Effects of dietary interventions on glutathione content and activity of glutathione-related enzymes in European sea bass (*Dicentrarchus labrax*)

E. Cotou, E. Fountoulaki, F. Kokou, V. Karalazos

Presented by Dr. E. Cotou

Nutrition : ARRAINNA, 15 October 2014, 14:50
• The increasing demand, price & world supply fluctuation of FM have emphasized the need to look for alternative protein sources

• Much attention has been focused on PP

• Positive results of FM partially replacement by PP on cultured species (e.g. Atlantic salmon, sea bream etc)

• Lack of information as regard the effects of FM replacement by PP related to the optimal micronutrients’ balance demand for sea bass
• We investigate the effects of FM replacement partially by PP focus on various levels of Premix* (cocktail of micronutrients) using specific biomarkers* at their functional* level...........

• To find an optimal micronutrient balance for sea bass
MATERIALS AND METHODS
Experimental design

- Fish species: European sea bass *Dicentrarchus labrax*
- Weight: ~ 8.5 g (initial), ~ 40 g (final)
- Temperature: ~ 26 °C
- Rearing system: open flow (25 fish/180 l)
- Duration: ~ 2.6 months
- Treatments: 7 diets plus one control (triplicate tanks/diet)
- Tissue: liver
- End point: specific biochemical biomarkers responses functional level

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MATERIALS AND METHODS
Preparation of Diets

• Formulations of diets for the different Premix levels were based on dietary requirements of Atlantic salmon (NCR, 2011)

• All diets were produced by BIOMAR
MATERIALS AND METHODS

Selection of Biomarkers (I)

I. Cellular Respiration
   Oxidative Burst → \( \text{O}_2^- \) → \( \text{H}_2\text{O}_2 \) → \( \text{H}_2\text{O} + \text{O}_2 \) → \( \text{H}_2\text{O} \)

II. Catalase
   \( \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2 \)

III. GPx
   \( \text{GSH} \rightarrow \text{GSSG} \)

IV. GRed
   \( \text{OH}^- \) → Protein Peroxidation, DNA Damage, Lipid Peroxidation

Environmental Factors
### MATERIALS AND METHODS

#### Diets’ composition/formulation

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
<th>Diet 7</th>
<th>Diet 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>10 %</td>
<td>10 %</td>
<td>10 %</td>
<td>10 %</td>
<td>10 %</td>
<td>10 %</td>
<td>10 %</td>
<td>62 %</td>
</tr>
<tr>
<td>Plant protein</td>
<td>50 %</td>
<td>50 %</td>
<td>50 %</td>
<td>50 %</td>
<td>50 %</td>
<td>50 %</td>
<td>50 %</td>
<td>-</td>
</tr>
<tr>
<td>Krill</td>
<td>2 %</td>
<td>2 %</td>
<td>2 %</td>
<td>2 %</td>
<td>2 %</td>
<td>2 %</td>
<td>2 %</td>
<td>-</td>
</tr>
<tr>
<td>Premix **</td>
<td>0%</td>
<td>25%</td>
<td>50%</td>
<td>100%</td>
<td>150%</td>
<td>200%</td>
<td>400%</td>
<td>25 % *</td>
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**Note:**
- * indicates a specific percentage adjustment.
- ** indicates a specific percentage adjustment for premix.

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## MATERIALS AND METHODS
### Composition of PREMIX

<table>
<thead>
<tr>
<th></th>
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<th>Diet 7</th>
<th>Diet 8</th>
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<tbody>
<tr>
<td>Se</td>
<td>0,21</td>
<td>0,27</td>
<td>0,32</td>
<td>0,44</td>
<td>0,55</td>
<td>0,66</td>
<td>1,12</td>
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<tr>
<td>Fe</td>
<td>118,89</td>
<td>127,14</td>
<td>135,29</td>
<td>151,70</td>
<td>168,02</td>
<td>184,38</td>
<td>249,83</td>
<td>260,51</td>
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<td>Mn</td>
<td>14,80</td>
<td>17,87</td>
<td>20,88</td>
<td>26,95</td>
<td>32,96</td>
<td>39,01</td>
<td>63,18</td>
<td>30,06</td>
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<tr>
<td>Zn</td>
<td>33,88</td>
<td>50,66</td>
<td>67,39</td>
<td>100,90</td>
<td>134,35</td>
<td>167,83</td>
<td>301,76</td>
<td>125,44</td>
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<tr>
<td>Copper, Iodine, Cobalt</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Amino acids &amp; derivatives</td>
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</table>

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RESULTS
Minerals in Diets and Fish

Se mg/kg

Fe mg/kg

Mn mg/kg

Zn mg/kg

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RESULTS
Glutathione-related enzymes activity & Glutathione content

- **RESULTS**
  - **non Se-GPx activity**
  - **Se-GPx activity**
  - **Total GPx activity**
  - **GST activity**
  - **Total SOD activity**
  - **GSH (total content)**

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DISCUSSION

• Trace metals concentrations and antioxidant activities of the formulated diets were varied.
• Each enzyme activity in each diet was not proportional to trace metal concentration or GSH content.

However,

• Increases of antioxidants activities & GSH content indicate defence against increase ROS production & oxidative stress while,
• Reduction of antioxidants activities & GSH content indicate reduced ability to protect against oxidative stress and damage (Stephensen et al., 2002).
CONCLUSION

- Using antioxidant activities and GSH content as biomarkers in comparison to control diet (diet 8) the most successful formulated diet was the diet 5 (150% of the commercial Premix’s quantity).
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Thank you for your attention

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