Chapter 2
Developments in Feeds in Aquaculture Sector: Contemporary Aspects

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Abstract Aquaculture, one of the largest protein food generating sectors, greatly relies on nutrition. Up to the present time, the dependency of the aqua feed sector on fish meal and fish oil as protein and lipid sources, respectively, was too high, which led to its inadequacy and over-exploitation of marine resources. Recently, numerous researches with locally available feed ingredients have been accomplished. There is urgency for a move from fish meal to plant/terrestrial animal/microbial proteins within the aquaculture industry as over-exploitation of wild fishes has negative ecological consequences. Plant proteins cannot successfully replace fish meal due to poor protein digestibility and essential amino acids imbalance, urging feed concerns to search for cheaper and nutritious fish meal alternatives from animal origin. In order to overcome the indiscriminate use of antimicrobial drugs, the concept of probiotics in aquaculture has received firm encouragement in recent years due to their wise inhibitory mechanisms and safeness. Negative perception for synthetic antioxidants among fish farmers and their unreliable nature has resulted in the research for non-synthetic, food derived antioxidants that could encounter and neutralize the detrimental effects of free radicals. In line with this, there is a strong captivation to research on the individual or synergistic effects of protein hydrolysates, peptide fragments, and free amino acids that could innately exhibit antioxidative activity. Commercialization of economical feeds with antioxidative feed ingredients could strengthen the profits of feed processors. Recent development in the aquaculture sector propelled by the application of biotechnological methods has clearly highlighted the need for development of functional feeds through incorporation of bioactive molecules. In the last few decades, the global aqua feed industry has witnessed milestones in the development of feed ingredients from waste raw materials and sustainable alternatives to antibiotics and chemicals used to tackle the disease outbreak in aquaculture. As the health and immunity of fishes

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primarily depend on their nutritional pattern, a great research interest is extended on incorporation of biomolecules like single-cell proteins, animal proteins, plant metabolites, biopolymers, and enzymes as feed ingredients to enhance the nutritive quality and immune tolerance in fishes. Besides the focus on the feed ingredients, the modification and development of fermentation strategies for producing probiotic-based feed and enzyme-assisted bioconversion into valuable feed ingredients is also gaining more importance. This chapter deals with the recent development in the aqua feed industry with specific reference to the incorporation of non-conventional feed ingredients like animal/microbial proteins, biopolymers, enzymes, and other immunostimulatory compounds in aqua feed and their impact analysis in improving the growth profile and pathogen tolerance in fishes.

Keywords Aqua feed · Probiotics · Fish meal · Biopolymers · Polyhydroxyalkanoates · Chitosan · Cellulose · Keratin · Single-cell protein · Immunostimulants · Enzymes

2.1 Introduction

The significance of inland aquaculture becomes more pronounced as the marine resources all over the world are depleting at alarming rates. The latest Food and Agriculture Organization (FAO) report depicts a domination of Asian countries in the arena of production of farmed aquatic animals. Asia has a share of 89 percent in inland aquaculture for the last two decades. China, India, Indonesia, Vietnam, Bangladesh, Egypt, Norway, and Chile form the major contributors. 63% of the world's farmed food fish production which consists of fish farms and inland natural water sources produces 51.3 million tonnes of aquatic animals by inland aquaculture. In global aquaculture, India holds second rank (FAO 2020). Among the many reasons for low productivity of many aquaculture farms in India are poor feed conversion ratio, fragmented holdings, lack of skilled personnel, etc.

One of the ways of improving fish productivity is by scientific fish feed formulation. When cultivated in large quantities and high densities, fishes require a high quality, nutritionally balanced diet for rapid and healthy growth. In this context, the quality of fish feed assumes great importance. One of the best ways to assess the efficiency of aquaculture farms is by calculating the "feed conversion ratio" (FCR), which is the weight of feed administered over the lifetime of an animal divided by the weight gain. Apart from proteins, lipids, and carbohydrates, there should be ample amounts of essential amino acids, minerals, and vitamins in the fish feed which can contribute to a better FCR. The past decade saw the emergence of many novel components in the feed compositions for aquaculture. Some of these newer components include probiotics, enzymes, single-cell proteins (SCP), and biopolymers like chitosan, cellulose, and keratin.

One of the grave concerns in aquaculture is the increasing problem of antimicrobial resistance among fish pathogens (Laxminarayan et al. 2013; Sattanathan et al. 2020a) which in turns drive to pump more and more antibiotics in the fish feed.

Antibiotics in fish feed presents one of the best routes for environmental exposure of these chemicals as the drug distribution through water can happen at faster rates with important ecosystem health implications (Liu et al. 2020; Lulijwa et al. 2020). For these reasons, there is a global demand for alternative ways of improving the immunity of the fish population. One of the upcoming trends is the use of probiotics, the details of which would be discussed in detail. Biopolymers are also emerging as novel components in fish feed. There is more thrust given to the production of biopolymers like chitosan, keratin, etc. produced from farm wastes as these set perfect examples for recycling of organic matter in the ecosystem. For the production of biopolymers, various kinds of agricultural and industrial wastes can be used as substrates. This will have multiple advantages—prevention of excess agro by-products going to waste and chance of making waste from one sector as substrate for another sector, thereby reducing the cost for procuring fresh raw materials. Effective ways of waste valorization is an area of active research these days as it can help achieve “zero waste” targets and biopolymer production from agro wastes can be seen as a perfect example of this. The task before us is to find out means of having standardized and cost-effective methods of achieving this. In fact, if standardized and accomplished in the proper manner, biopolymer production and incorporation in fish feed would indeed cater to the sustainable goals of the United Nations. Probiotics, prebiotics, and enzymes added to the fish feed will definitely result in better feed conversion ratio by helping the proliferation of friendly microorganisms, increased digestibility of food, thereby resulting in better returns to farmers. The present article discusses the latest developments in fish feed formulation for cost-effective aquaculture and environmental sustainability. The 2018 observed all-time record of 178.5 million tonnes of global fish, mollusks, crustaceans, and other aquatic animals’ production, excluding aquatic plants, was a 3% growth compared to 2017. A 401 US billion dollar worth global fish production was estimated in the first sale out of which aquaculture contributed 250 US billion dollars. The top ten producers of aquaculture (Fig. 2.1) produced 72.8 million tonnes collectively. By quantity, this is 88.7% of the global aquaculture production of 2018 which consisted of finfish (54.3 million tonnes), mollusks (17.5 million tonnes), crustaceans (9.4 million tonnes), and other aquatic species (0.9 million tonnes) (Fig. 2.2) (Food and Agriculture Organization 2021).

Over the current estimation (2015) of 73 million tonnes, global aqua feed output is forecast to rise in 2025 by 33 percent to 101,3 million tonnes, closely aligned with the targeted 101.8 million tonnes worldwide aquaculture production (Salin et al. 2018). Feed is the single most important input in aquaculture. As a result, the expansion of aquaculture practices is dependent on the expansion of the aqua-feed industry in order to achieve the projected fish yield. Traditionally, fish meal has been used as a primary source of dietary protein in the process. However, in the past few years, a high requirement and lack of supply of the fish meal resulted in an increase of price, and an absolute reliance on fish meal is not suggested (Chakraborty et al. 2019). Modern aqua feed is a complex, inventive mix of raw materials which supply the nutritional needs to make aquaculture species more intense and productive. Commodity meals, oils, vitamins, pigments, minerals, and concentrates are

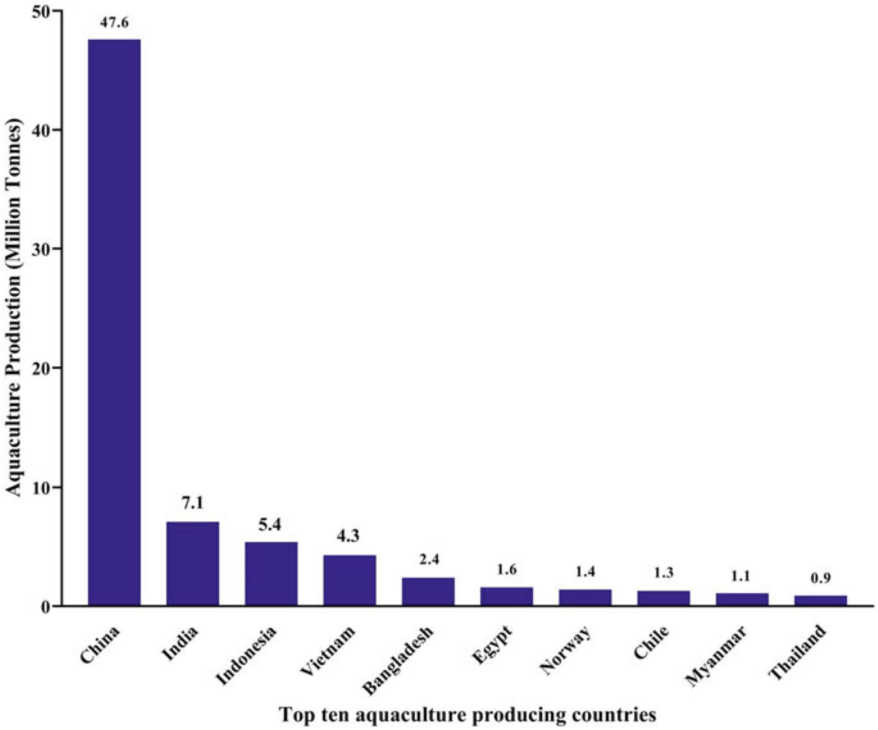

Fig. 2.1 Global aquaculture production in Million tonnes by top ten aquaculture producing countries (Food and Agriculture Organization 2021)

examples of raw ingredients that, when combined, meet an organism's macronutrient and micronutrient requirements. Furthermore, these ingredients promote rapid development, promote animal health, and, most importantly, produce a product with sensory and quality properties that meet consumer expectations (Hua et al. 2019). Over the last 20 years the fish meal and fish oil produced from forage fishes has declined steadily. The ratio of these important ingredients in the aqua feed for many crustaceans and carnivorous fishes are decreasing (Turchini et al. 2019).

2.2 Fish Meal and Its Demand

Aquaculture, one of the largest protein food generating sectors, greatly relies on nutrition. It is a process of farming aquatic organisms to promote its productivity. Fish meal (FM) could be simply defined as wild-caught fishes being used as feed for farmed fishes. It is a prime, dominant, and most expensive proteinaceous feed ingredient that provides good quality protein and essential nutrients for farmed fishes. Wild fish as FM is not only used by aquaculture feed production sectors,

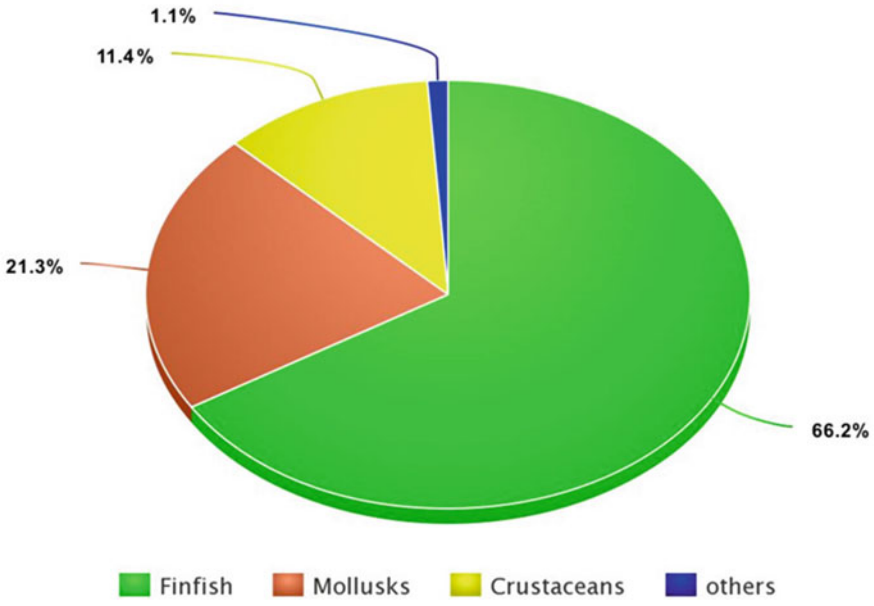

Fig. 2.2 Percentage of aquatic species contributed to global aquaculture production in 2018 (Food and Agriculture Organization 2021)

but also serves as protein source in pet animal and livestock feeds. Multi-use of these wild-caught fish for feed reduces its supply for human consumption. About 4–5 tons of whole wild fishes are required to generate 1 ton of dry FM (Allan 2004). Healthy expansion of aquaculture would not be possible if FM is relied upon as the prominent source of protein in feeds. Moreover, FM is risky due to its thiaminase activity, role as a vector of infectious diseases and rancidity at storage for their marked levels of lipid content. Hence, efforts to research substitutes for fish-derived feed ingredients (plant, animal, and microbial proteins) are highly focused in recent years.

2.2.1 Fish Meal Replacers

The suitable approach in feed formulations is to use high-quality feedstuffs that could meet the nutritional requirements of the fishes. This in turn depends on the amino acid profile in their protein. Due to the expensive nature of FM, there remains long-term scarcity for good quality proteins. Recently, numerous researches with locally available feed ingredients have been worked out. There is urgency for a move from FM to plant/terrestrial animal/microbial proteins within the aquaculture industry as over-exploitation of wild fishes has negative ecological consequences. Plant proteins cannot successfully replace FM due to poor protein digestibility and essential amino acids imbalance, urging feed concerns to search for cheaper and nutritious FM alternatives from animal origin.

FM replacers such as shrimp head waste (Oliveira Cavalheiro et al. 2007), chicken viscera and cryfish meal (Soltan and El-Laithy 2008), *Arthrospira platensis* (*Spirulina*), dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) and fermented *Cladophora* (Ali et al. 2019 ; Kumar et al. 2012a ; El-Sheekh et al. 2014), fermented animal protein blend (Samaddar et al. 2015), duckweed (*Lemna minor*) meal (Ma et al. 2017), *Chlorella vulgaris* (Radhakrishnan et al. 2014), and *Arthrospira platensis* (Radhakrishnan et al. 2016); algae (Sattanathan et al. 2020b) have been researched succesfully. Similarly, animal by-product meal, feather meal, meat and bone meals, poultry meal, chicken offal meal, and poultry by-product meal have been reported to substitute FM by many researchers (Sumathi and Sekaran 2010; Thazeem et al. 2015, 2016, 2017). Move towards animal and microbe-derived proteins as FM replacers has marked appreciable encouragement among researchers, as plant proteins lack certain indispensable amino acids needed by fish for its growth and few plant metabolites are noticed to hinder absorption of nutrients into the aquatic animal's mono-gastric system. With this regard, an experimental study was conducted in which five isonitrogenous diets were formulated for *Labeo rohita,* where fermented tannery solid waste flour replaced FM by 25, 50, 75, and 100%. Appreciable growth performance, nutritional indices, and body carcass composition were evident in the fish group fed with diet formulated by replacing FM with 75% fermented tannery solid waste flour, followed by 50 and 25% replacement (Thazeem et al. 2018).

According to Hasan et al. (1997) and Thazeem et al. (2018), hydrolyzed poultry feather meal could substitute FM up to 20% in the diets of *Labeo rohita* fry without compromising growth and feed utilization. Poorest response was observed with 100% replacement. Feather meals and meat and bone meals were evaluated for their ability to replace FM in the diets of rainbow trout. Up to 15 and 24% replacement of feather meals and meat and bone meals, respectively, was evidenced with significant growth performance which proved their potency to replace FM in fish diets (Bureau et al. 2000). Protein sources such as *Leucaena* leaf meal, coffee pulp, torula yeast, and cottonseed meal have been investigated for Tilapia (Oliveira Cavalheiro et al. 2007). Possibility of substituting dietary FM with a protein combination of canola meal and corn gluten meal and to discover a substitute lipid source for milk product without affecting the fish growth rate was achieved by Umer and Ali 2009.

A 60-day feeding trial was conducted to investigate the possibility of using soybean meal protein for FM replacement for *Labeo rohita* fry (Khan et al. 2003; Jahan et al. 2012). Growth rates, feed conversion ratio, and protein efficiency ratio indicated that FM could be replaced up to 50% without supplementation of additional amino acids. Samaddar et al. (2015) concluded that up to 75% replacement of FM in the feeds by the fermented blend of fish offal and slaughterhouse blood had no negative effect on the growth of *Labeo rohita.* Moreover, muscle protein and amino acids content decreased at 75 and 100% FM replacement. Evaluation of nutritional quality and acceptability of duckweed (*Lemna minor*) meal as component in the diets of *Labeo rohita* fry up to 15% gave best results of growth in the fish; however, FM was completely non-replaceable (Ma et al. 2017).

Studies on earthworm meal as a complete replacer of FM in supplemented feeds for common carp—*Cyprinus carpio* was performed by Pucher et al. (2014). Earthworm meal acted as a partial FM replacer for major Indian carps such as *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* (Beg et al. 2016). Nyina-wamwiza et al. (2012) studied the effects of partial and total replacement of FM with agricultural by-products and gave a significant conclusion that there were no detrimental effects on the onset of sexual maturation in African catfish. Total replacement of FM with agricultural by-product diets had positive impacts on reproduction. In the recent past, the aquaculture industries could be transformed with the advent of nanotechnology, to promote the uptake of drugs and metal nanoparticles. Muralisankar et al. (2014) reported the inclusion of zinc nanoparticles (ZnNPs) up to 60 mg kg^{-1} in the diets of freshwater prawn, *Macrobrachium rosenbergii* post larvae with superior performance in survival, growth, digestive enzymes' activities, and sound immune responses.

2.3 Probiotics in Aquaculture

The term "friendly bacteria" is quite popularly referred to as probiotic microbes (Cruz et al. 2012). Disease outbreaks due to bacterial, fungal, and viral infections in aqua farms have resulted in stock mortality due to poor sanitation, improper nutrition, and toxin production in farmed animals. As a measure of prevention, use of veterinary medicines (antibiotics) is preferentially practiced. This has led to drastic risks in terms of drug resistance among pathogens, significantly retarded growth with poor marketing, and bioaccumulation in the aquatic animals as well as the consumers (Nomoto 2005). Administration of antibiotics has led to the development of antimicrobial resistance among fish pathogens, which may be possibly due to plasmids acquisition or chromosomal mutation (Balcazar et al. 2006).

In order to overcome the indiscriminate use of antimicrobial drugs, the concept of probiotics in aquaculture has received firm encouragement in recent years due to their wise inhibitory mechanisms and safeness. Probiotic bacteria enhance the host's digestive enzymes by serving as a source of nutrients; improve the quality of water; and stimulate the immune system by the mechanism of competitive exclusion. In addition to this, they modify the intestinal microbiota, compete with pathogens for adhesion and nutrition, create antitoxins, and secrete antimicrobial compounds (Nayak 2010). They significantly regulate allergic responses and reduce/prevent cancer proliferation in mammals. Probiotic microorganisms serve as growth promoters, improve reproductive health, and support farmed animals to tolerate stress conditions.

Administration of probiotics could be accomplished in terms of oral, water, or feed additive routes. In the case of prawns, oral routes are highly suitable whereas the latter routes are widely practiced in aquaculture farms. According to Nayak (2010), several probiotic bacteria in non-viable form could potentially provoke similar effects in hosts when compared to viable probiotics, as they are commonly found in transient state and could easily expel after feed withdrawal in hydrobionts. Non-viable probionts are not only involved in gut colonization but also ultimately boost up the immune system of host due to the presence of certain microbial components (peptidoglycans, polysaccharides, and lipoteichoic acids) that act as activators of piscine immune system (Secombes et al. 2001). Lactic acid (LA) bacteria (*Lactobacillus* sp.) are the most prevalent aquaculture probiotics. Possible modes of their administration are via feed and water. They are highly preferred as they multiply rapidly and inhibit the growth of pathogens by producing beneficial antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide (Gatesoupe 2008). Numerous reports on the probiotic effects of *Lactobacillus* sp. on various fishes and crustaceans are available (Balcazar et al. 2006; Kesarcodi-Watson et al. 2008). Commercial fish probiotic products are mostly available in liquid and powder forms. Nowadays, increased research in bacterial fermentation and its optimization processes has led to improved functionalities of probiotic bacteria with significant results in growth performances of aquatic animals (Lacroix and Yildirim 2007). Kesarcodi-Watson et al. (2008) elaborately reviewed on the need, principles and mechanisms of action and screening processes of probiotics in aquaculture, concluding that the ever-increasing demand for aquaculture could be met with usage of probiotic formulations and this could act as substitute to hazardous antibiotics.

Nayak (2010) stated that probiotics are emerging as an important part of aquaculture practices for increasing production. *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus,* and *Saccharomyces* species are the probiotics which are generally used in aquaculture practices. In fishes, the participation of probiotics in nutrition, resistance to certain diseases and other enhancing activities has proven beyond any doubt. Flores (2011) has stated that the probiotics can be used as functional additives in feeds. The establishment of a strong disease prevention program, including probiotic and good management practice, can be beneficial to raise aquatic organism production.

Few of the primary reasons for the use of probiotics in aquaculture practice are due to the need for healthy growth of aquatic organisms and proper feed efficiency. The practice of using probiotics has been there since 1986 which further improves water quality and also has positive effects on controlling bacterial infection (Cruz et al. 2012). For aquatic animal growth, survival, and health, probiotics are positive promoters. More intense works on probiotics will globally provide organic aquatic products, which are needed for the safe human consumption of food and health security (Hai 2015).

2.4 Antioxidants, Protein Hydrolysates, Peptides, and Amino Acids in Fish Feeds

Formation of free radicals and their drastic effects on cells and tissues are unavoidable in living systems (Sheriff et al. 2014). With this context, synthetic antioxidants are one of the supplementary feed ingredients, as dietary antioxidants substantially promote animal health by decreasing the load of free radicals in its body. Additionally, presence of synthetic antioxidants could prevent oxidation during feed storage and could reduce the destruction of essential amino acids and vitamins, enabling maximum nutrient supply to the animal. They preserve flavor, taste, texture, and freshness of feeds. Essential criteria for selection of an antioxidative compound in feeds are—it must be efficient at lower concentrations, non-toxic, and economically viable. Ethoxyquin, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) at 150, 200, and 200 ppm, respectively, are the levels of synthetic antioxidants permitted by the U.S. Food and Drug Administration.

However, negative perception for synthetic antioxidants among fish farmers and their unreliable nature has resulted in the research for non-synthetic, food derived antioxidants that could encounter and neutralize the detrimental effects of free radicals. Disadvantageous consequences of synthetic dietary antioxidants may range from weight loss, notable unhealthy changes in liver, kidney, urinary bladder, alimentary tract, and mitochondria, lethargy, anemia, colored skin/urine, decreased survival rates, undermined immunity, condition factor fluctuation, allergy, and bioaccumulation in farmed fishes, subsequently harming the human health (Błaszczyk et al. 2013). In line with this, there is a strong interest in research on the individual or synergistic effects of protein hydrolysates, peptide fragments, and free amino acids in fermented end products that could innately exhibit antioxidative activity. Commercialization of economical feeds with antioxidative feed ingredients could strengthen the profits of feed processors.

From the viewpoint of fish nutrition, amino acids are classified as nutritionally indispensable (essential), dispensable (non-essential), and conditionally essential amino acids. Conditionally essential amino acids are provided via diets under dreadful conditions where the rate of protein synthesis and muscles build up is lower than the rate of feed utilization. Amino acids play a vital role in fish nutrition. They help in reproduction, metamorphosis, pigmentation, appetite and osmoregulation, immunity and antioxidative activity (Li et al. 2009). Major expensive component of formulated fish feeds is the dietary protein. In the recent past, attraction towards the search for feedstuffs consisting of protein hydrolysates, finer peptides, or free amino acids has gained eminence over crude intact proteins. This could differentially influence the growth of the animal as smaller hydrolysates could be feasibly digested and assimilated within the body than crude proteins. Protein hydrolysates and peptide fractions are known to exhibit antimicrobial and antioxidative properties (Balakrishnan et al. 2011). Reports on bio-functional molecules with antibacterial and antioxidant activities through microbial fermentation are abundant (Sachindra and Bhaskar 2008).

Antioxidant property of a peptide depends on the intrinsic characteristics of peptides (amino acid nature and sequence). Antioxidant activity was shown by protein hydrolysates from the shrimp wastes and the same has been attributed to the peptides and water-soluble protein seen in the material. Due to the low costs, safety, and the high nutritional values, the need for the utilization of amino acids, peptides, or proteins as antioxidants in food materials is rising. Since the intestinal absorption of farm animals appears to be more effective due to the protein hydrolysates, they are considered physiologically better than the intact protein (Kumar Rai et al. 2010).

Effective methods for the treatment of wastes generated from food industries including meat and fish along with applications of such reclaimed wastes have been recently reviewed. Microbial remediation is one of the present-day technologies recommended to reuse industrial wastes, as it is a greener technology which alleviates unsafe waste disposal methods (Umesh et al. 2021). Also, the protein hydrolysates acquired from wastes and by-products of the animal and fish processing industry are stated to show several bioactivities such as antioxidative, antihypertensive and immunomodulatory properties (Balakrishnan et al. 2011). To protect animal feed from lipid peroxidation, Ethoxyquin (EQ, 6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline) is excessively used in it. For human use (except spices, e.g., chili), EQ cannot be used in any food but human beings can be revealed to this antioxidant by carrying it from feed to farmed fish, poultry, and eggs (Błaszczyk et al. 2013).

Due to the increasing interest in finding antioxidants from natural sources which may have less potential hazard than synthetic ones, research on fish protein hydrolysates exerting antioxidant activity has gained an increased interest. Antioxidants are generally employed to prevent lipid oxidation in foods in order to avoid the formation of toxic compounds and undesirable flavors and odors. In the last decade, several authors have reported a strong antioxidant activity for fish protein hydrolysates obtained from different species such as black scabbard fish (*Aphanopus carbo*), sardinelle (*Sardinella aurita*), saithe (*Pollachius virens*), yellowfin sole (*Limanda aspera*), mackerel (*Scomber austriasicus*), and herring (*Clupea harengus*). García-Moreno et al. (2013) have reported the production of antioxidant activity exhibiting fish protein hydrolysates from discarded species in the Alboran Sea.

Protein recovery from industrial solid wastes and their biotransformation into fish feed ingredients through biotechnological interventions is a promising field of interest in recent years (Basheer and Umesh 2018). In line with this, Thazeem et al. (2020) statistically optimized the fermentation medium that consisted of tannery solid waste. Through lactic acid fermentation, the underutilized tannery solid waste was efficiently bio-converted into a proteinaceous feed ingredient that exhibited potent in vitro antioxidant and antimicrobial activities.

2.5 Amino Acids and Fish Nutrition

Increasing proof from studies on both aquatic and terrestrial animals have proved that several amino acids control important metabolic pathways which are important for the maintenance, growth, reproduction, and immune responses of fishes and are called "functional amino acids." Restoring food intake and growth as dietary protein is the important and highly expensive component of formulated aqua feeds since identification and dietary supplementation of those amino acids or their biologically active metabolites are believed to counteract adverse effects of substitution of fishmeal from aqua feeds (Wilson 2003).

Recent studies shows that some amino acids and their metabolites are significant controllers of key metabolic pathways that are fundamental for support, development, feed admission, supplement usage, insusceptibility, conduct, larval transformation, multiplication, just as protection from ecological stressors and pathogenic life forms in different fishes. Amino acids assume significant and adaptable parts in fish nutrition and metabolism. These functions include cell signaling (e.g., arginine, glutamine, leucine, proline, and polyamines); appetite stimulation (e.g., alanine, glutamate, proline, and serine); growth and development regulation (e.g., arginine, glutamine, hydroxyproline, leucine); energy utilization (e.g., carnitine); immunity (e.g., arginine, glutamine, and dopamine); osmoregulation (e.g., glycine, taurine, b-alanine, and arginine); ammonia detoxification (e.g., glutamate, glutamine, and citrulline); antioxidative defense (e.g., glutathione, cysteine, glutamine, glycine, and taurine); metamorphosis (e.g., tyrosine); pigmentation (e.g., melanin); gut development (e.g., taurine, glutamine, arginine, threonine, and polyamines); neuronal development (e.g., arginine, taurine, and creatine); stress responses (e.g., tryptophan, serotonin, branched-chain amino acids and glutamine); reproduction (e.g., polyamines, arginine, melatonin, and hydroxyproline); and suppression of aggressive behavior (e.g., tryptophan and serotonin) in aquatic animals. Also, certain amino acids (glutamate, histidine, and glycine) impact taste, texture, and even post-mortem seafood quality. Advances in amino acids nutrition technologies and their application to formulate functional and environmentally oriented aqua feeds can be observed in the coming decade (Li et al. 2009).

2.6 Single-Cell Protein (SCP) as Aqua Feed Additive

Single-cell proteins are microbial cells including bacteria, fungi, molds, and microalgae that can be used as an alternative source of proteins (Umesh et al. 2019). There are many advantages of SCP over plant-based protein sources. One of these is that these microbes can grow on cheaper carbon sources without compromising on the protein content (Asmamaw and Fassil 2014). For instance, yeast could be cultivated in sugarcane bagasse-based media yielding 250 tonnes of biomass protein within 24 h. 20 tonnes of algal biomass could be harvested per year from a simple open pond culture system with minimum investment. On the other hand, plants require a considerable area and time to grow and successfully survive any diseases or environmental stress (Guedes et al. 2015). SCP are also a storehouse of essential amino acids like lysine, methionine, and threonine that cannot be supplied by plant-based proteins (Asmamaw and Fassil 2014; Sharif et al. 2021). SCP has been used as a source of food for space travellers due to their ability to provide substantial amounts of energy. The major sources of SCP are discussed below in brief.

2.6.1 Microalgae

Microalgae are single-celled autotrophic organisms that can live on both fresh water and in marine water. The common examples of microalgae routinely used as SCP include *Chlorella* sp. and *Spirulina* sp. (Radhakrishnan et al. 2014). The commercial cultivation of microalgae to serve as SCP began in the 1960s with *Chlorella* sp. being the first commercial cultivar. Presently annual dry biomass of microalgae produced globally is around 19,000 tonnes (Jacob-Lopes et al. 2019), out of which only 0.7% is available for use in aquaculture (Hua et al. 2019). This suggests that more innovations and development are needed to produce the required amount of microalgae to meet their increasing demand in aquaculture as a protein supplement. Microalgae are rich in polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). They also contain appreciable amounts of vitamins like vitamin A, B, C, E, folic acid, and pantothenic acid. They are also rich in essential amino acids, carbohydrates, and important fatty acids like linolenic acid (Jovanovic et al. 2021; Das et al. 2015; Jovanovic et al. 2021). Microalgae contain 60–70% protein and are mainly used for the production of omega-3-fatty acids (Jones et al. 2020). Cultivation of microalgae is safe and environmentally sustainable. They are known for their promising antioxidant, antimicrobial, and immunostimulatory effects which are of great significance in aquaculture. Microalgae contain pigments like carotenoids and astaxanthin which helps to bring flesh color to salmonids and other fishes (Dineshbabu et al. 2019).

2.6.2 Bacteria

Bacteria are another important source of SCP production. Bacteria contain >80% of protein in its biomass (Hua et al. 2019). It also contains important vitamins and phospholipids. The main advantage of bacterial biomass cultivation is that they can grow over cheap carbon sources and are amenable to process control strategies for increasing biomass yield. Bacteria can utilize methane, methanol, syngas, carbon dioxide, and H_2 as cheap substrates for SCP production. This offers an added advantage that along with biomass cultivation, pollutants and greenhouse gases can also be removed (Jones et al. 2020). Several bacteria including purple non-sulfur bacteria are used for SCP production. They contain a substantial amount of carotenoids and vitamins and are observed to provide pathogen resistance in hosts. Purple non-sulfur bacteria are known to contain factors that resist ammonia stress as well which is of great significance in aquaculture (Alloul et al. 2021). Hence if incorporated in fish feed, this attribute can be expected to transfer to the fishes feeding on SCP thereby enhancing their stress tolerance. The main advantage of culturing purple non-sulfur bacteria is that they could be grown in various growth conditions. They may be photoautotrophic or photoheterotrophic, anaerobic or microaerobic which grows in light conditions, or heterotrophic and aerobic under dark conditions (Chumpol et al. 2018). Another potential source of bacterial SCP is purple phototrophic bacteria. They contain >60% crude proteins and important components like polyhydroxyalkanoates (PHA). The major feature which makes bacterial biomass a good source of SCP is the digestibility of its cell wall. Due to this feature fishes can easily feed on bacterial biomass. Also, the amino acid profile of microbial protein is comparable to that of commercial fish feed (Delamare-Deboutteville et al. 2019).

2.6.3 Fungi and Yeast

Yeast and fungi are good sources of SCP (Umesh et al. 2017). They contain 30–50% protein and contain valuable amino acids and vitamin B-complex (Jones et al. 2020). The cell wall of yeast and fungi contain substantial amounts of β-glucan which was reported to provide immunity. Brewer's yeast is the most commonly used source of yeast SCP (Andrews et al. 2011). Fungi like *Saccharomyces cerevisiae*, *Aspergillus* sp., and *Fusarium venenatum* are commercially explored for feed formulation. *Candida utilis* biomass was found to replace salmon and shrimp diet protein requirement by 40% (Jones et al. 2020). Reports suggested that 1% yeast extract when given to fingerlings of *Labeo rohita* increased their survival rate and respiratory bust activity. It also increased their total leucocyte count, serum protein level, and globulin activity which accounts for rendering immune power to the fishes (Andrews et al. 2011).

2.6.4 Challenges in Using SCP as Aqua Feed Additive

There are many challenges faced when SCP is used as a source of protein alternative in aquaculture feeds. Microalgae when used in aqua feed can be limited only to filtering mollusk and other true phytoplankton feeders. Some fishes can consume microalgae only during their larval stage (Muller-Feuga 2013). Thus despite being a potential source of SCP, microalgae are limited to certain species or certain life stages of fishes. For microalgae feeders, the next challenge could be its digestibility.

This could create problems with lower feed intake and feed conversion ability. So, while using microalgae it is important that the cell wall needs to be removed using chemical, mechanical, or enzymatic methods. Though it is a simple process it can contribute to the increase in cost of the final product. Another disadvantage with microalgae is the presence of phytic acid. Phytic acid reduces phosphate solubilization making the phosphates unavailable for consumption. A feed preparation with phytate solubilizers can solve these problems, but more research needs to be done in order to check the implications of such a feed formulation in aquaculture (Jones et al. 2020). In case of bacteria and other microbes, media preparation without contamination and maintaining culture conditions could be a rigorous task. Much care should be given in downstream processing, contributing to an increase in the production cost. Also, ethical issues need to be cleared while formulating such a diet.

2.7 Keratin in Aqua Feeds

Keratin is the second largest biopolymer after collagen and is found in all vertebrates as outer covering called integument. Keratin protein is found in skin, hair, wool, feather, quill, horn, and nails. Despite being a widespread protein, it is very difficult to digest keratin to make the proteins available in animal or fish feed. The main reason for its structural stability is the presence of disulfide bonds between cysteine amino acids present in the keratin molecule (McKittrick et al. 2012). Keratin protein is so strong that only 16% of it is digestible (Estévez et al. 2006). It is one of the richest sources of essential amino acids, containing 18–20 different types of amino acids. These amino acids are locked inside hydrophobic, intra and intermolecular disulfide bonds, ionic bonds and hydrogen bonds so that it is not easily available for consumption (Bhushan 2010; Ayutthaya et al. 2015). Even though there are a wide range of keratin sources, the main source from which keratin protein is being extracted for feed formulation is feather waste. Feathers, especially chicken feather wastes, are easily available from slaughter houses and are also a cheaper source of keratin. They contain 85–90% keratin protein (Estévez et al. 2006). In addition to being a cheaper source of keratin, feathers also have a larger surface area when compared to other keratin sources like nails and wool which make it easy for absorption and for pretreatments. Amino acid profile of feather meal matches with the amino acids needed for fish meal which adds to the value of the product. About 60,00,000 tonnes of feather meal are produced annually to incorporate into animal and aquaculture feed (Adler et al. 2018). These feather meals are prepared using different strategies. Conventional methods use a combination of steam and pressure for digesting feathers as keratin is a very stable protein. Temperature-pressure treatments, chemical hydrolysis, and steam explosion have proved to be capable of breaking down feathers to a more digestible form (Zhang et al. 2014). The common practice is steam hydrolysis under 15 psi pressure at 140 °C incorporating an acid or an alkali treatment or in the presence of a disulfide reducing agent. Even though this method is effective, they may cause a loss of important amino acids from the protein

(Łaba et al. 2015). So, the recent research focuses on using fermented feather instead of hydrolyzed feather for feed formulation experiments. Over the past few decades the fermented feather meal was recognized as more advantageous than hydrolyzed feather meal. Fermentation increases digestibility of feather from 39.09% to 48.75% (Adelina et al. 2021). For fermentation, many agents can be used, the important one being the microbes itself. Enzyme treated feathers are found to be more digestible, palatable and the amino acids released from them are more bioavailable (Mendoza et al. 2001). Rendered feather meals have been used in salmonids feed for decades (Martínez-Alvarez et al. 2015). In order to replace the conventional plant or forage fish based protein sources, many experimental trials on keratin feeding have been done in the past few decades. One such feeding experiment was done on juvenile gilthead seabream *Sparus aurata* where 25%, 35%, and 50% of the fish feed was substituted with treated chicken feather meal. It was found that 50% treated feather meal was palatable and digestible and thus opened a promising window for incorporation of keratin in aqua feed. The amino acid profiling of the treated feather meal revealed that it contained high amounts of essential amino acids and polypeptides (Al-Souti et al. 2019). The work of Psofakis et al. (2020) proved that 25% addition of keratin protein in the feed of *Sparus aurata* maintained the digestive enzyme activity and other hematological parameters in its normal level suggesting that the replacement of usual protein meal with animal by-products will not affect the metabolism or other growth parameters of fishes (Psofakis et al. 2020). 76% replacement of commercial protein with keratin in aqua feed is acceptable and is expected to give the same result as that of the commercial feed (Campos et al. 2017). One of the added benefits of using keratin protein in aquafeed is that it contains a lot of elements like calcium (0.16%), phosphorous (0.04%), potassium (0.15%), and sodium (0.15%) that helps in various metabolic processes in fishes (Chor et al. 2013).

A feeding trial done on silver pompano *Trachinotus blochii* was successful with 20% feed addition with fermented keratin protein. It was found to increase growth rate, feed efficiency and protein efficiency ratio with a satisfactory proximate composition of fish caress (Adelina et al. 2021). An experiment to ferment keratin protein with *Bacillus* sp. proved that fermentation can result in the increased protein content in fish meal. An addition of 10 ml of bacterial inoculum to ferment 2 g of feather keratin was found to be optimum with 85–90% protein recovery (Mulia et al. 2016). A feeding trial on red tilapia *Oreochromis* sp. proved that 6% addition of keratin protein in fish meal of tilapia fish is optimum to meet the protein need of tilapia. The addition of 6% keratin protein was successful as it increased the fat content and protein content in fish carcasses. The proximate analysis of fish carcass revealed the crude lipid content to be 0.83%, crude fiber content to be 2.15%, protein content to be 82.36%, ash content to be 1.49%, moisture content to be 12.33% with 64.47% carbon content, 10.41% nitrogen content and 2.46% sulfur content (Nursinatrio and Nugroho 2019; Tesfaye et al. 2017). In addition to fermentation, irradiation was done on feather meal to improve its digestibility. The experiment was a random one and it was successful. The experiment gave its findings that irradiated feather meal could replace fish meal by 180 g/kg weight of fish meal. The feeding trial was done on largemouth bass *Micropterus salmoides* (Ren et al. 2020).

The main challenge in using keratin as a protein source in aquaculture is its digestibility. Keratin is a stubborn structural protein (Ayutthaya et al. 2015) and requires a lot of energy to release the amino acids locked up inside. But fermentation has proved to be an effective method to digest keratin so that its amino acids are available in feed without degradation. The second challenge is the availability of keratin sources. Presently feather waste, especially chicken feather wastes, are being used for isolation of keratin protein. Even though there are many other sources like wool, and hog hair, they require a lot of pretreatments before keratin digestion making the process to be economically non-viable. There are fewer reports on the negative effects of using keratin protein as well. A work done on Anguilla *bicolor* showed a negative effect on the growth when feather meals were incorporated in fish feed (Thamren et al. 2018). Also, growth rate of *Sparus aurata* was found to be negatively affected as feather keratin downregulated live gene expression for growth (Psofakis et al. 2020). Research on negative effects and the way to nullify the problem need to be done so that a better protein alternative for feed addition could be formulated.

2.8 Polyhydroxyalkanoates (PHAs) as Aqua Feed Additive

Immunostimulant usage has gained importance in recent years for prophylaxis and infection control, because of its ecofriendliness and biocompatible nature in aquaculture (Umesh and Santhosh 2021). The hosts are protected from infections by their immunostimulants modulating the immune system (Bricknell and Dalmo 2005). Different groups of chemicals, biologicals, secondary metabolites from plants and microbe derived compounds have proved their effectiveness as immunomodulators in aquaculture (Sakai 1999). Different heterotrophic microbes forming biofloc were reported to promote the immunomodulatory effects in the reared organism in the aquaculture system (Crab et al. 2012). Most of the immunomodulatory methods like phage therapy and vaccinations demand high application cost and labor-intensive procedures making them no longer sustainable. This reason triggers the use of Short Chain Fatty Acids (SCFAs) and their polymers as immunostimulants in aquaculture. These SCFAs are organic fatty acids containing 1–6 carbon atoms, usually derived from oligosaccharides, polysaccharides, peptides, proteins, and glycoproteins via fermentation (Hoseinifar et al. 2017). One of such SCFA polymer families is the Polyhydroxyalkanoates, which are accumulated under nutrient imbalance conditions in many microbes as an intracellular energy reserve (Anderson and Dawes 1990). These PHAs are polymers of hydroxyalkanoates. Among the PHA family polyhydroxybutyrate (PHB) is the most widely studied (Umesh and Basheer 2018; Umesh and Preethi 2017). These compounds are fully biodegradable and fully biocompatible, which is an ideal quality to choose for packaging, drug delivery, and in other biomedical applications (Umesh et al. 2018a; Chee et al. 2019; Umesh and Thazeem 2019). But when it comes to commercialization of PHA a huge block is in place due to the high cost for fermentation and downstream processes that accelerates the final product cost (Preethi and Vineetha 2015). With the emergence of production strategy based on agro waste and environment friendly down streaming strategies, the light on PHA research is brightening (Umesh et al. 2018b). Reports of strong immunomodulatory and growth promoting effects were obtained when PHAs were used in aquaculture, by incorporating into the aqua feed (Gao et al. 2019; Suguna et al. 2014).

2.8.1 PHA Biosynthesis and Enzymology

All microbes take up the carbon source to generate energy for metabolism, the most preferred carbon source by microbes are sugar molecules (Umesh and Thazeem 2019). Sugar molecules undergo glycolysis to get converted to pyruvates. The pyruvates are converted to acetyl CoA which normally enters the Krebs cycle to produce energy molecules. As discussed earlier, PHA production happens when the microbes are in stressed condition with excess sugar, so in such PHA producers, the acetyl CoA produced from pyruvate will not enter the Krebs cycle, by arresting the β Ketothiolase enzyme activity. This will cause accumulation of acetyl CoA. When this compound is excess in the cell, PHA biosynthesis is force started (Umesh et al. 2021). Detailed molecular studies into the PHA biosynthesis pathway proved the existence of multiple biosynthesis pathways of PHA production with small differences. The most accepted and most referred PHA biosynthesis pathway is observed in *Cupriavidus necator* (Liebergesell et al. 1993). In this microbe there are 3 genes which are responsible for PHA biosynthesis, namely: *pha* A, *pha* B, and *pha* C, which codes the key PHA synthesis enzymes β-Ketoacyl-CoA thiolase, acetoacetyl-CoA dehydrogenase, and PHA synthase enzyme, respectively. β-Ketoacyl-CoA converts acetyl CoA molecules to acetoacetyl CoA. By the action of NADPH dependent acetoacetyl-CoA dehydrogenase, the acetoacetyl CoA condenses to (R)-3-hydroxybutyrate monomer unit. These monomer units are polymerized into PHAby enzyme PHA synthase (Anderson and Dawes 1990; Tsuge et al. 2005).

Among the different known applications of PHA like food processing and biomedical, its applications in the field of aquaculture are at its dawn. The major areas of research on the topic of PHA in aquaculture are PHA as feed additive and PHA in biofloc technology as a denitrification system. Due to the high growth profiles and immunological responses of PHA fed shrimps and fishes, the application of PHA in aqua feed are being focussed by many researchers during the past decades. Although many reports of high immunomodulatory effects have been reported, the actual molecular mechanism to this cause is yet to be discovered (Umesh and Santhosh 2021). The larval stage of the target organism is selected for the study of effects of different PHA on the growth performance and immunity, as larval stages are more sensitive and responsive physiologically and physically as compared to the adults. The larval stages are also prone to many diseases as they lack the adaptive immunity and these stages can give results to any exposed or fed chemicals quickly. Hence they are preferred to study the toxic effects, immunomodulatory effects of any given compounds (Castro et al. 2016).

In the studies conducted using different aquatic organisms by supplementing the different PHA molecules in the diet reported that the organism showed higher survival rate, growth rate and other growth related results. When Zoea larvae were fed with PHB enriched live feed, a significant increase in the survival rate, growth qualities and osmotic shock tolerance were observed. The reason for the growth is attributed to the additional energy provided by the digestion of PHB; the PHB enhances the absorption capability of the colonic cells by providing this extra energy (Sui et al. 2014). The supplementation of PHB in the feed can increase the crude protein and crude lipid content in organisms like *Oncorhynchus mykiss* fingerlings (Yaqoob et al. 2018). The partial or full degradation of PHAs will release β-hydroxy short chain fatty acids which will enter the lipid biosynthesis pathway (Clements et al. 1994). PHB are short chain fatty acids which will obviously change the pH in the gut of the juvenile organisms easily. This can be the reason to alter and increase the digestive enzyme activities and hematological parameters (De Schryver et al. 2010). The decrease in the pH will also arrest the infection chances of opportunistic pathogens which enters the gut. The PHA will also alter the microbial community in the gut; it will promote the growth of certain useful microbes which have the PHA degrading enzymes.

When different species like *Oreochromis mossambicus, Penaeus monodon, Liza haematocheila*, and *Labeo rohita* were fed with different PHAs, it was found that the total immune responses showed significant increase (Suguna et al. 2014; Misra et al. 2006; Laranja et al. 2017; Qiao et al. 2019). There are reports that organisms fed directly with PHA as feed and fed with live feeds enriched with PHA showed increased immune activity (Suguna et al. 2014; Laranja et al. 2017). For studying the nonspecific immune responses the total antiprotease activity, peroxidase activity, and lysozyme activity are analyzed and for understanding the total immune activity the host organisms should be challenged with virulent strains of common infectious microbes. The antiprotease activity indicates the level of total serum protease level. When pathogens invade a tissue cell, lysing enzymes are released, most of them get activated only after chelation, and the serum proteases will block the chelation and suppress the invasion. The hosts are protected from both gram-negative and gram-positive bacteria by lysozymes. In aquatic organism lysozymes are directly correlated with the phagocytic neutrophils, so an increased lysozyme activity attribute to increased number of active neutrophils (Misra et al. 2006). Other immunostimulatory effect reported by feeding PHA is that the transcriptional activities of genes controlling prophenoloxidase gene (proPO), transglutaminase gene (TGase), heat shock protein 70, penicillin-binding protein A (pbpA), aldehyde oxidase (AOX), interleukin-8 (IL-8), and major histocompatibility complex class II (MHC II) are upregulated (Morimoto 1998; Amparyup et al. 2013; Maningas et al. 2008; Ishida et al. 2006; Baggiolini et al. 1993; Wright et al. 1997; Ryhänen et al. 1991). Another immunological activity which showed a significant increase is the antioxidant enzyme activity. The main antioxidant enzymes which play a crucial role in the immune system's first line of defense are superoxide dismutase, catalase, and total antioxidant capacity. Superoxide dismutase removes the excess of reactive oxygen species, thus reducing the chances of adverse reactions (Meng et al. 2013). Catalases prevent the hydroxyl radical toxicity and convert peroxides into water and oxygen (Bagnyukova et al. 2005). As discussed above PHA can change the pH and microbial community in the gut. Before the Soiny mullets were fed with PHA the microbial community present in the gut were *Lactococcus, Bacillus, Carnobacterium, Achromobacter,* and *Delftia.* After feeding with PHA there was a significant increase in the population of intestinal *Bacillus* spp. Furthermore when analyzed with Kyoto Encyclopedia of Genes and Genomes (KEGG) it was found that the genes which regulate some secondary metabolites biosynthesis, major metabolism pathways, signalling molecules and interaction, immune system, and other downregulated genes involved in disease pathways, were significantly upregulated in PHB fed organisms (Semova et al. 2012). Table 2.1 shows the effect of PHA supplementation in aquaculture.

By the use of PHA as immunostimulant and growth enhancer, it tends to replace the traditional antibiotic administration for resisting pathogens. More research is needed on a global basis to fully understand how the administration of PHA is upregulating the immune system and growth. The non-toxic, fully degrading nature of PHA in the host should be also given importance in research. PHAs may become an alternative to the antibiotics and other disinfectants used in aquaculture in the near future if proper studies are done.

2.9 Chitosan in Aquaculture

Chitosan is a biopolymer which is extracted by the deacetylation of chitin. Chitosan is a highly basic polysaccharide which exhibits various properties such as solubility in different media, mucoadhesive property, polyoxy salt formation, viscosity, polyelectrolyte behavior, potential to form films, metal chelating property, optical and structural characteristics (Shukla et al. 2013). Chitosan is also known for its antimicrobial properties and free-radical scavenging property, which increases its scope in pharmaceutical applications (Shariatinia 2019). These properties increase the potential of chitosan to be applied in different fields such as cosmetics, agriculture, food industry, textile industry, bioimaging, tissue engineering, and in several other biotechnological applications (Lodhi et al. 2014). In natural conditions, chitosan is present in the cell walls of fungi, green algae, yeast, and protozoa and also in the exoskeletons of crustaceans and cuticles of insects (Alishahi and Aïder 2012). Chitosan consists of glucosamine and acetyl-glucosamine units which are accessible in various grades depending upon the degree of acetylated moieties and the degree of acetylation controls several properties of chitosan (Sorlier et al. 2002). Deacetylation is a process which contains elimination of acetyl groups from the molecular chain of chitin in order to obtain chitosan.

Chitosan is a part of a linear polysaccharides family which is a linear copolymer of β-(1–4)-linked N-acetyl-2-amino-2-deoxy-d-glucose (acetylated, A-unit) and

Table 2.1 Effect of PHA supplementation in aquaculture

Scientific name	Common name of the host	Effects of PHA supplementation on the organism	References
Mytilus edulis	Blue mussel	Increased survival and improved growth and development	Van Hung et al. (2015)
		Enhanced larval development and survival	Thai et al. (2014))
Litopenaeus vannamei	White leg shrimp	When fed with PHB along with glucose, an increased survival rate and final weight was observed	Luo et al. (2019)
		Improved growth, survival and robustness of the larvae when exposed and not exposed to pathogenic *Vibrio anguillarum.*	Gao et al. (2019)
Artemia franciscana	Brine shrimp	Survival of the starved nauplii were increased when fed with PHA were challenged with *Vibrio campbellii*	Defoirdt et al. (2007)
		Remarkable survival was observed in *Artemia* fed with 100 mg l^{-1}, when challenged with *Vibrio campbellii*	Baruah et al. (2015)
		Better survival when challenged with *Vibrio campbellii*	Halet et al. (2007)
Oreochromis niloticus	Nile tilapia	Increased serum lysozyme activity, serum peroxidase activity and immune response as host showed resistance towards virulent *Aeromonas hydrophila* strain when feed was added with 5% PHB	Suguna et al. (2014)
		Increased survival rate by 20% and increased lipase activity was observed when the larvae fed with PHB was challenged with *Edwardsiella ictaluri* gly09R	Situmorang et al. (2016)
Dicentrarchus labrax	European sea bass	Survival was enhanced when fed with 2%, 5% and 10%, the maximum weight gain was observed when 5% PHB was fed and overall decrease in the pH was also observed.	De Schryver et al. (2010)
Oncorhynchus mykiss	Rainbow trout	When fed with 1% PHB, increased specific growth rate, weight gain and remarkably higher specific activity of the total protease and amylase were seen. Enhanced immunostimulation and survival when challenged with *Yersinia ruckeri.*	Najdegerami (2020)
Acipenser baerii	Siberian sturgeon	Accumulation of more whole-body lipid content and pepsin activity when fed with PHB and PHB-HUFA	Najdegerami et al. (2015)
		Enhanced specific growth rate, survival and weight gain when fed with 2% PHB	Najdegerami et al. (2012)
Penaeus monodon	Giant tiger prawn	Increased growth and survival of the larvae which were exposed and not exposed to pathogens. High survival was observed when exposed with ammonium chloride	Laranja et al. (2014)

(continued)

Table 2.1 (continued)

Scientific name	Common name of the host	Effects of PHA supplementation on the organism	References
Eriocheir sinensis	Chinese mitten crab	Increased osmotic stress tolerance, developmental rate, and survival.	Sui et al. (2014)
		Growth and survival were enhanced when challenged and not challenged with *Vibrio anguillarum*	Sui et al. (2012)

2-amino-2-deoxy-d-glucose (deacetylated, D-units) (Kaur and Dhillon 2014). This arrangement in chitosan ends up in showing a rigid crystalline structure through inter- and intramolecular hydrogen bonding (Roberts 1992). On every glycosidic residue of chitosan, there is one –NH2 group and two –OH groups which provide various chemical as well as biological properties to chitosan. Due to these properties chitosan is majorly used in pharmaceutical applications. Drug delivery systems which use chitosan are gaining interest as they act as carriers and are able to release contents such as small active molecules, proteins, peptides, vaccines, genes, and oligonucleotides which are released at specific rate and location in the body (Vatanparast and Shariatinia 2018). Chitosan also shows wound healing properties (Zhang et al. 2018) and several other applications in the pharmaceutical sector such as in making contact lenses (Anirudhan et al. 2016), bioimaging (Agrawal et al. 2010), etc.

The extraction of chitosan is majorly done by chemical and biological methods where the chemical methods involve removal of calcium carbonates and proteins, using strong acids and bases, demineralization and deproteinization in biological methods involves bacteria which produces lactic acid and proteases from bacteria and the process of deacetylation is performed using enzymatic methods by chitin deacetylase (El Knidri et al. 2018). Obtaining industrial scale chitosan with completely removed organic salts is the major advantage of using chemical methods of extraction along with the short processing time. Biological methods of extraction on the other hand are environmentally safe but have longer processing time than chemical methods. The three main steps involved in the extraction process of chitosan are demineralization, deproteinization, and deacetylation, where demineralization is the process of eliminating the calcium carbonate and calcium chloride, deproteinization is the step where proteins are removed, and deacetylation is the process where chitin is converted to chitosan by removal of acetyl groups (Table 2.2).

In recent years the commercial use of chitosan has been increasing and demanding. Due to the versatile feature and property of chitosan, research based on its use in the seafood industry and as an immunostimulant is showing an exponential growth. Chitosan has been studied for its antimicrobial activity; this property of the substance has been explored for its application to extend the shelf life of marine-based food products. Chitosan can be used as an antibacterial food additive in the different food industries (Cao et al. 2009). Researchers have studied the effect of chitosan as

Table 2.2 Comparison of the degree of deacetylation of various aquaculture substrates and different extraction process

Raw materials	Extraction methods	Deacetylation degree%	References
Shrimp shells	Demineralization: 1 M HCl, ratio 1:15, at optimal temperature Deproteinization: 1 M NaOH, ratio 1:15, at 100 °C for 8 h Deacetylation: 50% NaOH, ratio 1:15, at 100 °C for 8 h	74	Marei et al. (2016)
Squid gladius (*Loligo vulgaris*)	Demineralization: 1.5 M HCl, ratio 1:10, at 50 °C for 8 h, Enzyme/substrate 10 U/mg, 3 h at pH 8 at 50 °C using Alcalase Deacetylation: 50% NaOH, at 120 °C for 4 h	71	Abdelmalek et al. (2017)
Squid pens (*Doryteuthis* spp.)	Ultrasound-assisted deacetylation (USAD) of β-chitin in 40% NaOH with ratio 1:10, for 50 min at 60 °C. The process was performed twice.	95.7	Fiamingo et al. (2016)
Larvae (*Zophobas morio*)	Demineralization: 1 M HCl, ratio 1:20, 35 °C water bath for 30 min Deproteinization: 2 M NaOH, ratio 1:20, 80 °C water bath for 20 h Deacetylation: 50% NaOH, ratio 1:20, 90 °C water bath for 30 h	74.14	Soon et al. (2018)
Catharsius molossus residue	Demineralization: 1.3 M HCl, at 80 °C for 30 min, kept 12 h at room temperature. Deproteinization: 4 M NaOH, at 90 °C for 6 h Deacetylation: Chitin was soaked in 18 M NaOH, at room temperature for 24 h. Then heated at 90 °C for 7 h and the alkali solution was respectively replaced at 3, 5, 7 h	94.9	Ma et al. (2015)
Fish scales (*Labeo rohita*)	Demineralization: 1% HCl, 36 h at room temperature Deproteinization: 0.5 N NaOH, 18 h at room temperature Deacetylation: 50% NaOH, 80 °C in oil bath for 2 h	–	Kumari and Rath (2014)
Antarctic krill (*Euphausia superba*)	Demineralization: 1.7 M HCl, 6 h at ambient temperature Deacetylation: 2.5 M NaOH at 75 °C for 1 h	11.28	Wang et al. (2013)
Cephalothorax (*Macrobrachium rosenbergii*)	USAD of α-chitin in 40% NaOH, ratio 1: 44, alternation of irradiation and non-irradiation periods with total time 45 min and 30 min respectively	77.9	Birolli et al. (2016)

(continued)

Table 2.2 (continued)

Raw materials	Extraction methods	Deacetylation degree%	References
Cuttlefish pens (*Sepia* spp.)	Demineralization: 1.0 M HCl, ratio 1:40, at optimum temperature for 3 h Deproteinization: 1.0 M NaOH, ratio 1: 20, at 70 °C for 24 h Deacetylation: 45% NaOH in the ratio 1: 15, at 600 W for 15 minutes	93	Sagheer et al. (2009)
Metapenaeus stebbingi shells	Demineralization: 2.5 N NaOH, at 65 °C for 6 h Deproteinization: 1.7 N HCl, at 25 °C for 6 h Deacetylation: 50% NaOH, at 120 °C	92.19	Kucukgulmez et al. (2011)

food additives and it was observed that chitosan increased the shelf life of fish balls (Kok and Park 2007) . Fernandez-Saiz et al. on the other hand studied its effect on fish soup and reported a reduction in the growth of many bacteria when chitosan was used as a preserve (Fernandez-Saiz et al. 2010) . Chitosan possesses the property of an antioxidant which further assists with the preservation process. The seafood industry has used and researched on the various aspects of the application of chitosan for film-forming ability, gel enhancement, encapsulation, a tissue engineering scaffold, and more in order to improve seafood quality.

2.9.1 Biomedical Applications in Aquaculture

The use of nanotechnology has shown tremendous growth in recent years and its use for the creation of chitosan nanoparticles has been recently studied. Chitosan nanoparticle and its application in the biomedical and aquaculture field has shown potential advancement. The chitosan polysaccharide and its derivatives are widely used in the areas of fish biotechnology, fish genetics, fish reproduction, aquatic health, etc. (Sharma and Ahmad 2013). The potential application of chitosan on aquatics has been widely researched which can be utilized for animal health, production, prevention, and treatment of diseases. There are properties of the chitosan structure such as the NH2 and hydroxyl group which make it suitable for certain specific chemical reactions for its biomedical uses. Chitosan has been studied for its antimicrobial activity against gram-positive and -negative bacteria along with certain fungal fish pathogen as well (Luo et al. 2011). The antimicrobial activity contributes to its use in various other industries such as for water disinfection of fish farms, certain food processing industry, and other medical areas. Chitosan-based products have biomedical applications which extend from their use as a dietary supplement for the freshwater fish species. The study conducted by Cha et al. shows the effect of using chitosan coated diet supplements for olive flounder which further showed to improve the water quality as well (Cha et al. 2008). The use of these chitosan-based supplements provides an enhanced survival rate, better growth, and improved meat quality. One of the other applications of chitosan is its use as a vaccine for fishes (Ferosekhan et al. 2014). There are studies conducted that show the use of chitosan as a drug oral vaccine delivery, the primary reason for it to be used as a delivery system is due to its high solubility, bioavailability, and its ability to penetrate through tissues (Shi et al. 2010). The proprietary use of nanoencapsulation technology for the release of bioactive ingredients has also incorporated the use of chitosan as a carrier. The most common encapsulated ingredients that have been studied are vitamins (Alishahi et al. 2011), hormones (Wisdom et al. 2018), enzymes, bioactive ingredients, and more. Researchers like Fernandes et al. have studied the use of chitosan as a fish disease diagnostic method. *Aeromonas* spp. which is a fish pathogen can be detected using chitosan-based nanoparticles (Fernandes et al. 2015). Thus, although there are few areas in the biomedical application that need to be studied thoroughly the use of chitosan in fish medicine has shown tremendous potential with the promising application as carriers, dietary supplements, antimicrobial activities, disease diagnosis, and more.

2.10 Cellulose as Aqua Feed Additive

With the advent of plant components to the fish feed have consequently made the addition of fiber inevitable (Sun et al. 2019). Cellulose is one such fiber that is available in abundance. It is generally found in the cell wall of plants, particularly in the stalk, trunk, and woody portion of the plant tissue (O'sullivan 1997). It is a straight chain polymer composed of glucose molecules linked together by β 1–4 glycosidic bonds (Hansen and Storebakken 2007). Cellulose from plants can be isolated in a series of steps, which includes dewaxing (Floros et al. 1987), alkali treatment (García et al. 2013), and bleaching (Rehman et al. 2018). It can be recovered in different forms such as microcrystals, nanofibers, and so on. There are even numerous applications of cellulose in various sectors. In aquaculture, cellulose is also utilized as an aerogel for wastewater treatments other than being an aquafeed additive (Darabitabar et al. 2020). However, most fishes lack the ability to digest cellulose due to the absence of enzyme cellulase (Sun et al. 2019). Due to its inability to digest, cellulose in fishes is even considered to be non-nutritional if the addition of cellulose in feed exceeds 7%. Despite this fact, there is adequate other research that suggests cellulose in fish can be above 7% and the capability varies within the fish species. The studies moreover showed that the cellulose fed to the fishes have shown a positive influence in growth rate (Ashraf et al. 2014). Therefore cellulose can be utilized as a successful aqua feed additive to an extent.

2.10.1 Effect of Cellulose in Fishes

The expansion of intensive aquaculture is an effective tool to meet the human consumption rate. Compound feed is one of the bases for intensive aquaculture. However, the high price for aqua feed makes it unaffordable for some fish farmers. In this regard, a compound feed with balanced nutrition at an affordable rate has to be assessed for fulfilling the aquaculture requirements. Concerning this aspect, addition of fiber can be included to the feed from naturally available plants, since addition of plant components in fish feed formulations is progressing. With reference to plant fiber, cellulose is one of the major plant components that are widely available. It is a polysaccharide composed of glucose and accounts for more than 50% of carbon content (Sinha et al. 2011). Also, the current research suggests that the addition of cellulose in feed improves feed stickiness, stimulates digestive tract peristalsis, and promotes digestion and absorption (Sun et al. 2019). Therefore, an optimal amount of cellulose content in fish feed needs to be assessed as it differs among various fish species which are discussed here.

Misgurnus anguillicaudatus, commonly known as pond loach, is a freshwater fish that belongs to the family Cobitidae of the order Cypriniformes. The richness of nutrients and pleasant taste makes the fish popular among the Chinese, with its market demand expanding every year. Prior to the experiment, the juvenile Taiwanese loaches were fed initially with commercial feed to acclimatize the fish to the experimental environment. The wheat bran was modulated to attain different fiber levels. The six groups of feed contained crude fiber content of 4.70%, 4.92%, 5.15%, 5.44%, 5.79% and 6.06% respectively. The results indicated that the feed containing crude fiber had significant effects on loaches, in reference to feeding rate, protein efficiency ratio, and feed conversion rate. However, there was no difference in specific growth rate. Also research indicated high fiber content will have a negative effect in utilization of other nutrients. This was due to the excessive amount of cellulose that makes the nutrients pass at a faster rate through the digestive tract. This eventually leads to a shorter digestion time and therefore lower digestibility rate (Krogdahl et al. 2005; Sun et al. 2019). Hence, appropriate fiber content is always recommended to improve the digestive efficiency as well as to yield enough nutrients and energy in loach. Therefore the optimal level of cellulose fiber applicable for Taiwanese loach ranges from 5.52% to 5.65%. In addition, the fiber content could also improve the antioxidant ability of Taiwanese loach. This was due to the presence of beta glucan in the fiber, which is also known to enhance the immunity level in loaches (Guzmán-Villanueva et al. 2014; Zhao et al. 2012; Sun et al. 2019). While in juvenile Tilapia (*Oreochromis mossambicus*) the optimal level of cellulose fiber was much lower than the loaches. Initially the juvenile tilapia was acclimatized to experimental conditions for about a week. The diet formulation either contained a minimum amount of fiber or without any fiber at all. The results indicated, the tilapia fed with 2.5% or 5% cellulose showed a better growth rate than those fed with a higher fiber or those without cellulose. Also, the survival rate declined as the cellulose content increased above the optimal level. Similarly the protein efficiency ratio increased with cellulose fiber up to 2.5%, beyond which it decreased (Dioundick and Stom 1990). Another study on tilapia however contradicts with the optimal level of fiber, as the study confirms a 10% dietary fiber is applicable (Anderson et al. 1984). This higher amount of cellulose fiber did not show reduction in growth rate which therefore indicates the optimal level could be beyond 5%. Research on tilapia even suggested that the long digestive tract allows them to utilize cellulose and other carbohydrates at high efficiency rates. Also, the extreme low pH in the stomach of tilapia becomes an additional factor for cellulose hydrolysis in the digestive tract. Generally the pH value for most fishes ranges between 2 and 2.2. While in tilapia it can reach up to 1.25 or 1. This extreme low pH facilitates the cellulolytic enzymes from the gut microflora to the fiber (Dioundick and Stom 1990). Therefore, these factors allow tilapia to take up the fiber content up to 10%. Moreover, the optimal fiber content in Nile tilapia (*Oreochromis niloticus*) partially supports the results of both (Dioundick and Stom 1990; Anderson et al. 1984). The Nile tilapia was fed with three diets, each containing α-cellulose from barley husk at 5%, 10%, and 15% respectively. The fish fed with all three diets had a survival ratio of 100%. Also, the average value of weight gain of the fish fed with 5% cellulose was similar to those fed with 10%. While those fed with 15% cellulose showed a lower value than the other two. Although the weight gain of fish was similar in the feed containing 5% and 10% cellulose, the best results for specific growth rate and feed conversion ratio were obtained from the fish feed containing 5% cellulose. In addition, the results also confirmed, the diet formulations should not exceed 10% cellulose in Nile tilapia (Ighwela et al. 2015). Certain other fishes like red sea breams and yellowtails also exhibit a better growth at 10% cellulose (Kono et al. 1987). Similar results were observed in sea bass juveniles, which can uptake up to 10–20% cellulose without affecting the growth parameters (Bromley and Adkins 1984).

Another freshwater fish, Rohu (*Labeo rohita*) is known to be one of the most important fish. The significance is due to consumer preference and it fetches the fish farmers a higher price when marketed. Therefore, the fish farmers are likely to stock this species among their major aquaculture species. On an average it contributes 35% of total stocking and produces 23% of total aquaculture production. In terms of growth among the Indian major carps, rohu comes after catla. Rohu gains maturity in 2 years from spawning and breeds by hypophysation from June to July. In regard to cellulose content, the fish is able to tolerate up to 16% α-cellulose. However, 12% α-cellulose is optimal in terms of growth rate and digestibility of nutrients; beyond this level the growth rate gradually decreases (Ashraf et al. 2014).

While the Atlantic cod (*Gadus morhua* L.) juveniles were able to retain up to 18% cellulose fiber. Generally, the natural diet of cod contains high levels of chitin (Link et al. 2000) which allows the cod to retain high levels of cellulose as well, since both share a β-1,4 glycosidic bonds between the monomers (Lekva et al. 2010). Two dietary mixtures were prepared for the experiment. One diet contained protein from both plant and fish meal while the other contained fish meal alone. Both the diets were supplemented with increasing amounts of cellulose (0%, 6%, 12%, and 18%). Minerals and vitamin sources were also added to the diets. The results confirmed the α-cellulose has not induced a negative effect on protein utilization at any concentration level. The protein efficiency ratio, protein productive value, and fillet yield were found to be equal in both the diets. Indeed, the growth was improved by the addition of cellulose. The addition of cellulose had a positive impact on the fish except the digestibility of fat and dry matter, which decreased upon increase in cellulose. Similarly the liver and muscle size were not influenced by the addition of cellulose. Therefore, as per the results the cod could retain a greater amount of cellulose up to 18% without affecting the growth parameters (Lekva et al. 2010).

A much higher fiber was found to be applicable in rainbow trout with a significant influence on its growth rate. Six experimental diets were prepared containing cellulose ranging from 0% to 50%. Each of these diets was tested for 51 days and the food intake was recorded on a daily basis. Also, the initial and final weight of the fish were analyzed. Prior to weighing, the fish were narcotized in 2-phenoxyethanol and the excess of water was removed. The total weight of food uptake increased due to the addition of dietary cellulose content. Also, there was an occurrence of rapid growth in fish having 0–30% dietary cellulose. The results also confirmed, the fish that took 40–50% dietary cellulose were comparatively smaller in size and had half growth in reference to those having up to 30% cellulose. However, there was a significant increase in stomach size of the fish fed with 40–50% cellulose. Slight variation in weight of hindgut was present due to the influence of dietary cellulose. Yet the liver did not show any size difference, rather it was relatively stable irrespective of cellulose in the diet. Lipid level, protein, and energy gains did have an impact by a low amount of cellulose that is 0–30%. Also, the nutrient energy conversion efficiency and protein conversion efficiency were found to be stable upon a diet fed with 0–30% cellulose; however had a decline when the cellulose content was beyond 30%. Thus the results confirmed, the trout could retain 30% cellulose providing a greater impact on its growth than those fed with 40–50% cellulose in the diet. The diets fed with 40–50% cellulose, though influenced the size of stomach, however could not increase the nutrient intake (Bromley and Adkins 1984). Although, there were discrepancies regarding the same species, as various other research suggested the trout can incorporate cellulose up to 8%, beyond which the growth rate decreased (Hilton et al. 1983; Poston 1986). There was still variation on this aspect, as certain other studies indicated that up to 15% cellulose inclusion in trout does not influence the digestibility of main nutrients (Hansen and Storebakken 2007). Data regarding the contradictory results are inadequate; however the discrepancies may probably be due to quality of feed and variation in the type of cellulose used.

2.11 Enzymes in Aqua Feed: Factors to Consider

Using enzymes in feeds, to improve feed utilization is a concept that has been well researched in both terrestrial and aquatic animal nutrition. The preliminary objective of application of enzymes in feeds is to enhance digestibility. It is suggested that providing an extra supply of enzymes in the feeds would boost the digestive processes, resulting in increased efficiency of the feeds. The aquatic animal population lacks some digestive enzymes during their early developmental stage or throughout their lives. The nutrient fractions digested by the application of these enzymes can be utilized by the aquatic animal that lacks the digestive enzymes (Ghosh and Mukhopadhyay 2006). Phytase, carbohydrases such as amylase, mannanase, galactosidase, xylanase, cellulase, and pectinase, protease, and lipase are the most widely used enzyme additives (Abishag and Betsy 2018).

2.11.1 Anti-Nutritional Factors

Based on existing feed formulations, the amount of the wild fish that are caught is inaccessible, and confusion about the potential access to the resort is a key problem. In 24 years, the supply of pelagic products for fish oil and meals has not increased in a sustainable way (Shepherd and Jackson 2013). As an alternative the plant- and animal-based by-products as sources of proteins in aquafeed are being used. With significant drawbacks and multi anti-nutritional factors, the plant-based proteins are still an important ingredient in the aqua feed (Malcorps et al. 2019). The dry matter indigestion of plant-based nutrients is higher than fish meals. This leads to a large contribution of waste product due to undigested nutrient excretion. The indigestion is mostly due to the non-starch polysaccharides, fibers, and other anti-nutritional factors. Anti-nutritional factors are categorized as organic materials that affect the feed consumption, utilization, growth, and normal functioning of internal organs (Altan and Korkut 2011; Kokou and Fountoulaki 2018). The antinutritional factors are phytates, saponins, non- starch polysaccharides, lectins, tannins, cyanogenic glycosides, and gossypols (Krogdahl et al. 2010).

2.11.2 Phytates

Phytates are the major storage form of phosphorus in plant grains and seeds that monogastric animals as well as humans cannot degrade. Since phytate is a potent chelator of mineral ions, it is linked with a variety of health problems (Kumar and Sinha 2018). The phytates interact with minerals and other nutrients, insoluble complexes are formed in the small intestines and it does not accord with any absorbable requisite elements. The digestibility and utilization of proteins and amino acids are disrupted by phytates in fishes and higher organisms. For example, in acidic Nile tilapia stomach the negatively charged phosphate moiety binds at the lysine amino group, imidazole groups on histidine and guanidyl groups on arginine in soluble proteins. In the alkaline intestines of Nile tilapia, ternary complexes are formed. These complexes are resistant to proteolysis (Kumar et al. 2012).

2.11.3 Non-starch Polysaccharides

Non-starch polysaccharides (NSPs) present in the aqua feed as part of 90% of the plant cell wall. These consist of cellulose, hemicelluloses, and pectin polysaccharides, non-cellulosic polymers such as arabinoxylans, mannans, xyloglucan and mixed-linked b-glucans. The interaction of the non-starch polysaccharides depends upon the structural linkages between the sugar residues and the sugar residues itself. The NSPs are also present in the aqua feeds as soluble purified form, for example, guar gums used for the stability of the feed pellets (Sinha et al. 2011). The most utilized ingredient in aqua feeds are soybean meals. Approximately 200 g/kg of NSPs are there in raw soybeans and cereals contain about 100-200 g/kg of soluble and insoluble forms of NSPs (Felix et al. 2018). The increase in NSPs causes the higher level of digested viscosity which will affect the emulsification reducing lipolysis. The entrapment of bile salts by NSPs also impairs solubilization efficiency of fats. This will all lead to reduced lipid absorption and utilization. The NSP induced digesta viscosity also hinders the absorption of minerals (Sinha et al. 2011).

2.11.4 Protease Inhibitors

The relatively low quality protein content of plant-based feedstuffs is one of the key drawbacks of using high inclusions of plant-based feedstuffs. Protease inhibitors (PIs) are prevalent in storage organs such as seeds and tubers, accounting for 1–10% of total protein in almost all plants. PIs account for 6% of the protein in soybeans, and residual levels can persist despite the feed processing. Protease inhibitors reduce the proteolytic digestive enzyme activity. Proteases are enzymes that catalyze the hydrolysis of peptide bonds in proteins. The protease inhibitors bind to the protein and reduce the activity of protease enzymes, such as trypsin and chymotrypsin, along the digestive tract. This leads to the hindered protein utilization (Felix et al. 2018).

2.11.5 Phytase Enzymes

Most commonly used plant grains and seeds in aqua feed contain phytates stored as indigestible phosphorus. Phytases are enzymes which can cleave the phytates and convert it to inorganic phosphorus inositol. The feed ingredients undergo a pretreatment with phytase enzymes to improve digestibility. The studies have proved the enhanced phosphorus digestibility and increased absorption of minerals. Some studies report the increase and utilization of protein and lipids (Lemos and Tacon 2017). In Nile tilapia, 50% phosphorus added with 500 or 1000 units of phytase per kg increases the body weight, protein content, and lipid content. The phytase supplementation also increases the feed utilization efficiency than that of the fish population diet without the addition of phytases (Abo Norag et al. 2018).

2.11.6 Protease Enzyme

The most essential ingredient of feedstuffs is protein. Increasing the protein content of feeds can boost fish productivity, but too much protein is metabolized as an energy source, resulting in further nitrogen discharge into the water (Xue et al. 2012). Proteases (EC 3.4) can catalyze the reaction of hydrolysis which degrade protein molecules to peptides and ultimately to free amino acids (Ramos and Malcata 2017). Exogenous proteases introduced to feed may compensate for endogenous protease deficiency, allowing macromolecular protein to be hydrolyzed into smaller molecular peptides, peptones, and a variety of amino acids which can be easily digested and absorbed, thus decreasing stimulation, barriers to nutrition, improved feed utilization and promotion of growth (Shi et al. 2016). Neutral and acid proteases are widely used feed industry proteases. In a study by (Liu et al. 2018) upon addition of protease in a lower protein diet, the dietary protein requirement of juvenile gibel carp (*Carassius auratus gibelio*), was decreased. The feed conversion ratio, digestibility of protein and lipids, and the protein efficiency ratio were also enhanced considerably.

2.11.7 Carbohydrase Enzymes

Carbohydrates are one of the primary constituents of the aquafeed and are used by the fish as an energy source. Two main components of carbohydrates are monosaccharides which are easily digestible (disaccharides and oligosaccharides) and the indigestible, insoluble polysaccharides (cellulose and hemicellulose). The carbohydrate supplement in many species is required as it promotes utilization of proteins and lipids and stimulates growth. Therefore the substitution of plant-based proteins has gained interest for the expansion of the global aquaculture industry. The antinutritional factors like phytates, non-starch polysaccharides, and protein inhibitors in plant-based nutrition negatively affect absorption of nutrients and fish health (Francis et al. 2001; Malcorps et al. 2019). Amylase, β glucanases and β xylanases, cellulase and pectinases are the carbohydrases widely used in food industry. The functions of carbohydrases differ upon its types, yet two main enzymes, xylanase and glucanase, account for more than 80% of the global carbohydrate enzyme market. All feed-relevant carbohydrases are the members of the family hydrolase/glycosidase which converts polymeric carbohydrates into low molecular oligosaccharides or polysaccharides (Zheng et al. 2020). The linear polysaccharides are digested by the xylanase enzymes, breaking down the hemicellulose which is a main component of the plant cell wall. The use of xylanases in maize-soy-based diet

Table 2.3 Beneficiary effects of distinct enzymes on aquatic animals

Enzymes	Species	Effects	References
Bovine trypsin	*Cyprinus carpio*	Enhanced proteolytic activity	Dabrowski and Glogowski (1977)
Cellulase	*Ctenopharyngodon idella*	Increased growth performance Enhanced endogenous enzyme activity Intestinal microbiota changes	Zhou et al. (2013)
	Carassius auratus	Gain in weight Increase in feed intake and trypsin activity	Shi et al. (2017)
Lipase	*Sparus aurata*	Significant absorption of glycerol trioleate in 45 day old juvenile	Koven et al. (1993)
Phytase	*Ictalurus punctatus*	Bone ash increment Increase in bone phosphorus Gain in weight Increase in bioavailability of phytate phosphorus	Jackson et al. (1996)
	Carassius carassius	Weight gain by 25%	Yu (2000)
	Oreochromis sp.	Dry matter energy digestibility coefficients of palm kernel meal diet (40%)	Ng and Chong (2002)
	Channa micropeltes	40% substitution of fish meal with soya bean meal with corresponding reduction of feed cost.	Hien et al. (2015)
	Macrobrachium rosenbergii	Improved protein efficiency ratio Enhanced growth	Biradar et al. (2017)
Protease	*Oncorhynchus mykiss*	Feed efficiency in canola pea diet	Drew et al. (2005)
	Salmonids	Improved carbohydrate and protein digestibility	Chowdhury et al. (2014)
	Litopenaeus vannamei	Weight gain Less feed conversion ratio	Li et al. (2016)
	Carassius auratus gibelio	Increased protease activity	Liu et al. (2018)
Amylase	*Labeo rohita*	Increased growth rate Improved dry matter digestibility	Kumar et al. (2006a, b)
Mannanase	*Oreochromis niloticus*	Weight gain WBC count increment	Chen et al. (2016)

helps to impair the plant cell walls to degrade to allow hydration for the endogenous enzymes to perform its activity for a better breakdown of starch and proteins. Xylanases also help in releasing the proteins from the aleurone layer which is rich in xylan in wheat. Supplementing xylanase enhances bile acid conjugate activity in intestines and increases size of the small intestinal villi. It also leads to improved weight gain, feed intake, and feed efficiency. Liver vitamin E also increases by the addition of xylanases (Ganguly et al. 2013) (Table 2.3).

2.12 Conclusion

Sustainable development with sound socioeconomic benefits in aquaculture could be achieved only when there is a decrease in the dependency on fish meal and fish oil in aqua feeds. A comprehensive research on finding effective alternatives for fish meal has been well recognized by the aqua feed industries, which in turn depends upon the economics and environmental effects. Another threat to the growth of aquaculture is the recurrent disease outbreaks among fishes due to unhealthy practices and antibiotic resistance. Development of functional aqua feeds; crafted aqua feeds; and the emergence of aquaponics have paved the way to replace the roles of conventional feeds. Fish farming has now become an efficient practice to produce animal proteins. Single-cell protein, fish processing waste, novel industrial by-products, and probiotics have transformed the aquaculture sector into a fish meal- and antibiotics-independent platform, supporting wide-scale fish farming. Continued research will promise to uplift the economic and environmental status of aqua feed industry, enabling its healthy expansion.

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