ENHANCING AQUACULTURE HEALTH AND PERFORMANCE: EXPLORING THE APPLICATIONS OF PROBIOTICS AND PREBIOTICS

Abstract

Aquaculture is an increasingly vital component of global food production, providing a significant source of seafood for human consumption. Maintaining a healthy microbial community in the water and the digestive tracts of aquatic species is crucial for the well-being and efficiency of aquaculture systems. Probiotics and prebiotics have shown promise in supporting the health, growth, and disease resistance of aquaculture species. This chapter reviews the uses of probiotics and prebiotics in aquaculture, emphasising their potential advantages, modes of action, and usefulness. Probiotics are used to modulate the intestinal microbiota of fish and shellfish. These include beneficial microorganisms such as lactic acid bacteria, Bacillus species, and yeasts. They support better growth performance overall, illness resistance, and enhanced digestion and nutrition absorption. Probiotics can also improve water quality by competitively excluding harmful microbes, which lessens the need for antibiotics. In contrast, prebiotics function as substrates that specifically promote the development and activity of advantageous microbes within the gastrointestinal tract. Inulin, fructooligosaccharides (FOS), and mannooligosaccharides (MOS) are common prebiotics used in aquaculture. These substances improve the general health of aquatic species' guts by encouraging the growth of probiotic bacteria. The continued research and development of prebiotics and probiotics in aquaculture will contribute to more sustainable and efficient fish and shellfish production, ultimately benefitting the industry and consumers.

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1. Introduction

Aquaculture, currently the world's fastest-growing food production, is expanding and intensifying in new directions. Numerous difficulties arise as aquaculture production becomes more intensive and commercialised. These include managing water quality, developing suitable feedstuffs and feeding mechanisms, improving and domesticating broodstock, and fighting diseases and epizootics. Of these, disease outbreaks are one of the major issues affecting aquaculture productivity, impeding social and economic advancement in many nations (Qi *et al.*, 2009). Many antimicrobial agents, including antibiotics and chemotherapeutics, have been employed to address this issue. However, antibiotics use during fish production can lead to other problems such as bacterial drug resistance, food safety, and water contamination, and its accumulation may be detrimental. This means that creating a different approach to managing infections brought on by various pathogenic agents, including bacteria, viruses, parasites, and many more, is necessary. In aquaculture, a range of helpful feed additives, such as probiotics, prebiotics, synbiotics, and other immunostimulants with positive effects on the host, have been used to improve their growth (size and weight gain), to combat diseases by providing supplements that also act as an alternative antimicrobial compounds (Irianto & Austin, 2002), as well as to stimulate immunity response of the host.

Prebiotics are non-digestible forage supplements that increase the activity or quantity of good gut bacteria, whereas probiotics are live microbial feed additives that alter gastrointestinal microbial ecosystems. Both of these have drawn much attention, as they demonstrate aquatic animals' improved production, health and disease resistance (Dimitroglou *et al.,* 2011). Aquaculture has extensively used their application to manage disease, boost immune response, contribute nutritionally and enzymatically to the host's digestion, and improve water quality. Probiotics and prebiotics, which inhibit bacteria via a number of methods, are being considered as potential antibiotic substitutes. This chapter retrieved the studies on probiotics and prebiotics and evaluated their further applications in aquaculture.

2. Probiotics

Probiotics are becoming morewell-recognised as a different kind of preventative medicine that can be used to treat diseases linked to pathogens in humans and animals and for preventive purposes. The term probiotic means life it was derived from two Greek words “pro” and “bios” (Gismondo, *et al*., 1999). Probiotics are living bacteria that enhance the microbial balance and growth efficiency of the host's intestinal tract.

2.1 Probiotics Definitions and History

Since there is no official definition of probiotics that might undermine the legitimacy of probiotic products on the market, the scientific definition of the term has been up for controversy for many years. The host’s gastrointestinal (GI) tract has been the primary focus of probiotic research, while applications to other organs or host surfaces have also been somewhat studied. In the general term, probiotics are considered as the live microbial feed supplements that improve the intestinal balance of the host animal, which has a positive effect on it (Makridis *et al*., 2005).

Probiotics are the exact opposite of "antibiotics," having been coined by Lilley and Stillwell (1965) to refer to chemicals generated by one bacterium that supported the growth of another (Fuller, 1989). Later, Sperti utilised it to characterise "tissue extracts which stimulated microbial growth" in 1971 (Fuller, 1992). The name "probiotic" was originally used by Parker in 1974 and comes from the Greek word "Biotikos," which means "for life" (Gismondo *et al*., 1999). "Organisms and substances which contribute to intestinal microflora" is how he characterised probiotics (Fuller *et al*., 1992). The preposition pro, which in Latin means "for" or "in front of/before" in Greek, and the Greek word biotic, which refers to the noun bios, which means "life," are combined to form the term probiotic. Although the general meaning is that it is "for life," it is also possible to interpret it to mean that a probiotic is a substance that supports life and increases vitality when activated in the environmen.

The term "viable mono- or mixed culture of microorganisms, which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microbiota" was later expanded by Havenaaer and Huis in't Veld (1992) with reference to the host and habitat of the microbial flora. As defined by Moriarty (1998), probiotics in the context of aquaculture are defined as bacteria that, when added to water, enhance its quality or prevent disease growth. As "microbial cells administered in a certain way, which reaches the gastrointestinal tract and remain alive to improve health," (Gatesoupe, 1999) was how they were described.

Despite these conclusions, a panel of experts agreed upon the concept of "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" in a 2002 FAO/WHO report. The term "direct-fed microbials" was used in 1988 by the US Food and Drug Administration (FDA) to describe live microorganisms used in animal feed.

2.2 Key Facts about Probiotic

1. "Probiotika" is the word from which "probiotic" originated.
2. Term Probiotic have been coined by – Lilly and Stillwell (Fuller, 1989)
3. Elic Hetchnikoff's conducted the initial studies on probiotics.
4. *Lactobacillus* sp. was the first probiotic to be identified. (Sahu *et al*., 2008)

2.3 Probiotics Microorganisms

The lactic acid-producing bacteria *Lactobacillus* sp. was the first probiotic to be found in history. During the 1980s, *Lactobacillus*, *Streptococcus*, *Bacillus*, and *Saccharomyces* spp. were the most widely used bacterial and yeast genera in probiotics for animal feed.

Thereafter, many probiotics such as *Aeromonas hydrophila*, *A. media*, *Altermonas* sp., *Bacillus subtilis*, *Carnobacterium inhibens*, *Debaryomyces hansenii*, *Enterococcus faecium*, *Lactobacillus helveticus*, *L. plantarum*, *L. rhamnosus*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Roseobacter* sp., *Streptococcus thermopilus*, *Saccharomyces cerevisiae*, *S.*

exiguous, *Vibrio alginolyticus*, *V. fluvialis*, *Tetraselmis suecica*, and *Weissella helenica* were considered for use in aquaculture.

2.4 Probiotic Selection Criteria

Diverse viewpoints from numerous scholars have been published regarding the crucial traits for choosing probionts for aquaculture applications (Spanggaard *et al*. 2001; Balcázar *et al*. 2006). These qualities were compiled and expanded upon by Merrifield et al. (2010) to create the following extensive set of criteria (where F denotes a desirable criterion and E an essential criterion). The probiont criterion are listed here (table 1):

Sl.No.	Favourable criterion (F)	Essential criterion (E)
1.	need to be accepted as safe for usage as an ingredient in feed	must be able to withstand low pH and bile salts
2.	should be able to colonise and attach to the surface of the intestinal epithelium	must not be pathogenic
3.	should have hostile characteristics against one or more important infections	must be devoid of antibiotic resistance genes encoded by plasmids

2.5 Types of Probiotics

The different types of probiotics are tabulated below in the table 2: (Nageswara and Babu, 2006)

Water probiotics	Gut probiotics
It can multiply in an water medium, using up all the available nutrients to keep the harmful bacteria out	It can be added to meals and taken orally to improve the beneficial microbiota in the gut

2.6 Mechanisms of Probiotics

Probiotics have only recently been used in aquaculture, which is a novel idea in comparison to its usage in mammals and other terrestrial livestock including cattle, pigs, and poultry. The mechanisms via which probiotics regulate microbiota, increase feed efficiency, or provide disease resistance have been the subject of several investigations.

1. **Competition for Adhesion Sites:** Pathogenic microorganisms cannot colonise the digestive tract due to competitive adhesion. The individual probiotics and infections, as well as the mucosal location, appear to affect the capacity to suppress pathogen adherence (Collado *et al*., 2007).
2. **Colonization Capacity:** Probiotics cling to and proliferate in intestinal mucus, inhabiting and colonising digestive tracts, especially the GI mucosal epithelium (Merrifield *et al*., 2010a).
3. **Antagonistic Activity**: Probiotics are employed in place of preemptive use of chemicals and antibiotics. To fight for location and nutrition, they created antibiotic-like chemicals

(Moriarty, 1998). Probiotics caused a pH decrease and enough organic acid production to combat numerous harmful bacteria.

4. **Bactericidal Activity:** By fermenting lactose to lactic acid, lactobacilli lower the pH to a level that is intolerant to pathogenic bacteria. Additionally, hydrogen peroxide is generated, which stops gram-negative bacteria from growing. Antibiotics are also said to be produced by lactic acid-producing bacteria belonging to the Lactobacillus and Streptococcus species (Klose *et al.,* 2010).
5. **Elevate Health Status and Disease Resistance:** Probiotics are a good means of preventing infections and have been demonstrated to be resistant to disease. Probiotics are essential for building immunity against infectious diseases and for generating antibacterial substances that keep harmful bacteria out of organisms.
6. **Produces Inhibitory Substances:** The growth of pathogens in the intestine may be inhibited by the molecules that probiotics create. Competition for nutrients that support the growth of probiotic strains or the expression of their inhibitory effects in the gastrointestinal tract can lead to antagonistic relationships (Gatesoupe, 1999).

2.7 Mode of Administration of Probiotics

Probiotics can be given intravenously through feed, albeit binders may occasionally be needed for stabilisation (Kolndadacha *et al.,* 2011). Probiotics are frequently added to feed in aquaculture with the goal of introducing live probiotic cells into the host animal's gut to create a balanced gastrointestinal microbial flora, enhance immune system responses, and improve digestion.

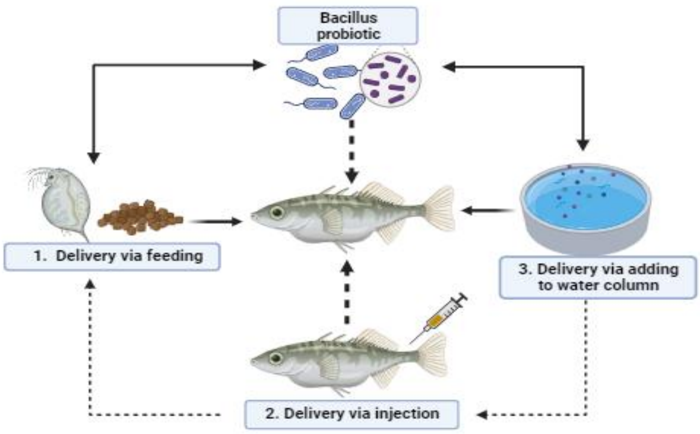


Figure 1: Mode of administrations (created with Biorender.com)

Furthermore, probiotics have been added straight to culture ponds to enhance water quality and increase the longevity of the animals housed there (Boyd and Cross, 1998; Moriarty, 1998). The microbial ecology of the water and sediment is enhanced by bioaugmentation or biocontrol mechanisms, which account for the effectiveness of probiotics (Rengpipat, 2005).
Additionally, fish immune responses against bacterial pathogenic infections have been boosted by injecting probiotic items into aquatic animals (Anderson and Siwicki, 1994). Because the probiotics from the host animal activate the immune system by encouraging the development of antibodies, freeze-dried probiotics can also be employed as immunisations in

fish (Austin *et al.*, 1995). However, administering probiotics to a large number of fish in this manner and injecting them into cultured fish can be challenging, particularly in small animals.

2.8 Application of Probiotic in Aquaculture

Table 3: Studies on different applications of probiotics in aquaculture (Martínez Cruz *et al*., 2012)

Application	Probiotic bacteria	aquatic species	References
Pathogen inhibition	*Bacillus sp.* *Pseudomonas fluorescens*	Penaeids *Oncorhynchus mykiss*	Moriarty, 1998 Gram *et al*., 1999
Growth promoter	*Lactobacillus helveticus* *Bacillus* sp.	*Scophthalmus maximus* Catfish	Gatesoupe, 1999 Queiroz *et al*., 1998
Stress Tolerance	*Bacillus sp.*	*Penaeus monodon*	Shishehchian *et al*., 2001
Water quality	*Lactobacillus acidophilus*	*Clarias gariepinus*	Dohail *et al*., 2009

2.9 Benefits of Probiotic in Aquaculture

1. It is cost effective.
2. Animals fed probiotic feed exhibit higher growth performance than animals fed regular diets (Mclntosh *et al.*, 2000).
3. By denaturing the possibly indigestible components of the food via hydrolytic enzymes like amylase and protease, probiotics may also help detoxify the potentially toxic substances in feeds.
4. Probiotics lower the amount of feed required for animal growth, which lowers production costs and, as a result, feed conversion ratio and utilisation (Ouwehand *et al.*, 2002).
5. Probiotics may also increase appetite and enhance nutrition through the synthesis of vitamins, the removal of harmful substances from the diet, and the breakdown of indigestible substances (Abd El-rhman *et al.*, 2009).
6. When animals are provided an enhanced diet containing probiotics, their protein digestibility increases.
7. Probiotics prevent pathogen colonisation and have a positive impact on the growth and stability of the host's natural microbiota.
8. Probiotics can also be a suitable substitute for antibiotics in the management of intestinal infections or in alleviating the symptoms of antibiotic-associated diseases. They can also speed up growth and improve feed conversion efficiency (Young-Hyo *et al.*, 2001).

2.10 Constraints of Probiotic in Aquaculture

1. The strains' inability to be generated commercially and, as a result, their large-scale demonstration.

2. The challenge of demonstrating performance at the farm level.
3. The incapacity of businesses to carry out in-depth study on how to create products especially for aquaculture.

2 Prebiotics

Prebiotics are substances found in some foods that encourage the development and activity of bacteria and fungus, as well as other helpful microorganisms, in the gastrointestinal system. These substances are critical for immunological response, digestion, general health, and preserving a balanced gut flora.

Prebiotics are specific plant fibres that aid in promoting the development and activity of good bacteria in the digestive system. In contrast to probiotics, which are live microorganisms (such as yeasts or beneficial bacteria) that you can eat, prebiotics are indigestible substances found in various foods. Prebiotics feed the good bacteria in your stomach by entering the colon undamaged after consumption. The primary role of prebiotics is to help maintain a healthy balance of the gut microbiota, which can have numerous positive effects on overall health. They serve as a food source for these beneficial bacteria and help support a healthy balance of the gut microbiota. Prebiotics are typically types of dietary fiber, including:

1. **Inulin:** Inulin is a naturally occurring polysaccharide found in various plants, such as chicory root, garlic, and onions. It's commonly used as a prebiotic ingredient in various food products.
2. **Fructooligosaccharides (FOS):** FOS are short-chain carbohydrates that occur naturally in foods like bananas, artichokes, and asparagus.
3. **Galactooligosaccharides (GOS):** GOS are another type of prebiotic found in foods like legumes and certain grains.
4. **Resistant Starch:** Resistant starch is a type that resists digestion in the small intestine. It can be found in green bananas, uncooked potatoes, and legumes.

The human body does not digest prebiotics; instead, they are fermented by gut bacteria in the colon after passing through the stomach and small intestine. Short-chain fatty acids (SCFAs), which are produced during this fermentation process, provide a number of health advantages, including better immune system performance, gastrointestinal health, and possible weight management impacts.

Eating a diet high in foods high in prebiotics might encourage the growth of good bacteria in the gut, such as Lactobacillus and Bifidobacterium, which can enhance gut health in general and possibly have a positive impact on several human health issues. Prebiotics and probiotics—live, helpful microorganisms—are frequently used together to support a healthy gut microbiota.

3.1 The Mode of Action of Prebiotics Involves Several Key Mechanisms

1. **Fermentation:** Prebiotics, typically in the form of complex carbohydrates like inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS), are not fully digested or absorbed in the small intestine. Instead, they pass into the colon intact. There, they

serve as a source of nutrition for specific beneficial bacteria, such as Bifidobacteria and Lactobacilli.

2. **Selective Promotion:** Prebiotics favour the growth and activity of some good microorganisms over harmful ones. Prebiotics can be fermented by these helpful bacteria, yielding short-chain fatty acids (SCFAs) such as butyrate, acetate, and propionate. Among the many health advantages of SCFAs is their ability to modulate the immune system and maintain the integrity of the gut lining.
3. **Increased Microbial Diversity:** Prebiotics can increase the diversity of the gut microbiota by giving different bacterial species distinct substrates to grow on. A varied microbiome is linked to improved general health and a lower chance of developing certain diseases..
4. **Gut Barrier Function:** Prebiotics can strengthen the intestinal barrier. They promote the synthesis of mucin, a material that acts as a barrier to stop harmful germs from sticking to the intestinal lining, and they aid in maintaining the mucus layer in the intestine.
5. **Immune System Modulation:** Prebiotics support a healthy gut flora, which helps control the immune system. It may enhance immunological responses and lessen inflammation, both beneficial to general health.
6. **Enhanced Nutrient Absorption:** Prebiotics encourage the growth of beneficial bacteria that increase the bioavailability of certain elements, like calcium and magnesium; they can boost the absorption of certain minerals.

Notably, prebiotics' efficacy may differ depending on personal characteristics, including dietary choices and the makeup of an individual's gut microbiota. A balanced, varied diet rich in prebiotic-rich foods such as fruits, vegetables, whole grains, and legumes is necessary to reap the full advantages of prebiotics. In addition, prebiotic supplements are available for people looking for a more specialised approach to gut health; however, before using them, it is advised to speak with a healthcare provider. (Gatlin *et al*., 2022).

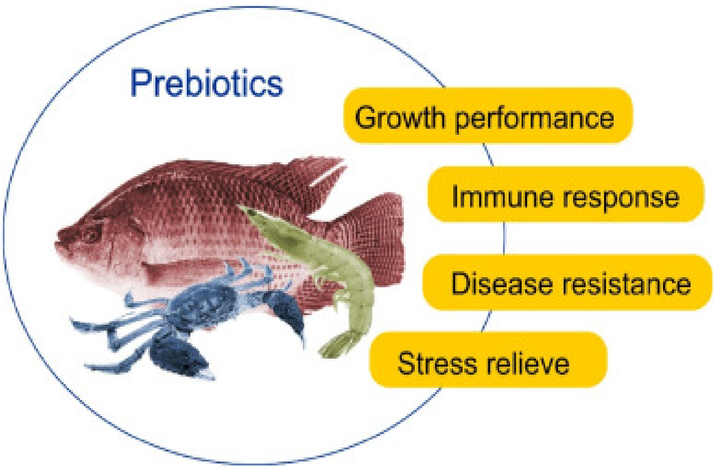
3.2 Applications of Prebiotics in Aquaculture

Prebiotics are useful in aquaculture for several reasons, including promoting healthy gut microbes in aquatic species, comparable to their role in supporting gut health in humans and other land animals. Prebiotics have the following uses in aquaculture:

1. **Improved Gut Health:** Fish and prawns are examples of aquatic creatures whose gut health can be improved by prebiotics. Prebiotics enhance general digestive health, preserve the integrity of the gut, and stop harmful pathogens from colonising the gut by encouraging the growth of good gut bacteria.
2. **Disease Prevention:** A healthy gut microbiome can help aquatic species resist infections and diseases. Prebiotics play a role in strengthening the immune system of fish and other aquatic organisms, making them more resistant to various pathogens.
3. **Enhanced Nutrient Utilization**: Prebiotics can enhance nutrient uptake and utilisation in aquaculture. They can help with nutrient absorption and digestion, improving growth rates and feed conversion efficiency by promoting the growth of good gut flora.
4. **Water Quality Management:** Consuming prebiotic foods can lower nutrient excretion, improving the water quality in aquaculture systems. This is particularly significant in closed-system aquaculture facilities where maintaining water quality is essential.

5. **Stress Reduction:** Prebiotics can aid aquatic life in adjusting to stressors such as alterations in water temperature or environmental factors. Enhanced flexibility and resilience to stress are correlated with a robust gut microbiome.
6. **Larval and Juvenile Health:** Aquaculture can benefit from the usage of prebiotics in its early phases, especially for prawns and fish that are larval or juvenile. Better development and survival rates can be achieved by assisting in establishing a healthy gut flora early.
7. **Reduced Antibiotic Use:** In aquaculture, prebiotics can be applied early on, especially to prawns and fish that are larval or juvenile. They can aid in the early establishment of a healthy gut microbiota, promoting faster development and survival rates.
8. **Cost Savings:** By improving feed efficiency and reducing the incidence of diseases, prebiotics can lead to cost savings for aquaculture operations.

It's crucial to remember that the precise prebiotics utilised in aquaculture can change based on the target species and the organisms' dietary needs. Prebiotics such fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin are frequently utilised in aquaculture (Ringø *et al.*, 2004).
It is important to thoroughly assess and incorporate the use of prebiotics in aquaculture into a more comprehensive management plan. For the greatest outcomes in aquaculture operations, proper feeding, water quality control, and disease management procedures should be taken into consideration.



(Source: wee *et al*., 2022)

Figure 2: Uses of Prebiotics in Aquaculture

3.3 Limitations of Prebiotics

Prebiotics have certain drawbacks and things to remember even though they are very beneficial to health and essential for maintaining a healthy gut microbiota.

1. **Individual Variability:** Everybody reacts differently to prebiotics. Prebiotics' ability to encourage the growth of good gut bacteria may vary depending on the individual's overall health and gut microbiota composition.
2. **Gastrointestinal Distress:** Consuming a large amount of prebiotics may occasionally result in gastrointestinal distress, which includes gas, bloating, and diarrhoea. This is

especially true when someone is first starting to take more prebiotics. Adding foods high in prebiotics to your diet gradually could help reduce these symptoms.

3. **Dosage and Timing:** It can be difficult to decide on the best amount and time to take prebiotics. Individual tolerance may vary, so weighing the advantages of prebiotics against any potential discomfort is crucial.
4. **Possibility of Overconsumption:** If prebiotics are used excessively, some gut bacteria may overgrow, resulting in imbalances in the gut microbiota. When including prebiotics in your diet, moderation is crucial.
5. **Limited Food Sources:** Although some foods are high in prebiotics, not everyone may regularly eat them. Prebiotic supplements are offered since it can be difficult to obtain prebiotics from natural sources.
6. **Interaction with Medications:** Before increasing your prebiotic consumption, speak with a healthcare provider if you have any specific medical conditions or medications you take. Prebiotics may influence or interfere with certain drugs or medical conditions.
7. **Potential for Weight Gain:** If prebiotics are not taken into account in the context of your total diet, they may occasionally serve as a source of extra calories, which could result in weight gain.
8. **Limited Scientific Understanding:** Although prebiotics are becoming increasingly well-known for their health advantages, research is still being conducted into their complete range of impacts. More research is required to comprehend prebiotic intake's long-term effects completely.

Incorporating prebiotics into a well-balanced diet while considering your unique tolerance and requirements is imperative. To ensure that prebiotics are suitable for your particular circumstances, speak with a medical expert or registered dietitian if you have any concerns or specific health conditions before making any big dietary changes. In conclusion, prebiotics are indigestible substances in some meals that nourish good gut flora, promoting a diversified and well-functioning gut microbiome. Including prebiotic-rich foods in your diet can be a proactive step towards maintaining gut health and overall well-being.

4. Conclusions

Because it provides high-quality animal protein that promotes nutritional and food security, aquaculture has become the sector with the quickest rate of growth. A disease outbreak is one of the many limitations to this increased and intensified aquaculture production. Thus, as a practical substitute for sustainable aquaculture, probiotics and prebiotics herald a new age in contemporary aquaculture. Numerous types of bacteria have been investigated as probiotics for potential application in aquaculture. It is obvious that some cultures help the host grow more quickly and have fewer illnesses. A possible indirect advantage could be a decrease in the utilisation of pharmaceutical compounds. We are aware that feeding costs account for 60–80% of operating expenses in intensive aquaculture practices. By improving feed utilisation and therefore growth performance, probiotics and prebiotics can help control feeding costs. By using them, aquaculture species become healthier and more resilient to pathogenic microorganisms.

5. Future Perspectives

Attempts to discover more productive strains are underway. In particular, strains that can bind to gut epithelial cells, strains that are heat stable, and strains with a high rate of development in the colon (Fuller, 1989). To further understand the mechanisms of action, a thorough investigation into probiotics and prebiotics should concentrate on alternative molecular techniques. Additionally, studies should be conducted to observe the interactions between probiotics and pathogens in fish digestive tracts, as well as important endogenous reactions and interactions. These studies should involve isolating the gut cells of salmonids fed probiotics and evaluating the expression of anti-microbial proteins, pattern recognition receptors, and immune activatory or immuno-regulatory cytokines.

When probiotics and prebiotics are taken together, the bacteria may have a better chance of surviving to reach the upper portion of the gastrointestinal tract and exerting their beneficial effects in the large bowel. Furthermore, the benefits of prebiotics and probiotics may even be synergistic or additive. According to Roberfroid (2000), this has been the case. In the near future, process technology optimisation will also be vital as we explore the best ways to incorporate prebiotic samples and probiotic strains into feed that maintain their efficacy even after intense heat processing.

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