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Probiotic in fish: IT effect O34 N fish performance and immunity

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Abstract

Aquaculture provides both protein and income and serves as a substitute for capture fisheries, thereby reducing stress on wild fish populations. Nonetheless, the industry faces several sustainability challenges, such as overreliance on fishmeal, high associated costs, negative environmental impacts, and the potential harm of antibiotics to aquatic ecosystems. To address these concerns, strategies are being developed to improve aquaculture sustainability. One promising approach involves the use of functional feed additives, particularly probiotics, in feed formulations. Probiotics are dietary supplements added to feed to fulfill nutritional needs beyond traditional feed by supporting growth, immunity, and overall health, while also offering economic advantages. This review highlights the role of probiotics as functional feed additives in enhancing fish performance and immune response. It also provides comprehensive insights into the benefits and applications of these additives, their contributions to sustainable aquaculture, and the obstacles faced in their implementation.

Introduction

The nutritional importance of fish feed has contributed to the expansion of fish farming, which is seen as a potential solution to global protein shortages. Advancing fish farming practices requires a deeper understanding of nutrition and the use of feed additives to achieve high yields at low costs with minimal negative impacts **(Onomu *et al.*, 2024).** Key challenges in aquaculture include promoting fish growth and enhancing disease resistance. A broad range of antibiotics and antibacterial, antiparasitic, and growth-promoting agents, such as ionophores and anabolic compounds, originally used for land animals, have been investigated for their potential use in fish farming **(Ansari *et al.*, 2021).**

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Feed represents over 50% of total production expenses in modern intensive aquaculture. Improving feed efficiency—particularly through better nutrient absorption—is critical for cost reduction and profitability **(Lieke *et al.*, 2020).**
Probiotics are live microbial feed supplements known to enhance the health of both fish and land animals. In fish, the gut microbiota is strongly influenced by the surrounding water, making it unique compared to terrestrial animals. Some probiotic products are not originally designed for dietary supplementation but rather for treating rearing environments. However, the idea of probiotics has expanded to include their dietary application, especially when the administered microbes can survive in the fish gastrointestinal tract **(Amenyogbe *et al.*, 2020).**

A poor growth rate might implies that the immune responsiveness would be markedly impaired. Few studies, however, have been concerned with the effect of nutrients on the immune response. A dietary deficiency leads to immune suppression in fish (Van Doan *et al.*, 2020). Inducing a protective immune response to a pathogenic organism before the individual fish becomes naturally exposed to it seems at first sight an eminently sensible way of preventing an infectious. The widespread use of antibiotics for both treatment and prevention poses a significant risk by encouraging the development of antibiotic-resistant bacteria, such as Aeromonas hydrophila, which can infect both fish and humans **(Kim *et al.*, 2018).**

Enhancing feed efficiency, nutrient absorption, and immunity, while reducing mortality rates in fish farms, is a top priority in modern animal production. Strengthening fish immunity to combat various diseases helps lower production costs and boosts fish yields, all while cutting feed expenses. Even minor feed cost reductions can significantly improve aquaculture profitability **(Leistikow *et al.*, 2022).**
Therefore, this study aimed to examine how probiotics affect fish immunity, particularly in combating diseases under Egyptian aquaculture conditions.

Definition of Feed Additives:

Feed additives are not essential nutrients for animals, but their absence can lead to poor productivity and profitability. When properly used, these substances can enhance animal growth and farm performance. In the United States, feed additives are regulated by the Food and Drug Administration (FDA), and adherence to these regulations is critical. Official guidelines are provided in the Feed Additive Compendium. For most users, appropriate and updated information comes directly from manufacturers, especially when additives are already mixed into commercial feeds **(Kim *et al.*, 2018).**

Effect of probiotics as feed additive on productive and economic efficiency.

Interest in using probiotic bacteria in aquaculture arose as part of ongoing efforts to find preventive measures, offering an alternative to chemotherapy and vaccination in controlling aquatic infectious diseases. These beneficial bacteria quickly gained attention for their ability to suppress harmful bacterial strains responsible for fish illnesses (Van Doan *et al.*, 2020).

Definition of probiotics:-

Probiotic bacteria are not just used as food but also serve as biological agents that help manage fish diseases and support nutrient absorption **(Amenyogbe *et al.*, 2020).**
Probiotics are widely recognized as functional feed additives in aquaculture. The term "probiotic" was introduced by (**Parker in 1974)**, who described them as organisms and substances that promote intestinal balance. Although there has been debate over its definition, **Fuller (1989)** refined it to mean "live microbial supplements that benefit the host by improving intestinal health." According to the **FAO and WHO**, probiotics are defined as "live microorganisms which, when administered in adequate amounts, provide health benefits to the host" **(FAO *et al.*, 2001).** These definitions highlight their role in promoting health, improving immunity, and enhancing the overall quality of aquaculture production.

Method of administration of probiotics

Probiotics can be delivered to animals in multiple forms, depending on the intended use and the specific probiotic strain. They can be mixed into pelleted feed or provided as capsules, pastes, powders, or granules, which may be administered directly or through the animals' diet. Continuous feeding is considered the most effective method, as it helps maintain a high probiotic concentration in the gut, allowing them to metabolize and exert their beneficial effects **(Amenyogbe *et al.*, 2020).** Nucleotides derived from RNA have been shown to boost non-specific immune responses in carp (Cyprinus carpio) when administered orally for three consecutive days, leading to improved immune function in the fish **(Van Doan *et al.*, 2020).**

Lactic acid bacteria as probiotic feed additive with special reference to bactocell (*Pediococcus acidilactici*):-

Eissa *et al.* (2022) reported that feeding fish with Lactobacillus casei and Streptococcus salivarius helped protect them from Salmonella typhimurium. The protective effect of L. casei was mainly due to its stimulation of IgA secretion in the intestines. Moreover, Lactobacillus fermentum strain 104r produces substances in its culture fluid that prevent the attachment of enterotoxigenic Escherichia coli K88.Also, **Anadón *et al.* (2019)** also confirmed that probiotics are safe and can be readily used in all fish farms and hatcheries. For example, they examined the probiotic potential of seven lactic acid bacteria species for human and animal use by evaluating their ability to adhere to and penetrate fish mucus, as well as their ability to resist fish pathogens and tolerate fish bile. One of these strains, Lactobacillus rhamnosus, showed positive effects against Furuncolosis in rainbow trout **(Büyükdevici *et al.*, 2018).**

Pediococcus acidilactici (marketed as Bactocell) is considered a valuable probiotic for fish larvae due to its role in promoting growth **(EFSA, 2019).**

The most favorable feed conversion ratios (FCR) were observed when Biogen® probiotics were added to diets. These probiotics enhanced the efficiency of feed utilization. When used in diets for tilapia fingerlings, the commercial probiotic additives significantly ($P<0.05$) improved feed efficiency. This means less feed was needed to support animal growth, which can lower production costs. The reduction in feed cost per kilogram of weight gain was most noticeable with higher levels of Biogen® (0.1%) in diets for mono-sex fingerling Nile tilapia **(Kim *et al.*, 2018).**

The use of Pediococcus acidilactici (Bactocell) has also shown promising outcomes in enhancing the survival and development of shrimp larvae by boosting their disease resistance. However, further research is required to better understand how Pediococcus acidilactici interacts with harmful bacteria. So far, its addition to feed has shown a clear positive effect **(EFSA, 2016).**

In probiotics, the bacteria produce lactic acid there by lowering the pH that weakens the growth of most pathogenic bacteria and favors acid producers. Lactic acid bacteria are characterized as Gram positive usually non-motile, non-sporulating, bacteria that produce lactic acid as a major or sole product of fermentative metabolism **(Büyükdeveci *et al.*, 2018)**.

EFSA (2019) demonstrated that the addition of Bactocell (Pediococcus acidilactici MA 18/5M) in fish food improves the quality of the final animal product. Bactocell has also been shown, through a number of trials reported here and in other dossiers submitted previously for authorisation, to be safe both for the handler of the additive, the environment and the target animal species. The Food and Drug Administration (FDA) considers Pediococcus acidilactici, the active substance in Bactocell, as Generally Recognized as Safe (GRAS). The use of Bactocell (Pediococcus acidilactici MA 18/5M) as a feed additive is therefore considered safe for animals and the environment with no apparent concerns regarding worker and consumer safety. The results showed that feeding compound feed supplemented with *Pediococcus acidilactici* MA 18/5M at the minimum inclusion rate of 10^9cfu/kg feed significantly ($P < 0.05$) improved the final fish quality **(Büyükdeveci *et al.*, 2018).**

Mechanism of action of probiotics:-

Effective probiotics should remain stable and viable for extended periods during storage and under field conditions **(Amenyogbe *et al.*, 2020).** Common mechanisms through which probiotics act include competitive exclusion, where they inhibit harmful pathogens by producing inhibitory substances or by competing for nutrients and binding sites in the digestive tract. They can also modify microbial metabolism by altering enzyme activity levels and stimulate both humoral and cellular immune responses. In addition, probiotics can kill harmful bacteria by converting lactose into lactic acid, which lowers the pH, and by producing hydrogen peroxide that suppresses Gram-negative bacterial growth. It has also been found that lactic acid-producing bacteria, such as Streptococcus and Lactobacillus species, may generate antibiotics or antibodies **(Büyükkardeş *et al.*, 2018).**

Non-pathogenic bacteria have successfully been encapsulated in rotifers and Artemia using short-term enrichment methods. Some probiotic strains are capable of inhibiting the growth of bacterial pathogens. In fact, two strains similar to Lactobacillus were shown to boost the population of rotifers. Moreover, growth enhancement can be achieved by applying bacterial additives under co-culture and low-density conditions **(Van Doan *et al.*, 2020).**

Impact of Probiotics on Absorption, Metabolism, and Feed Distribution in Fish

The gastrointestinal (GI) tract in fish and shellfish is populated by both native (autochthonous) and non-native (allochthonous) bacteria. These microbial communities play a key role in promoting metabolic functions, supporting GI tract development, enhancing digestion, boosting the immune system, and defending against harmful bacteria and diseases. They also influence the development of metabolic syndromes, contribute to host adaptability, vitamin production, and overall health. The gut's immune defenses operate through three main mechanisms: (i) gut barrier integrity, (ii) innate immunity, and (iii) acquired or adaptive immunity, all of which collaborate to strengthen disease resistance **(Li *et al.*, 2019).**

According to **Ringø *et al.* (2022),** gut microbiota and probiotics influence lipid, carbohydrate, protein, and amino acid metabolism in fish and shrimp. Among the various models, zebrafish (Danio rerio) is the most widely used for probiotic research. Findings show probiotics regulate glucose levels, reduce fat accumulation, increase short-chain fatty acids, and reduce the expression of genes related to oxidative stress. Probiotics also contribute to the lengthening of intestinal villi and increase the expression of nutrient transporters in fish intestines.

This review evaluates how intestinal microbes and probiotics affect nutrient metabolism and immune response in fish and shrimp under stress. It also incorporates relevant data from warm-blooded animals for a broader understanding. Differences in probiotic response may be linked to the distinct roles of upper and lower epithelial tissues in food intake and chemical signaling.

<u>Impact of Probiotics on Growth</u>

Using Lactobacillus delbrueckii delbrueckii as a probiotic has shown beneficial effects on the welfare and growth of young sea bass. These probiotics helped lower cortisol levels in the treated fish and influenced the activity of genes related to growth, such as IGF-I and MSTN. Specifically, IGF-I expression increased, while MSTN (which inhibits growth) expression was reduced in the treated group. These genetic changes led to a notable increase in body weight among the treated fish.

All above- mentioned changes resulted in a sharp increase of body weight of treated animals and so minimize the costs, increase production and profit. These results may be of great importance for the development of an environment-friendly aquaculture as well as a valid alternative to the use of drugs and antibiotics in marine aquaculture **(Li *et al.*, 2019).**

Bacterial probiotics such as Lactobacillus gasseri PI41 and Lacticaseibacillus rhamnosus PI48could answer the requirement of controlling potential pathogens and protecting fish health, preventing them from diseases, increasing the survival rates and welfare of reared fish so improve fish production and economic efficiency **(Torres-Maravilla *et al., 2024*).**

<u>Impact of probiotics on feed utilization:-</u>

Shao *et al.* *(2018)* studied the growth rate, the content of growth rate and the activity of digestive enzyme in *Litopenaeus vannamei* under six feeding regimens which included either of freshly hatched Artemia naupil, an artificial diet and algae or their combinations. The result reported that trypsin-like activity was higher (up to 10 times) in post larval fed Artemia and an artificial diet either alone or plus algae. The probiotics improved utilization of feed which may be achieved by increased efficiency of existing digestive processes or by promoting the digestion of previously indigestible substances.

Effect of probiotics on fish immunity

Probiotics are known to aid in preventing diseases and enhancing immunity in fish. For instance, when European sea bass (D. labrax) were given a diet supplemented with B. velezensis at 10^7 CFU/g for 30 days, they showed increased serum antibacterial and lysozyme activity as well as nitric oxide levels compared to fish infected with V. anguillarum **(Monzón-Antienza *et al.*, 2022).**

Fish receiving B. velezensis supplements were in better health than the unsupplemented control group, as indicated by blood-related immune indicators. Similarly, fish exposed to Vibrio anguillarum showed higher survival rates when fed B. velezensis compared to controls. Abalone (Haliotis discus hannai) fed with B. licheniformis displayed enhanced immune cell counts and nitric oxide levels compared to controls. Those fed doses of 10^8 and 10^7 CFU/mL had stronger phagocytic responses than those fed 10^6 CFU/mL or untreated. After V. parahaemolyticus infection, these abalone had better immune responses and lower death rates. However, fish fed with lower doses (10^8 CFU/mL) experienced greater mortality **(Gao *et al.*, 2018).**

Alagawany *et al.* (2020) suggested that the performance of birds can be improved by supplementing amino acids like methionine, which is often deficient in animal and poultry feeds. Increased lymphoid organ weight may result from better body growth due to methionine, possibly boosting lymphocyte production and increasing weights of thymus, spleen, and bursa.

Reda *et al.* (2020) found that raising methionine and lysine levels led to improved blood parameters and organ weights at 21 days. However, adding methionine and lysine didn't significantly affect hematocrit, and actually lowered uric acid levels **(Gao *et al.*, 2018).**

Singh and Rani (2019) examined the role of methionine-enkephalin (Met-enk) in fish, which is well studied in mammals but less explored in cold-blooded animals. Their research showed that Met-enk influences immune functions like phagocytosis and spleen activity in freshwater fish. Met-enk enhanced both phagocytic and respiratory burst activity, although its effect varied by concentration and time of exposure, being most effective after 10 days but least effective in boosting superoxide production at the same concentration after a month.

Choudhury and Kamilya (2019) reported that combining multiple probiotic strains (like Aeromonas hydrophila, Carnobacterium species, etc.) was helpful in controlling Furunculosis in rainbow trout. Probiotics such as B. subtilis and B. licheniformis (Bioplus 2B) could aid in treating bacterial infections like Yersinia ruckeri.

Gonçalves *et al.* (2022) suggested that oral vaccination of fish with vibrio vulnificus bacterin without a coating of acid resistant film was a very effective method to prevent the infection of fish with vibrio vulnificus in the aquaculture industry and there by prevent an outbreak of vibrio septicemia in humans. The inhibitory effect of two probiotics bacterial strains, (*Enterococcus faecium* SF68 and *Bacillus toyoi*), which were isolated from the commercial products in reducing *Edwardsiella tarda* in cultured European eel (*Anguilla anguilla L*). They found that E. faecium SF68 suppressed the growth of E. tarda in vitro; only of its initial inoculums was much higher than that of E. tarda.

Chan *et al.* (2024) investigated three probiotic strains—Lacticaseibacillus rhamnosus FS3051, Limosilactobacillus reuteri FS3052, and Bacillus subtilis natto NTU-18—for their effectiveness against N. seriolae and their enzyme-secreting capabilities in laboratory tests. They divided 144 grey mullet into four groups: a control group and three groups each receiving one of the probiotics. After being fed these diets for 28 days, the fish were examined for immune gene activity and short-term growth, then exposed to N. seriolae. Their survival was monitored for 35 days.

The study also looked at the gut microbiota to understand its role in growth and resistance to infection. Findings revealed that L. rhamnosus FS3051 and L. reuteri FS3052 successfully suppressed N. seriolae, whereas B. subtilis NTU-18 did not. The probiotics also demonstrated the ability to produce hydrolytic enzymes. Fish fed with probiotics showed better weight gain, feed efficiency, and specific growth rate—especially those fed B. subtilis NTU-18. Probiotic treatment, particularly with L. rhamnosus FS3051, boosted immune gene activity including IL-8, IL-1β, TNF-α, IFN-γ, and MHCI. Survival rates improved significantly in fish fed L. rhamnosus. Moreover, this strain positively influenced gut microbiota by increasing beneficial bacteria such as Lactobacillus, and reducing harmful types like Mycoplasma and Rhodobacter, ultimately enhancing disease resistance and gut health in the grey mullet.

References

Amenyogbe, E.; Chen, G.; Wang, Z.; Huang, B. and Li, H. (2020). The Exploitation of Probiotics, Prebiotics and Synbiotics in Aquaculture: Present Study, Limitations and Future Directions: A Review. Aquac. Int. 2020, 28, 1017–1041.

Alagawany, M.; Elnesr, S.S.; Farag, M.R.; Tiwari, R.; Yatoo, M.I.; Karthik, K.; Michalak, I. and Dhama, K. (2020). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health - a comprehensive review. Vet Q. 2020 Dec 7;41(1):1-29.

Anadón, A.; Ares, I.; Martínez-Larrañaga, M.R. and Martínez, M.A. (2019). Prebiotics and Probiotics in Feed and Animal Health. In Nutraceuticals in Veterinary Medicine; Gupta, R., Srivastava, A., Lall, R., Eds.; Springer: Cham, Switzerland, 2019; pp. 261–285.

Ansari, F.A.; Nasr, M.; Rawat, I. and Bux, F. (2021). Artificial neural network and techno-economic estimation with algae-based tertiary wastewater treatment. Journal of Water Process Engineering, 40, Article 101761

Büyükdeveci, M.E.; Balcázar, J.L.; Demirkale, İ.; Dikel, S. (2018). Effects of Garlic-Supplemented Diet on Growth Performance and Intestinal Microbiota of Rainbow Trout (Oncorhynchus mykiss). Aquaculture 2018, 486, 170–174.

Chan, C.H.; Chen, L.H. and Chen, K.Y. (2024). Single-strain probiotics enhance growth, anti-pathogen immunity, and resistance to *Nocardia seriolae* in grey mullet (*Mugil cephalus*) via gut microbiota modulation. anim microbiome **6**, 67 (2024). https://doi.org/10.1186/s42523-024-00353-0.

Choudhury, T.G. and Kamilya, D. (2019). Paraprobiotics: an aquaculture perspective. Rev.916. Aquacult. 11, 1258-1270.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), (2016). Scientific opinion on the safety and efficacy of Bactocell PA (*Pediococcus acidilactici* CNCM MA 18/5M) for pigs for fattening, minor porcine species, chickens for fattening and minor avian species. EFSA Journal 2016;14(6):4483, 15 pp

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Bampidis, V.; Azimonti, G.; de Lourdes Bastos, M.; Christensen, H.; Dusemund, B.; Kouba, M.; Kos Durjava, M.; López-Alonso, M.; López Puente, S.; Marcon, F.; Mayo, B.; Pechová, A.; Petkova, M.; Ramos, F.; Sanz, Y.; Villa, R.E.; Woutersen, R.; Anguita, M.; Galobart, J.;

Manini, P.; Pizzo, F.; Tarrés-Call, J. and Holczknecht, O. (2019). Assessment of the application for renewal of authorisation of Bactocell® (*Pediococcus acidilactici* CNCM I-4622) as a feed additive for weaned piglets, pigs for fattening, minor porcine species (weaned and for fattening), chickens for fattening, laying hens and minor avian species for fattening and for laying and its extension of use to all growing pigs and all avian species. EFSA J. 2019 May 15;17(5):e05690.

Eissa, E.S.H.; Baghdady, E.S.; Gaafar, A.Y.; El-Badawi, A.A.; Bazina, W.K.; Abd Al-Kareem, O.M.; Abd El-Hamed, N.N.B. Assessing the Influence of Dietary *Pediococcus Acidilactici* Probiotic Supplementation in the Feed of European Sea Bass (Dicentrarchus labrax L.) (Linnaeus, 1758) on Farm Water Quality, Growth, Feed Utilization, Survival Rate, Body Composition, Blood Biochemical Parameters, and Intestinal Histology. Aquac. Nutr. 2022, 2022, 1–11.

FAO/WHO (2001). Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria; Food and Agriculture Organization of the United Nations: Rome, Italy, 2001.

Fiorentini, M.; Kinchla, A.J. and Nolden, A.A. (2020). Role of Sensory Evaluation in Consumer Acceptance of Plant-Based Meat Analogs and Meat Extenders: A Scoping Review. Foods 2020, 9, 1334.

Fuller, R. (1989). Probiotics in Man and Animals. J. Appl. Bacteriol. **1989**, 66, 365–378.

Gao, X.; Zhang, M.; Li, X.; Han, Y.; Wu, F. and Liu, Y. (2018). Effects of a Probiotic (*Bacillus licheniformis)* on the Growth, Immunity, and Disease Resistance of Haliotis Discus Hannai Ino. Fish Shellfish Immunol. 2018, 76, 143–152.

Gonçalves, G.; Santos, R.A.; Coutinho, F.; Pedrosa, N.; Curado, M.; Machado, M.; Costas, B.; Bonneville, L.; Serrano, M.; Carvalho, A.P.; Díaz-Rosales, P.; Oliva-Teles, A.; Couto, A. and Serra, C.R. (2022). Oral vaccination of fish against vibriosis using spore-display technology. Front Immunol. 2022 Oct 13;13:1012301.

Kim, Y.; Keogh, J.B. and Clifton, P.M. (2018). Probiotics, prebiotics, synbiotics and insulin sensitivity. Nutr. Res. Rev. 2018, 31, 35–51.

Leistikow, K.R.; Beattie, R.E. and Hristova, K.R. (2022). Probiotics beyond the Farm: Benefits, Costs, and Considerations of Using Antibiotic Alternatives in Livestock. Front. Antibiot. 2022, 1, 1003912.

Li, X.; Ringø, E.; Hoseinifar, S.H.; Lauzon, H.; Birkbeck, H. and Yang, D. (2019). Adherence and colonisation of microorganisms in the fish gastrointestinal tract. Rev. Aquac. 2019;11:603–618.

Lieke, T.; Meinelt, T.; Hoseinifar, S.H.; Pan, B.; Straus, D.L. and Steinberg, C.E. (2020). Sustainable aquaculture requires environmental friendly treatment strategies for fish diseases. Rev. Aquac., 12: 943-965.

Monzón-Atienza, L.; Bravo, J.; Fernández-Montero, Á.; Charlie-Silva, I.; Montero, D.; Ramos-Vivas, J.; Galindo-Villegas, J. and Acosta, F. (2022). Dietary Supplementation of Bacillus Velezensis Improves *Vibrio anguillarum* Clearance in European Sea Bass by Activating Essential Innate Immune Mechanisms. Fish Shellfish Immunol. 2022, 124, 244–253.

Onomu, A.J. and Okuthe, G.E. (2024). The Role of Functional Feed Additives in Enhancing Aquaculture Sustainability. Fishes 2024, 9, 167. https://doi.org/10.3390/fishes9050167.

Parker, R.B. (1974). Probiotics, the Other Half of the Antibiotic Story. Anim. Nutr.

Health 1974, 29, 4–8.

Reda, F.M.; Swelum, A.A.; Hussein, E.O.S.; Elnesr, S.S.; Alhimaidi, A.R. and Alagawany, M. (2020). Effects of Varying Dietary DL-Methionine Levels on Productive and Reproductive Performance, Egg Quality, and Blood Biochemical Parameters of Quail Breeders. Animals (Basel). 2020 Oct 9;10 (10):1839.

Ringø, E.; Harikrishnan, R.; Soltani, M. and Ghosh, K. (2022). The Effect of Gut Microbiota and Probiotics on Metabolism in Fish and Shrimp. Animals 2022, 12, 3016.

Shao, J.; Zhao, W.; Liu, X. and Wang, L. (2018). Growth Performance, Digestive Enzymes, and TOR Signaling Pathway of *Litopenaeus vannamei* Are Not Significantly Affected by Dietary Protein Hydrolysates in Practical Conditions. Front Physiol. 2018 Aug 14; 9: 998.

Singh, R. and Rai, U. (2009). Delta opioid receptor-mediated immunoregulatory role of methionine-enkephalin in freshwater teleost Channa punctatus (Bloch.). Peptides. 2009 Jun;30(6):1158-64.

Torres-Maravilla, E.; Parra, M.; Maisey, K.; Vargas, R.A.; Cabezas-Cruz, A.; Gonzalez, A.; Tello, M. and Bermúdez-Humarán, L.G. (2024). Importance of Probiotics in Fish Aquaculture: Towards the Identification and Design of Novel Probiotics. Microorganisms, *12*(3), 626. https://doi.org/10.3390/microorganisms12030626.

Van Doan, H.; Hoseinifar, S.H.; Tapingkae, W.; Seel-Audom, M.; Jaturasitha, S.; Dawood, M.A.; Wongmaneeprateep, S.; Thu, T.T.N. and Esteban, M.Á. (2020). Boosted Growth Performance, Mucosal and Serum Immunity, and Disease Resistance Nile Tilapia (Oreochromis niloticus) Fingerlings Using Corncob-Derived Xylooligosaccharide and Lactobacillus Plantarum CR1T5. Probiotics Antimicrob. Proteins 2020, 12, 400–411.

Verschuere, L.; Rombaut, G.; Sorgeloos, P. and Verstraete, W. (2000). Probiotic Bacteria as Biological Control Agents in Aquaculture. Microbiol. Mol. Biol. Rev. 2000, 64, 655–671. [Google Scholar] [CrossRef].