Reduced inflammatory response to *Aeromonas hydrophila* infection in sea bass (*Dicentrarchus labrax*) fed with beta-glucan supplements.

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INTRODUCTION:

Aquatic microorganism develop antibiotic resistance, so the use of antibiotics against pathogens in aquaculture is ban in Europe and in USA. The solutions are **new alternatives** such as dietary supplemented of immunostimulants [1].

Immunostimulants (IS) enhance the immune response by increasing innate mechanisms and stimulating phagocytic activity of macrophages [2]; [3]. There are some suitable IS for aquaculture such as **β-glucan** that improve immune status controlling diseases in fish culture [4].

β-glucan derived from different sources have differences in their structure and on their activity [5]:

- Larger molecular weight → activated leukocytes stimulating their phagocytic, cytotoxic and antimicrobial activities.
- Low molecular weight → less cellular effects

IN THE EXPERIMENT we carry out an investigation about β-glucans effects in sea bass (*Dicentrarchus labrax*) fed with 4 diets:

- Control diet (**CTRL**)
- *Macrogard*© dose 0,1% (**MG01**)
- *Macrogard*© dose 0,2% (**MG02**)
- *Macrogard*© dose 0,5% (**MG05**)

→ ***Macrogard©*** :commercial formula based on β-glucans.

Fish were infected with ***Aeromonas hydrophila*** by an intraperitoneal injection to observe the immune response. This bacterium infection is a common disease in cultured fish [6].

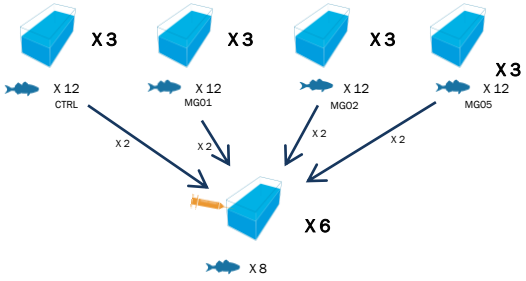
MATERIALS AND METHODS:

CONDITIONS:

Rearing period: 1,6 m^3 rectangular tanks at a density of 30 kg of fish *per* tank. Photothermal conditions remained constant (23 °C, 16L: 8O) and dissolved oxygen level were kept over 7 ppm. Fish were fed once a day with a commercial pellet food (Skretting®).

After breeding process: 144 distributed in 12 rectangular tanks (0,1 m^3) . Temperature oscillated between 21.6 °C to 23.9 °C. Oxygen level was maintained above 7 mg.L-1. Ammonia ranged between 0.043 to 0.26 mg.L-1, nitrite were between 0.041 to 0.158 mg.L-1 and nitrate fluctuated between 18.9 and 40.5 mg.L-1.

Challenge experiment: 48 fishes from the diet experiment were distributed in 6 new tanks (1,6m^3). In these tanks were performed the infection by intraperitoneal injection with *A. hydrophila*.



SAMPLING DATES:

FEEDING EXPERIMENT:

JUNE 17

15 DAYS: Tissue samples were collected from 4 fish in every tank

JULY 1

30 DAYS: Tissue samples were collected from the remaining fish

CHALLENGE EXPERIMENT:

JUNE 19

INTRAPERITONEAL INJECTION (17 DAYS)

JUNE 21

19 DAYS: Tissue samples were collected from all fishes used in challenge experiment

PROCEDURES:

RNA extraction (TriReagent Sigma) → **Quantification** (NanoDrop) All samples were put at the same concentration → **Agarose gel for RNA quality** → **Pool the samples** → **Reverse transcription** cDNA generation with 4µg of total RNA → qPCR:

Gene	Accession number	Sequence 5'-3'	Product size
EF-1α	AJ866727	F: GCTTCGAGGAAATCACCAAG R: CAACCTTCCATCCCTTGAAC	153 bp
IL-1β	AJ269472.1	F: GTGGTGGACAAGCCAGTCT R: TGTTCTCCTCTGCTGTGCTG	148 pb
TNFα	DQ070246.1	F: GAGTGGAAGGACGGTCAAGG R: AAGTAGAGGCCGGTTTGTGG	92 pb
COX-2	AJ630649	F: CATTCTTTGCCCAGCACTTCACC R: AGCTTGCCATCCTTGAAGAGTC	106 pb
IL-10	AM268529	F: ACCCCGTTCGCTTGCCA R: CATCTGGTGACATCACTC	164 pb
TGF-β	AM421619	F: GACCTGGGATGGAAGTGG R: CAGCTGCTCCACCTTGTG	225 pb

Table 1: Gene accession numbers and primer sequences: foward (F) and reverse (R), used in the real-time quantitative PCR for gene expression analysis.

RESULTS:

HEAD-KIDNEY:

Site of hematopoiesis that contains phagocytic cells.

Diet experiment: Diet **MG05** → an increase in the gene expression of:
- TNF-α1: induces the immune and inflammatory response.

Challenge experiment: We did not observe any significant change in the gene expression.

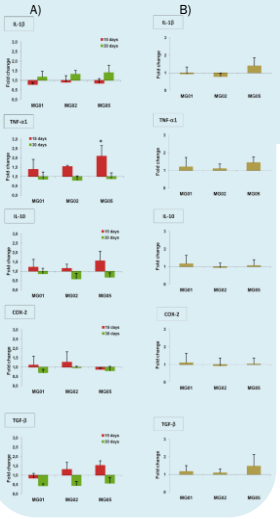


Fig.1. Effect of different doses of Macrogard: MG01, MG02 and MG05, on immune selected genes: IL-1β, TNF-α1, TGF-β, COX-2 and IL-10 in head-kidney of sea bass analyzed by RT-qPCR. **A)** after 15 days (red bars) and after 30 days (green bars). **B)** Sea bass infected with *Aeromonas hydrophila* 17 days after the experimental feeding. Significant differences are represented by * (P < 0,05).

GILLS:

Is in contact with the external environment and is one of the primary routes of entry for infectious agents

Diet experiment: We did not found any significant change in the gene expression.

Challenge experiment: Diet **MG01** → a significant change in gene expression of:
- COX-2: This enzyme mediates inflammatory processes and it is normally expressed after the inflammatory induction but in our results we did not observed that induction significantly.

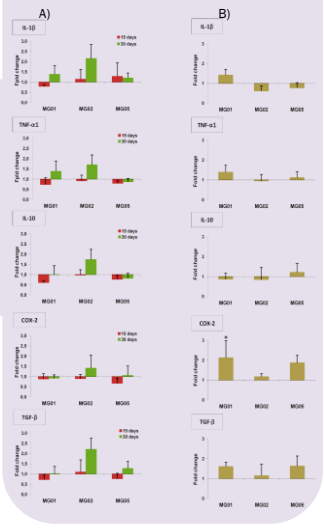
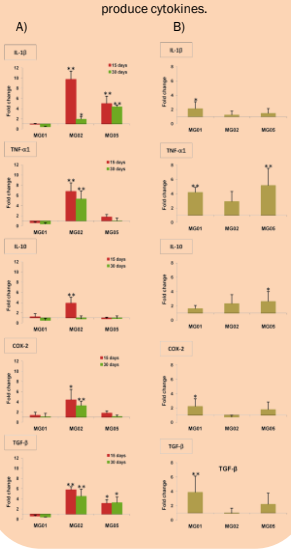


Fig.2. Effect of different doses of Macrogard: MG01, MG02 and MG05, on immune selected genes: IL-1β, TNF-α1, TGF-β, COX-2 and IL-10 in gills of sea bass analyzed by RT-qPCR. A) after 15 days (red bars) and after 30 days (green bars). B) Sea bass infected with *Aeromonas hydrophila* 17 days after the experimental feeding. Significant differences are represented by * (P < 0,05).

SPLEEN:

Is the biggest secondary lymphoid organ and numerous leukocytes are likely to produce cytokines.



Diet experiment: Diet **MG02** → different increases in the expression gene:
- IL-1β and TNF-α1: induce an inflammatory and immune response.
- COX-2: mediates inflammatory processes.
- TGF-β: anti-inflammatory cytokine that regulates the immune response.

We also observed an increase after 15 days of:
- IL-10: improves the regulation in the inflammatory response, promoting the production of antibodies and the inhibition of macrophage.

Challenge experiment: Diet **MG01** → increase the gene expression of:
- IL-1β and TNF-α1: induce an inflammatory and immune response.
- COX-2: mediates inflammatory processes.
- TGF-β: anti-inflammatory cytokine that regulates the immune response.

Diet **MG05** → increase the gene expression of:
- TNF-α1: induces the inflammatory response
- IL-10: improves the regulation in the inflammatory response, promoting the production of antibodies and the inhibition of macrophage.

Fig.3. Effect of different doses of Macrogard: MG01, MG02 and MG05, on immune selected genes: IL-1β, TNF-α1, TGF-β, COX-2 and IL-10 in spleen of sea bass analyzed by RT-qPCR. **A)** after 15 days (red bars) and after 30 days (green bars). **B)** Sea bass infected with *Aeromonas hydrophila* 17 days after the experimental feeding. Significant differences are represented by * (P < 0,05) and ** (P < 0,01).

CONCLUSIONS:

The initial hypothesis is not fulfilled. We had some significant results but we hoped to obtain more. We have to reconsider:
- The level of β-glucans in every dose of *Macrogard*©.
- The time of the feeding treatment.

The final results can not confirm that the diet supplemented with β-glucans enhance the innate immune response of sea bass. We could further analyzed the experiment in order to have better results:
- The effect of this treatment in different developmental stages of the sea bass.
- The effect of beta-glucans in different tissues and with other immune genes.

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