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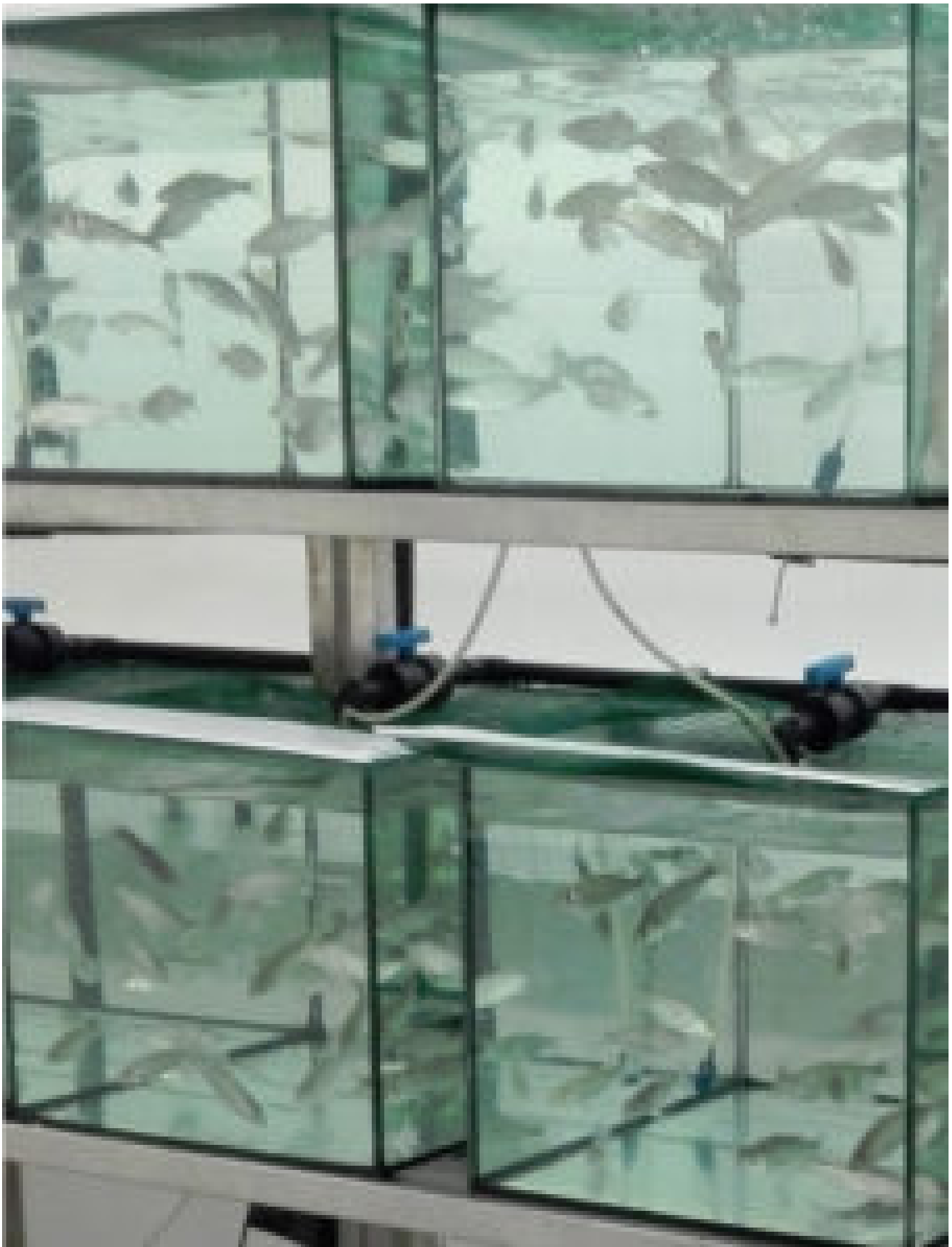
# Prebiotics and their role in sustainable aquaculture

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## Study evaluates effect of commercial product on juvenile European sea bass

The aquaculture industry's promising future will not come without challenges. Sustainability will accompany the goal of long-term profitability. Natural feed additives can make aquaculture production more efficient, reduce the need for medicated treatments and reduce waste discharges while improving farmed fish fillet quality.



Results of a study to evaluate a commercial prebiotic on growth of European sea bass juveniles indicates this product can improve fish growth performance.

## Aquaculture to feed billions

Aquaculture production, which offers a way to meet global demand while reducing pressure on wild capture fisheries, is expected to double by 2050. According to projections from the Food and Agriculture Organization of the United Nations (FAO), farmed raised fish will account for nearly two-thirds of seafood consumption worldwide by 2030. However, the intensification of rearing methods in aquaculture can lead to an overuse of antibiotic therapies and other less environmentally friendly practices.

This is of potential concern in several ways, including 1) development of antibiotic-resistant bacteria; 2) presence of antibiotic residues in seafood; 3) suppression of the organism's immune system that leads to diseases outbreaks; and 4) general environmental impact like the destruction of wild populations (from invertebrates to vertebrates), eutrophication, etc.

## The rise of sustainability

In recent years, several certification schemes were implemented to assess sustainable aquaculture practices and certify aquaculture products. Consumer demand and concerns about the environmental impacts of production are two factors that have driven the industry to increasingly embrace certification. Sustainable aquaculture production is important because it offers a way to feed a growing population and provides the benefits of global economic activity while limiting the environmental impacts. For producers, it can mean higher profitability and can open the door to higher-value markets. Sustainability is a complex topic, and solutions require multiple inputs and diverse perspectives.

## Prebiotics' role in a highly stressful rearing environment

In intensive culture operations, fish are exposed to a series of situations and interactions that are abnormal compared to what they would face in the wild. Temperature changes, manipulation of the photoperiod, salinity, transport, handling, crowding and hypoxia are environmental or anthropogenic factors that cause stress and induce immune suppression. Environmental conditions that induce stress can be the cause of some negative effects in a variety of physiological functions of teleost fishes, such as the immune responses related to the neuroendocrine system. Furthermore, the increased density used to culture fish promotes the proximity and contact between animals, which will favor potential disease outbreaks and the propagation of any pathogenic agents present throughout the rearing area.

Prebiotics are some of the leading candidates for environmentally friendly feed additives in the aquaculture industry. When beneficial bacteria (present in the gastrointestinal tract) ferment prebiotics, the byproducts of the reaction will be used to improve host health. So, applying prebiotics that modify the gastrointestinal conditions to help certain bacterial species that may enhance growth efficiency and reduce the susceptibility to pathogens of the host organism seems to be a very promising way to help support development of the industry.

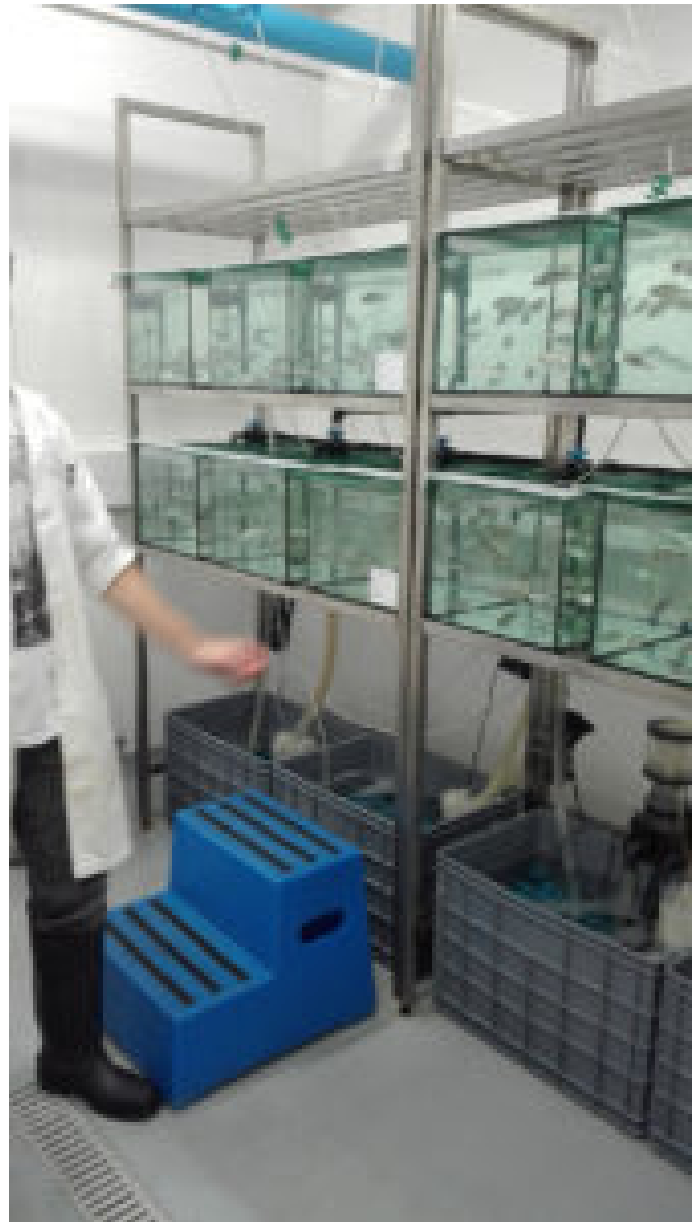
In this study we evaluated the effects of the commercial prebiotic Levabon® Aquagrow E (Biomin®, Austria) on the growth of juvenile European sea bass (*Dicentrarchus labrax*) under stressful conditions, and most specifically how fish responded to crowding events. This commercial prebiotic is composed

of autolyzed yeast (*Saccharomyces cerevisiae*) cells that contain potentially immunomodulatory substances, such as chitin, chitinase, nucleotides, mannan-sugars and manno-proteins.

## Study setup

Twenty-five fish (mean weight of  $11.67 \pm 2.96$  grams) were stocked in 60-liter aquaria, with three replicate aquaria: Control; Control plus Levabon®; Crowding stress; Crowding plus Levabon®. The supplement concentration was 6 g/Kg of feed. Fish were hand fed daily with three meals to apparent satiety based on visual observation of acceptance. The experiment lasted for 10 weeks and three samplings were performed during this period (first day, fifth week, and last day). The following conditions were monitored throughout the experiment: salinity ( $34 \pm 2$  PSU), temperature ( $19.6 \pm 0.4$  degrees-C), ammonia ( $\leq 0.5$  mg/L), nitrites ( $\leq 3$  mg/L), dissolved oxygen ( $7.01 \pm 0.17$  mg/L) above 90 percent saturation level, pH ( $7.5 \pm 0.3$ ), photoperiod (12h light/12h dark), and flow rate (6.6 L/min).

The stress challenges were applied three times a week and consisted of brief crowding of fish in one of the aquarium sides (60 seconds). A rectangular-shaped division with 5-mm holes, allowing water to flow through, was used to reduce the available volume to half of it.



View of the experimental setup used in the study.

## Results and discussion

A tendency for higher growth is visible for groups fed with the prebiotic in comparison to the groups fed without supplementation: control fish, as well as crowding-submitted fish fed with commercial prebiotic, were heavier than their counterparts fed without the supplement (Fig. 1).

This increased growth performance is also reflected in the tendency for higher daily growth index (DGI) and condition index in fish fed with the supplement, especially when considering crowding stress (Fig. 1; Table 1). The positive outcome of the prebiotic on the growth performance of juvenile sea bass might be related to the stimulatory effect of mannan-oligosaccharide (MOS) on the beneficial *Bifidobacteria* and *Lactobacilli*, resulting in better digestion and assimilation.

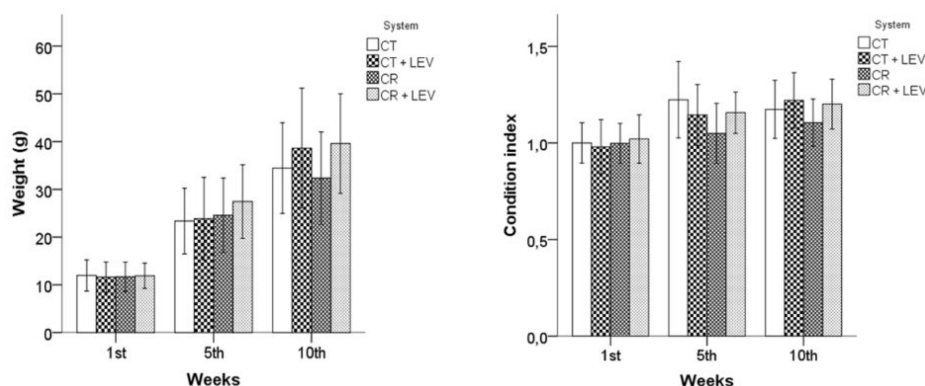


Fig. 1: European sea bass weight (left) and condition index (right) variations throughout the experiment.

Glucose plasma level is a good chronic stress indicator, as it peaks two hours after the stress, being less influenced by the samplings procedures than cortisol. By the fifth week, glucose was lower on fish from the commercial prebiotic treatments, but as time went on and stress was becoming more chronic, glucose rose on stressed fish fed with the supplement (Fig. 2). The cause for this increase is most likely related to the need for more energy in order to cope with the metabolic changes caused by stressful conditions, which is in agreement with the theories from Barton and Iwama (1991) and Wu et al. (2016).

## Passos, prebiotics, Table 1

Parameter	Control	Control + Levabon®	Crowding	Crowding + Levabon®
DGI (% BW/day)	0.70 ± 0.10	0.79 ± 0.07	0.65 ± 0.01	0.65 ± 0.01
FCR	2.11 ± 0.46	1.78 ± 0.32	2.15 ± 0.25	1.73 ± 0.13
VFI (% BW/day)	13.89 ± 0.80	12.90 ± 0.97	14.44 ± 1.11	13.14 ± 0.67

Table 1. Growth performance parameters (DGI - daily growth index; FCR - feed conversion ratio; VFI - voluntary feed intake) of European sea bass.

Alkaline phosphatase activity (ALP) is related to the hydrolysis of phosphate bonds to create phosphate ions that fight the stress condition and aid in coping with the higher metabolic rate. More enzymatic activity may indicate that fish were more stressed and, as such, results showed that fish subjected to crowding conditions were more stressed than control fish by the tenth week, and that from the fifth to the tenth week, sea bass may have grown accustomed to the stress, since its general levels decreased (Fig. 2).

Fig. 2: European sea bass plasma glucose (left) and alkaline phosphatase (right) variation throughout the experiment.

It has already been established that certain nutrients or immunostimulants can be introduced in the diets to modulate fish serum lysozyme activity. In this work, even though not significant, it can be assumed that the bioactive components of the product tested did affect the lysozyme activity to some degree, since both fifth- and tenth-week samplings had the same pattern: the prebiotic treated fish having a tendency for higher lysozyme activity than their counterparts (Fig. 3). Because lysozyme is the strongest bacteriolytic component involved on the humoral defense system, fish that were fed with the prebiotic in this experiment may have had a boosted resistance against bacterial infections.

Fig. 3: European sea bass plasma lysozyme activity throughout the experiment.

Regarding the oxidative stress biomarkers at the tenth week, some specific variations occurred. In the liver tissue, the prebiotic supplementation seems to have lessened lipid damage in the stressful rearing conditions, as seen in the difference between Crowding and Crowding + Levabon® treatments, the latter having less lipid peroxidation (LPO; Fig. 4). Several studies in the literature suggest that crowding stress can disrupt the balance between antioxidant systems and the production of ROS, causing oxidative damages such as LPO.

Fig. 4: European sea bass liver (left) and muscle (right) lipid peroxidation (LPO) levels after the 10-week experiment.

## Conclusions

Results of our study suggest that the fish fed with the commercial prebiotic Levabon® became generally healthier than their counterparts, which were only fed the basal diet. The higher glucose levels may have provided them with the energy to better cope with the stress conditions, and the relatively higher lysozyme could have made them more resilient against potential invasive pathogens.

Also, the mannan-oligosaccharides that make up the product tested may have contributed to an improved response against oxidative stress, resulting in less oxidative damage. All these positive outcomes enabled the fish to spend more energy in their somatic processes, and ultimately with better growth, higher hematocrit and a better condition index.

Based on our results, we believe this commercial prebiotic has potential to be a very profitable supplement to be included in aquaculture feeds, since it intervenes positively in two major aspects of a successful rearing process; and improving growth performance, allowing for the fish to reach the marketable size earlier, boosting the immune status and reducing the potential losses due to pathogens.

*References available from the corresponding author.*

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