

**Title:**

Evaluation of immune responses to oxytetracycline (OTC) in the red seabream (*Pagrus major*)

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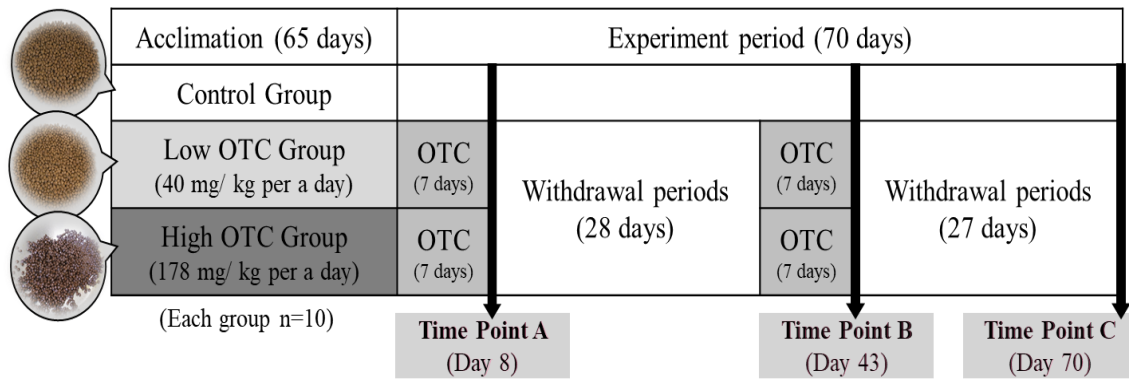
**Aim:**

Increasing seafood consumption results in intensive aquacultural system, which has been accompanied by an increased prevalence of the fish disease. The antibiotic oxytetracycline (OTC) is commonly used to prevent and treat bacterial diseases in fish. However, overuse of OTC has led to side effects, such as immune alteration. In view of this, the aim of present study is to reveal to immune effects by OTC administration in aquacultured red seabream (*Pagrus major*).

**Procedure:**

This presentation contains the results and discussions as follows;

- 1) *In vivo* OTC administration to the red seabream juveniles was performed. Three groups were established followed OTC administration concentration (0, 40 and 178 mg·kg<sup>-1</sup> diet), and three sampling times. Liver and spleen were collected followed by OTC feeding-withdrawal day cycle (Figure 1).



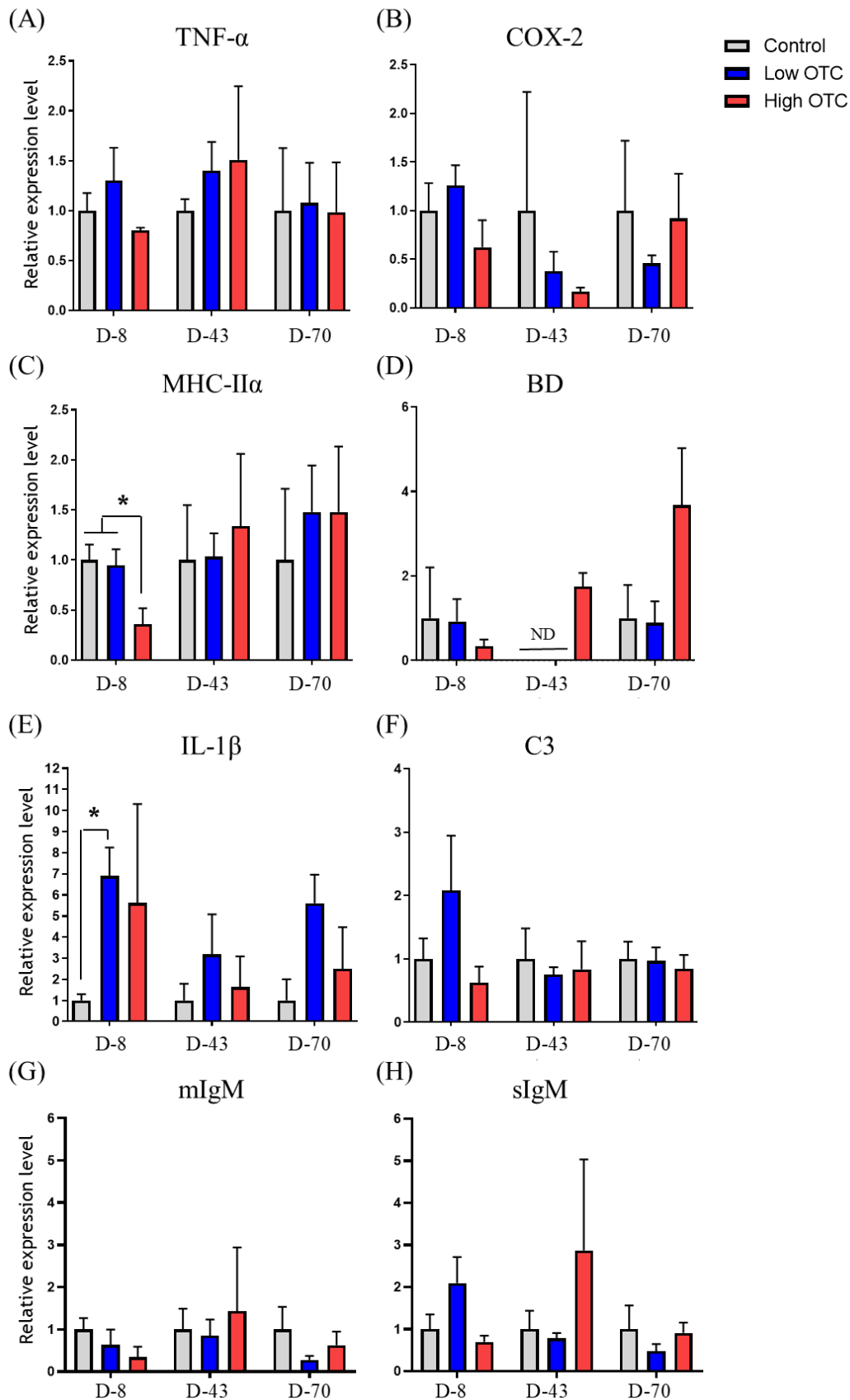
**Figure. 1** Experimental groups and sampling schedule.

2) The expression levels of immune-relevant genes were analyzed by real-time quantitative PCR (RT-PCR) in liver and spleen. Normalized using Ef-1 $\alpha$  gene expression level.

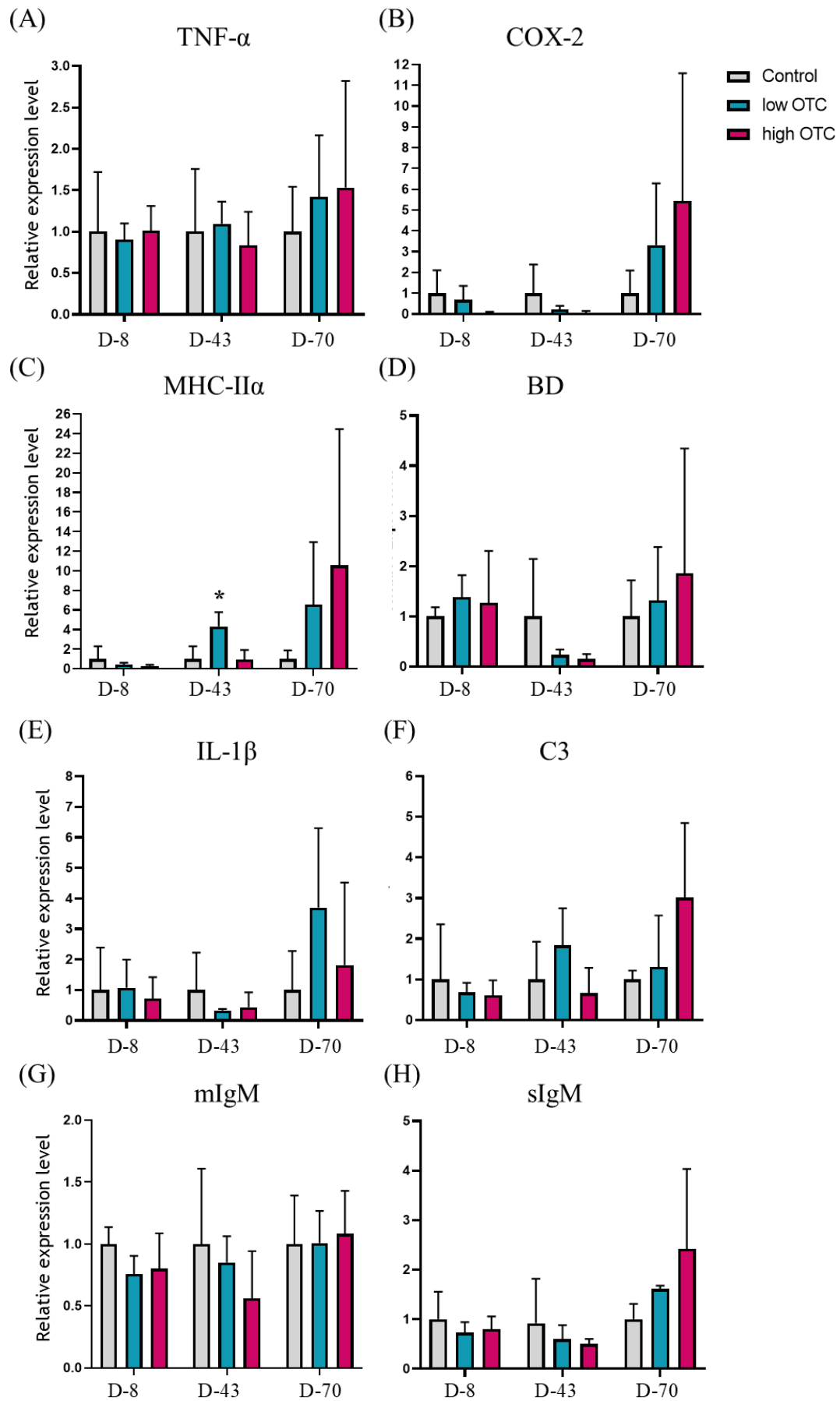
- Three proinflammatory process related genes (Interleukin-1 $\beta$ (IL- $\beta$ ), Tumor necrosis factor  $\alpha$ (TNF-  $\alpha$ ), Ciclooxygenase-2(COX-2)
- Five immune stimuli altered genes: Complement 3(C3), Major histocompatibility complex class II $\alpha$ (MHC-II $\alpha$ ),  $\beta$ -defensin(BD), Immunoglobulin M (IgM; membrane-bound IgM; mIgM and secreted IgM; sIgM)
- One reference gene: Elongation factor 1 $\alpha$ (Ef-1 $\alpha$ )

### Result:

In order to examine the effect of oxytetracycline on immune-related genes, RT-qPCR was performed (Figure 2 and 3). After first OTC administration (Day 8), proinflammatory process related genes, TNF- $\alpha$ , COX-2, IL-1 $\beta$ , C3 and secreted IgM were up-regulated in Low OTC-treated group's liver. MHC-II $\alpha$  gene was down-regulated by the high OTC in the liver at day 70. The OTC administration provoked immune stimulation in liver, and little effect on gene expression in spleen was observed. These are the opposite results from the paper of Guardiola et al., 2012, where the expression of the C3 gene in the head kidney of the gilthead seabream, *Sparus aurata* decreased according to the concentration of OTC, thereby suppressing the immune response.



**Figure 2.** Relative expression levels of immune related genes from OTC administrated red seabream liver. Asterisk indicated significantly difference compare to the control group at same sampling point using one-way analysis of variance followed by Dunnett's multiple comparison test ( $p < 0.05$ ). ND represented that not detectable due to the under the detection limit.



**Figure 3.** Relative expression levels of immune related genes from OTC administrated red seabream spleen. Asterisk indicated that significantly different compare to the control group at same sampling point using one-way analysis of variance followed by Dunnett's multiple comparison test ( $p < 0.05$ ).

### **Publication/conference presentation:**

In progress:

- With improved data, this study would be presented with other researchers in the conference and published as a paper.

### **Perspectives in future:**

This study could contribute to

- The understanding of the immunomodulatory effects in OTC administrated fish
- Provides basic immunological knowledge on fish disease prevention for the development of immunoactive agents
- The establishment of appropriate regulations and management of OTC usage in aquaculture.
- Solving the problem of drug-resistant bacteria occurrence caused by misuse and abuse of antibiotics
- Also by reducing the use of antibiotics, environmental and ecological side effects such as residual effects could be alleviated.

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### **Reference**

Guardiola, F. A., Cerezuela, R., Meseguer, J., & Esteban, M. A. (2012). Modulation of the immune parameters and expression of genes of gilthead seabream (*Sparus aurata L.*) by dietary administration of oxytetracycline. *Aquaculture*, 334, 51-57.