DIETARY USE OF PREBIOTICS IN GREATER AMBERJACK JUVENILES: EFFECTS ON GROWTH PERFORMANCE, IMMUNE GENE EXPRESSION AND DISEASE RESISTANCE AGAINST *Neobenedenia girellae*

Grupo de Investigación en Acuicultura

(GIA; www.giaqua.org)


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Research and Technology to enhance excellence in Aquaculture development under an Ecosystem approach
Importance of the genus *Seriola* for aquaculture (150,000 Tn; FAO 2010)

25% of the total aquaculture production in Japan, (Ohara *et al.*, 2005)

Purposed as new aquaculture species for Europe
INTRODUCTION

Biosanitary problems

Main bottleneck for *S. dumerili* production

Ectoparasites
(*Neobenedenia girellae*)

Secondary bacterial infections

Grup de Investigación en Acúicultura
Dietary MOS (mannan oligosaccharides) has been demonstrated to promote fish performance and disease resistance by stimulating the systemic and GALT immune system. Torrecillas et al., 2013
Introduction

MOS

• Not clear effects observed in skin immunology parameters in seabass (Torrecillas et al., 2011), but increase skin mucus production in rainbow trout (Rodriguez-Estrada et al., 2013)
Objective

Determine the effect of two commercial MOS on greater amberjack (*Seriola dumerili*) immune system and the impact on resistance to an experimental infection against the ectoparasite *Neobenedenia girellae*

Increase immune potential of greater amberjack (work package 25) for the start up of greater amberjack culture for the European aquaculture market in the 7th framework of the European project DIVERSIFY
Material & Methods

Experimental design
324 juveniles (331 ± 30g)

Randomly distributed into 18 1000l cylindroconical tanks

4 diets: C (control-non-supplemented), (MOS), (cMOS) & (MOS + cMOS)

- Growth
- Feed efficiency

Final sampling
- Gene expresión
- Skin mucus
- Serum

Parasite challenge
- Parasititation level
- Parasite samples of each tank

Start
30 days 60 days 90 days

Parasite challenge final sampling
100 days 115 days
Material & Methods

Final Sampling

- Extraction of gene expression samples
  - Posterior gut
  - Gill
  - Skin
  - Head kidney
  - Spleen

- Skin mucus and serum extraction

- Bactericidal activity
  (Against Photobacterium damsela)

- Lysozyme activity
  (Guardiola et al. 2014; Quade and Roth 1997)
Cohabitation in cages with experimental fish – 15 days

Parasitation level evaluation

Freshwater bath

Parasite measurements

Controlled HOST ATTACHMENT - 10 days

Highly parasited animals

Experimentally infested fish

ADULT

EGGS

NET

ONCOMIRACIDIA

Experimentally eggs culture
Material & Methods

Parasite challenge

- Parasitation level was made by direct observation by 3 different researchers following:

<table>
<thead>
<tr>
<th>parasitation level</th>
<th>number of parasites per fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1-5</td>
</tr>
<tr>
<td>2</td>
<td>5-15</td>
</tr>
<tr>
<td>3</td>
<td>&gt;15</td>
</tr>
</tbody>
</table>

- Nº of parasites per cm$^2$ Ohno et al., 2008

Total fish surface area (cm$^2$) =0.158 x (TL)$^{2.089}$ x2 ($r^2=0.937$)
Material & Methods

Gene expression analyses

- Total RNA extraction by TRIZOL method
- CDNA synthesis
- RTPCR (sybergreen)

Primers designed by Scottish Fish Immunology Research Center
RESULTS
Growth performance

(p > 0.05)

Results

Dimitroglou et al., 2010
Gültepe et al., 2011
Rodriguez-estrada et al., 2008

Salem et al., 2016
Samrongpang et al., 2008
Mucus and serum immunological parameters

(p>0.05)

Results

Serum Lysozyme activity

- Welker et al., 2011
- Ye et al., 2011
- Dimitroglou et al., 2011

Skin mucus Lysozyme activity

- Rodriguez-estrada et al., 2008
- Torrecillas et al., 2011
Mucus and serum immunological parameters

(p<0.05)

<table>
<thead>
<tr>
<th>Serum bactericidal activity</th>
<th>MOS</th>
<th>CMOS</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bactericidal activity</td>
<td>p=0.186</td>
<td>p=0.013</td>
<td>p=0.507</td>
</tr>
</tbody>
</table>

(p>0.05)

Torrecillas et al., 2011

Welker et al., 2011

Torrecillas et al., 2011

Rodriguez-estrada et al., 2008
Parasite Challenge

KW (p<0.05)

<table>
<thead>
<tr>
<th></th>
<th>DIET C</th>
<th>MOS</th>
<th>CMOS</th>
<th>MOS+CMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitation level (range)</td>
<td>2-3\textsuperscript{a}</td>
<td>2\textsuperscript{a}</td>
<td>1-2\textsuperscript{b}</td>
<td>1-2\textsuperscript{b}</td>
</tr>
</tbody>
</table>

(p<0.05)

Parasite total length

<table>
<thead>
<tr>
<th>P. Size</th>
<th>MOS</th>
<th>CMOS</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. Size</td>
<td>p=0.333</td>
<td>p=0.000</td>
<td>p=0.092</td>
</tr>
</tbody>
</table>

(p<0.05)

No of parasites/cm\textsuperscript{2} of fish surface area

<table>
<thead>
<tr>
<th>pxcm\textsuperscript{2}fish</th>
<th>MOS</th>
<th>CMOS</th>
<th>Interaction</th>
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</thead>
<tbody>
<tr>
<td>p=0.467</td>
<td>p=0.000</td>
<td>p=0.194</td>
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</table>
Results

Gene expression analyses

SKIN (INNATE IMMUNE PARAMETERS)

P<0.05

Skin iNOS

Skin TNFα

Skin IFNγ

Skin IFN 1
Gene expression analyses

SKIN (ADAPTIVE IMMUNE PARAMETERS, INFLAMMATORY RESPONSE AND MUCINE PRODUCTION)  p<0.05
Gene expression analyses

SKIN

- INNATE IMMUNE SYSTEM
- ADAPTIVE IMMUNE SYSTEM
- INTERLEUKINS

CMOS (PROINFLAMMATORY)

DIET C, MOS
Gene expression analyses

POSTERIOR GUT (INNATE IMMUNE PARAMETERS)

P<0.05

GUT Defensine

GUT Hepcidin

GUT IFN 1

Gut TNF α

Arbitrary units x 100

Arbitrary units

Arbitrary units

Arbitrary units

DIET C  MOS  CMOS  MOS+CMOS

DIET C  MOS  CMOS  MOS+CMOS

DIET C  MOS  CMOS  MOS+CMOS

DIET C  MOS  CMOS  MOS+CMOS

a

b

a

b

a

b

a

b

a

b

a

b

a

b

a

b
Gene expression analyses

POSTERIOR GUT (INTERLEUKINS)

P<0.05
Gene expression analyses

POSTERIOR GUT (ADAPTIVE IMMUNE PARAMETERS AND MUCINE PRODUCTION) p<0.05

### GUT IgM

<table>
<thead>
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<th>Condition</th>
<th>Arbitrary units x 10,000</th>
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<tbody>
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</tr>
<tr>
<td>MOS</td>
<td>b</td>
</tr>
<tr>
<td>CMOS</td>
<td>b</td>
</tr>
<tr>
<td>MOS+CMOS</td>
<td>ab</td>
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</table>

### GUT IgT

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<th>Condition</th>
<th>Arbitrary units</th>
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<tbody>
<tr>
<td>DIET C</td>
<td>a</td>
</tr>
<tr>
<td>MOS</td>
<td>b</td>
</tr>
<tr>
<td>CMOS</td>
<td>b</td>
</tr>
<tr>
<td>MOS+CMOS</td>
<td>ab</td>
</tr>
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</table>

### GUT MUC

<table>
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<th>Arbitrary units x 100</th>
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<tbody>
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<td>MOS</td>
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</tr>
<tr>
<td>CMOS</td>
<td>a</td>
</tr>
<tr>
<td>MOS+CMOS</td>
<td>a</td>
</tr>
</tbody>
</table>
Gene expression analyses

POSTERIOR GUT

INNATE IMMUNE SYSTEM
CMOS
DIET C

ADAPTATIVE IMMUNE SYSTEM
MOS
DIET C

INTERLEUKINS
CMOS
(ANTIINFLAMATORY)
MOS
(PROINFLAMATORY)
DIET C
Gene expression analyses

GILL

INNATE IMMUNE SYSTEM

CMOS

DIET C

ADAPTIVE IMMUNE SYSTEM

INTERLEUKINS
Gene expression analyses

Spleen and Head kidney

**INNATE IMMUNE SYSTEM**

MOS

**ADAPTIVE IMMUNE SYSTEM**

**INTERLEUKINS**

MOS (PROINFLAMMATORY)

DIET C, CMOS

**INNATE IMMUNE SYSTEM**

MOS

**ADAPTIVE IMMUNE SYSTEM**

**INTERLEUKINS**

MOS (PROINFLAMMATORY)

DIET C

**Spleen**

**Head kidney**

Diet C, CMOS
SUMMARY

1. Growth performance and feed conversion ratio were not altered by dietary MOS supplementation in *S. dumerili* at the concentration used.

2. Dietary cMOS supplementation stimulated serum bactericidal activity, however no effect was detected on lysozyme activity.

3. Dietary use of cMOS reduce *S. dumerilli* infestation by *N. girellae* after 3 months of supplementation.

4. cMOS presented a response more focus on ectomucoses (skin and gill), meanwhile MOS focused more the response in gut and systemic immunity (head kidney and spleen).
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