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#### Abstract

In the last decades, pollution of the environment by large scale use of antibiotics in agriculture and human medicine have led to increased antimicrobial resistance in both the environment and the host animal microbiome. Disturbances in the host microbiome can result in impaired immunity and reduced resilience of aquaculture species. Here, we investigated whether environmentally measured levels of the commonly used antibiotics ciprofloxacin and oxytetracycline influences the host microbiome and susceptibility toward saponin-induced immune stimulation in larval zebrafish. Firstly, neutrophil and macrophage reporter zebrafish larvae were exposed to different concentrations of soy saponin by immersion. A dose-dependent increase in neutrophil presence in the intestinal area was observed together with increased expression of immune genes *il1b*, *tnfa*, *il22* and *mmp9*. To investigate the effect of antibiotics, larval zebrafish were immersed in ciprofloxacin or oxytetracycline in the presence or absence of a low dose of saponin. In vivo imaging revealed that antibiotic treatment did not reduce the number of neutrophils that were recruited to the intestinal area upon saponin exposure, although it did tend to lower pro-inflammatory cytokine levels. Microbial sequencing of whole larvae revealed that exposure to a low dose of saponin already shifted the microbial composition. The combination of oxytetracycline and saponin significantly increased  $\alpha$ -diversity compared to the controls. In conclusion, the current study provides evidence that the combination of low levels of antibiotics with low levels of anti-nutritional factors (saponin) can induce inflammatory phenotypes and can modify the microbiota, which might lead to altered disease susceptibility.

**Keywords:** Zebrafish, microbiota, saponin, neutrophils, macrophages, antibiotics

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#### O-008.

##### Can passive immunization prevent disease outcome in gilthead sea bream exposed to *Enteromyxum leei*?

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#### Abstract

Passive immunization is used in humans for treating or preventing some infectious diseases, but it also constitutes an emerging field of interest in aquaculture, particularly with the restrictions for antibiotic use. Intracoelomically-injected antibodies can be detected in fish sera within the first 8 h and their half-life ranges from 7 to 22 days post-injection, depending on the species. The fish models studied so far used fast-acting pathogens such as virus, bacteria or ciliate parasites. The current work aimed to determine if passive immunization could help to prevent enteromyxosis in gilthead sea bream (GSB, *Sparus aurata*). *Enteromyxum leei* is a myxozoan intestinal parasite that invades the paracellular space of the intestinal epithelium, producing a slow-progressing disease, leading to anorexia, cachexia and mortalities. We have previously demonstrated that GSB that survive *E. leei* infection become resistant upon re-exposure, and this resistance is directly related to the presence of high levels of specific serum antibodies.

In the current study, we evaluated whether injection with sera from resistant animals would protect naïve fish when challenged by effluent exposure to the parasite. Serum from a pool of resistant (R) and naïve (N) animals (intact or heat inactivated, 10  $\mu$ l/g BW) was intracoelomically injected 24 h prior to the *E. leei*-effluent challenge and at 9 days post-

challenge (dpc). At 23 dpc, the different groups were allocated in separate tanks and the effluent exposure was terminated. A non-lethal parasite diagnosis was performed at 56 dpc. At the final sampling (100 dpc), blood, serum and tissues were collected for hematology, circulating antibodies, histological and molecular diagnosis and gene expression.

Groups injected with R sera had lower prevalence and intensity of infection than those with N sera, both in the intermediate and final samplings. At 100 dpc, the prevalence of infection in the PBS and N groups was 70%, whereas in R group it only reached 55%. Condition factor (CF) and specific growth rate (SGR), key parameters affected by enteromyxosis, were higher in R group. There was a significant correlation between prevalence of infection and SGR and CF. Immunohistochemistry and gene expression studies will reveal whether this partial protection was due to higher presence of specific antibodies or specific cell populations. These results show that, even with this long term disease, passive immunization can confer some degree of protection. The administration of specific antibodies during exposure, probably provided fish with time to activate the specific defenses before the parasite proliferated.

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**Keywords:** Passive immunization, Myxozoa, antibodies, gilthead sea bream, parasites.

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#### O-009.

##### Bacterial membrane vesicles as vaccines in aquaculture

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#### Abstract

Infections by two Gram-negative facultative intracellular bacterial pathogens, namely, *Piscirickettsia salmonis* and *Francisella noatunensis*, are causing major problems in aquaculture world-wide. *F. noatunensis* sp is one of the main factors hampering the development of fish farming based on Atlantic cod in Norway and is deleterious to tilapia, a farmed fish that is produced over 3.5 mill tons/year. *P. salmonis* infections have been devastating for salmon aquaculture. As of today no effective treatments are available against the diseases. The immunologically inaccessible intracellular location of *Francisella* and *Piscirickettsia* have until now complicated the development of protective measures. This is in stark contrast to the successful development of efficient vaccines that has been made possible against important extracellular bacterial infections in salmon based on whole inactivated bacteria injected with oil adjuvants. It has been shown that both *P. salmonis* and *F. noatunensis* secrete membrane vesicles (MV). Bacterial MVs has been shown to contain proteins, DNA and RNA and simulate the mother bacteria in a non-replicative form. Bacterial MV has been reported as potential vaccine candidates for a range of host including humans, mice and fish against infection caused by intracellular pathogenic bacteria as they induce both a humoral and cellular immunity. Here the characterization of MV isolated from *P. salmonis* and *F. noatunensis* is described, and their vaccine potential is verified in a zebrafish infection