

Influence of organic diets and probiotics on an experimental *Flavobacterium psychrophilum* infection in rainbow trout fry

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) is the dominant fish species produced in Danish aquaculture. The annual production in freshwater is around 30.000 tonnes, the organic production currently accounting for approximately 1 %. There is no production of organic fry, as the classification organic can only be given to fish that have been treated with antibiotics no more than twice in a lifetime. This is hard to achieve as recurrent disease outbreaks, especially with *Flavobacterium psychrophilum*, are seen during the fry stage. A study from 1998 showed that approximately 1/3 of all Danish rainbow trout fry died due to infections with this bacterium [1]. A further challenge for production of organic fry is that diets with high plant contents cause enteritis and injury to the intestine, which in the end will affect the overall health status of the fish and result in a higher risk of disease following exposure to pathogenic microorganisms.

The aim of the Danish project OPTIFISH is to optimize growth and survival for organic cultured rainbow trout. OPTIFISH investigates how organic vs. non-organic diet types as well as diets with or without probiotics affect the intestine, the intestinal microbiota and survival rates of rainbow trout following exposure to pathogens. The project consists of several work packages, among them the backbone of the project, namely the feed experiments with fry as well as sampling from the different diet groups. The samples will be used for studies of the bacterial microbiota in the fish by e.g. traditional bacteriology and molecular methods (16S rRNA PCR combined with next generation sequencing) as well as for the immunological investigation of the intestine by gene expression studies by quantitative RT-PCR. Fish health will be investigated by experimental exposure of fry to pathogens, e.g. *F. psychrophilum*.

In the current experiment four diet types were tested on fry, a conventional type (Inicio®, BioMar A/S) with and without probiotics, as well as an organic type with and without probiotic. Bactocell® (Lallemand) was used as the probiotic. Studies were done in triplicates, whereas the sampling and experimental infections were done in duplicates.

The rainbow trout were fed the different diet types from first feeding and onwards. Just before first feeding yolk sac fry were sampled for reference. Thereafter sampling was done when the fry had been fed for at least 10 days, just before experimental infection with *F. psychrophilum* as well as 5 and 26 days after infection. Until the fish reached average weight 5 g two more samplings were done.

An experimental bath infection model with *F. psychrophilum* was used [2]. A 48 hour old bacterial culture of the strain 950106-1/1 was diluted to 10⁷ CFU/ml in bore-hole water and

the fish were exposed to this dilution for 0.5 h. Thereafter the fish were moved to 100 l aquaria. During the whole feed experiment the water temperature was 12 °C.

The bath exposure challenge experiment was terminated after 35 days. The cumulative mortalities were less than 10 % in the groups exposed to the bacterium, whereas no mortalities were seen in the controls.

A traditional bacteriological examination of the microflora in the fry was done by taking out samples from inner organs for detecting potential pathogens as well as samples from the intestine to examine the intestinal microflora. Tryptone yeast extract salts (TYES) agar [3] was used for isolating *F. psychrophilum*. The random sampling done 5 days post bath exposure infection showed that *F. psychrophilum* could be isolated in 1 of 10 fry, 3 of 10 fry, 3 of 10 fry and 4 of 10 fry, respectively, in the four different diet groups, in total 27.5 % of the sampled fry. In all cases apart from one the bacterium was only isolated from the spleen. When the random sampling was repeated 26 days post infection, a total of 3 of 40 fish hosted the bacterium (each fish sampled from a different group), corresponding to 7.5 % of the sampled fish. The isolation site this time was spleen and kidney. *F. psychrophilum* was not isolated from the control groups.

The conclusions based on this study are that bath exposure under the given conditions resulted in low mortalities in diet groups no matter if the fish had been fed diets that were organic or conventional nor if the feed had been added probiotic. No significance in mortalities was seen between the diet groups. The bacteriological examinations showed that the bacterium had entered the fish, as it was found in inner organs in one fourth of the sampled fish 5 days post infection, but it seems that the fish were able to clear the infection, because the cumulative mortalities after 35 days were less than 10 percent in each group. 26 days post infection only 7.5 % of the sampled fish still harboured the bacterium.

Further analyses include next generation sequencing of samples from the diet groups and comparison of all data.

The OPTIFISH project is aiming at a production of a more robust and healthy rainbow trout fry/fingerling, so the production of organic fry can become a reality and the result can be a higher production in organic aquaculture.

To read more about OPTIFISH, see

http://www.icrofs.dk/Sider/Forskning/organicrdd_optifish.html
<http://www.vet.dtu.dk/Forskning/Projekter/OptiFish.aspx>

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