

Review

The Role of Functional Feed Additives in Enhancing Aquaculture Sustainability

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Abstract: Aquaculture serves as a source of protein and livelihood and is an alternative to capture fisheries, thereby reducing pressure on the wild. However, aquaculture tends to be limited by sustainability issues, which include overdependency on fishmeal, the high cost associated with fishmeal, the environmental impact of aquaculture activities, which may be detrimental to aquatic lives and the environment, and the use of antibiotics to treat diseases, which may have an adverse effect in their host or the environment. Efforts are being made toward attaining practical ways to enhance aquaculture sustainability. One such effort is using functional feed additives in feed formulation. Functional feed additives are dietary ingredients incorporated in feed formulations, not only for the usual provision of basic nutritional requirements as offered by traditional feed but also for growth and health enhancement; environmental and economic gain. This review emphasizes the importance of incorporating functional feed additives such as probiotics, prebiotics, symbiotics, and phytochemicals. This study evaluates and presents holistic information on functional additives, their roles in enhancing aquaculture sustainability, and the challenges encountered in their application.

Keywords: antibiotics; antiparasitic; feed efficiency; growth; immunity

Key Contribution: Aquaculture is of huge benefit to society. However, there are concerns regarding its sustainability. Functional feed additives are a catalyst for sustainable aquaculture. Their use in aqua feed formulation results in improved gut health and beneficial gut bacteria, the elimination of opportunist bacteria, increased enzyme production, and appetite stimulation, resulting in improved growth and immunity in their host. They also ameliorate the use of alternative proteins in aquafeed and improve water quality, thereby reducing the footprint of aquaculture on the environment. Therefore, functional feed additives are of great benefit in aquaculture, and their use should be encouraged by governments and stakeholders.



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1. Introduction

Aquaculture has been recognized as the fastest-growing sector worldwide regarding food production technology [1]. It contributes more fish biomass than capture fisheries, especially when considering the inclusion of non-consumable fish [1]. It serves as a nexus to household food security, a source of protein and livelihood, and also an alternative to capture fisheries, thereby reducing pressure on the wild [2–4]. However, concern has been increasingly raised about its sustainability.

The term “sustainability” refers to the systematic management of institutional, financial, technological, natural, and social resources to guarantee a continuous supply of necessities for people, both now and in the future [5]. Sustainability guarantees sustainable aquaculture; therefore, it is “the cost-effective production of aquatic organisms while maintaining a harmonious and continual interaction with the surrounding communities and ecosystems” [6].

Two-thirds or more of the crustacean and finfish produced from aquaculture are reared via fed aquaculture [7]. Feeds are made of protein and lipids to meet the nutritional requirements of aquatic animals. The protein and lipids are derived from fish meal and fish oil, with about 71% of the fish meal and fish oil prepared from wild-caught fish and the remainder from waste derived after processing aquatic animals [7]. This exerts a lot of pressure on wild-caught fish, thus negatively impacting its sustainability and deviating from the aim of aquaculture. Furthermore, the cost of feed accounts for about 40–60% of the cost of production, with protein (fish meal) being the most expensive nutrient in feed formulation [8]. Aquaculture, like agriculture, is affected by disease outbreaks emanating from its intensification and commercialization amidst other factors, such as environment and climate change [9,10]. Disease outbreaks are caused by viruses, bacteria, fungi, parasites, and unidentified pathogens, which are significant limitations to the culture of aquatic organisms, expansion, profitability, and sustainability of aquaculture ventures [11,12]. Additionally, the environmental impact of aquaculture activities, such as the disposal of untreated effluents (rich in nutrients) into aquatic bodies, is detrimental to the aquatic environment and the population therein [13–15].

Based on the several challenges associated with fed aquaculture and to ensure continuity in aquaculture, efforts are being made toward attaining practical ways to enhance aquaculture sustainability. Regarding reliance on and the costs associated with fish meal, alternative protein sources are being sought, which has led to studies on the potential of insects and plants (both terrestrial and aquatic) as a less expensive and partial/complete substitute for fishmeal [16–20]. To address the health of aquatic animals, antibiotics and chemicals have been used to combat diseases and parasites [21,22]. Integrated multi-trophic aquaculture (IMTA) has also been projected to reduce the nutrient load associated with aquaculture effluent, such that the waste from the fed species, which would have been discarded together with its nutrient, is used as a source of feed or fertilizer for the complementary species [23–27]. Similarly, photocatalysis, recirculatory aquaculture systems (RAS), and aquaponics have been used for the treatment of aquaculture wastewater. Photocatalysis has to do with the use of materials that break down toxic substances in the presence of sunlight and UV light for the remediation of aquaculture effluent [28]. RAS involves the recycling and reuse of wastewater after it undergoes filtration. In phytoremediation, such as the use of aquaponics, in which the wastewater from aquaculture is used to grow terrestrial plants, the plants make use of the nutrients, thereby bioremediating the water, and the discharged water is recirculated back to the aquaculture systems [29]. Consequently, the nutrient load and the impact of the resultant effluent discharged into the environment is reduced.

Although these interventions are helpful, they are open to limitations; for example, digestibility issues are associated with using some insect and plant species as protein substitutes. In line with this, reduced weight was reported for juvenile Atlantic salmon that were fed diets that had 80% of fishmeal substituted by plant protein [20]. Similarly, sea bass that were fed a diet in which fish meal had been totally substituted with plant protein experienced reduced growth [30–32]. Additionally, the nutrient composition of insect meal is unstable and varies according to the type of substrate used in rearing the insects [18,32,33]. The negative impact associated with the use of antibiotics/chemicals, such as residuals in the host, which may be passed on to the consumers, and its detriment to the environment has led to restrictions on their use [14,21,22,34]. Furthermore, integrated multitrophic aquaculture requires the knowledge of species selection, compatibility, and stocking density of the animals selected, preventing it from being readily adopted by amateurs [23].

The use of functional feed additives (FFA) in aquaculture is promising in addressing some sustainability challenges linked to aquaculture. Functional feed additives are dietary ingredients incorporated in feed formulations not only for the usual provision of basic nutritional requirements as offered by traditional feed but also for growth and health enhancement and environmental and economic gain [35]. They improve growth, immune

responses, and disease resistance [36–39]. Some functional additives used in aquaculture are probiotics, prebiotics, microalgae, cyanobacteria, enzymes, and immunostimulants. The attributes of FFA vary, and inclusion in feed formulations is directed at a specified purpose [40]. Functional feed additives, such as phytochemicals, symbiotics, probiotics, and prebiotics, improve intestinal health, feed ingredient digestibility, and disease and stress resistance and annul the adverse effects associated with antinutrients [40–45]. However, there is still a paucity of information on functional feed additives, especially regarding their relation to aquaculture sustainability. Moreover, available evidence shows that many aquaculturists have not optimized the use of functional feed additives, despite their significance in fed aquaculture; this is because the concept of functional feed in aquaculture is still in the developmental stage.

This study seeks to summarize past findings while contributing to the existing literature on functional feed additives.

2. Objectives

The specific objectives of this study are as follows:

- Itemize some common functional feed additives and their contribution to aquaculture sustainability;
- Identify challenges associated with the use of functional feed additives.

3. Methodology

Materials for this study were sourced from print books and different scholarly sites using various web browsers, including Google Chrome, Mozilla Firefox, Opera Browser, and Microsoft Edge. However, the literature used in this study was more web-based. The databases from which the literature was sourced include Science Direct, Research Gate, Wiley Online Library, SABINET, and SpringerLink. This review did not restrict literature reports on functional feed additives to specific locations/regions to obtain detailed information on the subject area.

4. Some Common Functional Feed Additives in Aquaculture

4.1. Probiotics

Probiotics are an aquafeed functional feed additive that is receiving global recognition. The term “probiotics” was initiated by [46] and was described as “organisms and substances that contribute to intestinal microbial balance. The inclusion of substances in the definition of probiotics was criticized by [47], who revised the definition as “a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance”. According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are “live micro-organisms which when administered in adequate amounts confer a health benefit on the host” [48]. These definitions of probiotics are appropriate for terrestrial animals and humans, but not for aquatic animals. This is because aquatic animals and microorganisms cohabit in the same aquatic environment; therefore, for aquatic animals, the interaction between microorganisms (probiotics inclusive) and the host does not only occur in the intestinal tract. Therefore, ref. [49] provided an appropriate definition for aquatic probiotics, which is “a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community or ensuring improved use of the feed or enhancing the nutritional value of the feed or enhancing the host response towards disease, or improving the quality of its ambient environment”.

Probiotics can be administered either via food or in the rearing water. Probiotic organisms used as food additives should not be pathogenic, should possess the ability to survive transit via the gut, and should withstand exposure to gastric juices and bile [50]. For probiotics to be effective, the probiotics must be able to multiply and colonize the digestive tract and be potent throughout and beyond the product’s shelf life [51,52]. The advantages of probiotics as feed additives include an improved immune response, enhanced

growth, the inhibition of pathogens via the production of siderophores, hydrogen peroxide, lysozyme, and organic acids, and improved food digestibility [53–56]. Probiotics also enhance the production of nutrients like biotin and vitamin B12 [57–60]. They can be categorized as bacterial or non-bacterial, spore-forming or non-spore-forming, multispecies or single-species, and allochthonous or autochthonous probiotics [61]. Multispecies probiotics contain a mix of species, while single probiotics contain only a single species of probiotics. Allochthonous probiotics are probiotics derived from microorganisms that are generally not found in the gastrointestinal tract, for example, yeasts, while for autochthonous probiotics, the microorganisms usually inhabit the gastrointestinal tract [62]. Examples of probiotics used in aquaculture include microalgae (*Tetraselmis*), gram-positive bacteria (*Lactococcus*, *Enterococcus*, *Streptococcus*, *Bacillus*, *Lactobacillus*, *Clostridium*), gram-negative bacteria (*Photobacterium*, *Pseudomonas* and *Alteromonas*), and yeast (*Saccharomyces* and *Debaryomyces*) [63,64].

4.2. Prebiotics

Prebiotics are indigestible feed additives, mainly oligosaccharides that enhance the host's health while stimulating and metabolizing beneficial microorganisms in the gastrointestinal tract [65–67]. They serve as energy and food sources for good gut bacteria, including probiotics [68]. For a feed additive to be categorized as prebiotic, it has to arrive at the colon undigested, possess resilience against gastric acidity, and be capable of being hydrolyzed by digestive enzymes and absorbed by the gastrointestinal tract [69,70]. The benefits obtained from using prebiotics as feed additives emanate from the byproducts obtained during fermentation by bacteria in the gut [71]. For prebiotics to promote the growth of gut microbiota, the particular microbiota needs to possess enzymes capable of fermenting the prebiotic; therefore, not all gut microbiota can be promoted by a particular prebiotic; hence, a mixture of prebiotics is encouraged [72,73]. The host's innate immune system is activated by prebiotics, either by improving the growth of gut bacteria or by stimulating the host's immune system [74]. Prebiotics naturally occur in animal dairy products and plants such as microalgae, fruits, vegetables, and seaweed [75,76]. The major type of prebiotics used in aquaculture include mannan oligosaccharide (MOS), fructooligosaccharides (FOS), galactooligosaccharide (GOS), arabinoxylan oligosaccharide (AXOS), inulin and β -glucan. The significant advantage of prebiotics over probiotics is that prebiotics are natural feed additives; hence, the regulatory restriction over their use is minimal.

4.3. Phytochemicals

Phytochemicals are a vast class of feed additives obtained from leaves, stems, roots, seeds, tubers, fruits, shrubs, and spices [35]. They can be used in dried, solid, or ground states or as extracts or essential oils [77]. Phytochemicals stimulate the appetite, enhance beneficial gut bacteria, and provide antioxidant, antimicrobial, anti-carcinogenic, analgesic, and antiparasitic effects in farmed aquatic animals [78]. Phytochemicals possess active compounds, such as phenols, flavonoids, alkaloids, terpenoids, saponins, and tannins [79,80]. Hence, information about the bioactive compounds and the proper dosage must be inquired before usage to prevent toxicity [81]. Examples of phytochemical feed additives are garlic (*Allium sativum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), and neem (*Azadirachta indica*).

Garlic (*A. sativum*) contains bioactive compounds such as ajoene, allicin, allin, phenol, polysaccharides, and saponin [38,82]. Garlic promotes growth and provides antioxidant, antiparasitic, and antimicrobial properties [38,82,83]. Garlic can be used in several forms, which include oil, powder, fresh mash, and aqueous extract. However, the powder form is the most widely used in aquaculture.

Neem is known to have a unique smell and bitter taste, which has been linked to tignic acid (5-methyl-2-butanic acid) and meliacine, respectively [84]. All the parts of the neem tree possess a broad range of pharmaceuticals that are effective against several bacterial, viral, and fungal diseases [85,86]. Neem also possesses anti-inflammatory and

anti-oxidative activity and hepato-protective and cancer chemo-preventive potential, and it acts as an anti-diabetic. One of the active chemicals that has gained much interest and is isolated from the neem tree is azadirachtin, which also possesses antibacterial, antiviral, and antifungal properties [38]. The major constraint with phyto-genic feed additives is that their properties and efficacy are highly variable and depend on the part of the plant used, the extraction technique and the concentration used, the harvest season, and the geographical location [77].

5. Aquaculture and Its Sustainability Issues

Aquaculture sustainability can be grouped into three categories, which are economic, environmental, and social sustainability [87]. Economic sustainability has to do with the ability of aquaculture to continue to support the livelihood of its practitioners. Environmental sustainability is the ability to perform aquaculture activities without causing harm to the environment, while social sustainability is the societal acceptance of aquaculture activities [87]. The economic sustainability issues include the unoptimized growth of animals in captivity, disease outbreaks, and parasite infection. Environmental sustainability issues include the discharge of nutrient-rich effluents in aquatic environments, which may eventually result in eutrophication, the escape of cultured animals into natural populations, potentially resulting in competition and interbreeding and which may lead to the genetic modification of the natural population, the transfer of diseases from aquaculture to the natural population/wild stock, the over-exploitation of wild stock for use as fishmeal and fish oil, and the use of antibiotics and chemotherapeutics to treat diseases in aquaculture [21,23,26,88–90]. The social sustainability issue includes the conversion of terrestrial habitats for aquatic farming, thereby competing for land with other commercial activities and destroying ecosystems, e.g., the use of mangroves for shrimp farming [91].

6. Functional Feed Additives and Their Sustainability Roles

Functional feed additives play sustainability roles in aquaculture, including feed efficiency improvement, encouraging sustainable resource utilization, and enhanced disease immunity. They also contribute to improving water quality and having antiparasitic effects. These roles discussed are discussed below.

6.1. Feed Efficiency Improvement

Feed efficiency refers to how effectively a consumed feed is converted into biomass. Feeds with good efficiencies are those that when consumed in smaller quantities, result in higher growth rates than inefficient counterparts [92]. A measure of feed efficiency is the feed conversion ratio (FCR), which is the ratio of the weight of feed consumed divided by the weight gained by the animal over a specific period, with a low FCR indicating more efficient growth [93]. Growth performance indicators are crucial components of aquaculture as they represent production yield. Growth performance indicators are influenced by genetic, environmental, and dietary factors [94]. Therefore, they are frequently utilized in evaluating the effectiveness of feeds [95]. The enhanced growth of cultured animals implies a reduced production cycle, enabling farmers to harvest and stock up their culture system in time, thereby increasing production efficiency. Enhanced growth can also result in large-sized animals often yielding higher prices when sold. Animal growth is crucial in aquaculture as it impacts profitability; as such, whatever innovation would lead to improved growth without compromising the health, well-being, and safety of farmed aquatic animals on consumption is highly embraced by aquaculturists.

Functional feed additives are well-known for enhancing their host's feed utilization and weight gain [96]. European sea bass, *Dicentrarchus labrax*, fed with probiotic *Pediococcus acidilactici*-supplemented diets at rates of 2, 2.5, and 3 g kg⁻¹ for 60 days had higher weight gain and specific growth rates (SGR) than the control [53]. Similarly, Nile tilapia, *Oreochromis niloticus*, that were fed diets containing *Saccharomyces cerevisiae* probiotics at a concentration of 4 g kg⁻¹ of feed had better FCR, specific growth rates (SGR), and protein efficiency

ratios (PER) and 44.99% higher weight gain than the control. However, fish fed 2 g kg^{-1} and 1 g kg^{-1} concentrations of *S. cerevisiae* had a similar response as the control regarding weight gain (%), SGR, PER, and FCR [43]. From the example above, it is evident that the efficacy of FFA is impacted by the dosage/concentration applied. The dosage needed for effective results is species- and strain-dependent, i.e., it depends on the animal species and probiotic strain used.

Probiotics mixed with various probiotics strains or prebiotics (symbiotics) result in better benefits in terms of growth and health compared to probiotics/prebiotics alone. The increased benefits derived from the use of multiple strains or symbiotics have been ascribed to these complementing each other, thereby broadening the range of benefits on the host [49,97]. For example, a mixture of probiotics *Bacillus subtilis*, *Lactobacillus Pentosus*, *L. fermentum*, and *S. cerevisiae* resulted in a higher FCR and weight gain in white shrimp *Litopenaeus vannamei* compared to a single strain of each of the probiotics and the control [98]. However, a mixture of probiotics does not always lead to improved growth, as is the case with the improved health of animals. Snakehead fish, *Channa argus*, that were fed diets supplemented with *Lactococcus lactis*, *Enterococcus faecalis*, and a mixture of *L. lactis* with *E. faecalis* (all at concentrations of $1.0 \times 10^8 \text{ cfu g}^{-1}$ diet) had higher final weights, SGR, and protein efficiency ratios (PER) and better FCR compared to the control. Within the probiotic-supplemented diets, those that were fed *L. lactis* had higher final weights, SGR, weight gain and better FCR than those that were fed *E. faecalis* or a mixture of *L. lactis* and *E. faecalis* [99]. A high concentration of probiotics does not necessarily translate into better growth. Ref. [54] fed abalone, *Haliotis discus hanna*, diets containing *B. licheniformis*; they were sprayed with 0, 10^3 , 10^5 , and 10^7 cfu/mL of *B. licheniformis* for 56 days. Abalone that were fed the 10^3 and 10^5 cfu/mL supplementation had higher survival rates than that of the control. Similarly, abalone that were fed the diet containing 10^5 cfu/mL of *B. licheniformis* had higher specific growth rates and feed intake than those fed 10^3 cfu/mL of *B. licheniformis* and the control. Likewise, abalone that were fed 10^5 cfu/mL of *B. licheniformis* had the lowest FCR (best) compared to those fed 10^3 or 10^7 cfu/mL *B. licheniformis* and the control.

Thin lip grey mullet (*Liza ramada*) that were fed with mannan oligosaccharide (MOS)-supplemented diets at 0.5, 1, and 2% for 56 days had higher final body weights, weight gain, SGR, and PER and lower FCR compared to the control [100]. Also, mannan oligosaccharide (MOS)-supplemented diets fed to Pacific white shrimp, *L. vannamei*, at inclusion levels of 1, 2, 4, 6, and 8 g kg^{-1} feed for 56 days resulted in significantly higher final body weights, weight gain, SGR and lower FCR compared to those fed a diet without MOS (control). Within the diets supplemented with MOS, those fed 2 g kg^{-1} feed had better weight gain, SGR, and FCR than those fed a 1 g kg^{-1} diet of MOS. The study showed that MOS supplementation at 2 g kg^{-1} feed was preferable to 4, 6, and 8 g kg^{-1} feed as the animals fed these diets (4, 6, and 8 g kg^{-1}) had similar responses in terms of weight gain, SGR, and FCR.

Phytogenics such as lemongrass, geranium, and garlic have been used to enhance the growth of aquatic animals. For example, ref. [101] used essential oils from lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) as a feed supplement for Nile tilapia, *Oreochromis niloticus*, for 84 days. The essential oils were used at a concentration of 200 and 400 mg kg^{-1} . Fish that were fed lemongrass-supplemented diets at 200 and 400 mg kg^{-1} and geranium oil at 400 mg kg^{-1} had lower FCR and higher protein efficiency ratios compared to the control. Likewise, fish that were fed lemongrass-supplemented diets at 200 and 400 mg kg^{-1} and geranium oil at 400 mg kg^{-1} had higher final weights and SGR than the control and those fed geranium at 200 mg kg^{-1} (Table 1). Garlic-supplemented diets (1%, 2%, and 3%) fed to rainbow trout (*Oncorhynchus mykiss*) for 120 days resulted in higher final weights, weight gain, and SGR compared to the control. However, FCR was similar between the garlic-supplemented diets and the control. Among the garlic-supplemented treatments, fish that were fed 3% garlic had the highest final weight and weight gain [102].

The improved growth indices recorded by the use of functional feed additives are achieved through the stimulation of appetite and palatability in their host, as well as the production of digestive enzymes, such as lipase, amylase, and protease, that aid in breaking down feed ingredients, thereby enhancing nutrient digestibility and making the nutrients available to the animal. Improved growth indices are also achieved via the provision of growth promoters, such as amino acids and vitamins, the amelioration of intestinal microbiota, the elimination of substances with the potential to cause harm from food, and boosting the immune system [103–106]. Efficient feed utilization provided by FFA implies that it reduces the nutrients in effluents discharged into the environment, thus reducing the aquaculture footprint and making aquaculture more environmentally friendly. Also, lower FCR implies that the amount of feed required for animal growth is reduced, which reduces production cost as less feed is required, thereby making the venture more profitable and sustainable.

Table 1. Effect of functional feed additives on growth and feed utilization.

| Animal Species | Types of Functional Feed Additives | Name of Strain | Concentration | Duration | Feed Conversion Ratio | Specific Growth Rate (%d ⁻¹) | Protein Efficiency Ratio | Reference |
|--|------------------------------------|---|---|----------|--|--|--|-----------|
| Tilapia (<i>Oreochromis niloticus</i> × <i>Oreochromis aureus</i>) | Probiotics | <i>Clostridium butyricum</i> | Control 0.5 g kg ⁻¹ diet 1 g 2 g 4 g 8 g kg ⁻¹ diet (1.5 × 10 ⁸) | 56 days | 1.14 ± 0.02 ^d 1.12 ± 0.01 ^{cd} 1.06 ± 0.02 ^{ab} 1.03 ± 0.03 ^a 1.07 ± 0.02 ^{abc} 1.10 ± 0.02 ^{bcd} | 3.54 ± 0.02 ^d 3.56 ± 0.02 ^{cd} 3.65 ± 0.03 ^{ab} 3.69 ± 0.05 ^a 3.63 ± 0.03 ^{abc} 3.59 ± 0.03 ^{bcd} | NP | [107] |
| Rainbow trout (<i>Oncorhynchus mykiss</i>) Broodstock | probiotics | Bio-Aqua® (<i>Pediococcus acidilactici</i> , <i>Enterococcus faecium</i> , <i>Bacillus subtilis</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus casei</i>) | Control 1 × 10 ⁹ (cfu/kg) 2 × 10 ⁹ 4 × 10 ⁹ | 56 days | 1.6 ± 0.0 ^a 1.5 ± 0.1 ^a 1.3 ± 0.1 ^{ab} 1.1 ± 0.0 ^b | 0.4 ± 0.0 0.4 ± 0.1 0.5 ± 0.0 0.6 ± 0.0 | NP | [108] |
| Catfish (<i>Clarias gariepinus</i>) | Probiotics | <i>Bacillus</i> NP5 | Control 1 × 10 ⁹ 1 × 10 ¹⁰ | 30 days | 2.14 ± 0.13 ^a 1.0 ± 1.4 ^b 1.09 ± 0.07 ^b | 1.49 ± 0.14 ^b 2.56 ± 0.08 ^a 2.44 ± 0.13 ^a | NP | [109] |
| Nile tilapia (<i>Oreochromis niloticus</i>) | Probiotics | <i>Saccharomyces cerevisiae</i> | Control 1 g kg ⁻¹ diet. 2 g 4 g | 60 days | 1.68 ± 0.01 ^b 1.39 ± 0.01 ^b 1.28 ± 0.01 ^{ab} 1.18 ± 0.01 ^a | 1.59 ± 0.25 ^a 1.67 ± 0.40 ^{ab} 1.70 ± 0.25 ^{ab} 2.10 ± 0.19 ^b | 2.11 ± 0.01 ^a 2.28 ± 0.01 ^{ab} 2.43 ± 0.01 ^{ab} 2.71 ± 0.01 ^b | [43] |
| White shrimp (<i>Litopenaeus vannamei</i>) | Probiotics | <i>Bacillus subtilis</i> <i>Lactobacillus pentosus</i> <i>Lactobacillus fermentum</i> <i>Saccharomyces cerevisiae</i> Mixture of all probiotics at 10 ⁷ , 10 ⁸ and 10 ⁹ Concentrations | 10 ⁹ cfu kg ⁻¹ diet. 10 ⁹ 10 ⁹ 10 ⁹ 10 ⁷ 10 ⁸ 10 ⁹ c control | 56 days | 1.64 ± 0.02 ^{ab} 1.67 ± 0.02 ^{ab} 1.75 ± 0.01 ^{bc} 1.82 ± 0.04 ^c 1.82 ± 0.03 ^c 1.67 ± 0.01 ^{ab} 1.53 ± 0.02 ^a 1.82 ± 0.01 ^c | NP | NP | [98] |
| Abalone (<i>Haliotis discus hannai</i>) | Probiotics | <i>Bacillus licheniformis</i> | 10 ³ cfu/mL 10 ⁵ 10 ⁷ Control | 56 days | 0.92 ± 0.01 ^{b*} 0.70 ± 0.01 ^{a*} 0.76 ± 0.01 ^{a*} 1.15 ± 0.01 ^{c*} | 0.22 ± 0.02 ^b 0.29 ± 0.02 ^a 0.26 ± 0.01 ^{ab} 0.17 ± 0.01 ^c | | [54] |

Table 1. Cont.

| Animal Species | Types of Functional Feed Additives | Name of Strain | Concentration | Duration | Feed Conversion Ratio | Specific Growth Rate (%d ⁻¹) | Protein Efficiency Ratio | Reference |
|--|--|--|--|----------|----------------------------|--|----------------------------|-----------|
| Pacific white shrimp (<i>Litopenaeus vannamei</i>) | Prebiotics | Mannan oligosaccharide | 1 g kg ⁻¹ feed. | 56 days | 1.55 ± 0.03 ^{bc} | 2.46 ± 0.03 ^{ab} | [110] | |
| | | | 2 g | | 1.44 ± 0.02 ^c | 2.59 ± 0.02 ^c | | |
| | | | 4 g | | 1.60 ± 0.07 ^b | 2.51 ± 0.05 ^c | | |
| | | | 6 g | | 1.58 ± 0.33 ^b | 2.51 ± 0.04 ^{bc} | | |
| | | | 8 g | | 1.61 ± 0.04 ^b | 2.51 ± 0.05 ^c | | |
| Control | 1.78 ± 0.05 ^a | 2.29 ± 0.05 ^a | | | | | | |
| Thinlip grey mullet (<i>Liza ramada</i>) | Prebiotics | Mannan oligosaccharide | 0.5% | 56 days | 1.22 ± 0.02 ^b | 2.57 ± 0.02 ^a | 2.71 ± 0.05 ^a | [100] |
| | | | 1% | | 1.21 ± 0.03 ^b | 2.54 ± 0.03 ^a | 2.75 ± 0.09 ^a | |
| | | | 2% | | 1.24 ± 0.01 ^b | 2.47 ± 0.02 ^b | 2.66 ± 0.03 ^b | |
| | | | Control | | 1.43 ± 0.10 ^a | 2.34 ± 0.04 ^c | 2.32 ± 0.15 ^c | |
| Rohu (<i>Labeo rohita</i>) | Prebiotics Probiotics Symbiotics | Fructo Oligosaccharide | 10 ⁷ cfu/g | 90 days | 2.22 ± 0.01 ^{d*} | 1.26 ± 0.01 ^{a*} | 1.29 ± 0.02 ^b | [111] |
| | | <i>Bacillus licheniformis</i> | | | 2.0 ± 0.01 ^{c*} | 1.34 ± 0.02 ^{b*} | 1.43 ± 0.01 ^{c*} | |
| | | <i>Bacillus methylotrophicus</i> | | | 2.02 ± 0.02 ^{c*} | 1.34 ± 0.01 ^{b*} | 1.41 ± 0.01 ^{c*} | |
| | | FOS + <i>Bacillus licheniformis</i> | | | 1.74 ± 0.01 ^{a*} | 1.42 ± 0.02 ^{c*} | 1.64 ± 0.02 ^{e*} | |
| | | FOS + <i>Bacillus licheniformis</i> | | | 1.84 ± 0.01 ^{b*} | 1.37 ± 0.01 ^{bc*} | 1.55 ± 0.02 ^{d*} | |
| | | FOS + <i>Bacillus methylotrophicus</i> | | | 2.4 ± 0.01 ^{e*} | 1.19 ± 0.02 ^{a*} | 1.19 ± 0.02 ^{a*} | |
| Nile tilapia (<i>Oreochromis niloticus</i>) | Probiotics, prebiotics and symbiotics | <i>Lactobacillus plantarum</i> CR1T5 | 10 ⁸ CFU g ⁻¹ | 84 days | 1.56 ± 0.01 ^c | 2.61 ± 0.01 ^b | [40] | |
| | | Xylooligosaccharides | 10 g kg ⁻¹ diet | | 1.55 ± 0.01 ^b | 2.59 ± 0.01 ^b | | |
| | | <i>Lactobacillus plantarum</i> + xylooligosaccharides | (10 ⁸ CFU g ⁻¹) + 10 g kg ⁻¹ | | 1.50 ± 0.01 ^b | 2.70 ± 0.03 ^a | | |
| | | Control | Control | | 1.62 ± 0.01 ^{a-} | 2.53 ± 0.02 ^c | | |
| Snakehead fish (<i>Channa argus</i>) | Probiotics and Symbiotics | <i>Enterococcus faecalis</i> | 1.0 × 10 ⁸ cfu/g of diet | 56 days | 1.29 ± 0.01 ^{b-} | 2.38 ± 0.03 ^b | 1.84 ± 0.03 ^b | [99] |
| | | <i>Lactococcus lactis</i> | | | 1.23 ± 0.03 ^{c-} | 2.51 ± 0.02 ^c | 1.93 ± 0.01 ^c | |
| | | <i>Enterococcus faecalis</i> + <i>Lactococcus lactis</i> | | | 1.27 ± 0.02 ^{b-} | 2.42 ± 0.01 ^{bc} | 1.88 ± 0.01 ^b | |
| | | Control | | | 1.34 ± 0.02 ^{a-} | 2.26 ± 0.03 ^a | 1.77 ± 0.02 ^a | |
| Nile tilapia (<i>Oreochromis niloticus</i>) | Phytogenics | Essential oil from Lemon grass | 200 mg kg ⁻¹ Lemon grass oil | 84 days | 1.79 ± 0.01 ^{bc*} | 3.90 ± 0.01 ^{a*} | 1.75 ± 0.01 ^{ab*} | [101] |
| | | (<i>Cymbopogon citratus</i>) | 400 mg kg ⁻¹ Lemon grass oil | | 1.75 ± 0.04 ^{bc*} | 3.88 ± 0.04 ^{a*} | 1.78 ± 0.04 ^{a*} | |
| | | Essential oil from geranium | 200 mg kg ⁻¹ geranium oil | | 1.86 ± 0.04 ^{ab*} | 3.78 ± 0.03 ^{b*} | 1.68 ± 0.03 ^{bc*} | |
| | | (<i>Pelargonium graveolens</i>) | 400 mg kg ⁻¹ geranium oil | | 1.77 ± 0.03 ^{c*} | 3.93 ± 0.03 ^{a*} | 1.76 ± 0.03 ^{ab*} | |
| | | Control | Control | | 1.89 ± 0.03 ^{a*} | 3.75 ± 0.04 ^{b*} | 1.65 ± 0.02 ^{c*} | |
| Rainbow trout (<i>Oncorhynchus mykiss</i>) | Phytogenics | Garlic (<i>Allium sativum</i>) | 1% | 120 days | 0.74 ± 0.02 ^a | 2.63 ± 0.00 ^b | [102] | |
| | | | 2% | | 0.73 ± 0.04 ^a | 2.66 ± 0.03 ^{bc} | | |
| | | | 3% | | 0.73 ± 0.03 ^a | 2.68 ± 0.04 ^c | | |
| | | | Control | | 0.76 ± 0.01 ^a | 2.60 ± 0.01 ^a | | |
| Rainbow trout (<i>Oncorhynchus mykiss</i>) | Phytogenics | Garlic (<i>Allium sativum</i>) | 5% | 90 days | 2.90 ± 0.0 ^a | 3.62 ± 0.03 ^a | [112] | |
| | | | 7% | | 1.90 ± 0.0 ^d | 3.68 ± 0.02 ^a | | |
| | | | 10% | | 2.60 ± 0.0 ^b | 3.65 ± 0.01 ^a | | |
| | | | Control | | 2.10 ± 0.0 ^c | 3.50 ± 0.02 ^b | | |

Columns with different superscripts represent a significant difference at $p < 0.05$. NP—not provided. Errors are presented in standard deviation, except where the * is used. *—represents standard error (S.E) and—represents calculated values.

6.2. Sustainable Resource Utilization

Feed is the most crucial input in aquaculture as it affects the growth and survival of aquatic animals, thereby stimulating the profitability of aquaculture. Fed aquaculture usually relies on supplying aquatic animals with formulated feeds. Formulated feeds are produced with various feed ingredients to meet the nutritional requirements of animals. Of these feed ingredients, fishmeal remains the most efficient protein source for farmed aquatic animals. Fishmeal and fish oil are the primary sources of omega-3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) [113]. They are more nutritious and digestible than any other feed ingredient for farmed aquatic animals [113]. Fishmeal and fish oil are mainly obtained from wild stock/natural populations. Among the several users of fishmeal and fish oil, such as animal production industries and humans (consumers of fish oil), aquaculture, which ought to promote the sustainability of wild stock, has become the major user. For example, 73 to 86% of the total annual fish meal and oil produced in 2020 were used in aquaculture feed production [114]. Aquaculture production via fed aquaculture is estimated to increase to 106 million tons by 2030, which is 22% higher than that of 2020 [113]. This growth in production forecast implies an increase in feed formulation; therefore, more fishmeal and fish oil will be needed.

The low supply of fishmeal and fish oil, the high demand, coupled with the consistently high price (which has been projected to be on an upward trajectory continually), and awareness of sustainability issues have led to a drive toward an alternative protein source for aquaculture. This has led to using terrestrial, plant-based protein as a partial or complete fishmeal replacement. Substituting fishmeal (partially/totally) in aquafeeds with alternative protein sources beyond a certain measure, either for economic or sustainability reasons, lowers performance in aquatic animals [30,32,42,115]. This is because plant-based protein contains anti-nutritional factors, including tannins, phytates, oligosaccharides, and trypsin inhibitors. The anti-nutritional factors compromise feed intake, digestibility, and efficiency, as well as the gastrointestinal tracts and health of animals [42]. Including functional feed additives to alternative protein sources can ameliorate the use of alternative proteins and minimize the detrimental effects associated with higher inclusion in aquafeed. Soyabean meal (SBM) is a plant-based protein with the potential to supplement fishmeal in formulated feed. However, supplementing SBM in fish diets at quantities above 15–20% has been reported to be detrimental to the growth and health of fish [42]. With FFA, soyabean meal can replace fishmeal above the benchmark (15–20%) without side effects and, in some cases, results in better animal growth than fishmeal diets. In some studies, higher supplementation of SBM, with the addition of FFA, led to improved growth compared to the control void of SBM. For example, ref. [42] reported similar final weights, SGR, feed intake, and FCR as the control when SBM was fermented with probiotics *B. subtilis*, *Lactobacillus*, and *S. cerevisiae* and supplemented at 30% in the feed of largemouth bass (*Micropterus salmoides*). Also, the utilization of FFA leads to increased nutrient utilization of SBM, such that the apparent digestibility coefficient of dry matter (ADDM), crude protein (ADCP), crude lipid (ADCL), protein and lipid retention of fish that were fed fermented soya bean meal (at 30% replacement) was reportedly similar to those of the control (without SBM) ([42], Table 2).

Also, ref. [115] reported that a 30% SBM supplemented with 1 g of heat-killed *L. plantarum* per kg of feed resulted in significantly higher final weights, weight gain, SGR, and feed intake in amber jack juvenile (*Seriola dumerili*) than the control. No adverse effect on the animal's health was recorded as the animal had similar glucose, hemoglobin, hematocrit, and triglycerides as those fed the control feed. Additionally, amberjack that were fed 30% SBM supplemented with *L. plantarum* displayed a higher serum bactericidal activity than the control.

For aquatic animals with freshwater habitats and omnivorous/herbivorous feeding habits, a total replacement of fish meal is possible without any adverse effect on growth. However, the animals' immunity may be compromised, making them susceptible to infection [116]. For example, Oriental river prawns (*Macrobrachium nipponense*) that were

fed SBM fermented with a combination of probiotics (*Pediococcus acidilactic*, *E. faecalis*, *S. cerevisiae*, *Candida utilis*, *B. subtilis*, *B. licheniformis*, *Rhodopseudomonas palustris*) and enzymes (protease, cellulase, and xylanase) completely replaced fish meal without any adverse effect on weight gain, specific growth rate, feed conversion ratio, or survival. Interestingly, the values of these indices were similar to the controls that were fed diets void of soyabean meal. However, when challenged with live *Aeromonas hydrophila*, the mortality rate of Oriental river prawns that were fed diets in which fishmeal was totally replaced had higher mortality than the control (Table 2). The higher mortality was due to compromised health status, as evidenced by a lower total hemocyte count and phagocytic activity, which are immune-related biomarkers [117]. Functional feed additives promote sustainable resource utilization, such as the use of alternative protein sources in fish feed production, resulting in reduced dependency on fish meal and a lower cost of production, thereby making aquaculture more sustainable.

Table 2. Functional feed additives and amelioration of alternative protein sources.

| Name of Species | Types of FFA | Strain | Experimental Design | Duration of Study | Effects | Reference |
|---|--------------|--|--|-------------------|---|-----------|
| Amberjack (<i>Seriola dumerili</i>) | Probiotics | Heat-killed <i>Lactobacillus plantarum</i> | Control (0% SBM), 15%, 30%, and 45% SBM supplemented with probiotics at 0% or 0.1% | 56 days | Higher final weight, weight gain, specific growth rate, feed ingestion rate, and protein retention in 30% SBM supplemented with probiotics. Similar hematocrit levels except for 30% SBM without probiotics supplementation. Similar hemoglobin levels, except for SBM 45%, without probiotic supplementation. Higher serum bactericidal activity in 30% SBM supplemented with probiotics. 30% SBM and 0% SBM exhibited higher tolerance to low-salinity stress. High ADCP in all treatments except for 40% SBM with and without probiotic supplementation. | [115] |
| Largemouth Bass (<i>Micropterus salmoides</i>) | Probiotics | Fermentation of SBM with <i>Bacillus subtilis</i> , <i>Lactobacillus</i> and <i>Saccharomyces cerevisiae</i> | Control (0% SBM), 15%, 30%, 45% and 60% SBM and FSBM | 56 days | Similar nutrient utilization of fish fed 35% FSBM with the control in terms of ADDM, ADCP, ADCL, protein retention, and lipid retention. Similar final weight, SGR, feed intake, and FCR of 35% FSBM with the control. No harm was evident in the intestinal epithelial mucosa in all treatment groups. Significantly lower intestinal villus height in 60% SBM group than the control. Significantly lower villus width of 45% and 60% SBM and 60% FSBM groups than that of the control group | [42] |

Table 2. Cont.

| Name of Species | Types of FFA | Strain | Experimental Design | Duration of Study | Effects | Reference |
|--|--------------|--|--|-------------------|---|-----------|
| Oriental river prawn (<i>Macrobrachium nipponense</i>) | Probiotics | Fermented SBM fortified with probiotics (<i>Pediococcus acidilactic</i> , <i>Enterococcus faecalis</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida utilis</i> , <i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Rhodopseudomonas palustris</i>) and enzymes (protease, cellulase, and xylanase) | Control 25% FSBM 50% FSBM 75% FSBM 100% FSBM | 56 days | Higher weight gain, SGR, and FCR in fish fed 25% SBM compared to other treatments and the control. Higher total hemocyte counts in control, 25% and 50% FSBM than other treatments. Higher mortality rate on exposure to <i>Aeromonas hydrophila</i> in 75 and 100% SBM than the control, 25 and 50% SBM. | [116] |
| Silver Barb (<i>Barbonymus gonionotus</i>) | Probiotics | SBM fermented with <i>Lactobacillus paracasei</i> | 20% FM + 20% FSBM 20%FM + 20%SBM 40% FSBM 40% SBM 40% FM (Control) | 90 days | Higher weight gain and SGR in fish fed the control, 20% FM + 20% FSBM and 20% FM + 20% SBM compared to fish fed 40% FSBM and 40% SBM. Lower hematocrit, hemoglobin, and erythrocyte counts in fish fed 40% SBM diet compared to those fed the control and 20% FM + 20% FSBM. | [118] |

SBM—soyabean meal; FSBM—fermented soyabean meal; ADDM—apparent digestibility coefficient of dry matter; ADCP—apparent digestibility coefficient of crude protein, ADCL—apparent digestibility coefficient of crude lipid; FCR—feed conversion ratio.

6.3. Enhanced Disease Resistance/Immunity

Organisms in aquatic habitats are constantly exposed to the risk of disease occurrence [119]. The risk of disease occurrence is higher in aquatic environments than in terrestrial habitats, as aquatic animals may ingest pathogens present in water via feeding [119]. Disease outbreak is a major constraint in aquaculture, accounting for about 40–60% of production losses experienced in fish and crustacean farming [120,121]. Various factors facilitate aquatic disease outbreaks. For example, the quest to meet the demand for aquatic animals and maximize profit has led to the intensification of aquaculture [122]. Aquaculture intensification via high stocking density acts as a breeding ground for pathogens/parasites, increasing the chances of the occurrence and spread of diseases [122]. Poor water quality, husbandry, handling, and nutrition lead to stress (acute/chronic) in farmed aquatic animals. Stress results in reduced growth performance and suppressed immune systems, eventually increasing the disease susceptibility of fish under culture.

Antibiotics have been included in aquatic feed to prevent or treat bacterial diseases. Antibiotics are known to eliminate intestinal bacteria, thus resulting in improved growth and feed efficiency in aquatic animals [123]. However, antibiotics and chemotherapeutics have been associated with the emergence of antibiotic-resistant bacterial strains, the elimination of non-target natural environment microbial, and antibiotic residues in reared animals intended for human consumption [124]. Apart from these detriments, antibiotics are usually expensive, adding to the cost of production. Probiotics, prebiotics, and phytonutrients have been used to prevent/reduce disease and boost host immunity. For example, European sea bass, *D. labrax*, that were fed a *B. velezensis*-supplemented diet at a concentration of 10^6 CFU g^{-1} feed for 30 days had higher serum bactericidal activity, lysozyme activity, and nitric oxide production in the serum than those of the control when challenged with *V. anguillarum* [125]. This indicates that fish that were fed *B. velezensis*-supplemented diets were healthier than the control as the health status of an animal can be assessed via hematological indices [83]. Likewise, after exposure to *Vibrio anguillarum*, the survival rates

of fish fed *B. velezensis*-supplemented diets were higher than that of the control. Abalone, *H. discus hanna*, that were fed *B. licheniformis*-supplemented diets had higher total hemocyte counts and nitric oxide from respiratory bursts than those of the control. Those fed *B. licheniformis* at 10^5 and 10^7 cfu/mL had higher phagocytic activity of the hemolymph compared to that of those fed a 10^3 cfu/mL concentration and the control. Fourteen days after infecting the abalone with *V. parahaemolyticus*, abalone fed probiotic-supplemented diets had lower mortality compared to the control. The dosage of *B. licheniformis* affected abalone mortality, with a lower dosage (10^3 cfu/mL) resulting in higher mortality compared to those fed 10^5 and 10^7 cfu/mL [54].

Prebiotics (xylooligosaccharides; 10 g kg^{-1} feed) derived from corn cob, probiotics (*L. plantarum*; 10^8 CFU g^{-1} feed), and symbiotics (xylooligosaccharides (10 g kg^{-1} feed) + *L. plantarum* (10^8 CFU g^{-1} feed)) were fed to Nile tilapia (*O. niloticus*) for 86 days. On challenging with *Streptococcus agalactiae*, fish that were fed the symbiotic diet had the highest survival (71.88%) compared to the control (31.25%), those that were fed a probiotic alone (59.38%), and those that were fed prebiotics alone (56.25%). Feeds supplemented with probiotics, prebiotics, and symbiotics had higher skin mucus lysozyme activity, skin mucus peroxidase activity, serum lysozyme activity, serum phagocytosis activity, serum peroxide activity, and alternative complement activity (aCH50) compared to the control group. However, fish that were fed symbiotics had higher skin mucus lysozyme activity, skin mucus peroxidase activity, and serum phagocytosis activity than those of fish that were fed probiotics alone or prebiotics alone [40].

Even though symbiotics result in better health and immunity benefits than diets containing probiotics or prebiotics alone, the concentration used in the symbiotics may impact the efficiency of the symbiotics in achieving the required benefits. Various combinations of *B. subtilis* and mannan oligosaccharide [(15% probiotics + 0.2% prebiotics); (5% probiotics + 0.6% prebiotics); (15% probiotics + 0.6% prebiotics)] were used as a feed supplement for mrigal carp (*Cirrhinus mrigala*) for 60 days. Mrigal carp supplemented with 15% probiotics + 0.6% prebiotics had increased lysozyme and respiratory burst activity and antioxidant enzymes compared to the control and other symbiotic treatment groups. Also, 15% probiotics + 0.6% prebiotics had the lowest mortality (20%) after the challenge with *Aeromonas hydrophilla* infection, while the control had 80% mortality [44]. Before a mixture of probiotics or symbiotics is used, it is advisable to evaluate the consequence of their interactions along with their distinct biological effects, as the combination of *B. subtilis* and *L. Plantarum* resulted in an antagonistic effect, leading to reduced immunity in mud crab, *Scylla paramamosain* [126].

Catfish, *Clarias gariepinus*, that were fed diets containing 0.5, 1, and 3% garlic powder had higher RBC counts, WBC counts, plasma protein, hemoglobin, and packed cell volumes compared to the control when fed for 12 weeks [83]. However, among the *C. gariepinus* fed garlic-supplemented diets, those fed a 0.5% garlic diet had significantly higher RBC counts, WBC counts, plasma protein, hemoglobin, and packed cell volumes [83]. Al-Sagheer et al. (2018) [101] used essential oils from lemongrass (*C. citratus*) and geranium (*P. graveolens*) as a feed supplement for Nile tilapia, *O. niloticus*, for 84 days. The essential oils were used at 200 and 400 mg kg^{-1} concentrations. The application of lemongrass oil and geranium oil reduced the total bacterial counts, coliform counts, *Escherichia coli* counts, and *Aeromonas* spp in the gastrointestinal tract of the fish compared to the control. Lemon grass oil at a concentration of 200 mg kg^{-1} led to a significant increase in serum catalase (CAT), plasma lysozyme activity, and immunoglobulin M (IgM) compared to other concentrations of lemongrass oil, geranium oil, and the control. The survival rate of tilapia after challenge with *A. hydrophilla* was 70, 95, 90, 85, and 95% for the control, 200 and 400 mg kg^{-1} lemon grass oil, and 200 and 400 mg kg^{-1} geranium oil, respectively. Advances in the use of FFA have occurred, being used as an immunostimulant and for the treatment of infections. The aqueous extract of neem leaves has been successfully used to treat *Citrobacter freundii* infection. According to [127], infected fish were treated by rearing them in sterile water containing 10 mg/l of the extract or by injecting intramuscularly. The

survival rate was recorded to be 80%, while those treated by injecting intramuscularly had a 70% survival rate. The efficacy of FFA is influenced by the strains/species of probiotics, prebiotics, or phytochemicals used, the dosage used, the duration of exposure, and the species of animals [128]. The FFA dosage may depend on the target benefits intended to be achieved. The dosage required for growth improvement may vary from disease immunity; for example, 10^8 cfu (kg^{-1} diet) of mixed probiotics is efficient for growth improvement in *L. vannamei*. However, 10^7 cfu (kg^{-1} diet) is more efficient in inducing disease resistance to *V. alginolyticus* [98]. The implication of the prolonged application of probiotics (throughout the culture period) is unknown. However, for prebiotics (β -glucan), a high dosage or prolonged use negatively impacts aquatic animals' immunity, leading to immunosuppression [129]. Moderate doses of β -glucan and an alternation of feeding regime (with basal diet void of β -glucan) are advised. Notwithstanding, [130] reported that a high concentration of β -glucan (10g kg^{-1} of feed) fed for the short term to gilthead seabream with an alternated feeding regime improved immunity against Pasteurellosis, while a low concentration of β -glucan (1g kg^{-1} of feed) fed for the long term with an alternated feeding regime resulted in higher immunity. The high concentration of β -glucan used for a long term resulted in immunosuppression as the mortality rate recorded was similar to that of the control. The discrepancies between the two studies mentioned above may be due to the different fish species and pathogens. Therefore, dosage and effectiveness against each species and pathogen need to be tested [130].

6.4. Antiparasitic

Parasite infestation is another limitation of aquaculture. Ectoparasites feed on the host's tissue, mucous, and blood, causing lesions on the host's skin. Parasite infestation affects the growth of the host, making the host vulnerable to a secondary infection by bacteria or fungi, which may result in mortality [9]. Parasite infestation also reduces the value of aquaculture products and reduces profitability [131]. Functional feed additives, such as phytochemicals, are also effective antiparasitics and help prevent or eliminate parasite infestation. Phytochemicals have been used in preventing or reducing the infection success of parasites, including *Ichthyophthirius multifiliis*, Trichodiniasis, and monogeneans [132–134].

Emmaectin benzoate, classified as avermectin, is safe and commonly used to treat all developmental stages of sea lice. However, the efficiency of emmaectin benzoate on sea lice is decreased due to its broad usage, resulting in parasite adaptation and, hence, increased tolerance [135,136]. Azadirachtin A extract from neem oil has been reported to effectively reduce sea lice infestation in salmonids [137]. Garlic-supplemented diets (50 and 150 mL of crushed garlic per kg of feed) fed to barramundi, *Lates calcalifer*, for ten days resulted in a similar infection prevalence (92–100%) to that of the control, regardless of the concentrations of garlic included in the feed. Garlic-supplemented diets (50 and 150 mL of crushed garlic per kg of feed) fed to barramundi, *Lates calcalifer*, for 30 days led to a lower infection success (about 10%) compared to the control [138]. Phytochemicals are effective alternatives in managing parasites in aquaculture because they are from natural sources, safe, environmentally friendly, and in line with sustainable production practices.

6.5. Improved Water Quality

Aquafeeds are rich in nutrients such as protein, carbohydrates, minerals, and vitamins. Protein, which comprises the bulk of nutrients in aquafeed, comprises carbon, nitrogen, phosphorous, and some other elements [139]. Of the nitrogen and phosphorous entering the culture system (via feed and fertilizer applications), only about 20–50% are retained in the animals. This implies that about 50–80% of these nutrients are remnants in the culture system. Therefore, the aquaculture culture unit is characterized by organic matter, nitrogenous and organic waste like ammonia and nitrite emanating from unconsumed feeds, and feces and other excretory products of aquatic animals. The buildup of these nutrients in the culture system is detrimental to the aquatic animals in such a system as it

deteriorates the water quality, increasing the biochemical and chemical oxygen demand, thus depleting the oxygen within the system.

Water quality refers to water's physical, chemical, and biological attributes, which influence its appropriateness for a specified use [140]. Water quality is critical in aquaculture because aquatic animals reside in water, and all their physiological functions, such as respiration, feeding, excretion, and reproduction, are carried out within the water medium. A strong link exists between water quality and the health of the animals in land-based culture systems [141]. Suboptimal water quality does not directly cause mortality per se but causes stress in aquatic animals, making them prone to disease [140]. It is necessary that the water quality be optimized, as to a great magnitude, it affects the growth, survival, reproduction, quality, and hence, the productivity and success of aquaculture ventures.

One practice that causes aquaculture sustainability to be frowned upon is effluent discharge into the aquatic ecosystem [87]. The aquatic ecosystem is the end point of aquaculture effluents, regardless of the production system used (be it open water or land-based systems). Untreated effluent is nutrient-dense and negatively impacts the receiving water body, which could result in excessive algal bloom and eutrophication. These may destroy aquatic biodiversity, reduce dissolved oxygen in the water, and destabilize ecosystem equilibrium [142].

Various techniques and systems have been developed to treat aquaculture wastewater and improve quality to reduce the water's nutrient load. They include bio floc technology, recirculating aquaculture systems, integrated multitrophic aquaculture, aquaponics, the use of biofilters, water exchange, photocatalysis, and chemicals such as Zeolite [23,26,27,143–145]. However, it is either that the systems/techniques are complicated, not cost-effective, or may result in bioaccumulation in the animal, which is detrimental to the consumer in the case of chemicals [146].

Functional additives can be applied to feed or water to enhance water quality. However, the application of functional additives to water yields better results in terms of water quality improvement, although application as feed additives has also produced some substantial results [146]. European sea bass (*D. labrax*) that were fed a *P. acidilactici*-supplemented diet at a rate of 2 g/kg, 2.5 g/kg, and 3 g/kg of feed for 60 days experienced a significantly reduced water pH and ammonia concentration in the tank receiving the probiotics diets compared to the control [53]. Similarly, the tank water of Crucian carp (*Carassius carassius*) receiving feed containing mixed probiotics (*B. megaterium* mixed with *B. subtilis*, *B. megaterium* mixed with *B. coagulans* at a concentration of 6.0×10^5 CFU/mL) had reduced NO₂-N, NO₃-N, and total phosphorous concentrations compared to the control after 15 days of application. Specifically, tanks receiving treatments *B. megaterium* + *B. subtilis* and *B. megaterium* + *B. coagulans* had 21.9% and 7.7% lower NH₄⁺-N concentrations, respectively, compared with the control [147].

When it comes to FFA application for water quality improvement, the temperature and exchange rate of the water are of critical concern so as not to kill the probiotic or render it ineffective by flushing out of the culture system [148]. The improvement of water quality through FFA offers numerous benefits for the health and welfare of farmed aquatic animals, such as stress reduction, enhanced survival and growth, reduced incidence of disease outbreaks, and minimized environmental impact, thereby increasing the sustainability of aquaculture (Table 3).

Table 3. The effect of functional feed additives on hematological indices and immunity.

| Animal Species | Types of Functional Feed Additives | Name of Strain | Concentration | Duration | Effect | Reference |
|--|------------------------------------|--|---|----------|---|-----------|
| Nile tilapia (<i>Oreochromis niloticus</i>) | Probiotics SD | <i>Saccharomyces cerevisiae</i> | 0 (control) 1 g kg ⁻¹ diet. 2 g kg ⁻¹ diet. 4 g kg ⁻¹ diet. | 60 days | Increased gut villus wall thickness, villus length, width, and area. | [43] |
| Catfish (<i>Clarias gariepinus</i>) | Probiotics | <i>Bacillus</i> NP5 | 0 (control) 1 × 10 ⁹ 1 × 10 ¹⁰ | 45 days | Increased levels of erythrocytes, leucocytes, hemoglobin, and phagocytic activity. Similar hematocrit count | [109] |
| Flounder fish (<i>Paralichthys olivaceus</i>) | Probiotics | <i>Lactococcus lactis</i> BFE920 <i>Lactobacillus plantarum</i> FGL0001 Mixture of both probiotics | 10 ⁷ cfu g ⁻¹ feed | 30 days | Increased skin lysozyme activity in flounders fed probiotics compared to the control. Increased skin lysozyme in flounders fed <i>L. plantarum</i> and a mixture of probiotics compared to <i>L. lactis</i> . Increased phagocytosis activity in flounders fed probiotic supplemented diets than the control (increased phagocytosis activity in mixed probiotics compared to single probiotics). Increased respiratory burst activity in Flounders fed probiotic diets compared to the control. Higher survival rate in flounder fed probiotics than] the control after exposure to <i>Streptococcus imiae</i> . | [96] |
| Abalone (<i>Haliotis discus hannai</i>) | Probiotics | <i>Bacillus licheniform</i> | 10 ³ cfu/mL 10 ⁵ cfu/mL 10 ⁷ cfu/mL Control | 56 days | Higher total hemocyte counts in probiotics supplemented diets than in control. Higher phagocytic activity in abalone fed diets supplemented with 10 ⁵ and 10 ⁷ probiotics than those fed diets containing 10 ³ probiotics and the control. Higher nitric oxide was produced from the respiratory bursts in abalone fed probiotics supplemented diets compared to the control. Lower mortality rate after exposure to <i>Vibrio parahaemolyticus</i> infection in abalone fed a probiotic-supplemented diet than the control. | [54] |
| European sea bass (<i>Dicentrarchus labrax</i>) | Probiotics | <i>Bacillus velezensis</i> | 10 ⁶ cfu g ⁻¹ feed | 30 days | Higher bactericidal activity, lysozyme activity, and nitric oxide production in fish fed <i>B. velezensis</i> -supplemented diets compared to the control. Higher survival rates after exposure to <i>Vibrio anguillarum</i> in fish fed <i>B. velezensis</i> -supplemented diets compared to the control. | [125] |

Table 3. Cont.

| Animal Species | Types of Functional Feed Additives | Name of Strain | Concentration | Duration | Effect | Reference |
|--|---------------------------------------|--|--|----------|--|-----------|
| White shrimp (<i>Litopenaeus vannamei</i>) | Probiotics | <i>Bacillus subtilis</i> <i>Lactobacillus pentosus</i> <i>Lactobacillus fermentum</i> <i>Saccharomyces cerevisiae</i> Mixture of all probiotics at 10^7 , 10^8 , and 10^9 Concentrations | 10^9 cfu kg diet ⁻¹ 10^9 10^9 10^9 10^7 10^8 10^9 Control | 56 days | Similar total hemocyte counts, superoxide dismutase, and phagocytic activity between the treatments and control. Increased respiratory burst in all mixtures of probiotics compared to the single strains and control. Reduced mortality on exposure to <i>Vibrio alginolyticus</i> and increased lysozyme in all probiotics diets (except single strain of <i>Saccharomyces cerevisiae</i>) compared to the control. | [98] |
| Mrigal carp (<i>Cirrhinus mrigala</i>) | Symbiotic (Probiotics + prebiotics) | <i>Bacillus subtilis</i> + Mannan oligosaccharide | 15% probiotics + 0.2% prebiotics 5% probiotics + 0.6% prebiotics 15% probiotics + 0.6% prebiotics (All at 10^7 cfu ml ⁻¹) Control (0%) | 60 days | Increased lysozyme and respiratory burst activity and antioxidant enzymes in 15% probiotics + 0.6% prebiotics compared to the control and other symbiotic treatment groups. Higher red blood cell and white blood cell counts in 15% probiotics + 0.6% prebiotics compared to the control pre-challenge and post-challenge. Lower mortality (20%) in 15% probiotics + 0.6% prebiotics after challenge with <i>Aeromonas hydrophilla</i> infection than the control, which had 80% mortality. | [44] |
| Nile tilapia (<i>Oreochromis niloticus</i>) | Probiotics, prebiotics and symbiotics | <i>Lactobacillus plantarum</i> CR1T5 Xylooligosaccharides <i>Lactobacillus plantarum</i> + xylooligosaccharides Control | 10^8 CFU g ⁻¹ 10 g kg ⁻¹ diet (10^8 CFU g ⁻¹ + 10 g kg ⁻¹) | 84 days | Fish fed feeds supplemented with probiotics, prebiotics, and symbiotics had higher skin mucus lysozyme activity, skin mucus peroxidase activity, serum lysozyme activity, serum phagocytosis activity, serum peroxide activity, and aCH50 alternative complement activity compared to the control group. Similar respiratory burst activity in all treatments, including the control. Fish fed symbiotic diet had the highest survival (71.88%) compared to the control (31.25%), probiotic alone (59.38%), and prebiotics alone (56.25%). | [40] |
| Caspian white fish (<i>Rutilus frisii kutum</i>) | Prebiotics | Galactooligosaccharides | 1% 2% 3% | 48 days | Fish fed diets containing 1% and 2% galactooligosaccharide had higher serum total immunoglobulin and lysozyme levels compared to 3% galactooligosaccharide and the control. Fish fed diets containing GOS had higher serum alternative hemolytic complement activity (ACH50) than the control. | [149] |

Table 3. Cont.

| Animal Species | Types of Functional Feed Additives | Name of Strain | Concentration | Duration | Effect | Reference |
|--|------------------------------------|---|--|----------|---|-----------|
| Nile tilapia (<i>Oreochromis niloticus</i>) | Phytogenics | Essential oil from Lemon grass (<i>Cymbopogon citratus</i>) | 200 mg kg ⁻¹ Lemon grass oil | 84 days | Increased survival rate in all treatments compared to the control after exposure to <i>Aeromonas hydrophila</i> . Increase in serum catalase enzyme, plasma lysozyme activity, and immunoglobulin M in fish fed diets supplemented with 200 mg kg ⁻¹ lemon grass oil compared to other treatments and the control. | [101] |
| | | Essential oil from geranium (<i>Pelargonium graveolens</i>) | 400 mg kg ⁻¹ Lemon grass oil 200 mg kg ⁻¹ geranium oil 400 mg kg ⁻¹ geranium oil Control | | | |
| Catfish (<i>Clarias gariepinus</i>) | Phytogenics | Garlic powder | 0.5% | 84 days | Increased leukocyte, erythrocyte, plasma protein, packed cell volume and hemoglobin values in fish fed the garlic supplemented diets compared to the control. Increased leukocyte, erythrocyte, plasma protein, packed cell volume, and hemoglobin values in fish fed the 0.5% garlic-supplemented diets compared to those fed other concentrations of garlic-supplemented diets. | [83] |
| | | | 1% 3% Control | | | |
| Asian seabass (<i>Lates calcarifer</i>) | Phytogenics | Neem leaf | 1 g kg ⁻¹ feed. 2 g 3 g 4 g 5 g | 28 days | Increased phagocytic activity, superoxide anion production, serum lysozyme, serum bactericidal activity, serum anti-protease activity in fish fed diets containing neem compared to the control. | [150] |

7. Adoption of Functional Feed Additives

Government policies, such as restrictions/bans on antibiotic use, led to the quest to develop safe and effective additives that can serve as an alternative to antibiotics, birthing the idea of functional additives in aquaculture. This validates the saying that necessity is the mother of invention. Identifying feed additives with exceptional properties started with research and expanded to field trials and ensuring that the feed additives used in feed formulation comply with the standards, limits, content requirements, and other specifications determined by regulation, the process of obtaining a license, and commercialization [151]. The functional feed industry has seen the rise of many commercial products for the various life stages of cultured animals. Some examples of commercial functional feeds are Z Pro by Zeigler and Bactocell by Lallemand Animal Nutrition (for finfish and shrimp), Hinter Aquafeed premix by Hinter (for finfish and shellfish), Sanacore*GM by Adisseo (for finfish), Leiber @ Bet-S by Leiber, and Nucleforce by Bioberica (both for finfish and crustaceans) [152]. The benefits of functional feed additives have gained awareness through the sensitization of aquaculture practitioners and stakeholders (seeking innovative solutions to enhance aquatic animals' growth, production, and health) via seminars and scientific publications. The demand for functional feed is rising; its growth is projected to increase at a compound annual growth rate (CAGR) of 4.3% between 2023 and 2030 [152]. The drivers

of functional feed demands are increased aquaculture product demand, the quest to enhance animal growth, profitability, disease prevention/resistance, and parasite prevention and termination. Functional feed additives are used in carp, shrimp, catfish, and almost all land-based aquatic farming.

Functional feeds have been widely adopted in Asia, Europe, and North America, probably because these are regions with established aquaculture industries. In China, functional feed additives are classified according to the particular function attributed to their use. These classifications include disease preventive feed, growth enhancement feed, health care feed, and fillet quality improvement feed [153]. For example, functional feed containing astaxanthin improves the color of fillets, leading to better acceptance by consumers. The color could cause the product to be graded as a premium, leading to higher prices and profitability. Thus, due to the benefits derived from functional feed, together with meeting sustainability requirements, functional feed may overtake traditional feed in aquaculture, especially in developed countries/large-scale aquaculture farms. However, in developing countries/small-scale farms, traditional feeds may persist due to the cost associated with functional feeds, which would increase the cost of the products to the point that consumers within the locality may not be able to afford them.

8. Challenges Associated with Functional Feed Additives

Although functional feed additive is beneficial to improving the growth performance, health, and overall immunity of the target species, it is not void of limitations. Functional feed additives contain specialized additives that may increase the feed cost and cost of production, making functional feeds more expensive than traditional feeds [154,155]. Formulating an effective functional feed requires a thorough understanding of the nutritional requirements of the target species. Hence, incorporating functional additives, such as probiotics and prebiotics, may require fish nutrition and feed formulation expertise. Ironically, the application of phytogenics, which seems less technical than other additives, may also require expertise. Moreover, the use of certain feed additives in aquaculture feeds may depend on regulatory approval; obtaining the approval for novel additives may be prolonged and complicated, delaying the commercialization of the feed. One of the reasons that such innovativeness is checked is to ascertain that the consumption of animals from such innovativeness is not detrimental to humans/consumers. Certain functional additives may be susceptible to environmental factors like temperature and humidity, thus impacting their stability and shelf life [148]. Maintaining the stability of these additives during the feed production process and durability during storage present a technical difficulty. This can be minimized using micro-encapsulation, which protects the additives during production and storage [156,157]. Functional feed additives are only effective when administered a few days or weeks before the commencement of a disease. One cannot foretell when a disease may occur, meaning that FFA needs to be used continuously/intermittently in farms to be effective because once the disease has commenced, FFA has limited ability to treat such disease. Due to this limitation in treating diseases, a high dependency on antibiotics and chemotherapeutics to treat diseases is still being recorded in aquatic farming. As in real-life situations, some functional feed may not be as palatable to some animals as traditional/conventional feed. This may result in inadequate feed intake, thus compromising the very essence of the functional feed [137]. The quantity of feed additive in functional feed is vital, as an excess may result in palatability issues, and too little may be inadequate to portray the attributes of the additive. Neem is known to have a bitter taste; hence, a high concentration in diets leads to reduced palatability [137]. To minimize feed palatability issues, functional feed could be introduced to animals early in life and not after the animal's taste buds are acquainted with a particular diet. Also, synthetic flavors, such as those obtained from amino acid mixtures (e.g., mixtures of arginine, alanine and glycine) or the mixture of water-soluble solvents such as propylene glycol with aromatic chemicals like trimethylamine, 2-acetylpyrazine, 2-acetylpyridine, and dimethyl sulfide, can be applied in feeds to reduce palatability issues [158,159].

Furthermore, the market acceptability of aquaculture products fed with functional diets may be influenced by the consumer's perception of functional feeds [160]. Hence, consumers need to be enlightened on the benefits associated with functional feeds. Moreover, functional diets may influence the sensory properties of aquaculture products [161]. For example, fish fed with diets supplemented with phytochemicals additives like garlic had the garlic flavor incorporated into their fillets (personal observation), which may be repulsive to consumers who are sensitive/allergic to garlic. The perception of aquaculture products affects both the demand and supply of the product. A diet switch from feed with phytochemical additives to feed void of additives can be performed in a few weeks before harvest to deplete the phytochemical concentration in the tissue. Furthermore, it is unknown if the use of probiotics will result in antimicrobial resistance, as probiotics have been reported to possess anti-microbial resistance genes or develop tendencies to result in antimicrobial resistance, which is one reason the use of antibiotics is discouraged [162].

9. Conclusions

This study expounds on the benefits of functional feed additives to aquaculture and how they help minimize the sustainability challenges associated with aquaculture. The various literature examined showed that the application of functional feed additives in aquaculture reduces stress, aids digestion, improves growth and water quality, increases the chances of survival of aquatic animals after exposure to infections, reduces parasitic infestation, and reduces the footprint of aquaculture on the environment. Feed additives, which provide all these benefits, are a plus to the farmer as they increase profitability, reduce reliance on antibiotics, and mitigate the cost of purchasing antibiotics, together with other effects associated with their use. All these benefits derived from functional feed additives make them superfoods. The initiative of functional feed additives remains a significant breakthrough for aquaculture; however, further research should be performed to determine the best functional feed additive combination and quantity that would result in more benefits than those attained presently.

10. Future Directions and Recommendations

The introduction of FFA in aquaculture is a welcome development, although it may not address all aquaculture sustainability challenges. Because FFA is a disease-preventive method rather than a treatment, future research is needed on natural alternative remedies to treat disease to further reduce reliance on antibiotics and chemotherapeutics. Further research should be conducted on the combinations, concentration, and duration of FFA applications to improve the effectiveness of FFA. Also, research should look into identifying cost-effective FFA, as the cost is a major constraint to adopting FFA. To address the various challenges with functional feed additives in aquaculture, collaboration among researchers, feed producers, regulatory bodies, and aquaculture practitioners is required. Continued research and innovation in feed technology and aquaculture practices are crucial to overcoming these challenges and advancing the sustainable use of functional feeds in aquaculture.

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