

# Organic diets are equally good for rainbow trout fry as conventional diets

With respect to rainbow trout, the OPTIFISH project has shown that feed composition is more important than whether the feed is of organic or conventional origin.

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Rainbow trout (*Oncorhynchus mykiss*) is the dominant fish species in Danish freshwater aquaculture and the annual production is about 30.000 tonnes. Only a minor part of this production is organic, but the proportion of farmed organic rainbow trout is continuously increasing. The aim of the project OPTIFISH has been to elucidate the effect of diet ingredients on fish health. Diets with either organic or non-organic ingredients were compared. The OPTIFISH project has shown that the ingredient type in the diet is more important for the bacterial intestinal microbiota of the fish than if the diet ingredients are of organic or conventional origin. Furthermore, fish fed organic diets appear to acquire the same health status as fish fed conventional diets.

OPTIFISH has become even more relevant for the aquaculture industry since a new EU Resolution (710/2009) requires that organic fish as from 2016 shall be 100 percent based on organic rainbow trout fry. The current requirement is that to be labelled organic, fish need only to be fed organic as from a weight of 25 g. In the future stricter regime, fish can only be sold with the organic label if they have been raised under organic measures throughout the production period and have been fed with diets approved for organic aquaculture.

## The background for the OPTIFISH project

To maintain its organic label, a fish may only be treated with antibiotics a limited number of times. Repeat-

ed outbreaks of Rainbow Trout Fry Syndrome (RTFS), caused by the bacterium *Flavobacterium psychrophilum*, increase the requirement for treatment and therefore increase the risk to the producer of losing the organic label. The main challenge is to avoid diseases. Disease prevention might be achieved with a diet, which strengthen the immune system and thereby reduces the risk of disease. Furthermore, it is known from salmon aquaculture that plant protein ingredients (e.g. soy meal) in the diet can affect the intestinal mucosa and increase the risk of infections that weaken the immune status of the fish. OPTIFISH focused on how organic diets with different levels of marine and plant ingredients, with or without probiotics, affect the intestine of the fish, the bacterial intestinal microbio-

ta as well as the survival of the fish in connection with infections.

## Diet experiments

The overall study was organized into two large feed experiments, in which groups of rainbow trout were fed with different diet types from first feeding till an average weight of 8 g. During Experiment I conventional marine diet types were compared with plant diets containing both rape seed oil and pea protein. The plant diets were shown to have a positive influence on the composition of the intestinal bacterial microbiota (higher amount of lactic acid bacteria) among the fish, but we did not determine whether this was due to the addition of rape seed oil or pea protein. Therefore, rape seed oil, pea protein or both ingredients were included in the diets tested in Experiment II (Table 1). The probiotic bacteria (approved for adding to fish feed) used in Experiment I did not have any clear effect in the tested dose and experimental design, neither on the composition of the intestinal

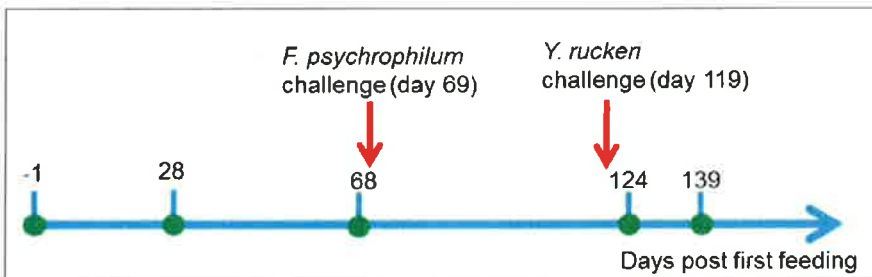


Rainbow trout is the dominant fish species in Danish freshwater aquaculture. Photo: BioMar A/S

**Table 1.** The composition of the diet types investigated in Experiment II.

	A	B	C	D	E
Fish meal	x				
Fish meal, trimmings *		x	x	x	x
Krill meal (MSC certified)	x				
Hydrolysed fish protein	x				
Pea protein				x	x
Wheat gluten	x				
Organic wheat gluten		x	x	x	x
Wheat flour	x				
Organic wheat flour		x	x	x	x
Fish oil	x				
Fish oil (wild and/or trimmings)*		x		x	
Organic rape seed oil			x		x
Premix	x				
Organic premix		x	x	x	x
Probiotics	x	x	x	x	x

\*=Approved for diets for organic fish



**Figure 1.** The samplings done during Experiment II. Experimental infections with either *Flavobacterium psychrophilum* or *Yersinia ruckeri* were done day 69 and 119, respectively, after first feeding.



Only a minor part of the production of rainbow trout in Denmark is organic, but the proportion of farmed organic rainbow trout is continuously increasing. Photo: Fiskehuset Thisted Aps.

bacterial microbiota nor in connection with experimental infection with bacterial fish pathogens. Since the probiotic is added as a standard in diet type A (the commercially sold fry diet Inicio Plus), it was added to all diets in Experiment II.

During the experiments, samples were collected to conduct a molecular investigation of the intestinal bacterial microbiota composition and to investigate the immune response in fry (Figure 1). Additionally, during both experiments sub-groups of fish from each treatment group were subjected to experimental infections with two pathogenic bacteria to investigate the effects of diet composition and organic origin on the progress of infection. Figure 1 shows a timeline of sampling and experimental challenges done during Experiment II.

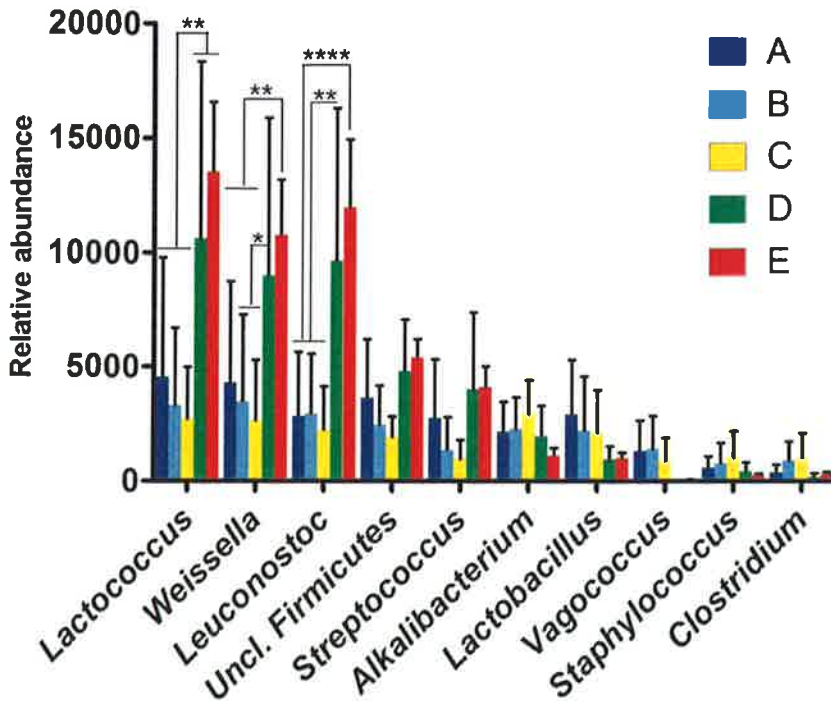
Concerning Experiment II, in the first experimental infection experiment 1.5 g fish were injected in the peritoneal cavity with *Flavobacterium psychrophilum*. The cumulative mortality was between 82 and 92 % in the different groups. The lowest mortality was seen for fish fed diet type B. In the second experimental infection, 4 g fish were bathed in a solution of the bacterium *Yersinia ruckeri*, the cause of enteric redmouth disease. The mortality among all treatment groups ranged from 53 to 61 %. There were no statistical differences among the mortalities in the diet groups in either infection experiment.

### Beneficial bacteria in the feed

Another task in the project OPTIFISH was to study how probiotic bacteria added to the diet influenced the composition of the intestinal bacterial microbiota and the health of the rainbow trout fry. Probiotic bacteria have been suggested to work in different ways. One of the mechanisms of action suggested is that growth and colonisation with probiotic bacteria may create a layer on the surface of the intestine, preventing the entry of undesirable bacteria into the host tissue. Another hypothesis is that the presence of probiotic bacteria may inhibit the growth of undesirable, pathogenic bacteria due to the production of inhibitory substances like bacteriocins and lactic acid leading to decreased pH in the intestine.

OPTIFISH examined whether addition of probiotic bacteria to the fish feed influenced the immune system and survival of the fish after challenge with the pathogenic bacteria *Flavobacterium psychrophilum* or *Yersinia ruckeri*. No effect of the probiotic

## Diets produced of marine/plant ingredients



**Figure 2.** The ten most abundant bacterial taxa on day 28 after first feeding for each of the five investigated diet types (A-E) in Experiment II.

was shown in this work with the used dosage and experimental design. Instead it seemed that the effect was much higher between diet types with differences in oil and protein contents as well as origin of the ingredients.

The results from Experiment I suggested a large influence on the intestinal bacterial microbiota in fish that had been fed the diet with pea protein and plant oil in comparison to fish fed the marine diet. Therefore Experiment II focused on diets that only varied with one ingredient. It was shown that the addition of pea protein (diet types D and E) seemed to be more important than the oil (Figure 2). This is a very relevant observation, and the results can be used in future design of fry feed (both organic and conventional diets). An increased occurrence of *Lactococcus* as seen for diet types D and E is desirable because of its well-known probiotic properties.

Results of the investigation of immunological parameters of intestinal samples from the fish showed that there was a high level of expression of innate immune genes, but there was no difference in this response in fish from the different diet groups.

Bacterial infections were also shown to have a high influence on the composition of the intestinal bacterial microbiota, and especially in Experiment II it was shown that the major part of the intestinal flora in the infected fish

consisted of the pathogenic bacterium *Yersinia ruckeri*.

### The feed ingredients are most important

The OPTIFISH project has shown that the composition of the diet is more important for the gut microbiota of the fish than whether the diet is of organic or conventional origin. There was a clear difference of the intestinal microbiota with diets containing pea protein, whereas the origin of the added oil (marine versus plant) seemed to be less important. Experimental infections showed that the diet type did not affect fish survival. Therefore

the investigated diets are believed to have the same effect on the fish health, according to the results on the parameters that we decided to investigate during the OPTIFISH project. According to OPTIFISH, organic diets for commercial use will lead to the same health status in fish as conventional diets. It has to be stressed that survival as a parameter for evaluating infection is quite a "rough" method to use. Therefore the project could not rule out that differences in the composition of the gut microbiota between the fish from the different diet groups might have had a minor, not "readable" influence on fish health.

OPTIFISH was a collaboration between universities and private companies. The project was headed by the National Veterinary Institute at DTU and the other partners were Faculty of Health and Medical Sciences, University of Copenhagen, BioMar A/S and Dansk Akvakultur (Danish Aquaculture Organization).

For more information read about the Organic RDD project OPTIFISH on the website: [http://www.icrofs.dk/Sider/Forskning/organicrdd\\_optifish.html](http://www.icrofs.dk/Sider/Forskning/organicrdd_optifish.html)

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A new EU resolution requires that organic fish must be 100 percent based on organic rainbow trout fry. Photo: OPTIFISH

