvae fed on Artemia radiolabelled with [U-14C] Protein hydrolisate

![Image of larvae]

**Evacuation into water**

<table>
<thead>
<tr>
<th>Time</th>
<th>NEA (U-14C-AA)</th>
<th>EA (U-14C-AA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>3 h</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>24 h</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Lipid inclusions within the enterocytes do not appear to affect total AA absorption efficiency.
Protein and amino acid absorption

Larvae fed on *Artemia* radiolabelled with [U-\(^{14}\)C] Protein hydrolisate

**Evacuation into water**

Faster AA absorption in larvae fed NEA ➞ higher food intake of NEA (related to gut transit time)
**Conclusions**

The long term feeding of a diet higher in neutral lipid may affect the capacity of larvae to efficiently absorb dietary FA. Lipid droplet accumulation in the enterocytes may function as a barrier for FA but not for AA absorption.

Lipid level in the diet may affect the rate of AA absorption and, consequently, evacuation rates (effects on food intake ??)

Accumulation of lipid droplets in the enterocytes may not be an obstacle for the efficient utilization of EFA (long chain PUFA - higher specificity of lipases, affinity of FABP and rate of esterification)
Food intake and absorption are affected by dietary lipid level and lipid source in seabream (*Sparus aurata* L.) larvae

Morais *et al.* (2005), submitted
1st Experiment: *Artemia* enriched in high and low doses of fish oil emulsion.

LF diet met minimum EFA requirements for growth.
Cold chase trial, at 33 DAH, with $^{14}$C-Artemia ($^{14}$C-OA liposomes)

HF ➤ significantly higher ingestion but lower nutrient absorption

*Inverse relationship between food intake and absorption efficiency (more rapid passage through the gut)*

*A higher neutral lipid level did not decrease larval food intake, as observed in older fish*
2nd Experiment: Co-feeding *Artemia* enriched in high and low doses of soybean oil emulsion + high or low lipid microdiet (MD)

Seabream, 34 DAH

Higher lipid diet (HS) ➔ significantly lower growth
Cold chase trial, at 32 DAH, with MD containing $^{14}$C-OA

The total neutral lipid level of the diet was inversely correlated with food intake.
Conclusions

In fish larvae food intake may not be regulated by dietary total lipid level and FA composition possibly has a more important role in controlling ingestion.

Possible explanations might be:

- FA digestibility, by influencing digestive and absorption efficiency, may affect the rate of gut clearance and subsequent refeeding;
- Palatability (mostly with microdiets);
- FA stimulation of release of gastrointestinal hormones (e.g. CCK);
General conclusions
**General conclusions**

Existing data seems to collectively indicate that enzymatic capacity is not a limiting factor for fish larvae to grow well on high lipid diets.

The main obstacle to high neutral lipid inclusion levels in diets for marine fish larvae may be at the absorption level.

Seabream larvae do not simply regulate their food intake according to total lipid level (i.e., energy content) and other factors must be involved.
Lipid level in diets for marine fish larvae may have an important impact in several factors influencing growth and development but it clearly cannot be dissociated of its FA composition, which appears to play a central role on the physiological impacts of dietary lipid, at the ingestion, digestion and absorption levels.
Acknowledgements

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