Effects of Microbial Feed Additives on Ornamental Fish

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Trade of ornamental fish has significantly increased. A rise in demand was observed, especially from top importing countries that contributed majorly to the growth of the market. Thus, there is a need to improve ornamental fish aguaculture, increasing the number of cultured species and limiting wild fish handling and transport stress losses. The use of microbial feed additives such as probiotics, prebiotics, and synbiotics, could help in improving the immune system and growth as well as increasing reproductive performance in captivity-bred species.

probiotic Microbial Feed Additives ornamental fish

1. Introduction

Ornamental fishes, due to their different and brilliant colors, shapes and behavior, are often referred to as living jewels and are kept in aquaria or garden pools for their beauty as well as entertainment. Ornamental fishes thank to their different and brilliant colors, shape and behavior, are often referred as living jewels and are kept in aquaria or garden pools for entertaining and fancy thus resulting, together with photography, among the most important hobbies worldwide. Owning and photography of ornamental fishes are popular hobbies worldwide. There is an increase in people who are more inclined towards purchasing attractive fish species for decorative purposes, citing their alluring features and differing characteristics. This has fueled the exponential growth of the ornamental fish market [1]. Moreover, the latest technological advancements in the industry, such as pet cameras and automatic filters, have further augmented the desire to adopt pets.

Therefore, in recent decades, the global trade of these "pets" has rapidly increased. In a recent research dealing with marine ornamental fish larviculture, Chen et al. [2] stated "each year, it is estimated that more than 20 million marine ornamental fish are collected from the wild and sold to over 2 million aguarium hobbyists world-wide", which indicates the dire need to expand this industry by increasing the number of new ornamental species 2.

Ornamental fish breeding started in China more than 1000 years ago when goldfish, a freshwater fish, was domesticated. Then, in the 1930s, in Sri Lanka, marine trade started as a result of the export of coral reefs for aguariums [3][4]. However, the fish industry only gained real economic importance from the 1950s onwards. Although the industry has expanded considerably, the production of fish declined in the late 1990s.

Nowadays, more than 7000 aquatic species are reared and marketed as ornamental fish. Of them, approximately 5000 are freshwater- and 1800 are marine species [2][5][6][7]. In contrast to marine ones, the majority of freshwater specimens are produced in captivity [8]. More than 120 countries are involved in the ornamental fish industry, in their import/export, led by Asian and developing countries, which produce approximately 60% of ornamental fish [9], with a global trade worth approximately of USD 15–30 billion each year [4][9].

Very recently, some comprehensive reviews on ornamental fish culture have been published [2][7][10], detailing the global interest in ornamental fish. To meet this increasing demand, the introduction of innovative and sustainable rearing practices could represent an improvement in the aquaculture sector, reducing the depletion of natural resources in many developing countries.

2. Effects of Microbial Feed Additives on Ornamental Fish Health

Disease outbreak is the major problem in ornamental fish production, causing a substantial economic loss of approximately 400 million US dollars [11]. Diseases can be either of parasitic, bacterial, viral or fungal origins and common symptoms include dropsy, ulcers, fin and tail rot, constipation, swim bladder inflammation, clamped fins, pop eyes, flip over disease, skin flukes, and cloudy eye. The resulting losses negatively affect the financial and socioeconomic status of the ornamental fish farming community.

Commercial aquaculture practices commonly used to prevent or heal damage in fish intended for human consumption, can represent a good starting point to reduce losses The aquaculture industry still represent a major area of antibiotic misuse [12] and so far, oxytetracycline hydrochloride and kanamycin have positively resolved a wide spectrum of fish bacterial diseases, including furunculosis, aeromonosis, pseudomonosis, lactococcosis, and vibriosis, being administered via feed, bath treatment or injection [13]. Excessive and unregulated use of antibiotics in aquaculture, as well as in the ornamental fish industry, has led to the development of gut antibiotic-resistant bacteria [14] and antimicrobial-resistant pathogens [15], subsequently affecting the immune system of fish.

Starting from the regulation of antibiotic use, researchers are now focused on considering and discussing valid alternatives and promising functional feed additives (vaccination, bacteriophages, quorum quenching, probiotics and prebiotics, chicken egg yolk antibody and medicinal plant derivative) that could also be successfully applied in ornamental fish culture [16].

Lilly and Stillwell [17] first proposed the term probiotics "to be used for substances that favors the growth of microorganisms". Since then, several definitions have been proposed and the most common is "live microorganisms that when administrated in adequate amounts, confer a health benefit to the host". proposed by the World Health Organization (WHO). Kozasa [18] was the first researcher who used probiotics in aquaculture and the very first review article on probiotics was published in 1998 [19]; since then, several reviews have been published [20][21][22][23][24][25][26][27][28]. Live bacteria as well as inactivated bacteria and spore formers have been used as probiotics in aquaculture [29]. Among microbial fish additives, lactic acid bacteria (LAB) and Bacillus

probiotics are the most used; however, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces*, *Vibrio*, microalgae (*Tetraselmis*), and yeast (*Debaryomyces*, *Phaffia*, and *Saccharomyces*) are also beneficial ^[28]. Probiotics can be administered via feed supplementation (single or in mixture) or dissolved in water.

Starting from the beginning of the 1980s, probiotics were used in aquaculture practices aiming at controlling bacterial infections and improving water quality. In teleost species, increasing evidence confirmed that probiotics can increase lipid and glucose levels [30][31][32], bone metabolism [33], microbiome composition [34], stress tolerance [35][36], and reproductive performance [21][37][38][39][40]. Nowadays, several commercial probiotics containing *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Carnobacterium* sp., and yeast *Saccharomyces cerevisiae* are used with strict safety measurements and careful management recommendations, which guarantee beneficial effects on production and health [41].

Lactic acid bacteria (LAB), a variety of Gram-positive bacteria, are the main microorganisms that ferment plants, vegetables, meats, fish, and dairy products in the intestine [42][43]. LAB are also commonly used to produce various compounds, such as small organic acids, vitamins, and biological peptides [44][45][46]. Within LAB, *L. acidophilus* is among the most industrially utilized strain in the manufacture of dairy products and dietary supplements [47][48]. Given that LAB may supply several organic molecules via various metabolic processes, these microbes could be utilized as valuable and specific sources of a wide range of enzymes with novel properties [49][50].

B. coagulans and *B. mesentericus*, used to enrich *Thermocyclops decipiens* cultures and administered via diet to *Puntius conchonius* post larvae, significantly changed gut microflora composition. Microbiota analysis further revealed that *B. coagulans* poorly adheres to the gut with respect to *B. mesentericus* [51].

The commercial mixture containing *B. subtilis*, *B. licheniformes*, *L. acidophilus*, and *S. cerevisiae* was beneficial in fish facing extreme conditions including handling and transport stress. Based on these results, these probiotic strains have been largely used in the trade of Cardinal tetra, *Paracheirodon axelrodi* [52] and the marbled hatchet fish, *Carnegiella strigata* [53], which showed a decrease in stress levels and related metabolite secretion. In these trials, a sensible improvement of water quality was also described.

Probiotics (*Bacillus* sp., *Lactobacillus* sp., and their mixtures) were used in the giant gourami, *Osphronemus* goramy, to produce higher-quality fish and to reduce the risk of diseases outbreak. Fish exposed to a mixture of *Bacillus* sp. and *Lactobacillus* sp. via water presented a higher survival rate. These species also improved the quality of the rearing water [54].

L. fermentum (KT183369) and B. subtilis sp. inaquasporium (KR816099) isolated from coconut were used in a feeding trial with the black molly, Poecilia sphenops. At the end, their adhesive properties towards the host cells were found, which led to the speculation that both strains could be used against Vibrio parahaemolyticus. The ability to fight V. parahaemolyticus was demonstrated by a challenge test. In addition, L. fermentum displayed a higher capacity to colonize the gut, suggesting that could be an excellent feed additive for ornamental aquaculture

species [55][56]. An additional challenge test against *V. anguillarum* was set up using *B. pumilus* RI06-95Sm and results showed its ability to colonize the molly's gut, reverse the negative impacts of antibiotic treatment and decrease the mortality rate [57].

3. Effects of Microbial Feed Additives on Ornamental Fish Growth

The main target of aquaculture practice is to acquire the most rapid growth and the lowest production cost. To achieve this goal, several means have been established to boost the growth rate and feed consumption by adding functional feed additives [58][59]. Probiotics are among those functional feed additives showing strong effects on growth, health, and well-being. In aquaculture, investigations on probiotic-containing diets demonstrated the role of these favorable bacteria in improving gut microflora balance and in the production of extracellular enzymes able to enhance feed utilization and increase growth performance [60][61]. Probiotics can increase the uptake of nutrients, the assimilation capacity, the feed conversion ratio and improve digestibility [61][62][63]. In addition, probiotics have been proven to promote the absorption of feed through the production of extracellular digestive enzymes, i.e., amylases, proteases and lipases or intestinal alterations, resulting in a better growth [20][64][65][66][67].

4. Effects of Microbial Feed Additives on Ornamental Fish Reproduction

The beneficial effect of probiotics in reproduction was demonstrated, thanks to their ability to produce vitamin B and certain unknown stimulants [68], which in turn could play a vital role in increasing the reproduction rate of the host [69]. One example is represented by *B. subtilis*, which is able to synthesize vitamins B1 and B12, responsible for the reduction of the number of abnormal and dead larvae [70]. The effects of one year of *B. subtilis* dietary supplementation were evaluated in five ornamental fish species—*Cirrhinus mrigala*, *P. reticulata*, *P. sphenops*, *X. helleri* and *X. maculatus*—and the results obtained highlighted better reproductive performances, as witnessed by the increase in the gonadosomatic index (GSI), fecundity and fertility rate in all species analyzed. In addition, fries presented higher survival rates as well as decreases of deformities [71]. Similarly, an Artemia diet enriched with *B. subtilis* significantly improved the reproductive performance of *P. latipinna*, in terms of fry production and survival [72]. The administration of *L. rhamnosus* strongly improved zebrafish reproductive performance, acting on both fertility and fecundity [21][37]. Probiotics acted at the gonadal level by inducing follicle maturation [38][73]. A similar effect of *L. rhamnosus* was also evidenced in killifish (*Fundulus heteroclitus*) [74].

5. Role of Microbial Feed Additives in Maintaining Good Water Quality of Ornamental Fish Holding Systems

Administration of probiotics in culture water can offer an advantage at any point in the species life cycle. This is of high importance, especially during larval stages, when their use can improve health conditions [75]. Probiotics in water can proliferate using available substrates and competitively exclude the pathogenic bacteria [76].

It has been suggested that water probiotics (*B. acidophilus*, *B. Subtilis*, *B. licheniformis*, *Nitrobacter* sp., *Aerobacter* sp., and *S. cerevisiae*) beneficially affect water quality through enhancing organic matter decomposition of the undesirable organic substances ^[77], increasing the population of food organisms, reducing pathogenic bacteria ^[78] and nitrogen and phosphorus concentrations and controlling ammonia, nitrite, hydrogen methane, etc., levels ^{[79][80]} [81][82]. Considering that fish feed and waste are two significant parameters of the aquaculture ecological footprint, it can be argued that probiotics can contribute to reduce the environmental impact of aquaculture ^[83].

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