Chapter 4
Nanotechnology: A Novel Tool for Aquaculture Feed Development

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Abbreviations

% WG	Percent weight gain
AgNPs	Silver nanoparticles
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CD- TiO_2	Carbon dots coupled with titanium dioxide
CD	Carbon dots
FCR	Feed conversion ratio
Fe	Iron
GIT	Gastrointestinal tract
GSH-Px	Glutathione peroxidase enzyme
Hb	Haemoglobin
IGF-1	Insulin-like growth factor 1
IgM	Immunoglobulin M
nFe	Iron nanoparticles
NPs	Nanoparticles
nSe	Selenium nanoparticles
$nTiO_2$	Titanium dioxide nanoparticles
PL	Post larvae
RBCs	Red blood cells
ROS	Reactive oxygen species

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V. K. Arivarasan et al. (eds.), *Nanotechnological Approaches to the Advancement of Innovations in Aquaculture*, Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-031-15519-2_4

Se	Selenium
SGR	Specific growth rate
TiO_2	Titanium dioxide
WBCs	White blood cells
Zn	Zinc
ZnO	Zinc oxide
ZnO-NP	Zinc oxide nanoparticles

4.1 Introduction

Aquaculture is an expanding area in the agriculture sector, contributing a total of 46%, i.e. 82 million tonnes in 2018. FAO 2020 reported around USD 250 billion from aquaculture production alone out of the total first-sale value of USD 401 billion from 179 million tonnes of global fish production. For billions of populations, this provides the best option for easily digestible protein and healthy fat, as well as many other essential micronutrients.

But many environmental issues are affecting the production of fish in water bodies. The extreme practice of aquaculture techniques is destructing the natural ecosystem through eutrophication, acidification, and chemical and biological contamination (Sarkar et al. 2021; Pudake et al. 2019). This results in an unsuitable environment for the rearing and breeding purposes of fish, which leads to the degradation of fish production. In this advanced world, the detection of contamination and toxicity levels in water bodies is tough and onerous work. Nutrients become unavailable in water bodies because of huge contamination by sewage inlets. Therefore, in an extensive aquaculture system, deficiency of nutrients has become dreadful, resulting in suboptimal breeding efficiency in juvenile and broodfish populations (Sarkar et al. 2021).

Nanotechnology can solve the recent problems in the agriculture and aquaculture sectors. It is the science of creating and using compounds with unique properties at nanoscale dimensions (1–100 nm) to enable new functionalities (Shaalan et al. 2016). The aquaculture industry can be transformed by nanotechnology through new tools for quick disease detection, nutrient delivery, improvement of the ability of fish to absorb vaccines and drugs, decreasing pollution, etc. (Pudake et al. 2019). In the aquaculture sector, nanotechnology has various practical purposes that help to improve management, such as wastewater treatment, fishpond sterilization, improving fish processing, avoiding nutritional deficiency, involvement in the feed industry, and health management. In this chapter, we focused on the role of nanotechnology in aquafeed development. The role of feed-in aquaculture has great importance because of its high input cost. The improvement in the fish feed will lead to high fish production, and nanotechnology has great possibilities to enhance the nutrient composition of feed, which will help to tackle the issues that originate from nutrient unavailability.

4.2 Role of Nanotechnology in Enhanced Feed Additive Preparation

Aquaculture has been fulfilling the demand for animal protein in a big way. The world population has been increasing, and so has its need for more food. Aquaculture has been contributing to the supply of animal protein, but as it gets more and more intensified, instances of stresses on the environment and on the fish species themselves are being witnessed. Diseases, harmful chemical accumulation, adverse effects on the ecosystem of the culture, incomplete and inadequate utilization of resources, faulty culture practices, incapable personnel involved in the culture, among others, limit the amount of produce we get through it. Nanotechnology is a branch of science that has very recently come into existence. It involves the study, use, manipulation, formation of particles that are in at least one of their dimensions between 1 and 100 nm. When bulky materials are changed to nanosize, then the surface chemistry and many other properties change. Nanomaterials are also naturally occurring in foods, such as proteins, fats, carbohydrates. They create their higher structures in our bodies. They have now been added intentionally also to our foods, feeds, etc. There are not many nanomaterials that are currently being applied as a feed additive, as it is a new field. General public perception on the fish produced on diets consisting of nanomaterials is still skeptical in many instances. Still, they are being researched and some are even substituting antibiotics (Peters et al. 2016). Nanomaterials may contain micronutrients. When fishes will consume them, the delivery of these nutrients will get precise. They can also change the density, shape, texture of the feed (Handy 2012). They will also improve the absorption and availability of nutrients and additives to the fish targeted (Chaudhry and Castle 2011). Due to their small size, the additives can be easily assimilated by cells and help in the rapid growth of fishes (Moges et al. 2020). Nanoparticles are prepared in several ways, which are influenced by the nature of the material, the stability of the material, its usage, etc. So, having nanotechnology as a tool to incorporate feed additives is possible. But risks that may be associated with the newly formulated materials must be considered. Safety evaluation is a must for them.

Nanoparticles can increase feed utilization by changing the colour, flavour, etc. Water-insoluble compounds such as some vitamins, minerals, etc. can be added to feeds, which makes them available to the fish (Sarkar et al. 2021). There is equipment available on the market that makes nanomaterials and adds them to the feed. Ubisol-AquaTM Delivery System Technology and NovaSOL developed by AQUANOVA are some of the examples which are creating the smallest of particles (Aklakur et al. 2016). Indian-origin companies are also producing equipment. Some of the examples are NannoCal Aqua (nano calcium), NanoPHOS (nano phosphorus), etc.

Nanoencapsulation is a process that protects the food from many factors, such as environmental stress, and masks inadequacies such as taste and odour. Also, it helps in bringing out the real taste of the feed (Fathi et al. 2012). Using different nanotechnology methods, lipids in solid form are also being encapsulated. Vitamin D and casein made micelles are made possible. Chitosan-based nanomolecules are enabling water-soluble entities such as ascorbic acid to also be capsulated (Jimenez-Fernández et al. 2014). Nanoparticles are in use in several shapes, which include nanospheres, nanotubes, nanocapsules, etc. (Shah and Mraz 2020). The shapes of the nanoparticles greatly affect their behavior. They can be elliptical, discoidal, conical, etc. (Bunglavan et al. 2014). Some of the common methods which are used to prepare nanoparticles are

- Cross-linking emulsion: A water-oil emulsion is made by vigorous shaking. Agents which can stabilize the product are also involved.
- Precipitation: Particles are produced by putting them in an alkaline solution. This process is also called 'blowing'. Purification is done by filtration and centrifugation, and then rinsing with cold and warm water.
- Spray drying: It is one of the easier processes. Drying is done. It is achieved by spraying them with compressed hot air. One chosen solvent is also used in this step, which evaporates instantly due to the high temperature of compressed air (Bunglavan et al. 2014).

A study was done in 2020 to determine the growth rate of silver catfish. Diphenyl diselenide nanocapsules were added to their feed. It resulted in the confirmation of the finding that growth was more in the group which had consumed the feed. In the experiment, Ph_2Se_2 added feed was produced using interfacial deposition of preformed polymers. For 1 hour, the organic component in it was kept at 40 °C temperatures in the water bath, then put into an aqueous phase and mixed for 10 minutes. Then, a rotatory evaporator was used to concentrate the mix and reduce the organic content. Later, the mixture was heated in a circulation oven for 24 hours, and the pellets were broken and stored (Baldissera et al. 2020).

Young carp showed faster growth when given iron nanoparticles in their feed. Nanoselenium is more potent than organic selenium for increasing selenium levels in muscles. It also improved the final weight, gain rate, antioxidant levels, etc. in the body (Sekhon 2014). Nutraceuticals are also being delivered using nanomaterials, which increase their potency, though the cost of feed preparation increases (Rather et al. 2011). Nanovaccines and nanoparticles used for gene therapy also involve the use of nanotechnology. In gene therapy, a carrier vehicle is designed to deliver DNAs (Sarkar et al. 2021). Compound Nano-863 is used in China for the growth and development of fish. They were produced by complementing nanomaterials exhibiting high-temperature sintering using a ceramic substance as a carrier and a strong light-absorbing capacity (Moges et al. 2020).

4.3 Types of Nanomaterial and Their Properties

According to their ability to relay various components and interact with diverse environmental circumstances, nanoparticles are categorized in various categories. Nanomaterials can be divided into four categories: carbon nanomaterials, inorganic-based nanomaterials, organic-based nanomaterials, and nanocomposites (Majhi and Yadav 2021; Khan and Khan 2020). Based on the chemical characteristics of the nanoparticle, it can be classified into inorganic and organic categories (FSAI 2008). The applications of nanoparticles are diverse, and they also help to improvise fish production in the aquaculture sector. It helps in disease detection, increases the drug absorption ability of fish, promotes vaccine development, upgrades water quality parameters, etc. (Pudake et al. 2019). The major cost in aquaculture is contributed by feed development, and nanoparticles help to improve the quality of feed by enhancing the nutrient composition, micronutrient delivery, feed encapsulation, and promoting the growth of fish. Some of the nanoparticles are categorized below based on feed improvements.

4.3.1 Inorganic Nanoparticles

These nanoparticles are manufactured by using inorganic ingredients at nanoscales already permitted for use in feed, e.g., the use of silver in poultry feed, which helps to improve the microbiota of chickens (Gangadoo et al. 2016). Metal-based inorganic nanomaterials comprise cadmium, silver, iron, gold, copper, aluminum, lead, and zinc nanomaterials, while titanium dioxide, iron oxide, cerium oxide, silica, zinc oxide, copper oxide, iron oxide, magnesium aluminum oxide, etc. are examples of metal oxide-based inorganic nanomaterials. Zinc helps to regulate energy consumption, metabolism of vitamin A and lipids, and protein synthesis when fed to animals along with feed (Pudake et al. 2019). nTiO2 is used to notice the growth and performance improvement of rainbow trout (Ramsden et al. 2009). It has been noticed that Nile tilapia (*Oreochromis niloticus*) and Prussian carp (*Carassius auratus gibelio*) fed on nSe as supplemented diets showed positive results such as a reduction in FCR with an increase in final weight and improved overall growth, respectively (Zhou et al. 2009; Deng and Cheng 2003).

4.3.2 Organic Nanoparticles

Organic-based nanomaterials are made up of organic compounds that do not contain carbon, such as liposomes, cyclodextrin, dendrimers, and micelles (Majhi and Yadav 2021). Organic nanoparticles are most likely utilized to enhance or modify food functionality to improve the nutritional value of food systems. These nanoparticles were aimed at providing vitamins and other nutrients without affecting the flavour or look of food and beverages. Such nanoparticles wrap the nutrients and transport them into the bloodstream via the gastrointestinal tract (GIT), enhancing their bioavailability (FSAI 2008). Micelles are organic nano particulates that an wrap nonpolar molecules like flavours, vitamins, antioxidants, lipids, and antimicrobials (Chen et al. 2006). Liposomes are used to facilitate the delivery of functional components to food such as antimicrobials, nutraceuticals, and flavouring properties (Peters et al. 2011).

4.3.3 Nanocomposites

Composite nanomaterials or nanocomposites are the amalgamations of carbon-based, metal-based, and metal oxide-based nanomaterials, and they have complex structures such as metal-organic structures (Majhi and Yadav 2021). These materials have been used by researchers to find various benefits of nanocomposites in the aquaculture sector. Abad-Álvaro et al. 2019 used two various clay nanocomposites (i.e. kaolinite-Ag and sepiolite-Ag) in which sepiolite and kaolinite act as carriers for silver nanoparticles (AgNPs) that are fed orally and found that only limited release of silver nanoparticles is done by carriers due to the formation of silver chloride during stomach stimulation (Abad-Álvaro et al. 2019). Various nanocomposites are also used during fish processing, and chitosan composites are one of them, which are used during the refrigeration of fish meat at 4 °C and help to inhibit bacterial growth and decrease the formation of volatile bases and oxidation products that spoil the fish during freezing (Ahmed et al. 2019). In aquaculture wastewater effluents, Louros et al. 2021 try to increase the photodegradation of antibiotics by solar irradiation using carbon dots (CD) coupled with titanium dioxide (TiO_2) (CD-TiO_2) and conclude that ecofriendly CD-TiO_2 hybrid materials photocatalyze efficiently and show sustainable as well as promising strategies to expedite the removal of antibiotics effectively from aquaculture effluents (Louros et al. 2021).

4.3.4 Carbon Nanomaterials

Carbon black, fullerene, multi-walled carbon nanotubes, graphene, single-walled carbon nanotubes, activated carbon, and carbon fiber are forms of carbon-based nanomaterials (Majhi and Yadav 2021). These materials can also be used to improve the quality of water. According to Baby et al. 2019, carbon nanomaterials can be used to absorb various harmful gases from the aquatic environment. It has been reported that Graphene, a type of carbon nanomaterial, can be used to capture CO_2 and H_2. The best-superior materials used for the elimination of organic contaminants from the water are based on graphene nano-adsorbents (Baby et al. 2019; Bradder et al. 2011).

Different types of nanomaterials have specific properties that help for good aquaculture management and maintain better animal health. Some of them are discussed in Table 4.1 along with their properties.

Table 4.1 Various properties of nanoparticles

Nanoparticles	Properties (as feed additives)	Reference
Silver	Antimicrobial agent in additives for animal feed Food supplements Act as nanocarriers to increase the absorption of nutrients in fish and shellfish	Abad-Álvaro et al. (2019), FSAI (2008), De Silva et al. (2021)
Nano-ZnO (nZnO)	Improve the specific growth rate (SGR), % weight gain, and feed conversion ratio (FCR) Antimicrobial activity Induced growth hormone level in serum Improve the concentration of total nitrogen and immunoglobin M	Chris et al. (2018), Tawfik et al. (2017)
Iron	Food supplement Modifies the appropriate performance of the central nervous system Improve the SGR, overall weight gain, and FCR Increase the level of IGF-1 in animals	FSAI (2008), Akbary and Jahanbakhshi (2019)
Copper	Improved body weight while improving FCR Decrease in mortality rate Improved growth and immunity performance	Gangadoo et al. (2016)
Selenium	Stimulate growth hormone production Regulate thyroid hormone production in fish Improve larval growth related to bone mineralization	Dawood et al. (2021)

4.4 Application of Nanoparticles in the Aquaculture Sector

The aquaculture sector is one of the world's most developing domains with high growth promise (Sarkar et al. 2021). In the racing sector, where progress in science and technology has proved sustainable and efficient development, the use of nanoparticles is one of the many advancements in this sector. Particles with diameters ranging from 1 to 100 nm are considered nanoparticles. The particles in the nanosize range demonstrate new chemical and physical phenomena (Márquez et al. 2018). Nanoparticles can be found in individual or aggregate form. These aggregates can have a size that extends over 100 nm and thus don't fall under the category of nanomaterials, but these will still have the properties of nanoparticles (Shah and Mraz 2020). Various experiments and research proved that nanomaterials have a broad range of uses in the aquaculture sector (Márquez et al. 2018). They can be used for the control of disease in fishes, water treatment, sterilization of pond water, processed nanofood, vaccine transport, etc. Mostly metal nanoparticles such as silver, selenium, copper, gold, etc. are used.

The nanoparticle can provide notable benefits in the transport of nutrients needed in minor concentrations and unstable components to the vital organs of the fish. Fatty acids, other highly soluble nutrients, and components that have limited uptake efficacy can be encapsulated using nanomaterials to avoid disassociation in the gut of the fish (Handy 2012). The nanoparticle can provide trace metal to fishes without causing much loss of faecal matter, which is generally related to mineral salt. Various nanoparticles can perform as immunomodulators and development enhancers when supplied with aquafeed in smaller quantities. Selenium nanoparticles, when induced into Crucian carp along with the feed, showed an increase in weight, muscle development, and antioxidant characteristics. Research showed that nanoparticles of silver given to *Danio rerio* (zebrafish) enhanced growth and increased metalloprotease and protease activity (Sarkar et al. 2021). Nanoparticles when used to integrate vitamin C along with the feed for *Onchorhychus mykiss* (Rainbow trout) showed an enhancement in active permanency for about 20 days, whereas active permanency was lost in the initial 3 days when the fishes were given conventional feed. The nanoparticle was also able to secure vitamin C from various acids and enzymes in the gut of the rainbow trout as it showed prolonged production of vitamin C in the gut epithelium, which improves the nonspecific immune response of the fish (Márquez et al. 2018). When iron nanoparticles and *Lactobacillus casei* were provided along with feed for rainbow trout, they showed remarkable growth parameters (Shah and Mraz 2020). Selenium, manganese, and zinc nanoparticles given to *Sparus aurata* (gilthead sea bream) in the initial feed showed an increase in bone mineralization and tolerance to stress (Shah and Mraz 2020). Along with increasing the firmness and biological accessibility of the feed components, nanomaterials can also be used to change the physical parameters of fish feed. The instability, poor buoyancy, and texture of pelleted feed usually are primary causes of food wastage, which may lead to water quality deterioration, which is a major issue. Adding nanomaterial in small quantities can alter the physical parameters of feed pellets (Handy 2012).

Another important aspect of the nanoparticle is its ability to control disease (Márquez et al. 2018). A few biomaterials cannot attach to the disease-causing microbes because of their lower specificity and thus can't be used as a major target of the cells of disease-causing microbes; this leads to the use of nanoparticles (Munawar 2021). The silver nanoparticle has a bacteria-killing ability (Márquez et al. 2018). Nanoparticles of copper (mostly n-copper oxides) have the ability for antifouling and bactericide, and they are also a good conductor of heat (Khosravi-Katuli et al. 2017). Nanoparticles of n-iron oxides have properties such as lower toxicity and special surface properties, which can be used in the aquaculture sector. NPs of iron oxides can be used for the repair of tissues, delivery of medicine, and cellular labeling (Khosravi-Katuli et al. 2017). Nanoparticles of titanium oxide could attain the required sterilization efficacy on various bacteria such as *Vibrio anguillarum, Aeromonas hydrophila*, and *E. coli*, and these particles can disintegrate the organic pollution-causing agents in water (Shiwen et al. 2015). Oral administration and injection are the most dependable and potent methods of vaccination, but these methods may lead to the death of fish (Shah and Mraz 2020). Traditional methods of drug delivery in fishes performed badly due to their lower efficiency and biological presence in aquatic mediums (Sarkar et al. 2021). To avoid this problem, researchers used nanoparticles for the administration of the vaccine in fishes (Shah and Mraz 2020). Alginate particle, extracted from brown algae or as polysaccharides in a few bacteria, is a copolymer of a-L-guluronic acid and b-D-mannuronic acid, which are the primary particles responsible for vaccine delivery to fishes orally (Shah and Mraz 2020). Antibiofilm and antimicrobial properties are associated with oxides of iron, zinc, copper, and silver nanoparticles (Sarkar et al. 2021). Graphene oxide shows a hampering response to crucial aquatic disease-causing microbes such as *E. coli*, *S. aureus*, *Vibrio harveyi*, and *P. aeruginosa* in association with other polymeric nanoparticles. A small DNA strand with nano-encapsulated particles is suspended in the water body, where it is absorbed by the fishes. Subsequently, DNA is released into the fish using ultrasound, resulting in an immunological response (Sarkar et al. 2021).

Nanotechnology has a wide range of applications for water quality enhancement to facilitate a suitable environment for aquaculture. Adsorption and photocatalysis are the methods of water treatment (Shah and Mraz 2020). Shrimp aquaculture showed that the nano-enabled devices were able to enhance the quality of water and decrease the water exchange rate. It also reduces the mortality of shrimp and increases yield (Shiwen et al. 2015). The discharge of municipal sewage, industrial waste, agricultural runoff, antibiotics, etc. into water has not only caused problems for humans by reducing the amount of pure groundwater but also affected aquatic animal health. Catalytic adsorbents and hydrogel biofilms based on various nanoparticles work efficiently in water treatment (Shah and Mraz 2020). The nanoparticle can also be used in packing technology and the preservation of seafood without any microbial damage (Shiwen et al. 2015). There are different methods for packaging, like using nanopolymers and coatings to increase the strength of the packaging, which protects the fish fillet from mechanical damages or other bruising (Handy 2012). Nanomaterials such as carbon nanotubes are extremely stronger than steel, so they can be used in gear and boatmaking. Carbon nanotubes are also used in the manufacture of nets and cages (Sarkar et al. 2021).

4.5 Nanotechnology in Aquaculture Feed Production

The development of feed contributes the maximum input into aquaculture and growth, as well as weight gain, depending on the feed consumption by fish. A small disbalance of nutrient composition in the feed will lead to the inappropriate growth and spread of diseases, and finally, the collapse of farming occurs. Fish suffer from nutritional deficiencies because of an imbalanced diet, a lack of food, or an abundance of dietary components. In aquaculture, one of the most important functions of nanotechnology is feed production, where nanoparticles are employed for growth enhancement, vitamin delivery, and feed encapsulation (Fig. 4.1) (Pudake et al. 2019). Nanoparticles are seen to be effective for (i) growth promotion (e.g., nFe, nSe, and ZnO), (ii) delivery of micronutrients (e.g., chitosan NPs), and (iii) production of feed amount per unit time (e.g., fullerenes (C60), and nTiO2), during aquafeed development.

The growth of aquatic animals is influenced by many factors such as water quality, temperature, vaccination, and well management, but notionally balanced

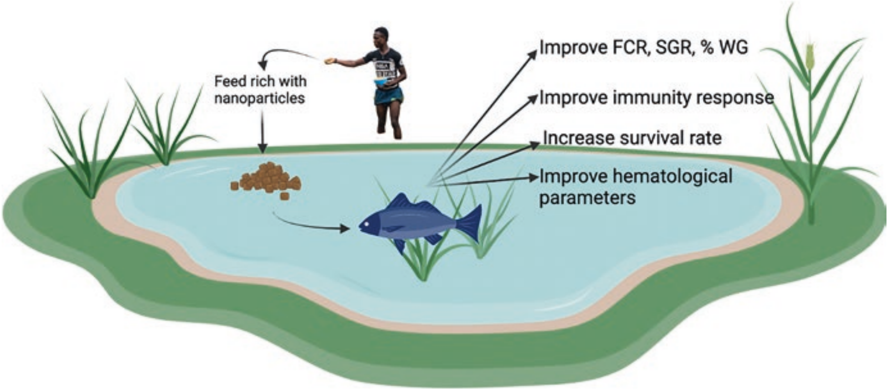

Fig. 4.1 Demonstration of the impact of nanoparticle-rich feed on fish health. (Created with BioRender https://biorender.com/, Farmer picture by https://www.usaid.gov/. Data from Table 4.2)

aquafeed is an important factor that determines good production by improving feed digestibility, health condition, and growth performance. A well-balanced feed formulation can be achieved by using both macro- and micronutrients (Dawood et al. 2021). The use of nanotechnology/nanoparticles as feed additives is the best way to achieve proper nutrients in feed. The application of various nanoparticles as feed additives has been examined by various researchers. According to Rathore et al. 2020, selenium (Se) can boost growth hormone production, resulting in increased fish growth. It's an important trace element for animals that can be fed to them. It is a constituent of the glutathione peroxidase enzyme (GSH-Px) that upholds the cell membranes through glutathione concentration reduction (Pudake et al. 2019). The use of Se nanoparticles as a feed additive is important because of its antioxidant defence properties (Sonkusre et al. 2014). Another function of Se used as a nanoparticle is to enhance larval growth by preventing skeleton anomalies and bone mineralization (Izquierdo et al. 2017).

Silver nanoparticles (AgNPs) are also used for aquafeed development like other nanoparticles, and they have great importance for animal health due to their antibacterial activity and better efficiency due to their small size and shape and large surface-to-volume ratio that enhances the antibacterial capacity (De Silva et al. 2021). The use of AgNPs as nanoparticles increases nutrient absorption in aquatic organisms via target delivery, controlled release, encapsulation, and as cargo on a nanocarrier. AgNPs, when working as nanocarriers, indicate improved adsorption and delivery of nutraceuticals needed by an aquatic organism for better growth. The best AgNPs are obtained when they are synthesized biologically because they contain biodegradable properties, which reduce the chances of toxicity (Rather et al. 2013).

Zinc (Zn) is an important micronutrient implicated in a variety of metabolic pathways, including protein synthesis, lipid metabolism, energy consumption, and vitamin A absorption (Muralisankar et al. 2014). It has been noticed that the supplementation of zinc nanoparticles in the diet of *M. rosenbergii* postlarvae (PL) improves the performance of digestive enzymes such as protease, amylase, and lipase, as well as survival and growth (Muralisankar et al. 2014). Faiz et al. (2015) showed that zinc oxide acts as a supply of dietary Zn that demonstrates a better response of the immune system and grass carp growth improvement. A considerable increase in the concentration of antioxidant enzymes, protein content, and improved body weight of freshwater prawns (*Macrobrachium rosenbergii*) has been noticed when organisms feeding on feed improved with nZnO (Muralisankar et al. 2014).

Chitosan is indeed a polysaccharide-type nanoparticle that is frequently employed in animal feed production due to its antimicrobial capabilities, low toxicity, and low immunogenicity (Khosravi-Katuli et al. 2017). A novel application of chitosan nanoparticles is the supply of hydrosoluble or unstable micronutrients, which also increased the shelf life and liberation of vitamin C in the fish (Alishahi et al. 2014). It has been observed that *Oreochromis niloticus* shows a positive and improved growth rate and feed utilization during feeding with an improved diet with chitosan (Abd El-Naby et al. 2020). It is essential for the stimulation of phagocytic cell bactericidal activity and increased serum bactericidal activity, which results in the induction of several humoral components involved in innate and/or adaptive immunity, which successfully protect the fish from various infections (Maqsood et al. 2010). It is also used as an encapsulating agent to help release the nutrients from feed pellets into water, and it is easily degraded with contact with water (Khosravi-Katuli et al. 2017). Various nanoparticle along with the concentration used during the experiment as a feed additive for developing aquafeed has been elaborated in Table 4.2.

Table 4.2 Effect of nanoparticles used in feed and their concentration on various fish species

Nanoparticles	Fish species	Concentration	Effects	Reference
Selenium	Nile tilapia fingerlings (15.73 ± 0.05 g)	1 mg/kg	Higher weight gain, average daily weight gain, FCR, SGR	Rathore et al. (2020)
	Asian seabass (Lates calcarifer) (32.78 ± 2.23 g)	4 mg/kg	Higher feed consumption, weight gain, and specific growth Enhanced immune response	Longbaf Dezfouli et al. (2019)
	Gilthead seabream (5.10 ± 0.43 mm)	3 mg/kg	Prevent skeleton anomalies Improve larval and growth performance	Izquierdo et al. (2017)
Silver	Zebra fish (Danio rerio)	0.963 and 1.925 mg/L	Induced developmental and transcriptional distress in embryos development	Qiang et al. (2020)

(continued)

Table 4.2 (continued)

Nanoparticles	Fish species	Concentration	Effects	Reference
Zinc	Nile tilapia (*Oreochromis niloticus*)	60 mg/kg	High SGR rate Increase in growth hormone Increase in the concentration of total protein and IgM	Tawfik et al. (2017)
	Macrobrachium rosenbergii post larvae (PL)	10–60 mg/kg	Increased growth and digestive enzyme activities (protease, amylase, and lipase) Reduce the FCR	Muralisankar et al. (2014)
	Grass carp, (*Ctenopharyngodon Idella*)	30 and 60 mg/kg	Showed a higher growth rate Higher energy reserves available due to the higher availability of nutrients	Faiz et al. (2015)
	Labeo rohita (Hamilton) fingerlings	20 mg/kg	Improve weight gain and SGR	Mondal et al. (2020)
Chitosan [poly(1,4-β-D-glucopy-ranosamine)]	*Oreochromis niloticus* (4.97 ± 0.02 g)	5 g/kg	Enhanced the indicators related to growth performance, feed utilization Improved the concentration of WBC count, RBC count, and Hb values	Abd El-Naby et al. (2020)
	Common carp (*Cyprinus carpio*) (45 ± 2 g)	20.0 g/kg	Improved FCR and specific growth rate Enhancement of innate immune responses Reduced mortality percentage and high phagocytic activity	Maqsood et al. (2010)
	Penaeus monodon (mean initial wet weight 1.49 g)	4.0 g/kg	Increase the survival rate Improved weight gain Immunostimulant	Niu et al. (2013)

(continued)

Table 4.2 (continued)

Nanoparticles	Fish species	Concentration	Effects	Reference
Copper	White fish (*Rutilus frisii kutum*)	10 ppm Cu	Antifungal effects of copper nanoparticles to avoid serious losses in fish hatcheries	Kalatehjari et al. (2015)
	Mozambique tilapia (Tilapia mossambica)	2 mg/L and 15 mg/L	Significant improve tissues such as liver, gills, brain, and body weight Modify the enzyme performance in various organs, e.g., liver, gills, brain, etc. Overall reduction in protein level	Al Ghais et al. (2019)
Titanium	Rainbow trout (*Oncorhynchus mykiss*)	10 and 100 mg/kg	Significantly body weight gain, but mean final weight approximately same for all group No major haematological disturbances	Ramsden et al. (2009)
	Common carp (Cyprinus carpio) (15.18 ± 0.22 g)	0.125 mg/L	No significant effect on blood parameters such as haemoglobin concentrations The immune parameters of serum and mucus significantly decrease	Hajirezaee et al. (2020)

(continued)

Table 4.2 (continued)

Nanoparticles	Fish species	Concentration	Effects	Reference
Iron	Goldfish (*Carassius auratus*) (4.3 g)	0.5 g/kg	Significantly improved feed conversion ratio and specific growth rates Increase the concentrations of alkaline phosphatase in plasma Upregulated IGF-1 and ghrelin gene expressions	Akbary and Jahanbakhshi (2019)
	Labeo rohita	10.0 mg/kg	Improve the weight gain, survival rate, SGR, and FCR Increase the concentration of carbohydrates in the liver, gills, muscles of the fish	Thangapandiyan et al. (2020)
	Bagridae catfish (*Clarias batrachus*) (5.23 ± 0.07 g)	40 mg/kg	Highest growth and feed utilization performance has been observed Significantly increase the total protein and lipid content of fish muscle Serum total protein, cholesterol, and triglyceride were found to increase	Akter et al. (2018)

Feed formulation along with nanoparticles can be done by various methods. Faiz et al. (2015) formed the zinc nanoparticle-rich feed. The inorganic dietary sources of Zn, $ZnSO_4$, and ZnO were used to synthesize zinc oxide nanoparticles (ZnO-NP), and it is ground along with other feed ingredients (dry) to obtain a fine powder that is mixed with oil. The semisolid paste is prepared by adding water in the optimal quantity and passing it through a meat grinder to obtain an unbroken noodle-like shape that changes into small, uniform-size pellets. At low light and room temperature, the pellets were dried to avoid oxidation and stored in an airtight container at 4 °C. It is important to note that the manufacturing of zinc nanoparticle-rich feed is done by grinding ingredients as per respective diets and mixing with inorganic sources of Zn after and before being blended with oil (Faiz et al. 2015). Thangapandiyan et al. (2020) used the leaves of *Amaranthus tricolor* for the green synthesis of iron oxide nanoparticles and mixed them with ingredients for achieving the desired feed diet. Ramsden et al. (2009) formulate a diet rich in titanium dioxide. One kilogram of feed was placed in the commercial feed mixer and sprayed with the required TiO_2 nanoparticle solution. The spraying of a 10% bovine gelatine solution on ingredients leads to the TiO_2 nanoparticles being rapidly covered and sealing the feed. The gelatin coating was permitted to dry before the feed was moved to airtight storage containers (Ramsden et al. 2009). Maqsood et al. (2010) ground all desired ingredients separately in an electric grinder and mixed them properly, and water was added in the optimal quantity. The well-mixed mixture of ingredients was steamed for 20–25 minutes. The chitosan along with the mineral mixture at the required quantities was added and mixed thoroughly for making dough. The dough of the desired consistency passed through a hand pelletizer with the desired size of the die, and the resultant pellets were put under shade for air-drying. The dried pellets were stored at a temperature of −20 °C after being packed in airtight polythene. After achieving the pellets along with the desired nanoparticles, they can be used for feeding the aquatic organism.

4.6 Nanoparticle Exposure to Aquatic Animals and Health Improvement

The field of nanotechnology is still growing in aquaculture. Recent observations proved that the role of nanoparticles (NPs) in the aquaculture sector is increasing gradually. Various companies related to nanotechnology tools are endeavouring the use of nanotechnology-based tools to eradicate the obstacles around aquatic food, reproduction, species culture, growth, health, and treatment of water to upsurge the rates of overall aquaculture production (Khosravi-Katuli et al. 2017).

In the fishery and its related industries, NPs are developed for various direct and indirect purposes, as shown in Fig. 4.2. Fishpond sterilization, treatment of wastewater, and fish processing for commercialization such as tagging and barcoding are some of the indirect uses; direct uses include animal health management like controlling fish disease and the feed industry (Khosravi-Katuli et al. 2017).

4.6.1 Silver Nanoparticles

Silver nanoparticles effectively control several diseases caused by viruses, fungi, bacteria, and other single-cellular microorganisms. It holds back the reproduction of various disease-causing microorganisms. Reproduction and growth of those microorganisms responsible for the contagion are inhibited by bad odour, inflammation, and blisters. AgNPs are noticed to act quickly and to be nontoxic, freshening, highly efficient, non-stimulating, tolerance-free, nonallergic, and hydrophilic, thus making them very efficient for bacterial resistance. Hence, AgNPs act as disinfectants in aquaculture to disinfect and thwart diseases (Deshmukh et al. 2019).

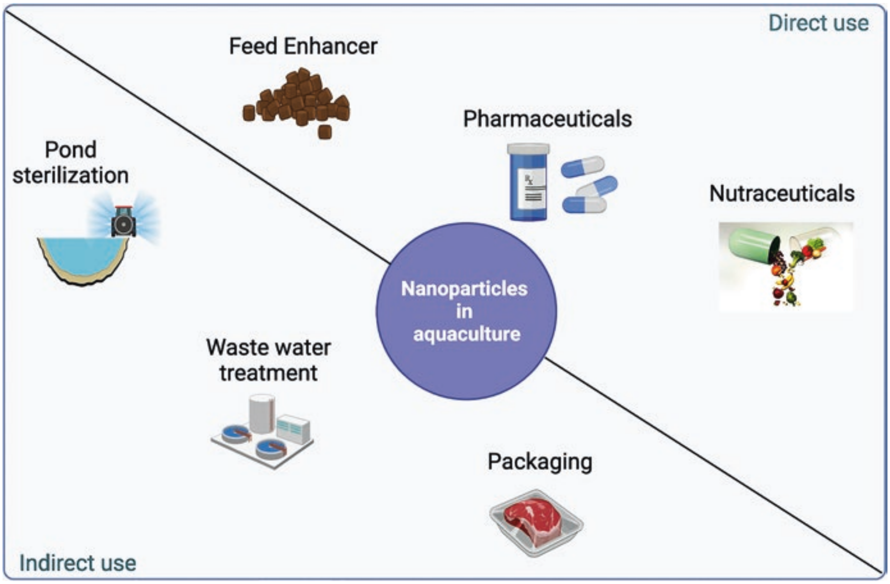

Fig. 4.2 Uses of nanoparticles in the aquaculture sector. (Data from Khosravi-Katuli et al. (2017); created with BioRender.com)

Various researchers investigated the antibacterial mechanisms of AgNPs. Silver can interact with the membranes of a bacterial cell, which comprise proteins and amino acids which contain sulphur and are found on both the inside and outside of the cell membrane, resulting in the inactivation of the bacterial cell. Furthermore, silver ions released from AgNPs inhibit enzyme activities when interacting with sulphur-containing proteins as well as with phosphorus in DNA. AgNPs engage in various roles such as antimicrobial and anti-adhesive agents to restrain bacterial adsorption, growth, and attachment on the surface of the membrane, resulting in the prevention of biofilm formation on the membrane (Deshmukh et al. 2019). Banach et al. in 2016 reported that AgNPs are efficient in obliterating a wide range of Gram-positive (+) and Gram-negative (−) bacteria. Gram-positive bacteria consist of genera such as *Clostridium*, *Enterococcus*, *Bacillus*, *Listeria*, *Streptococcus*, and *Staphylococcus*, while Gram-negative (−) bacteria consist of the genera *Pseudomonas*, *Acinetobacter*, *Vibrio*, *Salmonella*, and *Escherichia* (Banach et al. 2016).

4.6.2 Chitosan Nanoparticle

Aquatic organisms exposed to pesticide pollutants demonstrate an inequity between cell-produced reactive oxygen species (ROS) endogenously as well as exogenously, which can then encourage a decline in antioxidant defence systems or instigate oxidative impairment in the cells of organisms. Enzymatic and nonenzymatic antioxidants are crucial for preventing the destruction of the cellular membrane by ROS and re-establishing normal function and cellular metabolism. Vitamin C, or ascorbic acid, is one of the chief antioxidants, nonenzymatic in nature that can transform ROS to stop their destructive effects, thus shielding tissue from impairment (Naiel et al. 2020). Özkan et al. 2012 mentioned that vitamin C can preserve both cytosolic cells and membrane elements from oxidative impairment. Numerous researchers have stated that diets enhanced with vitamin C vetoed the bad effects of stress for aquatic animals, decreased the destructive effects of water toxicity contamination, and boosted immune defence mechanisms (Naiel et al. 2020).

A natural deacetylation polymer, chitosan, is changed from chitin and separated mostly from the crustacean exoskeleton. Chitosan and its reforms have been fruitfully used for the purging of metal phenols, dyes, ions, pesticides, fungicides, and humic constituents through adsorption (Naiel et al. 2020). Chitosan has an advantage over activated carbon and other sorbents due to its low cost, a prominent affinity for numerous toxins (because of the existence of amino and hydroxyl groups), high reactivity, chemical constancy, and selectivity concerning pollution. Besides the above-mentioned reasons, chitosan-supplemented diets with nanoparticle interpretation play a critical role in the stoppage of detrimental toxicological outcomes of pesticides in aquatic environments (Naiel et al. 2020).

4.6.3 Selenium (Se)

Selenium (Se) is an imperative microelement, necessary for the healthier performance and well-growth of aquatic animals by enhancing immunity response and tolerance of aquatic species to infectious diseases (Dawood et al. 2019, 2021). Adequate Se add-on is essential for various metabolic processes, such as the production of thyroid hormone, fecundity, DNA synthesis, and cytokine formation (Dawood et al. 2021). Supplementation of Se in fish feed was widely conventional since Se from feed and ambient water alone could not provide the optimal level needed by cultured aquatic species (Dawood et al. 2019). They act as a precursor for synthesizing antioxidative enzymes, resulting in high antioxidative ability. Se insufficiency reduced appetite and growth as well as root peroxidative destruction to cell membranes, resulting in elevated mortality in rare cases, while extreme Se lessened the efficiency of feeding and brought about tissue destruction, reproductive failure, and teratogenic abnormalities of organs (e.g., mouth, spine, etc.) (Watanabe et al. 1997).

Therefore, an ideal dietary level of Se is required for diverse cultures of various fish species. Selenium can shield the oxidation damage of animal cells caused by various stressors due to poor water quality, high density, transportation, and infectious diseases by fostering the activity of thyroid hormone metabolism and reproduction and antioxidant-related enzymes (Pacitti et al. 2016; Hefnawy and Tórtora-Pérez 2010). The aquatic organisms demand more precise diets which consist of the micro and macro ingredients to meet their high rate of metabolism coupled with their improved growth rate. The supplementation of Se in fish feed can be influenced by Se structure, feed formulation, and the species and size of fish (Dawood et al. 2019). Recently, the Se nanoparticle (Se-NP) form was used for fish diet due to its low harmfulness and high bioavailability. Additionally, nano-Se enhanced the growth of the animal, feed utilization, and antioxidant defence ability of several cultured fish (Dawood et al. 2019).

4.6.4 Zinc (Zn)

Fish require dietary zinc for their healthy growth and development. It acts as an antimicrobial and regulates both the reproductive and immune systems of animals, in addition to promoting growth. As Zn cannot be deposited in the bodies of animals, regular dietary intake is a must. Failure of this could lead to repeated infections and a poor appetite (Chris et al. 2018).

4.6.5 Iron (Fe)

The role of iron in physiological processes includes transporting oxygen molecules, lipid oxidation reactions, cell respiration, defence against infections, and immune system functioning. Iron portrays a vital role in aquatic species. Most of the natural iron sources do not meet the dietary requirements of fish even if they are found abundant due to their low bioavailability and solubility; thus, dietary iron supplementation became essential. Significant progress in the growth, survivability, and hematological parameters of fish is detected when nurtured with a nano-iron-supplemented diet (Chris et al. 2018).

4.7 Future Perspectives

Nanotechnology is still evolving and paving the way in aquaculture with its very few applications in feed enhancement, pharmaceuticals, and nutraceuticals. Nanoparticles, in general, are used to improve vitality, immune mechanism, growth, fecundity, reproductive ability, cell defence, etc. as they hold various properties such as antimicrobial, antibacterial, antioxidant, and disinfectant. As the chapter focuses on the interpretation of nanotechnology in feed development, only a few researches are successful in this area due to many disadvantages. Each culture species requires a specific feed ameliorated with an ideal concentration of nanoparticles, and this was successfully derived theoretically. Practical implementation has made it difficult to produce a feed that is ideal for a wide range of aquatic organisms in various water conditions with different salinity ranges. Hence, the field of nanotechnology still requires a lot of expertise to develop advanced products.

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