Recent advances in the study of Systemic Granulomatosis in meagre (*Argyrosomus regius*)

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Definition of the granuloma...

Localized inflammatory reactions

- Bacterial, fungal, and viral infections (Tuberculosis)
- Non-infectious inflammatory diseases (Chron’s disease, sarcoidosis)
Systemic granulomatosis in meagre

• Bottleneck for meagre production

• Affects 100% of the population

• Multiple granulomas in all soft tissues

• Aetiology of the disease is unknown
### Hypotheses

<table>
<thead>
<tr>
<th>Metabolic disorder (Katharios et al., 2011)</th>
<th>Disease caused by pathogens (Elkesh et al., 2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Visceral granulomas in seabream</td>
<td>• Nocardia/mycobacteria</td>
</tr>
<tr>
<td>• Visceral granulomas in brook trout</td>
<td>• Other granuloma-inducing pathogens</td>
</tr>
<tr>
<td>• Renal granulomas in turbot</td>
<td>(fungi, bacteria, intracellular parasites…)</td>
</tr>
<tr>
<td>• Some reports in cichlids and goldfish</td>
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</tbody>
</table>
Aim...

Feeding trials to identify potential nutritional causes of SG

Monitoring meagre populations from various locations in Greece to isolate and identify *Nocardia* spp.
“Metabolic disorder” hypothesis
Material and methods

• 3 feeding trials
  ▪ Vitamin D₃
    (4 diets with increasing levels of vitamin D₃)
  ▪ Ca/P levels
    (9 nine experimental diets with different levels of Ca and P)
  ▪ Plant ingredients
    (4 experimental diets with 60% and 14% FM and increasing levels of P in the diets with 14% FM)
Material and methods

- 3-month old (0.5-2 g) meagre
- Each trial lasted 3 months
- Histology
- Semi-quantitative ordinal-scale scoring system

<table>
<thead>
<tr>
<th>State</th>
<th>Score</th>
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<tbody>
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<td>Granulomas visible only with microscope</td>
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</tr>
<tr>
<td>Granulomas visible macroscopically</td>
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<tr>
<td>Tissue calcification</td>
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Material and methods

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<tr>
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<th>Weight</th>
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<th>Liver</th>
<th>Gonads</th>
<th>Intestine</th>
<th>Spleen</th>
<th>Peritoneum</th>
<th>Swimm Bladder</th>
<th>Kidney</th>
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</tbody>
</table>
The effect of Phosphorus

- The medians of the groups with high and low P content are significant different.
The effect of Phosphorus

- Statistical significant difference exists between the diets and the development of granulomatosis for both tissues

“Metabolic disorder” hypothesis
The effect of plant protein

- FM median was significant different from PP median

“Metabolic disorder” hypothesis
The effect of plant protein in combination with P inclusion

- A statistical significant difference exists only for the liver
“Metabolic disorder” hypothesis
“Metabolic disorder” hypothesis
“Metabolic disorder” hypothesis
Conclusions

• Vitamin D did not affect the development of the disease
• High P in the diet seems to improve the condition
• Plant protein negatively affects the condition
• The overall pathology was not different to that described by Katharios et al. 2011
“Pathogen” hypothesis
Material and methods
Material and methods

• Microbiological techniques
  (General and selective nutrient media)

• Molecular techniques
  (PCR for *Nocardia* spp)

• Histological techniques
  (standard and special staining techniques)
Results

• All fish examined had granulomas in their tissues

• In most of the cases no bacterial growth was observed

• In total we purified approximately 25 isolates

• None of the isolated bacteria had phenotypes consistent to *Nocardiia* spp.

• Sequencing confirmed that none of the isolates belonged to the *Nocardia* genus

• More likely environmental strains
Results

PCR analysis directly on SG-affected tissues using specific primers against *Nocardia* spp. were negative for all samples assayed except....
• 2 fish from Astakos, West Greece
• Severe dermal lesions and ulceration of the skin
• PCR for *Nocardia* spp. was positive in 4 out of the 6 different organs
BLAST analysis showed a 100% identity with *Nocardia seriolae*.
“Pathogen” hypothesis
Conclusions

- Meagre does not seem to be very sensitive to bacterial infections

- Nocardiosis is present in Greece but it is not the cause of SG
## Conclusions

<table>
<thead>
<tr>
<th>Metabolic disorder</th>
<th>Disease caused by pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement of SG by change in the diet</td>
<td>• Absence of pathogens in SG-affected population</td>
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<tr>
<td></td>
<td>• Only a single case of nocardiosis</td>
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</table>
Thank you for your attention!!!!!!