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Beneficial roles of nutrients as immunostimulants in aquaculture: A review

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ABSTRACT

Protein deficiency is a major difficulty for human needs in the past few decades, while different types of aquatic species are rich in high quality protein. Therefore, aquaculture is considered as the main developing food production sector globally. Bacterial infections are the main problem for aquaculture, and their outbreaks have a great impact on productivity, and previously indiscriminate use of antibiotics to control them. However, the emergence of multidrug-resistant pathogens might lead to sudden infectious disease outbreaks resulting in serious economic loss. Immunostimulants application is an effective technique to protect and enhance the immune system of aquatic animals and therefore improve aquaculture production. Nutrient immunostimulants such as essential fatty acids, amino acids, vitamins, and minerals are the most important responsibility to improve aquaculture production, as well as the cost of this method, which is effective, non-toxic, and environment friendly. These nutrient immunostimulants are supportive to increase the immune system, antioxidant, anti-inflammatory, and infection resistance of aquatic animals. In addition, nutritional feed additives improved feed palatability and the excellence of aquatic products and also enhance gut functions. Some information is available on nutrient immunostimulants in aquaculture applications, and this review provides information on different kinds of nutritional administration used in aquaculture to enhance positive impacts on aquatic animals' health as well as feed quality development. This review will provide theoretical references for the application of nutrient immunostimulants in aquatic feeds.

1. Introduction

Fish and fishery products are the essential basis of proteins and vital micronutrients that are necessary for human health. Different types of aquatic products like finfish, crustaceans, mollusks, and algal plants have increased production in aquaculture for the last three decades (Rana, 1997). Aquaculture is rapidly growing into the modern approach to the progress of aqua production worldwide (Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014). The fast growth of modern aquaculture is determined by different types of factors, which comprise the rising use of mixture aqua feeds and the strengthening of the culture systems (Dawood, El-Dakar, Mohsen, Abdelraouf, Koshio, & Ishikawa 2014; Tal et al., 2009). At present existence, diseases arising to reduce aquatic production, and moderation developments are essential to get better healthy aqua production and also impeding both economic and social development in many countries (Bondad-Reantaso et al., 2005). Infectious diseases may turn out inside the country in many ways, for instance by the foreword of recognized exotic diseases by unexpected alteration in the pattern of existing endemic diseases or by the look of prior unidentified infections. Possibility development, early-on caution, and near-the-beginning response are significant to the efficient administration of such infection emergencies (FAO, 2006). In recent years disease avoidance and control have led to considerable improvement in the employ of veterinary medicines and chemical additives. The use of antimicrobial elements as a defensive assessment against pathogenic microbes (Nomoto, 2005). Additionally, there are environmental efforts linked to chemical additives (Wang & Xu, 2004).

Aquaculture is rapidly growing worldwide, but diseases are a threat to aquaculture. Dietary nutrients are new tools to enhance the immune response of aquatic animals and reduce diseases; the dietary nutrient additive approach is safe, non-toxic, cost-effective, and eco-friendly (Boyd, 1971). Immunostimulants and vaccines are novel techniques for fish farming and controlling infections. Currently, due to collective consumers' discussion on antibiotics in farmed animals, consideration

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has focused on immune protective perspectives such as immunostimulants to avoid the outbreak of infections in aquaculture (Cuesta & Esteban, 2021). Nutrition is the biochemical and physiological development of organisms that utilizes food to support their life, and nutrient elements or factors are used to enhance growth, survival, and reproduction. There are some important nutrients (carbohydrates, dietary fiber, fats, proteins, minerals, and vitamins), and nutrients can be classified into two types, macronutrients (carbohydrates, dietary fiber, fats, and proteins) and micronutrients (vitamins and minerals needed in milligram or microgram quantities) (National Geographic Society, 2017). In sustenance, the diet of a life form is the addition of foods it eats, which is mainly firm with the accessibility and deliciousness of foods. Numerous herbivores animals rely on bacterial fermentation to produce digestible nutrients from hard-to-digest plant cellulose, though carnivores must eat diets/meats to get positive vitamins or nutrients their bodies cannot otherwise produce. Animals usually have an elevated condition of energy in assessment to plants (Hawkey, Lopez-Viso, Brameld, Parr, & Salter, 2021; Singh, Baidya, Das, & Biswas, 2021). Dietary incorporation of carbohydrate additives can regulate glucose metabolism in fish to an elevated level. Dietary carbohydrate supplements significantly enhanced growth performance compared with the control group. Balanced diet macronutrients induce the emission and action of some hormones at inconsistent levels, mainly vital being insulin and glucagon/GLPs. In addition, secretion of growth hormones and IGF are also regulated by environmental factors. Additionally, through direct oxidation of key tissues including the brain, glucose utilization is helpful for the synthesis of pentoses, thus following the production of nucleic acids essential for general growth phenomena, and the regeneration of NADPH by the lipogenic enzyme systems (Hemre, Mommsen, & Krogdahl, 2002). Dietary protein is the essential supplement for fish, dietary protein requirements ranged between 24 and 70% of the diets, depending on species and life stanzas. Dietary protein intake was linearly related to weight gain, while protein retention was not affected, averaging 187 g protein kg^{-1} wt gain. On average, fish need a protein intake of 624 g kg^{-1} wt gain and dietary protein retention efficiency is close to 32% (Teles, Couto, Enes, & Peres, 2020). Dietary supplementation of protein and lipid requirements is essential to maintain growth performance in fish and also is cost-effective (Sankian, Khosravi, Kim, & Lee, 2017). Dissimilar kinds of nutrient immunostimulants are expanded aquaculture production (Dawood, 2021). The present review addressed to provide insights into the nutrients and immune responses of aquatic animals.

2. Nutrients as immunostimulants in aquaculture

The nonspecific immune system is capable to remove foreign substances including microorganisms and toxins based on the capability to distinguish self from nonself cells directly using various cellular and humoral substances (Magnadóttir, 2006). Thrombocytes main vital protective cells of fish and are a reaction for phagocytosis (Tavares-Dias et al., 1999). The neutrophils and macrophages are important function in the phagocytosis response of fish species and remove pathogens via the production of reactive oxygen species (ROS). The humoral immune reaction is helpful to recognize specificities as well as these factors remove or protect the growth and spread of harmful microbes in many ways. Immunostimulants and vaccine carrier are important techniques for fish farming as they assume the role of controlling infection losses in their facilities. Comparatively small investigations have been completed with these factors. Many investigations compact with support that to enhance the strength of the effect, to improve the speed, and to extend the period of the specific immune reaction (Anderson, 1992).

Earlier studies have investigated that different types of immunostimulants are used to enhance the immune response and disease resistance, as also gut microbial colonization of different aquatic animals (Balcazar, De Blas, Ruiz-Zarzuela, Cunningham, Vendrell,& Muzquiz 2006; Kesarcodi-Watson, Kaspar, Lategan, & Gibson, 2008; Merrifield, Dimitroglou, Foey, Davies, Baker,& Bøgwald 2010; Ringø, Løvmo, Kristiansen, Bakken, Salinas, & Myklebust 2010a; Ringø, Olsen, Gifstad, Dalmo, Amlund,& Hemre, 2010b). Immunostimulants are non-toxic and they do not have harmful side effects when administered to aquatic animals (Ali et al., 2021). Simultaneously, the use of immunostimulants is mainly capable of innovative production of immunostimulants namely, macronutrients, vitamins, chitosan, polysaccharides, nano elements, and different biological factors (Koch, de Oliveira, & Zanuzzo, 2021). Immunostimulating feed additives improved immune response to infections in aquatic animals (Sakai, Hikima, & Kono, 2020), and also enhanced humoral and cellular immunity in blood, head-kidney, and gene expression (Mandujano-Tinoco, Sultan, Ottolenghi, Gershoni-Yahalom, & Rosental, 2021). Among different elements and extracts examined as dietary supplements, macroalgae and macroalgal are derived in rich polysaccharides (e.g. alginic acid, laminarin, fucoidan) and have several bioactive molecules (e.g. vitamins or polyphenols) recognized to boost growth activity and health condition of diverse fish species (Ahmadifar, Jalali, Soudagar, Azari, & Mohammadi, 2009; Fleurence, 1999; Gupta & Abu-Ghannam, 2011; Heidarieh, Mirvaghefi, Akbari, Farahmand, Sheikhzadeh, & Shahbazfar 2012; Holdt & Kraan, 2011; Sheikhzadeh et al., 2012). Studies have revealed that functional feed additives stimulate the natural defense process of aquatic animals in two ways: (i) regulating the commensal gut microbiota and then shaping the immunity or (ii) directly activating the innate (nonspecific) and adaptive immune systems (Thépot, Campbell, Rimmer, & Paul, 2021).

Currently, due to collective consumers' discussion on the utilization of antibiotics in farmed animals. The use of nutrient based immunostimulants helpful to avoid the outbreak of infection in aquaculture (Chotigeat, Tongsupa, Supamataya, & Phongdara, 2004; Gatesoupe, 1999; Montero-Rocha, McIntosh, Sanchez-Merino, & Flores, 2006). Numerous study reports examined those dissimilar kinds of immunostimulants that increase infection confrontation of farmed aquatic species (Azad et al., 2005; Chiu, Tsai, Hsu, Liu, & Cheng, 2008; Heidarieh et al., 2010; Smith, Brown, & Hauton, 2003). Different types of elements such as amino acids, vitamins, and minerals are recognized to perform as immunostimulants and improve aquaculture production in a cost-effective manner (Raa, 1992; Siwicki et al., 1998). (See Fig. 1)

2.1. Functional amino acid

2.1.1. Glutamate and glutamine

The function of amino acids as immunostimulants in aquatic animals is summarised in Table 1.

Glutamine is the main power factor for leukocytes and a major rule of cytokine and nitric oxide, as well as glutamate, was the most vital responsibility in the resistant system of turbot (*Scophthalmus Maximus*) and also dietary glutamine additive notably decreased the expression of interleukin 8, interleukin-1 beta and tumor necrosis factor α genes, nuclear and cytosolic NF-κB p65 abundance levels in a dose-dependent manner as well as increased the IκBα protein level but decreased its phosphorylation, and down-regulated the IKKα/β and phosphorylated IKKα/β levels (Gu, Pan, Deng, et al., 2021). Glutamine has protected the enterocytes in opposition to oxidative damage, upturned the boost in lipid peroxidation, lactic acid dehydrogenase action, and protein oxidation as replace ROS effect reduces the action of intestinal enzymes (Ranasinghe & Bola, 2021). Glutamine supply is important for the synthesis of purine and pyrimidines, nucleotides, amino sugars, protein, and nicotinamide adenine dinucleotide (NAD) (Hirabara et al., 2021). The most significant role of glutamine and glutamate (3%) in fish is improving growth performance, specific and non specific immune response, and skeletal muscle development, as well as glutamine and glutamate, were the potential feed supplement to decrease the employ of fishmeal while acquisition the productivity of total aquaculture production. (Li, Zheng, & Wu, 2020). These nutrients are improved intestinal health, defend against oxidative damage, and enhanced the growth

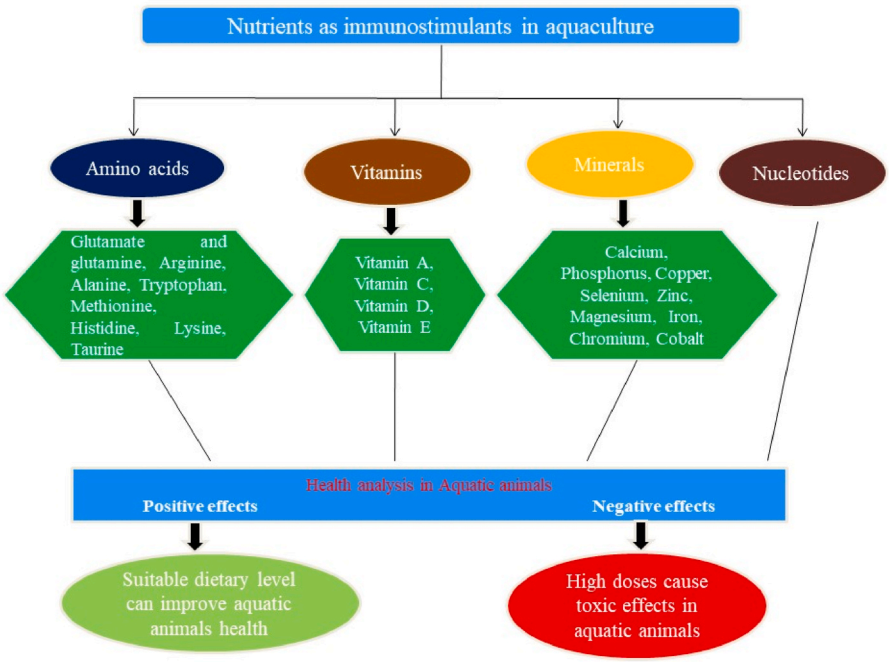

Fig. 1. Nutrients as immunostimulants in aquatic feeds.

Table 1
Functions of amino acids as immunostimulants in aquaculture.

Immuno stimulants	Aquatic organisms	Doses	Administration time	Responses	References
Glutamate and glutamine	*Senegalese sole larvae*	0.5 g/L^{-1} (GLN)	22-day	Improved growth performance and enterocyte height at the intestinal epithelium of sole post-larvae	Viegas (2020)
	juvenile giant trahira	4.83 and 5.42 g/kg	12 weeks	improve growth performance and intestinal histomorphometry	Ramos et al. (2022)
Arginine	channel catfish	2.0%	9weeks	Increased survival percentage	Wang et al. (2021)
	Tilapia	1% or 2%	8 weeks	regulating the expression of genes for lipid metabolism	Li, Wang, and Wu (2021)
Alanine	young grass carp	14.0 g/kg	8 weeks	Improved intestinal mucosal immune system and regulation of immune genes	Hu et al. (2021)
Trypthophan	*C. mrigala* fingerlings	1.36%	60 days	Reduces stress	Varghese et al. (2021)
	sea cucumber	3%	75 days	Increased non specific immune response, growth and intestinal enzyme activities	Zhang et al. (2018)
Methionine	grass carp	6.07 g/kg diet	8weeks	Increased the intestinal mucosal immunity	Zhang, Duan, et al. (2021)
	European sea bass	0·63 and 0·88%	12 weeks	Enhanced the innate immunity	Machado et al. (2020)
Histdine	grass carp	7.9 g/kg diet	8 weeks	Improved fish growth	Wu et al. (2020)
	juvenile Persian sturgeon	1.32% dry weight	8 weeks	Improve fish growth	Arab, Shamsaei Mehrgan, Furuya, Vieira, Paulino, and Michelato (2021)
Lysine	grass carp	13.51–14.55 g/kg	60 days	Increased immune activity, growth and intestinal expansion	Hu et al. (2021)
	rainbow trout	2.75% and 2.23%	12-week	Improved fish growth and protein retention	Lee et al. (2020)
Taurine	red sea bream	1%	20 weeks	Improved fish growth performance and feed utilization	Gunathilaka et al. (2019)

activity of turbot (Gu, Pan, Deng, et al., 2021). Supplementation of glutamine and glutamic acid (10.6 g/kg) enhanced GI improvement and growth performance, but dietary additives revealed no effect on survival, feed intake, and body composition in Nile tilapia (*Oreochromis niloticus*) fingerlings (Macêdo et al., 2021). The combined supplement of glutamine and alanine (2%) enhanced the immunological response and intestinal improvement but considerably reduced the expression levels of *ikkβ*, *myd88*, *nf-κb p65*, *TNF-α*, *ifn-α*, *il-1β*, and *hsp70* genes in juvenile hybrid groupers (*Mycteroperca tigris*) (He et al., 2021). Dietary glutamine (0.5 g/L^{-1}) improved intestinal immunity, growth, and survival function but in contrast, no notable difference in the total activity of amino peptidase, BB alkaline phosphatase, and trypsin was revealed. In addition, acid phosphatase exact action was not affected by dietary L-glutamine in Senegalese sole (*Solea senegalensis*) (Viegas, 2020).

2.1.2. *Arginine*

Arginine is an essential amino acid (EAA) in humans and warm-blooded animals, as well as fish. Glutamate, glutamine, and pyroline can be converted in the structure of citrulline through the middle pyrroline-5-carboxylate (P5C) in the enterocytes. Citrulline can be converted form of arginine in the kidneys of healthy individuals and transported to the liver where it is metabolized via the urea cycle and used for the creation of polyamines and creatine (Wu, Meininger, McNeal, Bazer, & Rhoads, 2021). Arginine plays a significant responsibility in the nonspecific and specific immune structure of fish species. Arginine has an endocrine role in fish and also is an effective stimulator for insulin-free glucose in fish, and the nutritional additive of arginine (2%) in channel catfish diets increased survival levels and disease resistance towards *Edwardsiella ictaluri* (Wang, Xu, & Ai, 2021). Arginine (2.39%) is most important immune regulators in Nile tilapia fingerlings, arginine insufficiency can cause immune-related infection in several fish species and reduced phagocytosis, lysozyme and complement performance, nitric oxide and immunoglobulin production, leukocyte, superoxide anion, and haemagglutination (Vianna et al., 2020). Arginine insufficiency decreased resistance against *Aeromonas hydrophila* in Nile tilapia fingerlings (Varghese et al., 2020). The nutritional additive of arginine (0.5%, 1%, and 1.68%) enhanced immunity, growth, disease resistance, and anti-oxidative capacity in several fish species, and these amino acids concerned with nitric oxide (NO) production, polyamine synthesis, inflammation, and innate immune responses as well as dietary additives of arginine1.68% was notably reduced total mortality after *Vibrio parahaemolyticus* challenge (Azeredo et al., 2020; Yu et al., 2022). Arginine injection (0.03–6.6 μ mol/g body weight) increased plasma insulin concentration along with decreased plasma glucose and performance to get better glucose uptake in Coho salmon (*Oncorhynchus kisutch*) (Chandhini et al., 2021). According to the study observed in the screening of liver cells in high diet Atlantic salmon (*Salmo salar*) as well as the nutritional additive of arginine (Mullins et al., 2020). Dietary arginine additive (1% or 2%) increased immune response without side effects and decreased lipid content in the intestinal region of Nile tilapia (Li, Wang, & Wu, 2021). Major facts from the fish model pointed out that nutritional supplementation of arginine (1%) improved lymphocyte development, and the excess amount of arginine improves immune function in gilthead sea bream (*Sparus aurata*) (Ramos-Pinto et al., 2020). Nutritional supplementation of arginine improved the humoral and cellular immunity of fish as well as arginine can regulate the mRNA expression of the TOR pathway, inflammatory cytokines, and antioxidant-related signaling molecules (Habte-Tsion, 2020).

2.1.3. Alanine, β-alanine, aspartate and asparagine

Alanine and aspartate are the main important glycogenic precursor and vital energy factors for fish species. Consequently, aspartate is necessary for the synthesis of purine nucleotides in the entire cell type. In addition, alanine is a favoured transporter of nitrogen for inter-organ amino acid metabolism in yellowtail (*Seriola quinquerdiata*) (Shin et al., 2021). β-alanine is an element of anserine, carnosine and carcinine. Carnosine and anserine are having antioxidant activity and important buffers in the skeletal muscle of aquatic animals, particularly migratory pelagic marine fishes (Wang et al., 2021). de Oliveira Coelho and Pinto (2020) distinguished that the nutritional additive of β-alanine enhanced the intramuscular concentration of carnosine, but not anserine in the yellowtail. Alanine aminotransferase was a considerable presence in Nile tilapia and the liver function of fish fed Sangrovit® was not affected compared to control diet-fed fish (Hassaan et al., 2021). Phenylalanine was revealed as a good dietary supplement (4.12 g/kg diet) in grass carp (*Ctenopharyngodon Idella*) by boosting lysozyme and ACP activities and C3 content in the intestinal segments, as well as phenylalanine, is playing the main role in the natural immune response of fish (Hu et al., 2021). Tie et al. (2021) revealed that the dietary additive of phenylalanine (11.5 and 14.0 g/kg) improved intestinal immune reaction and increased antibacterial components concentration and down-regulated pro-inflammatory cytokines (IL-8 and TNF-a), and up-regulated anti--inflammatory cytokine (IL-10 and TGF-b1) expression in young grass carp.

2.1.4. Tryptophan

Nair (2020) revealed that tryptophan is important for the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) for fish. Supplementation level of tryptophan at 0.38 g/100 g dry weight to silver barb (*Barbonymus gonionotus*) diet improved growth performance, and reduced erythrocyte counts in tryptophan fed group (Sahu et al., 2020). Moreover, supplementation of tryptophan (3.8 g/kg) reduced stress, decreased cortisol level, and improved the growth status but no negative effects on whole-body composition and amino acid profile of Nile tilapia (Prabu, Felix, Uma, Ahilan, & Antony, 2020). In addition, a nutritional additive of 0.25% tryptophan enhanced fish growth under chronic stressful conditions, the immune system, and amino acid reserves in meager (*Argyrosomus regius*) and there is no negative response after tryptophan feeding (Herrera et al., 2020). Furthermore, supplementation of tryptophan (1.28% and 2.56%) to the rohu diet improved growth and increased thermal and salinity stress resistance in the fish (Vieira et al., 2021). The nutritional additive of tryptophan (0.4%) to silver barb (*Barbonymus gonionotus*) improved growth, immune function, and reproduction (Singh et al., 2021). Tryptophan (1.36%) improved growth performance and salinity stress in mrigal (*Cirrhinus mrigala*) fingerlings (Varghese, SanalEbeneeza, & Pal, 2021). The additive of tryptophan (0.38 g/100 g dry diet) is linked with several performance patterns like stress reaction, aggression, feeding, social dominance, and sex behaviour in fish (Sahu et al., 2021). Tryptophan (0.5%) plays a very important responsibility to control the immune system response and anti-stress action and dietary tryptophan treatment can considerably decrease feed conversion ratio in rainbow trout (*Oncorhynchus mykiss*) (Hoseini et al., 2020). Dietary supplements of tryptophan (6.35 g/kg) positively affected the intestinal immune action and antioxidant status in crucian carp (*Carassius carassius*) as well as ruled the natural immune reaction in sea cucumber (*Apostichopus japonicas selenka*), respectively (Fu et al., 2021). Zhang, Dong, Wang, Tian, and Gao (2018) revealed that dietary supplementation of tryptophan (3%) enhanced the specific growth rates (SGR), and intestinal enzyme response; increase the immune reaction, and cannot improve superoxide dismutase (SOD) activity of sea cucumber (class *Holothuroidea*). Similarly, tryptophan (0.40%) in grass carp diet increased intestinal immunity by activating the antioxidant enzymes as well as up-regulating the immune-related and protein binding genes (IL-10, transforming growth factor-b1, occludin, zonula occludens 1, claudin-b, claudin-c, and claudin-3, SOD1, GPx and NFE2-related factor 2, and Nrf2) and down-regulating the pro-inflammatory genes (TNF-a, IL-8 and TOR) (Li, Han, Zheng, & Wu, 2021).

2.1.5. Methionine

Methionine is an EAA and can synthesize cysteine, and they form the sulphur amino acids (SAAs). Methionine is the key limited amino acid in many fish diets having a high inclusion level of plant protein sources like peanut meal, soybean meal, and copra meal (Irm et al., 2021). Zhang, Duan, et al. (2021) concluded that the nutritional additive of methionine (6.07 g/kg diet) in grass carp for an 8-week trial period increased the intestinal immunity, tight junction proteins, and the expression of p38MAPK/NF-jB, TOR, JNK, Nrf2, and MLCK genes, reducing the intestinal oxidative status, and apoptosis. Methionine supplementation (0.6%–0.9%) is revealed to have a synergistic impact on growth performance and the natural immune response of rohu (Noor, Noor, et al., 2021). Methionine (0.84%) addition to a juvenile blunt snout bream (*Megalobrama amblycephala*) diet showed immunostimulating action and antioxidant activity and also increased the innate immune response by proliferation and differentiation of T cells, inducing PI3K/Akt/Nrf2 pathway and inhibiting NF-κB pathway in the fish (Ji et al., 2020). Li, Zheng, and Wu (2020) reported that DL-methionine (1.45% of dry matter) improved serum lysozyme activity, haemoagglutination titer, and immunoglobulin M (IgM) in juvenile hybrid grouper. Machado et al. (2018) revealed that methionine (0·63 and 0·88%) enhanced cellular immune status in European sea bass (*Dicentrarchus labrax*) after 12 weeks of feeding without triggering an inflammatory response. In addition, methionine (6.4 g/kg diet) enhanced the intercellular arrangement integrity of immune organs in young grass carp (Wu et al., 2018).

2.1.6. Histidine

Histidine is rich in the plasma protein of fish (Thalacker-Mercer & Gheller, 2020). Dietary administration of histidine (8.09 g/kg) increased growth, muscle development, gene expression in Nile tilapia, body composition, survival, feed intake, hepatosomatic index, and blood parameters of glucose, total protein, cholesterol, hematocrit, triglycerides but hemoglobin was not affected by dietary treatments (Zaminhan-Hassemer et al., 2020). A dietary additive of histidine (7.9 g/kg diet) improved growth, digestive enzyme activities, and up-regulated expression of *Keap1*, total and nuclear Nrf2 protein levels, phosphorylated TOR levels in muscle, and the His-insufficient diet down-regulated *CuZn* CAT, SOD and signalling factors *Nrf2*, *TOR* and *S6K1* genes expression in grass carp (Wu et al., 2020). Saavedra et al. (2020) suggested that histidine (3%) has a novel function to increase muscle fiber recruitment and muscle growth and decrease the level of stress in terms of cortisol levels in juvenile meager. In a study using juvenile Persian sturgeon (*Acipenser persicus*), histidine supplementation (1.32% dry weight) regulated the immune system, and antioxidant activity and also increase protein synthesis, tissue formation, and repair in Persian sturgeon juveniles, but histidine insufficiency reduced growth activity and feed utilization in the juvenile Persian sturgeon (Arab, Shamsaei Mehrgan, Foroudi, Soltani, & Chamani, 2020).

2.1.7. Lysine

Lysine (21.60 g/kg) in plant-based protein diets enhanced crude protein and lysine levels and reduced fat content in the body, improved immune response, and growth, and up-regulated antioxidant activity but no significant response in amylase, lipase, and SOD, cholesterol, triglycerides after dietary inclusion of lysine was revealed in rainbow trout (Ahmed & Ahmad, 2021). Lee, Small, Patro, Overturf, and Hardy (2020) reported that lysine added at 2.75% and 2.23% levels to the rainbow trout diet improved weight gain and lower feed conversion ratio. Al-Humairi, Al-Noor, and Al-Tameemi (2021) reported that dietary inclusion of lysine (3.2–6.2%) enhanced growth performance in common carp (*Cyprinus carpio*) compared to control groups. Prabu, Felix, Uma, and Praveenraj (2020) reported that dietary lysine (19 g/kg) positively affected the hematocrit, leukocyte count, muscle growth, and muscle-related genes expression of Nile tilapia, but no changes in hemoglobin and red blood cell counts. Lysine (1.22 g–1.94 g/kg dry diet) deficiency cause loss of appetite and reduced fish growth of young spotted scat (*Scatophagus argus*) (Mac Nhu et al., 2021). Huang et al. (2021) suggested that 2.44% lysine enhanced immunity, and intestinal antioxidants factors including catalase and glutathione peroxidase, activating the target of rapamycin (TOR), p38 mitogen-activated protein kinase (p38MAPK) signaling pathways, and growth of grass carp fry. Lysine dietary supplement (15.4–15.6 g/kg of diet) improved body weight gain and protein efficiency rate but not affected the biochemical response in *Colossoma macropomum* (Cuvier, 1818) juveniles (da Silva Liebl et al., 2022).

2.1.8. Taurine

Taurine, a sulphur-containing nitrogenous complex (NH2–CH2–CH2–SO3H), is reported in numerous marine migratory fishes, for example, red sea bream (*Pagrus major*) and yellowtail, and taurine is important to improve fish health (Zhang Lu, Qin, & Nie, 2020). In addition, in marine fish species, certain invertebrates, and higher vertebrates, intracellular taurine revealed an important function in the modulation of cell volume as a necessary osmolyte (Mersman, Zaidi, Syed, & Xu, 2020). In addition, dietary additives of taurine (1%) improved the immune system in red sea bream (Gunathilaka et al., 2019). Dietary additives of 1% taurine improved weight gain and feed efficiency ratio, high level of taurine dietary additive showed that there is no notable variation in feed efficiency (FE), weight gain, hepatosomatic index (HSI), whole-body composition, intraperitoneal fat ratio (IPF ratio), or muscle yield in striped bass (*Morone chrysops x M. saxatilis*) (Suehs, & Gatlin III, 2021). Taurine additive (3%) improved the growth, biochemical and immune gene expression of sea cucumbers, but the total amino acids and total essential amino acids were significantly lower than in the control group (Shi, Zhao, Wei, Zhai, Ren, & Han. 2021).

2.2. Vitamins (A, C, D, and E)

Vitamin A (0; 20,000 and 40,000 IU/kg/diet) enhanced the immune system and resistance mechanism and notably decreased feed conversion in Atlantic salmon and other fish species (Mishra, 2020). The nutritional additive of vitamin A increased the anti-protease and bactericidal activity of Atlantic salmon and anti-protease is important for the neutralization of extracellular protease during infection of *Aeromonas salmonicida* (Hernandez & Hardy, 2020), and Jaxion-Harm (2021) revealed that supplementation of vitamin A improved feed intake and disease resistance in Atlantic salmon.

Vitamin C (400 or 600 mg/kg diet) improved phagocytic activity, serum bactericidal activity, lysozyme activity, antibody levels, and whole-body crude lipid content notably decreased compared to the control group in Nile tilapia (El Basuini et al., 2021). Accordingly, Abdo et al. (2021) reported that dietary supplementation of vitamin C (0.8 mg VC/kg) to Nile tilapia enhanced the phagocytic cells, immune system hematological parameters, and histopathological alterations, but decreased the weight gain ratio compared to the control group. The dietary additive of 200 mg kg^{-1} vitamin C for 8 weeks enhanced nonspecific immunity and infection resistance against *Aeromonas hydrophila* and *Edwardsiella tarda*, in addition to enhanced growth and feed efficiency ratio in rohu (Gupta et al., 2021).

Barrea et al. (2021) revealed that the nutritional additive of vitamin D increased the immune system in mammals. In a study using gilthead sea bream, supplementation of vitamin D3 (11.6 IU g^{-1}) enhanced the immune system and anti-oxidation functions, and also dietary vitamin D3 levels up to (11.6 IU g^{-1}) may decrease the occurrence of skeletal anomalies, mainly caudal and maxillary anomalies in fish (Dominguez et al., 2021). Yang et al. (2019) noted that Vitamin D3 (10,000 IU/kg) improved the immune system and antioxidant capacity in oysters (*Ostreidae*). Luqman, Fatima, Shah, Afzal, and Bilal (2021) revealed that an elevated quantity of dietary vitamin D3 can damage the immune function, resistance to stress, and disease resistance in mrigal fingerlings. Liu, Zhou, et al. (2021) examined that dietary incorporation of vitamin D3 (400 IU/kg) increased the bactericidal activity against *E. tarda*, regulated T and B lymphocytes, and reduced the mortality rate in turbot.

Vitamin E, also known as alpha-tocopherol, is a collection of biologically active phenolic substances. The addition of vitamin E (123.8 and 136.4 mg/kg) in grass carp diets improved cytotoxicity and phagocytosis activity, but vitamin E insufficiency caused decreased SOD, oxidative damage, glutathione reductase (GR) activities glutathione peroxidase (GPx), and catalase (CAT), along with downregulated the mRNA levels of antioxidant enzymes and signaling molecules Nrf2 (Pan et al., 2017). The dietary supplementation of vitamin E notably enhanced growth performance, and immune response to prevent muscle degeneration, and disease resistance, and improves reproductive efficiency in Japanese flounder (*Paralichthys olivaceus*) and shrimp (El-Sayed & Izquierdo, 2021). Vitamin E supplements improved macrophage phagocytosis response in channel catfish (*Ictalurus punctatus*) (Li, Zheng, & Wu, 2021) and turbot (Huang, Wang, Wang, & Jia, 2019). Vitamin E deficiency affects the nonspecific immune system defense act and adaptive immune response in rainbow trout (Harsij, Kanani, & Adineh, 2020). Nazeemashahul, Prasad Sahu, Sardar, Fawole, and Kumar (2020) reported that vitamin E (131.91 mg/kg) improved growth performance, carcass composition, and erythrocyte fragility in rohu (*Labeo rohita*). Enhanced immune system function and infection resistance to harmful microbes were also exhibited in diverse shrimp species fed diets containing vitamins A, C, D, and E supplements (Javanmardi, Tavabe, Rosentrater, Solgi, & Bahadori, 2020; Mohammadidust et al., 2020; Plaipetch & Ngamphongsai, 2021).

The functions of vitamins as immunostimulants in aquatic animals are summarised in Table 2.

2.3. Minerals (macro and micro)

The small components or trace minerals, such as calcium, phosphorus, sodium, chromium, cobalt, copper, iodine, iron, manganese, selenium, and zinc, are essential in growth and metabolism. Trace minerals have significant functions to regulate physiological and hematological activity (Antony Jesu Prabhu et al., 2018). All trace minerals have their role in the immunity of cultured animals, but the vital trace metals that have been positively correlated with an improvement in immunity or role that sustain immunity are Zn, Mn, Cu, and Se (Arshad, Ebeid, & Hassan, 2021; Lall & Kaushik, 2021). Trace elements play a vital role in maintaining the immune system, dietary supplementation of trace minerals has been reported to improve the immune system, antioxidant, growth, and disease resistance in aquatic animals (El-Shafai et al., 2021; Ghazi, Diab, Khalafalla, & Mohamed, 2022).

The functions of minerals as immunostimulants in aquatic animals are summarised in Table 3.

2.3.1. Calcium

Ca is important for tissue development, nerve transmission, muscle contraction, blood clotting, and osmoregulation, and as a cofactor for the enzymatic procession (Javith et al., 2020). Sanderson, Derry, and Hendry (2021) revealed that nutrient Ca (0.34% or less) condition is improved by the water chemistry, species variation such as giant croaker (*Larimichthys crocea*), red lip mullet (*Liza haematocheilus*), scorpionfish (Scorpaenidae), marine fish tiger puffer (*Takifugu rubripes*), blue tilapia (*Oreochromis aureus*), channel catfish and the freshwater fish American cichlid (*Cichlasoma Notophthalmus*). Dietary Ca supplement has important physiological functions for aquatic animals. Fish gut absorption of Ca is a significant role and essential needs like gonadal maturation and growth spurts (Srivastav, Mishra, Srivastav, Suzuki, & Srivastav, 2020), especially in Ca-free water (Glover & Goss, 2021). Pan et al. (2021) observed that Ca uptake from seawater is not sufficient for appropriate growth and feed utilization of scorpionfish (Scorpaenidae) fingerlings. Even though this fish may develop tricalcium phosphate (TCP) as a nutrient Ca source, a high TCP additive to the diet reduced weight gain, and feed competence, and decreases trace mineral substances in tissues. Accordingly, an eagerly accessible Ca (mono or dibasic) additive to the nutrient is essential for scorpion fish fingerlings. Musharraf and Khan (2020) pointed out that matter with enough level of calcium in dietary for optimizing growth probable and boost minerals in the whole body in rohu fingerling. Hence, dietetic Ca requirement levels between (5.16 and 5.48 g/kg) in diets with (6.5 g/kg) phosphorus are mainly positive and are recommended for fingerling rohu (in water with at least 22 mg Ca L^{-1}). Calcium phosphate components help in long-lasting and steady immune function in fish. Calcium phosphate is a component that enhances identical immune responses and there are no negative effects in fish (Biswas, 2020). Xu, Ye, Zhou, and Su (2020) recommended that antigen-absorbed calcium phosphate factors can activate innate and adaptive immune responses via parental immunization without negative effects on fish health. The calcium sensor (Ca SR) is a very important role in gut barrier and resistance as well as control of gut microbiota. Insufficiency of Ca SR reduces the intestinal barrier role, mishappen composition and division of the gut bacteria, and unstable immunity with a pro-inflammatory reaction (Yang & Cong, 2021), and the authors strongly suggested that Ca SR plays an imperative role in the epithelial layer triggering immune activation and gut inflammation.

2.3.2. Phosphorus

Phosphorus investigation is the main concern in aquaculture; phosphorus level increases in the feed not only increase cost but are also concerned with severe environmental impacts (Sugiura, 2018). Suarez-Bregua, Pirraco, Hernández-Urcera, Reis, and Rotllant (2021) demonstrated that phosphorus mineral levels utilizing the circulating level of minerals as indicators, information on post-prandial substitute as affected by nutritional factors is necessary. Lall and Kaushik (2021) pointed out that phosphorus is the mainly significant macro mineral, this mineral is comprehensively studied. This mineral plays an important role in the proper growth of fish and bone mineralization and carbohydrate and fat metabolism. Phosphorus insufficiency can cause severe health problems in fish species like poor growth, reduced feed efficiency, poor bone mineralization, skeletal deformities, low ash, and high lipid content in the whole body (Wang et al., 2021). Phosphorus (17.4 g/kg) improved growth performance in Atlantic salmon (Sambraus et al., 2020). In fish, growth is linked to the immune role of the immune organs (Liu, Xu, et al., 2020). The nutritional additive of low phosphorus (0.6–1.0%) in the Atlantic salmon diet revealed a decrease in Ca, P, and Mg levels of the whole body, bone, skin, and scales, and slowly evolved abnormally soft and deformed bones (Lall, 2022). The nutritional additive of phosphorus in diverse concentrations (6.56, 6.58, 6.56, 8.02, and 8.44 g/kg) improved growth and bone mineralization with dietary phosphorus supplement was considerably affected alkaline phosphatase activity, and serum phosphorus concentration of rohu (Musharraf & Khan, 2019a, 2019b, 2019c). The fish can utilize the limited level of phosphorus in the water. The dietary additive of phosphorus improved the growth of various fish species such as common carp (0.7%), rainbow trout (0.34–0.8%), Atlantic salmon (0.6–1.0%), and sunshine bass (*Morone chrysops*) (0.65%) (Chen et al., 2017; Saha, Nayak, & Giri, 2020; Sujatha, Nallusamy, Senthilkumaar, Panimathy, & Silambarasan, 2021). Yu, Chen, et al. (2021) revealed that (1%) phosphorus dietary additive increased weight gain rate, specific growth rate, protein efficiency ratio and decreased feed conversion ratio, SOD activity of several commercially important blunt snout bream (*Megalobrama amblycephala*) (Yu, Chen, et al., 2021; Furuya Vieira, Paulino, & Michelato, 2021). Yang et al. (2021) recommended that a nutritional

Table 2
Functions of vitamins as immunostimulants in aquaculture.

Immunostimulants	Aquatic organisms	Doses	Administration time	Responses	References
Vitamins A	Atlantic salmon	6500 and 50,000 (IU/kg)	8 months	Essential nutrient for normal growth and reproduction	Jaxion-Harm (2021)
Vitamins C	Nile tilapia	400 mg/kg^{-1}	10weeks	Improved growth performance, antioxidant activity and enhance intestinal histomorphology	Ibrahim et al. (2020)
	Rohu	200 mg kg^{-1}	8 weeks	Enhanced innate immune response	Gupta et al. (2021)
Vitamins D	Oyster	10,000 IU/kg	30 days	Improved immunity and antioxidant activity	Yang et al. (2019)
	Turbot	400 IU/kg	8 weeks	Reduced mortality, increased bactericidal activity and regulate T and B lymphocytes	Liu, Zhou, et al. (2021)
Vitamins E	Rohu	131.91 mg/kg	3months	Improved growth and reduce stress	Nazeemashahul et al. (2020)

Table 3
Functions of minerals as immunostimulants in aquaculture.

Immunostimulants	Aquatic organisms	Doses	Administration time	Responses	References
Calcium	Channel catfish	1500 mg/L	96 h	Modulating Ca^{2+} regulatory pathways and histomorphological alterations	Limbaugh et al. (2021)
	fingerling rohu	5.16–5.48 g kg−1	8 weeks	Increased serum alkaline phosphatase activity	Musharraf and Khan (2020)
Phosphorus	juvenile red drum	0.8%	7 weeks	Improved growth performance and increase non specific immune system	Xu and Gatlin (2018)
	fingerling rohu	8.44 g/kg	8 weeks	Improved serum phosphorous level	Musharraf and Khan (2019a, 2019b, 2019c)
Sodium	yellowfin seabream fingerling	5 g/kg	8 weeks	Improved growth and digestive enzyme activities	Sotoudeh, Saghaei, and Dehghani (2020)
	juvenile common carp	5 g kg^{-1}	8 weeks	Improved growth performance and thermal stock resistance	Yusefi, Mohammandiazarm and Salati (2022)
Copper	mud crab	162 mg/kg	8 weeks	Up regulated stress related genes	Luo, Zhu, et al. (2020)
	common carp	0.16 and 0.53 mg/L	21 days	Improve haematological indices	Afaghi and Zare (2020)
Selenium	Nile tilapia	1.0 mg Se/kg	8 weeks	Improved growth performance, immune response and disease resistance	Wangkahart et al. (2021)
Zinc	fingerling rohu	47.85–52.93 mg/kg	8weeks	Optimal growth and haematological parameter	Musharraf and Khan (2019a, 2019b, 2019c
	Siberian sturgeon juvenile	46.4 mg/kg	8weeks	Improved growth and feed utilization	Moazenzadeh et al. (2017)
Magnesium	rohu fingerlings	0.44–0.45 g/kg	16:00 h	Increased serum alkaline phosphatase activity	Musharraf and Khan (2018)
	juvenile grass carp	0.65–0.79 g/kg	–	Improved growth and feed utilization	Zafar and Khan (2021)
Iron	rohu fingerlings	211–265 mg kg^{-1}	8-week	Maintain normal tissue iron concentration and improved antioxidant activity	Musharraf and Khan (2019a, 2019b, 2019c
Chromium	Tilapia	16.5–17.2 mg kJ^{-1}	110 days	Improved growth performance	Peres et al. (2022)
Cobalt	Tilapia	0.05–1 mg/kg	12 weeks	Improved growth performance and feed conversion ratio	Makwinja and Geremew (2020)
	gold spond mullet	0.5 and 1	90 days	Improved growth performance and survival	Vishwakarma et al. (2020)

additive (11.5 g/kg) of phosphorus in juvenile blunt snout bream (*Megalobrama amblycephala*) enhanced growth, antioxidant capacity, the immune capability of the liver, and reduced body lipid. A nutritional additive (0.8%) of phosphorus in juvenile red drum (*Sciaenops ocellatus*) improved growth performance and natural immunity (Xu & Gatlin, 2018).

2.3.3. Copper

Copper is a vital trace component for animals including fish (Chen, Yamamoto, & Gatlin III, 2020), as it plays an imperative function in biological development including the synthesis of hemoglobin, arrangement of bone, protection of myelin inside the nervous system, and as the main factor of key enzymes, such as cytochrome oxidase, dopamine hydroxylase ferroxidase, and tyrosinase (Zafar & Khan, 2020a, 2020b). The system following the responsibility of Cu in enhancing the resistance of fish is not clear right now, but Cu insufficiency is managed for the immunosuppression and decrease of T cell proliferation and cytokine production (Dawood et al., 2020; Pecora, Persico, Argentiero, Neglia, & Esposito, 2020). Dietary additives of copper (162 mg/kg) upregulated the expression of stress response-related genes and reduced the expression/activities of anti-oxidation genes/enzymes in Nile tilapia (Luo, Zhu, et al., 2020). Luo, Zhu, et al. (2020) noted that dietary copper (20.78–40.34 mg/kg) improved immunity and infection resistance in crabs, as well as copper, is enhancing phenoloxidase (PO) activity, total hemocyte count (THC) values, serum SOD activity, oxyhemocyanin (OxyHc) concentrations in mud crab (*Scylla paramamosain*). Some recent studies revealed that supplementation of copper (12.4, 49.8, and 50.0 mg/kg) increased immune responses and antioxidation abilities along with dietary copper mainly affected amino acid and glycerol phospholipid metabolism. Moreover, two considerably changed pathways (phagosome and IL-17 signaling pathway) related to the immune system were identified in shrimp fed the Cu diet (Shi, Yuan, et al., 2021). Dietary additives of copper (1000 mg Cu/kg) in a common carp-fed diet improved hematological indices like hematocrit, hemoglobin, red blood cell, white blood cells, neutrophils, and reduced lymphocytes in fish species (Afaghi & Zare, 2020; Musharraf & Khan, 2022). Ray et al. (2020) mentioned that dietary additives of copper can enhance the physiological parameters, including several immune parameters during stressful state in carps.

2.3.4. Selenium

Selenium (Se) is an essential trace factor for the normal growth of numerous cultured fish species and has been revealed as a vital micronutrient for sustainable aquaculture (Dawood et al., 2021). Selenium is an element of the enzyme GPx, which protects the cell membrane from oxidative stress, and it's a significant function in biological response in the animal body (Seyedi, Kalbassi, Esmaeilbeigi, Tayemeh, & Moghadam, 2021). Some studies displayed that supplementation of selenium (0.39–0.43 mg/kg) improved the antioxidant activity and immune response to reducing oxidative stress in fish (Du et al., 2021; Guo et al., 2020). Dietary administration of Se (1 mg/kg of feed) enhanced growth performance, immune response, antioxidative reaction, and infection resistance against *A. hydrophila* and *E. coli* in Nile tilapia and rohu (Pavithra, Darthiqueen, Karthik, & Ramasubramanian, 2021; Rathore et al., 2020). Recently, selenium has been developed in organic and inorganic forms, organic form (1.0 mg/kg) enhanced health-promoting properties compared with the inorganic form as well as dietary supplementation with Se improved growth, antioxidant status, immune response, disease resistance, and decreased feed conversion ratio in Nile tilapia (Wangkahart et al., 2021). Consequently, a dietary additive of selenium (1.0 mg Se/kg) improved growth and feed utilization in Nile tilapia (Rathore et al., 2020; Wang et al., 2021). Dietary Selenium (0.5–1.0 mg/kg) improved growth, feed intake, and immunocompetence in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) (Ning et al., 2021), rainbow trout (Fontagné-Dicharry et al., 2020) and marron (*Cherax cainii*) (Wang et al., 2021). Barbosa et al. (2020) pointed out that selenium (0.6 mg/kg) improved red blood cell, thrombocyte, and leukocyte levels and improved glutathione peroxidase activity in red blood cells in common carp. A dietary additive of Se-chitosan (2 g/kg) improved the growth, and immunity, enhances the survival level, and protect against *A. hydrophila* infection and the expression of the myostatin gene was down-regulated when compared with the control group in Nile tilapia (Ibrahim, El-Gendi, Ahmed, El-Haroun, & Hassaan, 2021).

2.3.5. Zinc

Zinc is the most significant growth factor for aquatic animals, dietary additives of zinc (33.5–64.6 mg/kg) play different cellular responses like cell proliferation, co-factor reproduction, immune response, and defense against free radicals (Mahboub, Shahin, Zaglool, Roushdy, & Ahmed, 2020; Nazari et al., 2021). Musharraf and Khan (2019a, 2019b, 2019c) revealed that different dietary zinc concentrations (51.42 mg/kg diet) considerably increased Hct, RBCs, and Hb, and dietary zinc (29.84 and 19.73 mg/kg) levels improved MCV and MCH in fingerling rohu and also notably affected magnesium and iron contents in the whole body, liver, scales, and vertebrae. Dietary zinc (60 mg kg^{-1}) enhanced hematological parameters such as erythrocyte numbers, hemoglobin, and hematocrit values and decreased the activities of alanine and aspartate aminotransferase (ALT and AST) in Nile tilapia (Ibrahim, El-Gendi, et al., 2021). Moazenzadeh, Rajabi Islami, Zamini, and Soltani (2017) noted that dietary zinc (46.4 mg/kg) improved erythrocyte numbers, hemoglobin, and hematocrit in Siberian sturgeon (*Acipenser baerii*). The additional defense of ZnO is helpful to enhance growth, feed intake, immune reaction, reproductive function of animals, response as an antimicrobial factor anti-oxidative status, and thermal tolerance (Kumar, Chandan, Wakchaure, & Singh, 2020; Kumar, Krishnani, Kumar, Jha, et al., 2017; Kumar, Krishnani, Kumar, & Singh, 2017). The importance of Zn in enhancing survival, feed utilization, growth, immune reaction, antimicrobial performance, and stress resistance has been described in surf parrotfish (*Siganus rivulatus*) (Sallam, Mansour, Alsaqufi, Salem, & El-Feky, 2020).

2.3.6. Magnesium

Magnesium is one of the most important minerals in cells; magnesium and its mixture are mainly involved in different roles like metabolic pathways of protein, enzymatic reaction, carbohydrate, and lipid (Zhang, Duan, et al., 2021). Magnesium is the one of the most efficient minerals in aquatic animals, this mineral quantity level is low in freshwater compared to marine water (1–4 mg/L), and intake of low quantity waterborne magnesium in freshwater fishes, this mineral deficiency mainly affected total metabolic pathways (Hansen et al., 2021; Mooney et al., 2020). Therefore, signs of magnesium deficiency have been reported in some fish species which include poor growth, anorexia, high mortality, sluggishness, and reduced bone and body magnesium concentration (Lall & Kaushik, 2021). Galkanda-Arachchige, Roy, and Davis (2021) revealed that dietary additives of Mg (3 g/L) reduced oxidative stress and free radical generation, and improved meat quality, growth, survival, hemolymph osmolality of Pacific white shrimp (*Litopenaeus vannamei*) and grass carp (Monto et al., 2021). Zafar and Khan (2021) demonstrated that dietary additives of magnesium (0.4–0.6 g/kg) enhanced the immune system, growth, bone mineralization, and antioxidant response in juvenile grass carp. Consequently, a dietary additive of magnesium (0.44–0.45 g/kg) enhanced the growth performance, tissue magnesium concentration, and antioxidant response of rohu (Musharraf & Khan, 2018).

2.3.7. Iron

Iron is one of the most important minerals and its plays a very important function in the organs and tissue development of higher animals including fish, because of its important responsibility in oxygen transport and cellular respiration. Iron is one of the primarily important micronutrients in terms of its result on immune system reaction and host defense against infections (Noor, Piscopo, & Gasmi, 2021; Valenzuela-Muñoz et al., 2020). Iron, like ferrous sulfate, has been used to avoid the toxicity of free gossypol in diets of monogastric, terrestrial animals (Bai & Reddy, 2020). Dietary additives of iron (10.0 mg/kg) enhanced the physiological, biochemical, hematological, and enzymatic response of rohu fingerlings (Chandrapalan & Kwong, 2020; Thangapandiyan, Alisha, & Anidha, 2020). The dietary additive of iron enhanced growth, feed efficiency, hematological values and immune response of juvenile channel catfish (*Ictalurus punctatus*) has been determined to be about 30 mg/kg diet (Yu, Chen, et al., 2021). Reda, Nasr, Ismail, and Moustafa (2021) suggested that nutritional additives of SBM with iron up to 671 mg/kg enhanced the nutritional value of the diet. This investigational diet range (203–283 mg/kg diet) maintains normal immune function and defense against enteric septicemia of catfish caused by *E. ictaluri*. Musharraf and Khan (2019a, 2019b, 2019c) concluded that dietary iron enhanced growth, hematological parameters, maintaining tissue iron concentration, and better antioxidant activities of rohu fingerling, and suggested that dietary iron necessity range (211–265 mg/kg) of rohu fingerlings.

2.3.8. Chromium

Akter, Jahan, Rohani, Akter, and Shahjahan (2021) showed that Cr supplementation (8 mg kg^{-1}) enhanced the growth and increased feed intake activity of striped catfish (*Pangasianodon hypophthalmu*) as well as chromium dietary additive notably reduced in hemoglobin, RBC, and blood glucose level. It was noted that the nutritional additive of Cr has improved the glucose utilization and regulation of endogenous insulin response as well as dietary chromium supplement was no changes in the whole-body compositions except the lipid content and also decrease the concentration of glucose, phosphoenol pyruvate carboxykinase activities in hepatopancreas than compared to the control diet in common carp (Cui, Cheng, & Sun, 2021). Azaza, Saidi, Dhraief, and El-Feki (2020) reported that an additive of chromium increased growth activity with good energy retention and liver glycogen deposition in tilapia. A chromium additive (16.5–17.2 mg kJ^{-1}) improved weight gain and energy deposition in Nile tilapia but decreased the protein and energy ratio in the whole body, muscle, visceral lipid content, and increased the fillet yield (Peres et al., 2022). Supplementation of Cr is a necessary nutrient for parasitic resistance in aquatic animals and maintains metabolic and immune responses, as well as deficiency in the Cr diet, which can affect animal immunity (Zhao et al., 2020). Lall and Kaushik (2021) showed that chromium supplements can change the immune reaction in rainbow trout and dietary organic chromium improved the natural disease resistance of rainbow trout. Dietary supplementation of chromium improved the plasma IgM of Korean rockfish (*Sebastes schlegelii*).

2.3.9. Cobalt

Cobalt is a factor of cyanocobalamin (vitamin B_{12}) constituting approximately 4.5% of its molecular weight (González-Montaña et al., 2020). Dietary cobalt chloride and cobalt nitrate improved growth and develop hemoglobin production in carp (*Cyprinus carpio*) (Mohd Khan & Khan, 2021). Dietary cobalt chloride enhanced growth and protein competence in Nile tilapia (Makwinja & Geremew, 2020). The dietary additive of cobalt mixture was a considerable result in the numerous feedstuffs (Saha & Pathak, 2021). Dietary supplementation of cobalt improved erythrocyte counts in carp and rainbow trout and enhanced survival and growth response in gold spend mullet (*Liza parsia*) and common carp fingerlings, embryonic stage development of trout eggs (Stubblefield et al., 2020; Vishwakarma, Sharma, Kala, Mohan, & Pandey, 2020). The dietary additive of cobalt was important in glucose metabolism; a higher quantity of cobalt may change physiological and hematological functions and cobalt deficiency reduced the synthesis of vitamin B_{12} in aquatic animals (Hoopes & Koutsos, 2021).

2.4. Nucleotides as immunostimulants in aquaculture

Nucleotides are intracellular biological compounds with low molecular mass, which contain important roles in the arrangement of nucleic acids and play a significant role in physiological and biochemical functions in diverse fish species (Guo, Tang, Sheng, Xing, & Zhan, 2017; Hossain, Koshio, & Kestemont, 2020; Novriadi, Ilham, Roigé, & Segarra, 2021). Research on nucleotide nutrition to give insights into the relations between nutrition and physiological activity is necessary for fish and shrimp as well as dietary additives of nucleotide showed that improved disease resistance of aquatic animals (Ding, Song, Liu, Xu, & Li, 2021; Novriadi et al., 2021). The roles of achievement through which nucleotides stimulate fish immunity to some extent remain unclear (Yaseen S et al., 2020), but Hastuti et al. (2021) revealed that immune cells can synthesize new nucleotides in most tissues and turn on exogenous absorption through the diet. Dietary inclusion of nucleotides is effective response against stressful condition in barramundi (*Lates calcarifer*). The regulatory movement of nutritional nucleotides on lymphocyte development, stimulation, and propagation, macrophage phagocytosis, immunoglobulin responses, gut microbiota as well as gene expression of certain cytokines have been described in aquatic organisms (El-Nokrashy et al., 2021). Prakash, Palod, Naik, and Shamsudeen (2020) mentioned that nucleotide feed additive improved growth, survival, and better immune responses in giant freshwater prawns (*Macrobrachium rosenbergii*), and a nutritional level of 1.5 g nucleotide per kg diet was favorable in freshwater prawn. In addition, the nutritional additive of nucleotide was also expressed to enhanced stress tolerance in Atlantic salmon (Mullins et al., 2020) and GI physiology and morphology of Nile tilapia (5.0–7.5 g/kg) (de Lima et al., 2020), and red drum (*Sciaenops ocellatus*) (Hossain et al., 2020). While nutritional supplementation (2 or 4 g/kg) of nucleotides might supply potential reaction from the immune system activity and for growth and cellular regeneration in red sea bream (Bae et al., 2020). Ringø, Olsen, Vecino, Wadsworth, and Song (2012) recommended an optimal dietary supplementation of nucleotides (2 and 5 g/kg) in the diets of various aquatic animals. Dietary supplements of nucleotides modulated the gut microbiota, immune function, and disease resistance against various pathogens such as *Vibrio anguillarum*, *A. salmonicida*, and infectious pancreatic necrosis (IPN), *infectious salmon anemia virus* (ISAV), rickettsia-like bacteria, *Flavobacterium*, *Moritella viscosus*. Dietary supplementation of nucleotides (500 mg/kg) is the optimum level for the best growth performance, health condition, and digestive organ development in European sea bass (Magouz et al., 2021).

3. Conclusion

This review is based on the last five-year literature data analysis and provides an enlightening direction for nutrient immunostimulant improvement in the aquaculture industry. As comprehensive studies are needed to improve nutrient immunostimulants application in aquaculture, this review concluded that various kinds of nutrient immunostimulants are effectively utilized to enhance health development of aquatic animals. The nutrients immunostimulant are positive responses for the improvement of healthy aquatic production and for increasing feed quality levels. The utilization of nutrient immunostimulants is strongly recommended for getting better aquatic animal health and aquatic environment enhancement. In the next steps we should give special attention to the application of those nutrient additives in different aquatic species and try to illustrate their possible mechanisms, this will surely guide the application of different nutrients in the aquatic feeds.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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