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Microfeed Incorporated with Probiotic for Aquaculture: A Review

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ABSTRACT

Ensuring the availability of high-quality larvae in sufficient quantities remains a significant bottleneck for the grow-out phase of aquaculture. Over the past century, various alternative dietary solutions for larval stages have been explored, encompassing bacteria, microalgal pastes, yeasts, and various inert microparticles, though with inconsistent outcomes. This review aimed to discuss the innovative integration of probiotics into microfeeds, highlighting encapsulation, coating, and fermentation techniques to propel aquaculture productivity. Microfeeds, which are often nutrient-rich and easily assimilated in powdered or liquid form, play a crucial role in larval fish nutrition. These can be classified into microencapsulated, dry, liquid, and live feeds. The choice of microfeed is pivotal, ensuring appeal, digestibility, and water stability tailored to each larval stage. As probiotics gain popularity in aquaculture for their potential to enhance growth, bolster disease resistance, and improve water quality, their administration methods have diversified. The probiotics can be administered through direct immersion and bath treatments to biofloc systems and feed additives. The results indicated that microfeed incorporated with probiotics showed a positive result impact on the aquaculture industry.

Keywords: Alternative diets, Aquaculture, Microfeed, Probiotics

INTRODUCTION

The aquaculture sector is in a solid foundation to address the escalating need for protein, a demand fuelled by the upward trajectory of user incomes, evolving lifestyles, and the expanding population. However, challenges in the aquaculture industry (such as regulatory framework, water quality management, and food production) of larval fish rearing limit the supply of sufficient numbers and high-quality larvae for grow-out production (Vadstein et al., 2018).

Feed is the most crucial yet often underutilized aspect of fish production, constituting over half of the expenses due to its high manufacturing costs and extra care (Iliyasu et al., 2016). The cost of producing live feed accounts for between 20% and 50% of a hatchery's overall operating expenditures. To find alternate or supplemental meals that might successfully reduce the dependency on live feeds for raising postlarvae, ongoing research remains active in this field (Nagappan et al., 2021). Over the past century, there has been much research on the usefulness of many alternative diets as food for larval stages, including bacteria, microalgal pastes, yeasts, and particular types of inert microparticles, with differing degrees of success (Albentosa et al., 2002; Enes et al., 2003; Ponis et al., 2003; Espinosa et al., 2006).

Micro-encapsulation is a method of enclosing liquids and particle food components within a finely designed wall (Nagappan et al., 2021). The biological system of the target fish or prawn larvae absorbs the internal nutrients in active locations. In the fish or prawn larval body, the wall or shell disintegrates by bacterial action, enzymatic action, pH shift, or rupture (Langdon, 2003). The capsule's wall could be made of a bio-degradable polymer, such as modified gelatine, with the nutrients inside being released by the animal's enzymatic processes or microflora in its gut. Capsules of the microfeed with a diameter of less than 20 microns have been reported, giving the culturist a wide variety of sizes of nutritionally diverse capsules to meet the different growth phases of the fish species (Vadstein et al., 2018).

Probiotics or beneficial microorganisms are believed to improve the fish immune system, especially in stressful environments, by influencing the gut colonization of probiotic bacterial strains and the generation of antibodies, acid phosphatase, lysozyme, and antimicrobial peptides (Salminen et al., 1999; Taoka et al., 2006; Panigrahi, 2007; Mohapathra et al., 2012a). Probiotics have direct immunostimulant properties, which can improve disease resistance, reduce stress response, and enhance gastrointestinal morphology (Ige, 2013). Benefits to fish producers and consumers include increased fish appetite, growth performance, feed utilization, carcass quality, meat quality, and fewer deformities

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(Ige, 2013). This review discussed the integration of microfeeds and probiotics, a practice increasingly adopted in the aquaculture industry to enhance growth index and health parameters.

MICROFEED

Microfeed is defined as a specific kind of feed designed to suit the particular nutritional needs (protein and lipid) of larval fish throughout their developing phases (Hamre et al., 2013). The type of fish being cultured and the particular nutritional needs of the larvae at various stages of development will determine the type of microfeed needed (Langdon, 2003). The nutritional needs of larvae during development would help enhance the quality of larvae and juveniles by optimizing diets and feeding procedures (Hamre et al., 2013). In 2017, fish and seafood intake accounted for 17% of protein altogether obtained from animals, and this fraction has been growing continuously (FAO, 2020).

Microfeeds for fish larvae commonly consist of very nutrient-dense, readily assimilated powdered or liquid diets (Langdon, 2003). Rotifers, artemia, copepods, and other kinds of phytoplankton are a few typical microfeeds for fish larvae (Dhont et al., 2013). For early-stage larvae, dry feeds like microparticles and powders are commonly used (Powell et al., 2017). Mid-stage larvae are usually fed with liquid feeds like emulsions and suspensions (Hua et al., 2019). For late-stage larvae, live feeds like algae and zooplankton are widely used (Murugesan et al., 2010).

The term “microencapsulated feed” refers to the sort of feed that has been specially formulated for the tiny mouths of fish larvae. Microencapsulated feeds offer a rich blend of easily digestible proteins, fats (lipids), and carbohydrates, complemented by vital minerals, vitamins, and micronutrients. Typically ranging from 50 to 500 mm in diameter, these feeds are sized optimally to ensure efficient consumption and digestion by larvae (Kolkovski, 2008). The feed components are encapsulated under a protective covering throughout production to increase stability and avoid nutrient loss (Temiz et al., 2018). This coating is often made of gelatine or other polymers (nylon and aramide, Temiz et al., 2018).

Dry feeds come in the form of microparticles and powders, designed especially in the early stages of larvae development (Jafari et al., 2008). For freshwater species like tilapia or catfish, the dry feeds can range from around 50 to 200 microns in size, while for marine fish larvae, which are often smaller than their freshwater counterparts, the dry feed size ranges from 20 to 100 microns in size, depending on the specific species, developmental stage and mouth size of the fish (Portella et al., 2008). Microparticles and powders (dry feed) for fish larvae can be fed directly into the larval rearing tanks or mixed into a solution to guarantee optimal distribution and consumption. To meet the larvae’s particular dietary needs, they consist of a well-balanced blend of proteins, lipids, carbs, vitamins, minerals, and other vital components. Dry feeds may be produced using various methods, including grinding, milling, extrusion, and spray drying (Jobling et al., 2001). The final product’s required physical qualities, target particle size, feed components, and processing technique are among the crucial considerations (Sørensen, 2012).

Liquid feed is another form of microfeed widely used for feeding mid-stage larvae in various applications, including aquaculture and insect rearing (Hua et al., 2019). In larval rearing, the emulsions technique is frequently produced by mixing lipid-based oils (fish oil, vegetable oil) with water-based solutions, including nutrients and other ingredients, compared to the suspensions technique that needs dispersing finely ground or micronized solid feed materials (fishmeal, microalgae, yeast) in water or another liquid media. Microalgae, which include *Chlorella* and *Nannochloropsis*, are small photosynthetic organisms that provide fish larvae with a suitable liquid diet. Large amounts of microalgae could possibly grow in specialized cultures. When compared to dry feeds, liquid feed is a more readily digestible source of nutrition, ensuring effective nutrient absorption.

Live feed is considered a microfeed for fish larvae. It includes algae and zooplankton that are commonly used as food sources for late-stage larvae in aquaculture and marine hatcheries (Das et al., 2007; Abbas et al., 2015). Omega-3 fatty acids, which are necessary for the proper growth of marine species, can be found in some microalgae (Adarme-Vega et al., 2012). Microalgae not only offer a natural food that closely reflects the creatures present in the larvae’s natural habitat (Mau et al., 2017), but it can also be maintained at different growth stages to fit the nutritional requirements of varied larval species. Another live feed source for late-stage larvae is zooplankton, which are tiny floating organisms commonly found in aquatic ecosystems (Dumont et al., 2013). Depending on the particular needs of the larvae being raised, zooplankton consists of a variety of species, such as copepods, rotifers, and brine shrimp (Das et al., 2012). As the primary food supply for most marine species in the larval stages, they (algae and zooplankton) play an important part in the marine food web to ensure that the larvae receive a nutritionally balanced diet (Rasdi et al., 2020a; Rasdi et al., 2020b).

SELECTION OF MICROFEED

There are some essential elements for selecting a microfeed for fish larvae. Depending on the type of fish being cultured, the particle size might change. Feed particle size varies depending on the species, age, and feeding behavior of the fish,

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as well as the aquaculture operation's output goals productions (Jobling et al., 2001). To ensure that fish larvae can effortlessly consume the feed particles, the particles need to be suitable for their small mouth.

To promote their growth (fish larvae), immune system, and general health, the microfeed should include high-quality proteins, necessary amino acids, vitamins, minerals, and fatty acids (Arney et al., 2015). The FAO reported that carnivorous species had dietary protein needs of 40–55 percent, whereas freshwater omnivore and herbivorous species have levels of 30–40 percent (FAO, 2010). For example, juvenile tilapia may need up to 40% protein daily for good growth (Luz et al., 2012). Lipids, contributing between 10% and 20%, provide the larvae with vital energy essential for their overall growth and optimal organ function (Lee et al., 2002). The development of the neurological system and visual abilities in fish larvae depends on omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Pilecky et al., 2021a). A potential strategy for improving dietary protein availability in compound feeds would be to prevent leaching by efficient encapsulation of feed particles that are supplemented with high levels of free amino acids (FAA), peptides, and soluble protein to mimic live feeds (Tonheim et al., 2007).

The term "digestibility" pertains to the extent to which something can be assimilated and broken down by the digestive system of fish (Tonheim et al., 2007). The higher digestibility indicates that more nutrients are accessible for the fish's growth and development (Tonheim et al., 2007). Fish larvae are categorized according to whether they have stomachs; a gastric fish lacks a stomach even as an adult (Rønnestad et al., 2013). The solubility of proteins, the source and level of lipids, the manufacturing processes used to make the feed, the presence of anti-nutritional factors that can reduce digestibility, and the feed's particle size each have an influence on the feed's digestibility for fish larvae.

Due to their frequent feeding demands and relatively high metabolic rates, fish larvae must be fed often (at least twice per day). The number of larvae per unit volume of water or the amount of food transferred per unit area or volume are the two common ways to represent the feeding density (Blancheton, 2000). The number of larvae per litter or cubic meter of water is generally utilized to represent the appropriate feeding density for fish larvae. To maintain the larvae's proper growth and survival, it is compulsory to develop an ideal feeding environment as well as temperature (warm water temperature), which affects metabolism with respect to protein and energy (Luz et al., 2012). Microfeeds that remain stable in water for an extended length of time are selected to give an assurance that the feed is accessible to fish larvae while not disintegrating or polluting the water excessively (Jobling et al., 2010). While the microfeed must be stable in water, it must also possess the right solubility properties. Dissolved nutrients from the microfeed can help the water become more nutrient-loaded, which might cause excessive ammonia levels, oxygen depletion, or algal blooms (NOAA, 2023). Less frequent feeding intervals are possible if the feed stays stable for a longer period of time, which reduces the labour and operational expenses linked to regular feed replenishment (Vivas et al., 2006).

Biosecurity is a vital part of fish larval rearing, including the feed used in the process (Kumar et al., 2022). One of the processes that may be employed in the creation of microfeed is to implement quarantine measures for a freshly obtained feed before introducing it into the larval rearing system. In order to maintain its quality and prevent contamination, fish larval feed must be handled and stored properly, for example, by selecting feed producers who follow good manufacturing procedures (GMP) and have strict quality control procedures to maintain the quality of production for the microfeed (Padilla, 2009).

FORMULATION OF MICROFEED

The properties of the feed should be appealing, stable in the water for a long period, and continued moving until the first feeding larvae can catch it. The standard diet size spans from 5 to 300 µm, however, the diet's particle size and specific gravity depend on the species being produced and its embryonic stage (Amirkolaie et al., 2006). Due to its increasing cost and erratic availability, fish meal must be replaced in fish feed with less expensive materials of plant origin (Bhosale et al., 2010). The basic ingredients processing the microfeed consist of soybean meal, milk powder, egg, corn flour, cod liver oil, agar powder, and vitamin mixture (vitamin B complex and vitamin E). The nutritional value for each of the mentioned ingredients is beyond doubt as it is far-reaching among manufacturers. To maximize the development and preservation of the larvae, the microfeed composition and feeding regimens must be optimized for each type of fish larvae. 4% vitamin premix is suggested to maintain ideal feed solubility and the best possible feed quality (Mau et al., 2017).

It is hard to formulate a compound diet suitable for fish larvae because standard nutritional techniques cannot be used to estimate the nutritional requirements of fish larvae and because the current available commercially prepared diets do not sustain larval development (Cahu et al., 2001). Improving comprehension of the digestive processes in larvae has resulted in suggested food compositions that satisfy their nutritional needs.

Alternative feed component formulations usually fulfill the nutritional needs of a specific species while staying economically competitive and efficient (Sørensen, 2012). Low molecular weight, water-soluble chemicals can be delivered via lipid coatings with little leaching into the surrounding water (Langdon, 2003). Recent research has found that larvae have distinct dietary needs (Cahu et al., 2001). However, sufficient proteins must be provided to maintain

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optimum growth because development is primarily protein deposition. The use of a ready-made diet allowed researchers to look into larvae's protein requirements, collect the most definitive data on lipid requirements, and examine glucidic and vitaminic nutrition.

Feed attractants should be included to induce a feeding response (Kolkovski et al., 2009). Physical and chemical characteristics, such as color, shape, size, movement, and smell stimulation at the molecular level, primarily influence the seeking, identification (of feed attractants), and ingesting processes. Some production of feeds fortified with astaxanthin, a carotenoid pigment, enhances the development of red coloration in the fillet of farmed charr in the weeks prior to harvest (Jobling et al., 2010). Using sustainable sources or naturally grown macroalgae in powder or meal form is a low-cost alternative to integrating feed attractants and stimulants (Kolkovski et al., 2009).

ADVANTAGES OF MICROFEED

The nutritionist puts forth considerable effort to reduce feed costs as part of an environmentally friendly aquaculture strategy. The reason is that they can spare protein and thus increase the cost-effectiveness of the diet, feedstuffs with relatively high quantities of carbohydrates are favored when formulating fish feed (Bhosale et al., 2010). The lipidic component of live prey significantly impacts the performance of larvae because it is more changeable and manageable. As a result, lipids and the essential fatty acids of fish larvae have been given more emphasis (Izquierdo et al., 2000).

The digestibility of a particular feed ingredient reflects the fish growth. The choice of dietary protein to be used in practical rations is an economic decision, which depends on the protein source as well as on the expected returns from fish growth and value (Bhosale et al., 2010). To obtain the best growth and development of fish larvae, DHA, EPA, and ARA should also be included in their (fish larvae) diet (Mejri et al., 2021). According to several studies, these fatty acids (DHA, EPA, and ARA) serve as a precursor for certain prostaglandins and other biologically active substances that control growth (de Mello et al., 2022). Therefore, protein and fatty acids contribute to satisfactory larvae fish growth.

Early-stage aquatic species have small mouths and limited feeding abilities, making it difficult for them to swallow normal feed particles. For their quick development and growth, it often contains high concentrations of proteins, and lipids, along with essential nutrients like vitamins and minerals (Siddik et al., 2023). Microfeed can assist optimum growth rates and general development by providing adequate nutrition in an accessible approach. Therefore, microfeeding is essential for their effective growth. In order to ensure that larvae and fry receive a sufficient amount of nutrients, microfeed is made to be small enough so that it can be easily digested. Besides, it will also increase the survival rates of the fish larvae indirectly as it has the right amount of feed consumed (Sardi et al., 2023)

Microfeed, which has been processed or ground to a smaller size than regular feed particles, has less of an impact on the environment (such as biodiversity loss and resource depletion). By giving animals small amounts of food on a regular schedule, microfeeding reduces feed wastage. It generates less trash and suspended particles, which can enhance water quality and reduce the danger of nutrient imbalances and decreased oxygen levels. For aquaculture systems to be healthy and productive, good water quality (pH from 6 to 9) must be maintained. It can enhance the overall economic effectiveness of animal production systems by reducing feed waste (Henriksson et al., 2021).

Besides, microfeeding has an improving effect on animal (fish larvae) welfare and wellness. Early-stage aquatic organisms fed on microfeed are more resistant to stressors and diseases because they deliver sufficient nutrition and sustain optimal health (Siddik et al., 2023). The particle size of microfeed also helps in lowering the possibility of feed loss, which can result in problems with the water supply and disease outbreaks in intensive culture systems (Siddik et al., 2023). It also contributes to a healthier gut microbiota and lowers the risk of digestive diseases by supplying an ongoing supply of nutrients, which helps maintain a stable gastrointestinal environment. Additionally, by fulfilling an animal's natural needs, regular feedings can reduce stress and improve performance (Siddik et al., 2023).

APPLICATION OF PROBIOTICS IN AQUACULTURE

In aquaculture, the application of probiotics has become increasingly prevalent (Table 1). These beneficial microorganisms serve as a powerful tool to protect aquatic organisms from diseases, foster growth, and enhance their immune capabilities, thus contributing significantly to the overall health and productivity of aquatic ecosystems. Generally, probiotics refer to the application of beneficial microorganisms as supplements for feed or water to improve the health and growth performance of aquatic organisms, such as fish, shrimp, and shellfish, in aquaculture systems. The most widely utilized probiotics are bacteria (such as *Bacillus*, *Lactobacillus*, and *Enterococcus* species) and yeast (such as *Saccharomyces cerevisiae*, Kurniawati et al., 2021). For instance, in carps, probiotics such as *Lactobacillus* spp., *Bacillus* spp., *Saccharomyces cerevisiae*, and *Lactococcus* spp. are among the most common species that are currently being utilized (Feng et al., 2019). Probiotics offer several potential benefits in aquaculture, including enhancement of digestion and nutrient absorption (Samat et al., 2021).

Probiotics can be administered to aquatic life in a variety of methods, including as feeding additives. Usually, probiotics are added to the meal in liquid or dry form. It can be included in the formulation of commercial aquafeed or

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prepared as a supplement to be manually added to the feed during the manufacturing process. To achieve optimum efficiency without having negative side effects, the amount of probiotics administered to the fish diet should be calculated based on the specific requirements of the aquatic organisms. In a study on keureling, *Tor tambra* (*Cyprinidae*) fish fry, the amount of probiotics that should be consumed is determined to be 10 ml kg^{-1} of feed (Muchlisin et al., 2017). Nevertheless, it is vital to comprehend that the recommended dose of probiotics for different species might differ (Muchlisin et al., 2017).

Another way to administer probiotics to aquatic organisms is by direct immersion in water containing probiotics. A concentrated solution of probiotics is prepared, typically by culturing the desired probiotic strains in a suitable medium range from a few minutes to several hours, depending on the species, size, and health condition of the aquatic organisms. The frequency of direct immersion might be a one-time treatment or repeated at regular intervals, such as daily or weekly, to maintain a consistent presence of beneficial microorganisms. A study found that submerging larvae of Nile tilapia in probiotic (*Lactobacillus plantarum)* led to considerably greater development and survival rates than the control group (Sherif et al., 2020).

Bath treatment is one of the applications of probiotic. The aquatic organisms are placed in a bath containing a concentrated solution of probiotics. The organisms come into direct contact with the probiotic solution, allowing the beneficial microorganisms to colonize their external surfaces and provide potential benefits, especially in the early stages of larval development, such as *Lactobacillus acidophilus* on the growth and survival of zebrafish (*Danio rerio*) larvae. Depending on the intended application, the particular probiotic strains utilized, and the tolerance of the aquatic organisms, bath treatments can last from 1 to 4 hours. It improves disease resistance and fish growth (Song-Lin et al., 2012). Wang et al. (2020) also stated that *Lactobacillus acidophilus* bath treatment had a positive effect on growth performance, digestive enzyme activity, and disease resistance in juvenile grass carp that persisted for at least 4 weeks after the treatment.

Biofloc systems are defined as a form of aquaculture that uses microbial communities by converting waste into protein-rich food for fish (Crab et al., 2012). Probiotics in biofloc systems can support effective nutrient cycling and limit the spread of harmful microorganisms. Enzymes and other substances produced by the biofloc system will help break down feed elements and increase the bioavailability of nutrients (Kumar et al.,2021). A study on the evaluation of the biofloctechnology system showed improvement in mean final weights, specific growth rates, feed conversion ratios, and total biomass in Nile tilapia (Mohammadi et al., 2020). Recently, He et al. (2023) conducted a study showing that the addition of *Bacillus* (*B.*) *subtilis* to biofloc systems enhanced the water quality, growth performance, and immune enzymes of *Litopenaeus vannamei*.

PROBIOTICS MODE OF ACTION

The mode of action or mechanisms of probiotics refers to the specific ways these live microorganisms exert good impacts on the host's health. It is crucial the particular mechanisms of probiotics can vary depending on the strains used and the individual's initial characteristics (Plaza-Diaz et al., 2019).

In the first place, probiotics compete with dangerous microbes for resources and space, assisting in retaining microbial communities in the gut. Physical barriers that stop dangerous bacteria from adhering to the intestinal wall are created by probiotics' adhesion to the intestinal lining and occupation of surface regions. By occupying this area, fewer dangerous bacteria have a chance to grow and establish a presence (Amara et al., 2015).

Besides, short-chain fatty acids and other metabolites that promote gut health and general well-being are created when probiotics ferment food fibers. During the fermentation process, probiotics break down these dietary fibers into various by-products, among the most essential being short-chain fatty acids (SCFAs). Probiotics can produce a variety of additional metabolites from the fermentation of dietary fibers in addition to SCFAs, including bioactive substances with potential health advantages and gases like carbon dioxide and hydrogen (Bamigbade et al., 2022).

Some probiotics are thought to influence the metabolism of certain nutrients, such as B vitamins and fatty acids. They may also aid in the absorption of minerals like calcium. It has been shown that some probiotic bacteria may ferment food substrates to create B vitamins, including folate, riboflavin (B2), and cobalamin (B12, Kaprasob et al., 2018). These vitamins are crucial for several metabolic activities, such as DNA synthesis, cell development, and energy generation (Indira et al., 2019).

To conclude, some probiotic strains have been proven to aid in the detoxification of toxic substances, possibly lowering the chance of contracting specific illnesses. Antioxidant qualities in some probiotics may help fight off damaging free radicals and lessen oxidative stress. Heavy metals may be chelated or bound to by certain probiotics, aiding in the removal of these potentially hazardous chemicals from the body (Chen et al., 2022).

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Table 1. The application of probiotics incorporated with microfeed using different methods toward different fish (larvae) species

Fish species	Probiotics	Technique	Outcomes	References
Nile tilapia (*Oreochromis* niloticus)	*Bacillus subtilis* and *Lactobacillus plantarum* (10^7 CFU/g respectively)	Fermentation (*Saccharomyces cerevisiae*)	higher amylase activity than the fish-fed control diet	Essa et al. (2010)
Gilthead sea bream (*Sparus aurata)*	*Bacillus subtilis, B. licheniformis and B. cereus* (solid form, 10 ppm per m^3)	Direct addition	Specific alkaline and acid protease activity was greatly boosted (for improved growth parameters and nutrient conditions).	Nihan et al. (2013)
European sea bass (*Dicentrarchus labrax L.)*	Lactic acid bacteria (*Pediococcus acidilactici*) (3.0g per kg, twice a day)	Direct addition	increases fish survivability and blood chemistry	Eissa et al. (2022)
Pike-perch larvae	*Lb. paracasei* subsp. *paracasei* BGHN14 (1.3g saline × volume and glycerol at 0.3 × volume per wet pellet weight)	Coating	supports skeleton development and improves fish growth.	Ljubobratović et al. (2021)
Nile tilapia (*Oreochromis niloticus)*	*Bacillus amyloliquefaciens* (10^6 CFU/ml) and *Bacillus subtilis* (10^6 CFU/ml)	Microencapsulated	enhances fish health and zootechnical aspects by showing how probiotics function as an immunomodulator.	de Moraes et al. (2022)
Seabream (*Sparus aurata)*	*Bacillus subtilis* (10^7cell/g)	Microencapsulated	improves the survival rates and length gain	El-Dakar et al. (2020)

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PROBIOTIC IN MICROFEED

Probiotic feed in aquaculture is an alternate protein source with a highly digestible protein and energy content as well as a healthy amino acid profile (Gomes et al., 2009; Nayak, 2010). A number of investigations have been conducted to evaluate the nutritional content of various foods (Gomes et al., 2009; Nayak, 2010). The incorporation of probiotics increases hemopoesis and produces nonspecific immunity in fish, according to the latest studies (Marzouk et al., 2008; Lazado et al., 2014). The lack of understanding of larval feeding contributes to the inability of microfeed development.

Foremost, probiotics can be incorporated into microfeed by direct addition. The probiotics are usually in the form of freeze-dried or lyophilized bacteria, which are mixed with the other ingredients of the microfeed before it is given to the aquatic organisms (Jalali et al., 2012). The type and amount of probiotics added must be examined to reduce the risk of probiotic degradation during processing or storage, which causes the probiotics to not survive the digestive process and limits their effectiveness. For instance, the microfeed is made by adding the probiotic bacteria containing 10^6 colony-forming units (CFU) of *B. subtilis* per gram of feed to the feed of juvenile Nile tilapia. It is proven that the microfeed incorporated with probiotics improved their growth performance, feed utilization, and immune response (Chen et al., 2014).

Another method is encapsulation allows disease-control drugs, such as antibiotics or probiotics, to be delivered to filter-feeding bivalves, improving disease management while reducing expenses, environmental harm, and threats to human health (Luzardo-Alvarez et al., 2010). It will create a capsule by enclosing the probiotics' core elements inside a wall material. In the process known as microencapsulation, the probiotics can be shielded from damaging environmental factors while being processed, stored, and transported through the gastrointestinal system (Rajam et al., 2022). Spray drying, lyophilization, emulsion, and extrusion are techniques used for encapsulation (Rajam et al., 2022). Spray drying is widely used in the aquaculture industry. It is cost-effective as it involves atomizing a solution containing the probiotics into a stream of hot air or nitrogen gas, forming small droplets that dry to form powder particles (Vivek et al., 2023).

Another approach is to coat the surface of the microfeed particles with probiotics. Although simple and cost-effective, the probiotics are not protected from harsh environmental conditions during digestion, which reduces their survival rate and effectiveness. Bahrami et al. (2023) proved the effectiveness of microencapsulated *Lactobacillus acidophilus* using an alginate/starch coating in Nile tilapia fry. The alginate-chitosan-probiotic-inulin layer is then applied to the microfeed particles after they have been submerged in this solution (Bahrami et al., 2023). This technique ensures that the probiotics can reach into the intestine, where they can exert their beneficial impacts while also helping to protect them from severe conditions (gut failure) of the gut (Bahrami et al., 2023).

Lastly, there is a fermentation technique process by utilizing microbial fermentation to produce a mixture of probiotics and microfeed. The probiotics are added to the feed, and the microorganisms break down the feed components and produce metabolites, which enhance the nutritional value of the feed and encourage the development of advantageous microorganisms (Soemarie et al.,2021). A study has been carried out on the Nile tilapia (*Oreochromis niloticus*) by analyzing their responses to supplemental probiotic brewer's yeast (*Saccharomyces* cerevisiae), which affects their development, feed consumption, and intestinal morphology (Islam et al., 2021). The fermentation that had been applied gave a positive result which improved feed conversion ratios and increased nutrient utilization (Islam et al., 2021). Another study is the utilization of lactide bacteria and yeast (for fermentation) with cellulase enzymes in the conversion of seaweed powder to seaweed silage, which was enriched with probiotics as food for fish larvae and rotifers showed better feed conversion and growth rates in Asian seabass after 56 days (Santhanaraju Vairappan, 2021).

CHALLENGE IN THE PRODUCTION OF PROBIOTIC-INCORPORATED MICRO FEED

Probiotics enhance digestion and nutrient absorption in the gut through the inclusion of beneficial bacteria into the feed diet increases feed utilization efficiency. While probiotics have been shown to have several benefits (on the digestive processes of aquatic animals) in aquaculture, incorporating them into microfeed can be challenging as it needs to be addressed to ensure their effectiveness and stability (El-Saadony et al.,2021).

It is needed to optimize the production process (preparation and preservation) to minimize the negative effect on probiotic viability. Probiotics may be subjected to a range of stressors during the microfeed manufacturing process, including heat, pressure, and mechanical forces (Fenster., 2019). Probiotics can be exposed to oxygen throughout the microfeed production and storage procedures, such as drying and packing, which may decrease their viability. It is necessary to find strains that are durable and resistant to the processing conditions, such as *B. subtilis*, *B. licheniformis*, and *Lactobacillus acidophilus* (Elshaghabee et al., 2017).

Probiotics have a limited shelf life, particularly when they are subjected to unfavorable factors like high temperatures, humidity, or light (Sun et al., 2023). An essential factor to take into consideration is extending the shelf life of probiotic-containing microfeed without affecting its viability. The effectiveness of probiotics in enhancing animal health, growth, and disease resistance will be impacted by their shelf life (Al-Shawi et al., 2020). Microfeed in fish feed

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has a shelf life ranging from a few months to a year. In order to maintain the shelf life of microfeed, packing and storage conditions are very important.

The cost-effectiveness of using probiotics as specialized additives in microfeed is being examined in light of the advantages of probiotics in terms of fish performance improvement (Al-Shawi et al., 2020). Since formulating and producing tiny particles requires additional effort, microfeed is often more expensive than standard feed. High-quality proteins, specialized components, marine-based components, and medicinal additives are all incorporated into the microfeed. The reason the microfeed is effective is that the nutrients required serve specific nutritional needs or treat particular medical issues for the fish larvae (Beski et al., 2015).

Developing suitable techniques or technologies, such as the entrapment of probiotic bacteria in a gel matrix of gellan, alginate, xanthan, and k-carrageenan for delivering and distributing probiotics in microfeed can be one of the challenging issues in microfeed production. To protect against moisture, light, and oxygen, proper packaging materials must be selected to keep the probiotic incorporated with microfeed from contamination (Fenster,2019). It helps to keep probiotics alive and extends their shelf life. However, it is undeniable that the microfeed depends on the region and the particular probiotic strains used; thus, there may be legal requirements and limitations on the use of probiotics in aquaculture (Fenster, 2019).

CONCLUSION

When microfeed combines with probiotics, it increases its use in aquaculture. It appears that it will offer additional benefits (improving fish growth and reproduction), especially when the live stream is replaced. It is without debate that probiotics are widely used in the aquaculture sector. Further study of microfeed incorporated with probiotics must be done for the sustainability of aquaculture, especially for microencapsulated feed, as the live feed is limited and high-cost.

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Authors' contributions

Nor Mala Yaslikan conducted literature reviews and wrote the original manuscript. Nur Jasmin assisted in manuscript preparation and revision. Nadiah Rasdi contributed to the manuscript revision. Murni Karim supervised the content, revision, and proofreading of the manuscript. All authors confirmed the final draft of the manuscript.

Ethical considerations

The authors declare and confirm that the manuscript is original, has no misconduct, has never been published in another journal, and is confirmed to be published in this journal.

Availability of data and materials

All data generated during the research are relevant and included in this published article.

Competing interests

The authors declare that they have no competing interests.

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