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Feature title: Use of probiotics in aquaculture: can these additives be useful?

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Use of probiotics in aquaculture: can these additives be useful?

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Probiotics are well known and routinely used additives in the main livestock species. They claim to improve gut health by stabilising gut flora being their effect reflected in a better overall health status, welfare and performance of the animals.

However, their use in fish production is still scarce, being nowadays only one additive registered to be used in the European Union.

Different factors might be behind the lack of this type of products for aquaculture:

1) Gut microbiota and physiology of fishes cultured are still being studied and seems to differ in a high extent from one species to another

2) Probiotics used in monogastric and ruminants available nowadays are mainly based on bacteria or yeast that need temperature enough to develop in the animals' gut. It can be difficult to reach taking into account that fish are poiquiloterms and in some specific productions water temperature is extremely low

3) Up today, it is not well known if the microorganisms from probiotics can develop multiply as well as modify fish gut flora in these environmental and gut conditions

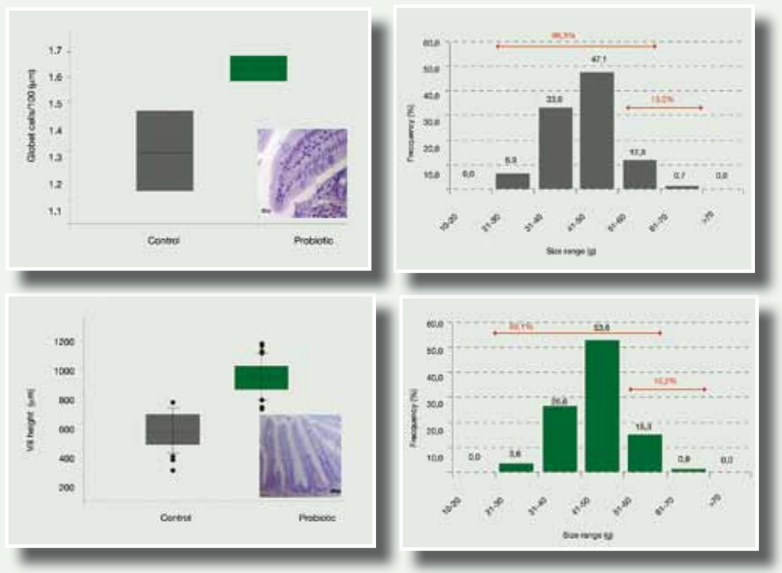
4) Feed fish processing is extremely hard in terms of temperature and pressure so, how to apply these alive microorganisms to fish pellets is still being studied. Their inclusion by coating after pelleting can be the solution, although the stability of this microorganism in this oily solutions as well as once reach water in tanks or sea needs still to be demonstrated

Despite all this, and taking into account the increasing importance of fish production all over the world, Rubinum SA is investing a lot of effort in this field. In this regard, it recently ran a trial in collaboration with IRTA to study the effect of the probiotic *Bacillus* cereus var. toyoi on rainbow trout (Oncorhynchus mykiss) fingerlings.

In the trial, fingerlings of rainbow trout (4.2 ± 0.1g) were fed two diets, a commercial diet (Aller FuturaTM from AllerAqua, Denmark) and the same diet containing the probiotic B. cereus var. toyoi at the final concentration of 2*104 UFC/g, during 93 days.

Each treatment was tested in triplicate (400-L tank, 125 fish/tank, initial density: 1.3kg/m3). During the trial, water temperature, conductivity, pH and dissolved oxygen were 13.2 ± 0.2°C, 1800 ± 200 µS/cm, 7.5 ± 0.01 and 8.0 ± 0.3 mg/L (mean ± S.D.), respectively.

Tanks were connected to a recirculation system (IRTAMAR®) which maintained adequate water quality parameters. Fish were fed at apparent satiation (3.3 percent) with automatic feeders (ARVO-TEC T-Drum-2000TM, Finland). The proximate biochemical composition of diets was 64 percent protein, 12 percent fat and 11 percent ash (2.0 mm pellet size).



All fish from each tank were measured for their body size, and 45 specimens per condition (15 per replicate) sacrificed for histology (size of intestinal villi and number of goblet cells), assessment of digestive system functionality and quantification of the intestinal microbiota by RFLP.

Results

At the end of the trial, fish fed the diet containing the probiotic were slightly significantly heavier and longer (43.9 ± 9.1 g, 14.4 ± 1.1cm) than those fed the control diet (42.5 ± 7.6g, 14.1 ± 1.1; n = 276).

Distribution of size classes in body weight were also affected by the incorporation of the probiotic in the diet.

The frequency of fish belonging to the 51-70g size class was higher in the group fed the probiotic (53.6 ± 1.1 vs. 47.1± 2.2%; t-test, $P < 0.05$; n = 3; see figure 1 and 2), whereas those trouts fed the control diet showed a higher frequency of smaller individuals (6.9 ± 0.5 vs 3.6 ± 0.7%; t-test, $P < 0.05$; n = 3).

The percentage of fish with intermediate weights (41-50 g) was also significantly higher among those fish fed the probiotic (16.2 ± 0.5 vs. 13.0 ± 1.5; t-test, $P < 0.05$; n = 3).

The above-mentioned changes in growth and size classes did not affect the proximal composition of fish fed both diets (protein: 40.0 ± 2.2%, lipids: 20.5 ± 2.0%, ash: 1.8 ± 0.5%).

The inclusion of the probiotic into the control diet did not affect the functionality of the digestive system, as indicated by the absence of significant differences in the specific activity of pancreatic (trypsin, chymotrypsin, total protease) and intestinal brush border (alkaline phosphatase, aminopeptidase-N, maltase) enzymes.

However, the number of goblet cells (1.6 ± 0.1 vs. 1.3 ± 0.2 cells/100 µm; n = 15) and height of villi (928.5 ± 137 vs. 527 ± 130 µm; t-test, $P < 0.001$; n = 15; see figure 3 and 4) in the intestinal mucosa was significantly higher in those fish fed the diet containing the probiotic. Goblet cells, or so-called mucous cells, reside throughout the intestine and are the main source of mucins production in the gut.

Mucins are considered to play important roles in host defense by forming a physical barrier between the host and the contents of the intestinal lumen.

Thus, these results indicated that the inclusion of the probiotic in the diet promoted goblet cell proliferation and possibly the immune response in the intestinal mucosa.

In addition, the intestinal microbiota was also affected by the diet, showing different RFLP results (clades) depending on the tested dietary group. These results indicate that the inclusion of the probiotic in the diet was able to modulate host microbiota, although the molecular techniques used in this study did not allow the identification of the bacterial genus or species.

Beneficial and advantageous

In conclusion, the inclusion of B. cereus var. toyoi at the final concentration of $2*10^4$ UFC/g in a commercial diet promoted growth in rainbow trout fingerlings, as well as the organisation of the intestinal mucosa (number of goblet cells and villi height), whereas did not affect the specific activity of selected pancreatic and intestinal digestive enzymes.

Therefore, the inclusion of this probiotic in trout feeds could be beneficial and advantageous in terms of the fish host, as well as for the intensive production of the species, although more studies are needed to study mode of action of Gram positive bacteria in the gut as well as the correct dosage to administer.

Photograph © Fred Jonny Hammeroe

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