IS IT POSSIBLE TO INFLUENCE METABOLIC PROCESS OF EUROPEAN SEA BASS JUVENILES BY A NUTRITIONAL CONDITIONING DURING LARVAL STAGE?

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INTRODUCTION

Natural fish stocks

Nowadays

Carnivorous marine fish are mainly fed with fish meals and oils.

BUT fish aquaculture and natural fish stocks

Substitution of fish lipid sources with vegetable lipid sources

Disadvantages of vegetable products:

- Not adapted to the requirement in essential fatty acids (HUFA): rich in C18 fatty acids (i.e. HUFA precursors) BUT poor in HUFA (EPA, DHA)

- Low capacity for marine fish to convert C18 FA into HUFA, in particular because of the low expression of the Delta-6 desaturase (D6D)
High interest in producing fish, which could better incorporate vegetable products.

But how?

Could it be possible using the concept of metabolic programming?
Adaptive process at a cellular, molecular and biochemical levels occurring during young stages, and then maintained in further developmental stages and potentially transmissible to the offspring (Lucas, 1998)

Is it possible in sea bass to orient physiological process of juveniles by a larval conditioning to dietary vegetable products, characterized by a low HUFA content?
1. Experimental design

**Larval stage**
- Mouth opening
  - 2 diet
  - 2 T°C
  - 
    - LH Low HUFA EPA+DHA = 0.8% DM
    - HH High HUFA EPA+DHA = 2.2% DM

**Juvenile stage**
- 1 commercial diet
  - 90 days
    - ex-LH16
    - ex-LH22
    - ex-HH16
    - ex-HH22
    - EPA+DHA = 2.7% DM
    - 19°C

- 1 HUFA-restricted diet
  - d-151-211
    - 60 days
    - ex-LH16
    - ex-LH22
    - ex-HH16
    - ex-HH22
    - EPA+DHA = 0.5% DM
    - 19°C
2. Growth performances

I- Nutritional conditioning

1. Larval stage

- C1

2. Juvenile stage

- R1

- Individual mean weight (g)

- Time (days)

- LH16
- HH16
- LH22
- HH22

- d-45

- T***

- diet ***

- 100% survival rate in all groups

- No significant effect of nutritional conditionning

- No effect of diet on the survival rate
5. Lipid metabolism in larvae

I- Nutritional conditioning 1

Diagrams showing:

- **D6D mRNA level at d-45**
  - Relative expression in relation to a housekeeping gene and to a referential condition HH22.
  - LH16, HH16, LH22 are compared.

- **D6D Desaturation product at d-45**: 18:3n-6 in PL
  - LH16, HH16, LH22, HH22 are compared.

**Stimulation of desaturation pathways for HUFA synthesis, without any effect of temperature**
I- Nutritional conditioning 1

5. Lipid metabolism in larvae

- AA % FAME PL
- EPA % FAME PL
- DHA % FAME PL

HUFA deficiency
6. Lipid metabolism in juveniles

I- Nutritional conditioning 1

D6 mRNA level - 1st part of the challenge

PL final composition in DHA

Relative expression in relation to an housekeeping gene

- Significant effect of larval conditioning
- BUT transient (30 days)

- Better capacity of ex-LH groups to adapt to a low-HUFA diet
- BUT with a low amplitude
- NS for EPA

% FAME
7. Conclusions

POSSIBLE to influence fatty acid desaturation pathways for HUFA synthesis in juveniles, using a nutritional conditioning during larval stage, without any effect of temperature on the capacity of FA desaturation (≈ 30% of HUFA requirement).

BUT this modulation seems to be limited

Is it possible to obtain a response with a larger magnitude?
II. Nutritional conditioning

2. Experimental design

<table>
<thead>
<tr>
<th>Stage</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
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</thead>
<tbody>
<tr>
<td>Larval</td>
<td>XLH1 0.5%</td>
<td>LH1 0.7%</td>
<td>HH1 1.7%</td>
<td>XH1 3.7%</td>
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<tr>
<td>Mouth opening</td>
<td>C1</td>
<td>XH</td>
<td>HH</td>
<td>XH</td>
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<tr>
<td>4 diets</td>
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<tr>
<td>30 days</td>
<td>XLH2</td>
<td>LH2</td>
<td>HH2</td>
<td>XH2</td>
</tr>
<tr>
<td>PI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 commercial diet</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>35 days</td>
<td>XLH2</td>
<td>LH2</td>
<td>HH2</td>
<td>XH2</td>
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<tr>
<td>R2</td>
<td></td>
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<tr>
<td>Juvenile stage</td>
<td></td>
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<tr>
<td>1 more HUFA restricted diet</td>
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<tr>
<td>d-83-118</td>
<td>XLH2</td>
<td>LH2</td>
<td>HH2</td>
<td>XH2</td>
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<tr>
<td>≠d151-211(R1)</td>
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<tr>
<td>35 days</td>
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</tr>
</tbody>
</table>

- XLH1 = commercial diet with EPA + DHA = 2.7% DM
- LH1 = 19°C
- HH1 = 19°C
- XH1 = 19°C

- XLH2 = 19°C
- LH2 = 19°C
- HH2 = 19°C
- XH2 = 19°C

- 4 diets: XLH1 = 0.5%, LH1 = 0.7%, HH1 = 1.7%, XH1 = 3.7%
- PI = 30 days ≠ 90 (C1)
- R2 = 35 days ≠ 35 days (R1)
- EPA + DHA in R2 = 0.3% DM

- Temperature: 19°C
II. Nutritional conditioning 2

3. Growth performances

No effect of diet on the survival rate

100% of survival rate in all groups

No significant effect of larval conditioning

Idem C1

Idem R1
II. Nutritional conditioning 2

4. Lipid metabolism in larvae

Stimulation of desaturation pathways for HUFA synthesis

D6D mRNA level at d-45

Relative expression in relation to an house-keeping gene

D6D desaturation product at d-45 in PL

18:3n-6 in % FAME

Stimulation of desaturation pathways for HUFA synthesis

Idem C1
II. Nutritional conditioning 2

4. Lipid metabolism in larvae

Idem C1

AA (% FAME in PL)

EPA (% FAME in PL)

DHA (% FAME in PL)

HUFA deficiency
II. Nutritional conditioning 2

5. Lipid metabolism in juveniles

- **D6D mRNA level**
  - Relative expression in relation to an house-keeping gene
  - DIET: ***
  - Diet **:
    - J-83, J-90, J-107, J-118
  - Significant effect of larval conditioning
  - D6D stimulation NON transient

- **PL final composition in DHA**
  - % FAME
  - NS
  - IDEM for EPA
  - Non significant effect of larval conditioning
Although no beneficial effect on growth performances and no clear higher HUFA content in PL, **POSSIBLE** to influence FA desaturation pathways for HUFA synthesis for a better incorporation of vegetable products, using a nutritional conditioning during larval stage (60% of the HUFA requirement).

Possible to use the concept of metabolic programming in sea bass

**BUT is it a:**
- Long-lasting adaptation?
- Transient acclimatation of juveniles to their nutritional environment?

Need to better investigate mechanisms involved in desaturation pathways in response to a low HUFA dietary content in larvae and in juveniles
Inhibition of desaturation pathways for HUFA synthesis

Implementation of metabolic programming using a nutritional conditioning during larval stage

Possible to influence the FA desaturation pathways for HUFA synthesis for a better incorporation of vegetable products

BUT NO transient stimulation

 transient stimulation

HUFA requirement at 0.7%

Additional factors: HUFA n-3 from the juvenile environment

Control of desaturation mechanisms previously gained

Control of the fish lipid composition
Was the metabolic programming really present?

Intermediate period: Stop or Non stop?

Transmission to the offspring?

Challenge extension: what will happen during adulthood?

IF YES

Application to other carnivorous species with high commercial value in aquaculture
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Thanks for your attention
### Récapitulatif

<table>
<thead>
<tr>
<th>XLH1</th>
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<th>LH</th>
<th>HH</th>
<th>HH1</th>
<th>XH1</th>
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<tr>
<td>0,5%</td>
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**LARVES**

- Stimulation des mécanismes de désaturation
- Adaptation à un régime carencé en HUFA n-3

**JUVENILES**

- Niveau ARNm D6 continue
- Niveau ARNm D6 transitoire
- Synthèse d’HUFA
- D6 fonctionnelle ? D6 non fonctionnelle ?
- R1 trop proche du besoin (0,7% EPA+DHA)
- Faible en DHA
<table>
<thead>
<tr>
<th>XLH1</th>
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<th>LH</th>
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**Stimulation des mécanismes de désaturation**

**Adaptation à un régime carencé en HUFA n-3**
HUFA = AGLPI (n-9, n-6 et n-3) = Acides gras insaturés à 20-22 atomes de carbone et au moins 4 doubles liaisons = Acides gras essentiels

Constituants principaux des PL de la bi-couche membranaire

Chez les poissons, majoritairement les HUFA n-3:
- Acide docosahéxaénoique 22:6n-3 (DHA)
- Acide écosapentaénoique 20:5n-3 (EPA)

Acide arachidonique 20:4n-6 (AA)

- Croissance et survie larvaire
- Développement de la vision et du cerveau
- Résistance au stress et aux maladies
- Qualité de la chair, ...
III- Hypothesis concerning the regulation of lipid metabolism in sea bass

**PPARs**

<table>
<thead>
<tr>
<th>Stage</th>
<th>XLH1 (0.5%)</th>
<th>LH1 (0.7%)</th>
<th>LH (0.8%)</th>
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<th>HH1 (2.2%)</th>
<th>XH1 (3.7%)</th>
</tr>
</thead>
</table>

**Larval stage d-45**
- D6D: +
- PPAR: +
- D6D: +
- PPAR: -
- D6D: D6D
- PPAR: PPAR

**Juvenile stage The end of the challenge**
- D6D: +
- PPAR: +
- D6D: +
- PPAR: -
- D6D: D6D
- PPAR: PPAR
Étude des PPAR

Peroxisome Proliferator-Activated Receptor, récepteurs nucléaires

Trois isoformes: \( \alpha \), \( \beta \) et \( \gamma \)

Décrits chez les mammifères et le bar

Facteur de transcription de la delta 6 chez les mammifères

Dans le contexte de programmation métabolique, les PPAR sont-ils impliqués dans la stimulation de la transcription du gène de la D6D chez le bar?
III- Hypothèses de régulation du métabolisme lipidique du bar

Mise en place d’une mémorisation au niveau moléculaire des mécanismes de désaturation des AG
IV- Conclusions & Perspectives

1. General conclusion

**1. Nutritional conditionning during larval stage**

**2. Nutritional environment**

**3. Previously gained mechanisms**

POSSIBLE to influence the fatty acid desaturation pathways for HUFA synthesis for a better incorporation of vegetable meals

Fish lipid composition

0.5 < HUFA dietary content < 0.7

PPAR + D6D transcription
Stimulation des mécanismes de désaturation

- HUFA
- PPAR

Transcription du gène de laΔ6-D

- +

Traduction de l’ARNm en protéine

- +

Synthèse d’AG précurseurs des HUFA

- +

Inhibition

- HUFA

Mise en place d’une programmation métabolique par le conditionnement larvaire

- Gène de la delta 6
Présence de cette programmation chez les juvéniles

Facteurs additionnels: teneur en HUFA n-3 de l’environnement nutritionnel

\[ R_2 = 0.3\% \quad Teneur \ en \ EPA+DHA \ du \ régime \ (%MS) \quad R_1 = 0.5\% \]

Contrôle des mécanismes de désaturation préalablement acquis

Transcription du gène de la D6D

Niveau ARNm D6 continue

Niveau ARNm D6 transitoire

Synthèse d’HUFA

- teneur en DHA

Faible en DHA

D6 fonctionnelle ?

D6 non fonctionnelle ?

dérivées