



INVESTIGATION OF THE EFFECTS OF HEAVY METALS (COPPER, COBALT, MANGANESE, SELENIUM, AND ZINC) ON FISH IMMUNE SYSTEMS – AN OVERVIEW

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Abstract

Aquaculture, as a strategic and developmental industry, plays an important role in ensuring food security and economic stability within countries. This crucial sector faces various influences, including infectious and nutritional diseases. Certain minerals function as essential nutrients, playing a main role in processes across all aquatic animals when present in appropriate dietary proportions. Studies showed that some heavy metals serve as a catalyst for various biological functions, including the maintenance of colloidal systems, acid-base balance, bone formation, and the regulation of fundamental natural elements such as vitamins, hormones, and minerals. Fish acquire essential elements crucial for their well-being through both diet and water sources. Adequate levels of essential elements, such as iron, zinc, copper, cobalt, manganese, and selenium, in the diet contribute to enhanced growth, increased survival rates, improved disease resistance, and heightened specific immunity in fish. When incorporated in appropriate quantities, these elements play a pivotal role in disease prevention, fostering the production of high-quality fish, minimizing economic losses, and solidifying aquaculture as a robust and profitable industry. This article aims to delve into the immunological effects induced by metal elements, specifically copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), and selenium (Se), in diverse fish species. The objective is to underscore the significance of this exploration, paving the way for the development of more potent immune supplements in the future. These supplements have the potential to improve growth, fortify immune responses, and enhance resilience against diseases in various fish species.

Key words: aquaculture, food security, essential minerals, heavy metals, immune responses

Aquaculture as an industry with an extended history, effectively contributes to the food security of countries by providing the high-quality protein. In recent years, aquaculture has emerged as the fastest-growing activity, playing a fundamental role in the economies of societies (Su et al., 2021). However, this developing industry encounters several risks that can compromise fish health by suppressing their immune systems.

Studies have shown that the immune system function of fish is pivotal in aquaculture, influencing disease resistance, stress response, vaccination strategies, nutritional support, water quality management, and selective breeding for enhanced resistance. Therefore, proactive measures to maintain fish health, including stress reduction and proper nutrition, are essential for successful and sustainable aquaculture practices (Dawood, 2021; Mendivil, 2021).

The immune system of fish comprises innate and acquired immunity. Innate immunity involves cellular elements like monocytes, neutrophils, and macrophages, along with humoral factors such as the complement

system, acute phase proteins, and cytokines (Delves and Roitt, 2000; Parkin and Cohen, 2001). Acquired immunity in fish includes antibody production by B cells and the development of immunological memory. The reliance on innate and acquired immunity varies among fish species, with more primitive species relying heavily on innate mechanisms (Parkin and Cohen, 2001). The adaptability of the fish immune system to environmental factors like water quality and temperature is essential for disease prevention and maintaining the health of fish populations in diverse aquatic environments.

Diet significantly influences the immune system function in fish. A well-balanced diet, rich in essential nutrients, proteins, antioxidants, omega-3 fatty acids, and supplemented with probiotics, enhances immune responses and disease resistance (Dawood, 2021; Mendivil, 2021). Studies have shown that minerals play a crucial role in modulating the immune system of fish, influencing various aspects of their immune responses. Essential minerals such as zinc, copper, selenium, and iron are known to impact the function and efficiency of immune cells,

including phagocytes and lymphocytes. These minerals serve as cofactors for enzymes involved in immune processes, contributing to the production of antibodies and the regulation of inflammatory responses. Adequate levels of minerals are essential for the development and activation of immune cells, and deficiencies can compromise the fish's ability to resist infections and diseases. On the other hand, excessive mineral concentrations, particularly heavy metals, can have immunosuppressive effects, impairing the fish's immune function (Chandrapalan and Kwong, 2021). Thus, maintaining an optimal balance of minerals is critical for ensuring a robust and effective immune system in fish. However, certain heavy metals, such as lead, mercury, and cadmium, can be harmful to the body even in small amounts. They are identified as non-biological elements and do not contribute to the essential functions of the body. Therefore, the exposure of fish to these heavy metals, whether through their water environment or food sources, can endanger their health.

Heavy metals are metals characterized by a density exceeding 5 grams per cubic centimeter. These metals are toxic and have a relatively stable nature in water. Thus, they have a substantial potential to induce toxicity and accumulate in the tissues of aquatic animals. Heavy metals are typically generated during activities such as electroplating, electrolytic depositing, milling, anodizing-cleaning, conversion-coating, and etching industries (Carolyn et al., 2017). These elements exhibit resistance to decomposition in the environment and are not easily metabolized, with toxicity levels ranging from low to high. Additionally, certain heavy metals are classified as trace elements (Bagheri et al., 2022; Kabata-Pendias, 2000; Mertz, 1993).

If the organism's detoxification system is unable to eliminate heavy metals from the body, it results in the accumulation of these metals in various tissues (Raeesazadeh et al., 2022). Metal elements persist in aqueous environments either dissolved or suspended, gradually settling over time. Aquatic organisms may absorb these heavy metals (Poopak et al., 2023). Subsequently, the metals accumulate in the food chain, affecting organisms from primary producers to final consumers (Baby et al., 2010; Sharma and Agrawal, 2005). However, certain heavy metals are essential in appropriate dietary quantities to facilitate essential bodily functions in the organism (Raeesazadeh et al., 2022).

Studies showed that fish require specific heavy elements in controlled amounts within their diet to support vital bodily functions (Shahjahan et al., 2022). Essential heavy metals include zinc, copper, iron, and manganese, which play vital roles in enzyme activities and other physiological processes. In addition to the aforementioned metals, there are other essential elements that influence the physiological functions of living organisms. The absence of these elements can significantly impact blood parameters and the immune system. Furthermore, the deficiency of these elements may be linked to the onset of certain aquatic animals' diseases (Hod-

son and Hilton, 1983; Kesbiç et al., 2020; Parrino et al., 2018).

Vital heavy elements play a crucial role in bolstering the immune system, enhancing its responses within living organisms. This fortification of the immune system ultimately improves the body's resistance, minimizing the occurrence of various diseases during periods of reproduction and cultivation. Therefore, strengthening the immune response through these elements serves as a protective strategy against ailments, optimizing the health and productivity of organisms. This study aimed to investigate the impact of important heavy metals, such as copper, cobalt, manganese, zinc, and selenium, on the immune system functions in different fish species.

Copper

The advantage of copper (Cu) in dietary supplements

Copper is recognized as a crucial trace element for cellular function in all animals (Lall and Kaushik, 2021). It functions as a cofactor for various enzymes involved in fundamental physiological processes. Copper is particularly vital for processes such as cellular respiration (cytochrome c oxidase), antioxidant defense (superoxide dismutase), connective tissue formation, and neurotransmitter synthesis (dopamine hydroxylase and peptidyl alpha-amidating monooxygenase). Moreover, it contributes to iron metabolism, melanin production (tyrosinase), collagen synthesis (lysyl oxidase), and the overall maintenance of a healthy immune system (Linder, 2002).

There is extensive information available regarding the quantity of copper (Cu) present in feed components or feed additives. According to research findings, copper has been established as a crucial trace element essential for the cellular function of animal body cells. The distinctive chemistry of copper ions arises from the existence of specific redox states, namely the reduced state (Cu^{3+}) or the oxidized state (Cu^{2+}). Free ionic copper is identified in physiological body fluids at very low concentrations. Copper absorption occurs through both the digestive system and gills in fish. The diet is recognized as the primary source of copper for developmental, growth, and essential physiological processes in the body, as highlighted by studies such as Bury et al. (2003) and Kamunde et al. (2002).

Enzymes containing copper in their structure, identified as multi-copper oxidases (MCOs) or ferroxidases, possess the capability to convert the iron ion Fe^{2+} to Fe^{3+} . This specific type of iron can then bind with the protein transferrin, facilitating its transport to the site where red blood cells are formed. The MCO family encompasses circulating and membrane-bound ceruloplasmin, along with two other proteins (Prohaska, 2011). Changes in the activity of these enzymes or metabolic changes in copper-dependent proteins can lead to pathophysiological conditions (Harris, 2003). Furthermore, copper plays a significant role in the functioning of one of the body's most crucial systems. Therefore, in this section, the research conducted in this field has been delved into to clarify the importance of this issue.

Rougier et al. (1994) observed immunomodulatory properties in zebrafish exposed to varying levels of copper (Cu). Their finding suggested the use of copper in formulating supplements could enhance the immune system. El Basuini et al. (2016) showed that oral administration of Cu supplements could increase immune system response, growth performance, and antioxidant status in red sea bream (*Pagrus major*). The study of Mohseni et al. (2014) demonstrated that appropriate levels of Cu in the diet could improve the growth performance and immune responses in juvenile beluga (*Huso huso*). Study of Afshari et al. (2021) on snow trout (*Schizothorax zarudnyi*) showed that oral administration of iron and copper nanoparticles could impact on fish growth, antioxidant response, and hemato-immunological indices. However, exposure to sub-lethal Cu levels could reduce the non-specific immune response in Nile tilapia (*Oreochromis niloticus*) (Gopi et al., 2019). In a 2022 study, Delavari

et al. (2022) found that combining Cu nanoparticles (Cu-NPs) with dietary vitamin C supplements significantly improved rainbow trout's resistance to pathogens, antioxidant capacity, and overall growth and health (Table 1).

The disadvantage of copper (Cu) in dietary supplements

The disadvantage of incorporating Cu in dietary supplements for fish lies in the potential for toxicity when present in excessive levels. Elevated copper concentrations can lead to adverse effects on fish, including reduced growth rates, impaired reproduction with decreased fertility and hatching success, abnormal behaviors such as altered swimming patterns and reduced feeding, damage to the gills affecting respiratory functions, disruption of essential enzymatic processes, and the risk of bioaccumulation in fish tissues (Weyh et al., 2022).

Table 1. The effects of copper on immune system

Species	Diet	Administration route	Year	Effects	References
Zebrafish (<i>Danio rerio</i>)	Fish exposed to 0.05, 0.15 and 0.25 mg l ⁻¹ zinc and 0.05, 0.10 and 0.15 mg l ⁻¹ copper during 7 days.	<i>In vivo</i> and <i>in vitro</i>	1994	Lower cytotoxic activity, an enhanced macrophage response and lower counts of kidney lymphocytes.	(Rougier et al., 1994)
Red seabream (<i>Pagrus major</i>)	Six diets were formulated containing CuSO ₄ or 2, 4, 6 or 8 mg supplemental Cu as NPs.	Oral	2016	Immunomodulatory effects. Significantly higher protease activity, lysozyme activity and total serum protein, improve fish growth, immune response and antioxidant defense system.	(El Basuini et al., 2016)
Juvenile beluga (<i>Huso huso</i>)	Eight semi-purified diets containing 1.1 (Cu ₁₀), 3.5 (Cu ₄₀), 7.1 (Cu ₇₀), 9.7 (Cu ₁₀₀), 13.1 (Cu ₁₃₀), 25.1 (Cu ₂₅₀), 49.9 (Cu ₅₀₀) and 195 (Cu ₁₉₅₀) mg Cu kg ⁻¹ diet in the form of CuSO ₄ ·5H ₂ O were fed.	Oral	2014	Aspartate aminotransferase, alanine aminotransferase and Cu accumulation in tissues increased with dietary Cu and in optimum range strengthens the immune system.	(Mohseni et al., 2014)
Nile tilapia (<i>O. niloticus</i>)	The first group was used as the control, while the 2nd and 3rd groups were exposed to (low) 40 µg L ⁻¹ and (high) 400 µg L ⁻¹ concentrations of Cu.	Added to water	2019	A significant (P≤0.05) reduction in the activities of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase activity (GPx), and glutathione (GST). Non-specific immune response of LYZ, RBA, and MPO activity in serum decreased significantly (P≤0.05) in Cu-exposed fish.	(Gopi et al., 2019)
Snow trout (<i>Schizothorax zarudnyi</i>)	Seven experimental diets supplemented with 0 mg copper and iron (Cu, Fe) (control), 150 mg inorganic iron (FeI), 3 mg inorganic copper (CuI), 150 mg FeI and 3 mg CuI (FeI + CuI), 150 mg nanoparticulate iron (FeNPs), 3 mg nanoparticulate copper (CuNPs), 150 mg FeNPs and 3 mg CuNPs (FeNPs+CuNPs) per kg of diet.	Oral	2021	Antioxidant, growth, antioxidant and hemato-immunological indices of fish were improved.	(Afshari et al., 2021)
Rainbow trout (<i>Onchorhynchus mykiss</i>)	Supplementation of Cu-NPs and VC as feed additives in the amount of 2/250–500 mg/kg.	Oral	2022	Antioxidant capacity, the growth and health of rainbow salmon and resistance to pathogens in rainbow significantly increased.	(Delavari et al., 2022)

However, dietary copper toxicity is uncommon in practical feeding scenarios, unless there are errors in feed mixing or feed contamination with high copper levels. Copper toxicity has been documented in various fish species, including Atlantic salmon, rainbow trout, Nile tilapia, rockfish (*Sebastes schlegeli*), and African walking catfish (*C. gariepinus*).

It is important to note that the tolerance to high copper levels varies among fish species (Adeyemi et al., 2022; Baker et al., 1998; Berntssen et al., 1999, 2003, 2017, 2018; Clearwater et al., 2002; Damasceno et al., 2016; Ghazi et al., 2022; Hansen et al., 2004; Kang et al., 2005; Kim and Kang, 2004; Lanno et al., 1985). Symptoms of copper poisoning may include decreased growth and feed consumption, lipid peroxidation, and alterations in tissue blood parameters.

Cobalt

The advantage of cobalt (Co) in dietary supplements

Cobalt constitutes a component of vitamin B₁₂, known as cobalamin. Vitamin B₁₂ is primarily synthesized by certain microorganisms, including bacteria, archaea, and certain algae.

Methylcobalamin and 5-deoxyadenosylcobalamin represent the active forms of vitamin B₁₂, while hydroxycobalamin and cyanocobalamin are transformed into these active forms. In ruminants, the digestive system facilitates the conversion of cobalt into vitamin B₁₂. However, animals with monogastric digestive systems, like fish, rely on dietary intake of preformed vitamin B₁₂ as they cannot synthesize it internally. The synthesis process involves complex biochemical pathways and requires collaboration between microorganisms and the host organism (Lall and Kaushik, 2021). Certain tropical fish species, such as Nile tilapia (Lovell and Limsuwan, 1982; Shi-Yen and Chun-Qui, 1993) and hybrid tilapia (Shi-Yen and Chun-Qui, 1993), appear to meet their vitamin B₁₂ requirements by synthesizing the vitamin through intestinal microorganisms. However, this is not the case for channel catfish (Limsuwan and Lovell, 1981).

In freshwater fish, cobalt (Co) is taken up through the main absorption routes, namely the intestine and gills (Baudin et al., 2000; Wood et al., 2012). Rainbow trout eggs also absorb some cobalt during embryonic development (Kunze et al., 1978). Cobalt plays various roles in the body, including influencing immune system activities. Recent studies investigating the impact of cobalt on immune responses have revealed its beneficial effects on the immune system. Younus and Zuberi (2022) demonstrated that feeding *Tor putitora* with diets contain cobalt-methionine (Co-Met) at a rate of 3 mg/kg resulted in the highest growth performance and improvement of hemato-immunological factors compared to other groups. Al-Khalaifah et al. (2020) demonstrated that administering Doum Palm Fruit Powder (DPFP) containing cobalt could enhance growth performance, immune response, hepatic antioxidant capacity, and disease resistance to *Aeromonas hydrophila* challenge. Similar effects were observed in

studies investigating the effect of DPFP in the diet on the antioxidant systems of gilthead seabream (Cerezuela et al., 2016; Esteban et al., 2014), European sea bass (Guardiola et al., 2016), and common carp (Hoseinifar et al., 2017).

The disadvantage of cobalt (Co) in dietary supplements

Although the acute toxicity of cobalt in freshwater fish is well-documented, there has been limited focus on the uptake, accumulation, and persistent toxicity of Co in these aquatic organisms (Lall and Kaushik, 2021). The use of cobalt in dietary supplements for fish can have potential disadvantages. One significant drawback is the risk of toxicity. Although cobalt is an essential trace element for some organisms, excessive levels can be harmful. High concentrations of cobalt in fish diets may lead to toxicity, negatively affecting fish health and performance. The importance of water and food as sources of Co is known. The uptake of cobalt by freshwater fish is significantly influenced by the waterborne concentration of cobalt and the calcium concentration in water.

Manganese

The advantage of manganese (Mn) in dietary supplements

Manganese is a crucial element with significant involvement in bone mineralization, energy and protein metabolism, cellular defense against free radicals, synthesis of glycosaminoglycans, and the regulation of metabolism (Aschner and Aschner, 2005).

Mn plays a crucial role in various physiological processes, including bone mineralization, energy and protein metabolism, glycosaminoglycan synthesis, cell defense against free radicals, and metabolic adjustments (Aschner and Aschner, 2005). Mn acts as an activator for enzyme involved in the biochemical processes such as oxidoreductases, ligases, kinases, hydrolases, and decarboxylases, and is a constituent of several metalloenzymes (Bidlack, 1997). The absorption of Mn in freshwater fish has been shown through the digestive system and gills (Miller et al., 1980; Rouleau et al., 1995). The dietary intake and absorption of Mn in monogastric animals, however, are generally limited (Spears and Hansen, 2008). Various Mn compounds, including manganese chloride (MnCl₂·4H₂O), manganese sulfate (MnSO₄·4H₂O or MnSO₄·H₂O), manganese oxide (MnO), manganese carbonate (MnCO₃), manganese amino acid complex and manganese methionine complex, manganese hydrogen phosphate (MnHPO₄·3H₂O), can be used in animal and fish feed. The bioavailability of manganese is contingent upon its chemical form.

The study investigated the impact of Mn on immune responses, the antioxidant defense system, and erythron profile modification in goldfish (*Carassius auratus*). The results indicated that Mn stimulates the antioxidant response, modulates the immune response, and modifies the erythron profile, enhancing the capacity of oxygen-carrying blood cells and overall fish health (Aliko et al., 2018). In a 2022 study, the bioaccumulation of Mn and its effects on oxidative stress and immune responses in

juvenile groupers (*Epinephelus moara*♀ × *E. lanceolatus*♂) were examined. The findings revealed that Mn can accumulate in fish, leading to oxidative stress and suppressed immune responses, adversely affecting grouper health (Wang et al., 2022).

Furthermore, the influence of Mn ions on the immunological responses of common carp (*Cyprinus carpio*) against *Yersinia ruckeri* was explored. The study observed that water containing 50 mg/l of Mn did not reduce the production of antibodies against *Yersinia ruckeri*, but it significantly decreased the hematocrit index (Cossarini-Dunier et al., 1988) (refer to Table 2).

The disadvantage of manganese (Mn) in dietary supplements

One potential disadvantage of Mn in dietary supplements for fish is the risk of over-supplementation, leading to toxicity. Excessive levels of dietary Mn can result in adverse health effects, such as impaired growth, altered reproduction, and oxidative stress. Manganese generally has low toxicity. However, elevated levels of dietary Mn supplement (1 g/kg) in grouper fish (*Epinephelus lanceolatus*) induced alterations in feeding behavior and reduced body iron concentration (Shaw and Handy, 2006). Toxic effects of dietary Mn on brain function and the olfactory system have been documented in humans, freshwater brown trout, and other animals (Hoyle et al., 2007).

Selenium

The advantage of selenium (Se) in dietary supplements

Selenium plays a vital role in various physiological functions within living aquatic organisms. Se is an es-

sential micronutrient, contributing to functions such as antioxidant defense, thyroid hormone metabolism, and immune system support. However, it is important to note that while selenium is crucial in appropriate amounts, excess levels can be toxic and have adverse effects on health (Lall and Kaushik, 2021; Weyh et al., 2022).

Se is recognized as a crucial micronutrient for fish (Su et al., 2021). Selenium in dietary sources exists in either organic forms, such as selenium-methionine (Se-Meth), selenized yeast, and analogs, or inorganic forms like selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}), and its metabolism, bioavailability, and toxicity vary significantly. Several freshwater and saltwater cultured species have demonstrated the essentiality of Se (Hilton et al., 1980; NRC, 2011; Poston et al., 1976). In general, inorganic Se takes four oxidation forms: selenide, selenite, selenite, and elemental Se. Within biological systems, these forms are converted into more bioavailable organic types, primarily in the forms of selenocysteine (SeC) and selenomethionine (SeMet).

In vertebrates, selenocysteine is found in active components of glutathione peroxidases, thioredoxin, diiodinases, iodothyronine reductases, and selenophosphate synthases, and is a significant constituent of various other selenoproteins (Prabhu et al., 2016; Hesketh, 2008; Jobling, 2016) and Labunsky et al. (2014) indicated that the digestive tract serves as the primary site for selenium absorption in fish. However, under specific circumstances, a minor quantity of selenium may also be absorbed through the gills and skin. One noteworthy role of Se in the body is its impact on the immune system. Numerous studies in recent years have explored the effects of Se on the immune system of various fish species, and these will be further examined.

Table 2. The effects of cobalt and manganese on immune system

Species	Diet	Administration route	Effects	References
<i>Tor putitora</i>	Control group, two other groups were fed graded levels (0.5–3 mg/kg; increment of 0.5 mg Co/group) of o-NPs and Co-Met supplemented diets.	Oral	The highest performance of growth and improvement of hemato-immunological factors compared to others.	(Younus and Zuberi, 2022)
African catfish (<i>C. gariepinus</i> (B.))	A control diet, and three other diets were prepared by supplementing 5, 10, or 15 g kg ⁻¹ DPFP feeding.	Oral	DPFP has beneficial effect on fish growth, intestinal histomorphology, immune response, hepatic (CAT) levels, SOD activity and GSH content and disease resistance to <i>Aeromonas hydrophila</i> challenge.	(Al-Khalaifah et al., 2020)
Goldfish (<i>Carassius auratus</i>)	Two doses of metal (3.88 ± 0.193 mg/L and 7.52 ± 0.234 mg/L Mn ²⁺) for 96 h.	Added to water	Stimulation in antioxidant response, modulate the immune response.	(Aliko et al., 2018)
Juvenile groupers (<i>Epinephelus moara</i> ♀ × <i>E. lanceolatus</i> ♂)	The groupers were exposed to Mn ²⁺ (0, 0.5, 1, 2, and 4 mg/L)	Added to water	Bioaccumulation of Mn and subsequently causing oxidative stress and immune responses, disturb the health of the grouper.	(Wang et al., 2022)
Common carp (<i>C. carpio</i>)	Mn supplementation	Added to water	Mn did not reduce the production of antibodies against <i>Yersinia ruckeri</i> , but the hematocrit significantly reduced.	(Cossarini-Dunier et al., 1988)

Yazhiniprabha et al. (2022) observed enhanced immune parameters in Mozambican tilapia (*Oreochromis mossambicus*) when fed dietary supplements of zinc oxide nanoparticles (ZnO-NP) and selenium nanoparticles (Se-NP). They reported the increased parameters in both serum and mucus included myeloperoxidase (MPO) activity, lysozyme (LYZ) activity, respiratory burst activity (RBA), alkaline phosphatase activity (ALP), natural complement activity (NAC), and serum anti-protease activity in fish exposed to *A. hydrophila*.

Jha et al. (2022) showed that AMLP-SeNPs, synthesized from *Avicennia marina* mangrove leaves, improved growth performance, and enhanced immune reactions, antioxidant defense, and resistance to *A. hydrophila* in common carp (*C. carpio*).

Wangkahart et al. (2022) found that Se could increase antioxidant enzyme activity (catalase, lysozyme, myeloperoxidase, glutathione peroxidase, and superoxide dismutase) in Nile tilapia (*O. niloticus*). Furthermore, they showed that Se significantly boosted the tilapia's resistance against *Streptococcus agalactiae*, leading to

improvements in growth, antioxidant defenses, and immune parameters. The effects of Se on redox enzymes and humoral innate immune parameters were investigated in African catfish (*Clarias gariepinus*) (Adeyemi et al., 2022). The results of this study showed that infection with *Serratia marcescens* could disrupt the immune system and redox homeostasis in *C. gariepinus*. However, Se had the ability to improve the physiological well-being of fish and exerted an influence on bacteria through its antioxidant properties (Table 3).

The disadvantage of selenium (Se) in dietary supplements

Although selenium is an essential micronutrient, excessive levels can lead to selenium toxicity, causing adverse effects on fish health. Studies have shown that incorporating selenium (Se) into fish dietary supplements may lead to cytotoxic effects, reduced growth performance, poor food efficiency, and increased mortality. Over-accumulation of selenium in fish tissues can result in tissue damage, and even mortality (Lall and Kaushik, 2021; Weyh et al., 2022).

Table 3. The effects of selenium on immune system

Species	Diet	Administration route	Effects	References
Mozambique tilapia (<i>O. mossambicus</i>)	Fed with Mb-ZnO NPs and Mb-Se NPs supplemented diet at different concentrations (0.5 mg/kg, 1 mg/kg and 2 mg/kg).	Oral	Enhancement of immune parameters in the serum and mucus of fish fed with Mb-Se NPs supplemented diet determined by MPO, RBA, LYZ, ALP, serum antiprotease activity and NAC.	(Yazhiniprabha et al., 2022; Lall and Kaushik, 2021)
Common carp (<i>C. carpio</i>)	One group was control and the other groups were dosed with AMLP (0.2 g/kg diet), AMLP-SeNPs (2 mg/kg diet), and AMLP-AgNPs (0.2 mg/kg diet).	Oral	The CAT, SOD, GPx activity, total protein and globulin contents were significantly ($p < 0.05$) higher and albumin concentration was significantly lower in fish that received 2 mg/kg of AMLP-SeNPs as compared to control. Inclusion of AMLP-SeNPs improved immune response and the growth performance and antioxidant defense system, and provided resistance against <i>Aeromonas hydrophila</i> in common carp	(Jha et al., 2022)
Nile tilapia (<i>O. niloticus</i>)	A control group and organic Se (L-selenomethionine; SeMet) and inorganic Se (sodium selenite; Na_2SeO_3) were each added to the basal diet at 1, 3 and 5 mg Se/kg.	Oral	The antioxidant enzyme activities such as LYZ, CAT, myeloperoxidase, SPO and GPx significantly increased ($P < 0.05$). Nile tilapia resistance against <i>Streptococcus agalactiae</i> infection enhanced ($P < 0.05$).	(Wangkahart et al., 2022)
African catfish (<i>C. gariepinus</i>)	Fish were pre-treated with 50 $\mu\text{g/L}$ selenium for 14 days after being challenged with 5×10^3 CFU/mL of <i>S. marcescens</i> .	Added to water	Significant increase in GPx. Significant reduction in nitric oxide, lipid peroxidation, respiratory burst, and CAT. Improving fish physiological status challenged with bacteria	(Adeyemi et al., 2022)

In cases of Se poisoning, the activity of a series of enzymes is affected. There is a limited difference between nutritional requirements and toxicity in vertebrates and fish (Hodson and Hilton, 1983; Labunskyy et al., 2014; Ye et al., 2009). Toxicity of Se in catfish and rainbow trout was reported when dietary selenium levels exceeded 15 and 13 mg/kg of dry food, respectively (Hodson and Hilton, 1983; Horning et al., 2015). Furthermore, high concentrations of selenite and Se-Meth in diets can result in decreased growth, along with other negative effects including reduced energy conservation (Gatlin III and Wilson, 1984; Hosnedlova et al., 2017), decreased egg viability (De Riu et al., 2014), compromised immunological functions (Zee et al., 2016), diminished swimming activity (Schultz and Hermanutz, 1990), pathological changes in the liver, kidney, and ovaries (Choi et al., 2013; Tashjian et al., 2006), and skeletal malformations (Berntssen et al., 1999, 2018; Hamilton, 2004; Hicks et al., 1984; Kupsco and Schlenk, 2016).

Zinc

The advantage of zinc (Zn) in dietary supplements

Zinc is an essential trace element that serves crucial roles in biological systems. It is a cofactor for a diverse range of enzymes, influencing processes such as DNA synthesis, cell division, and protein metabolism (Luo et al., 2021). Moreover, zinc plays a vital role in immune function. Moreover, the effect of zinc on the immune system of fish is multifaceted, as this essential micronutrient participates in various physiological processes crucial for maintaining overall health (Hasnat et al., 2012; Maage et al., 2001). Zinc serves as a component in enzymes like superoxide dismutase and metallothionein, actively neutralizing free radicals and safeguarding cells from oxidative stress. An efficient antioxidant defense system is vital for sustaining the robustness of the immune response in fish.

Furthermore, zinc plays a pivotal role in cell-mediated immunity, particularly in the development and function of T lymphocytes. These immune cells are essential for recognizing and eliminating infected or abnormal cells, forming a critical part of the immune defense mechanism. In the realm of humoral immunity, zinc is implicated in the production and activity of antibodies by B lymphocytes. This aspect is crucial for recognizing and neutralizing pathogens, thus contributing to the overall effectiveness of the immune response (Kumar et al., 2017, 2018). Zinc's involvement in modulating the inflammatory response and its role in wound healing further emphasize its significance in fortifying the immune system of fish (Kishawy et al., 2020). Achieving an optimal concentration of zinc in fish diets is essential, as both deficiency and excess can have detrimental effects on immune function. Thus, maintaining a balanced and appropriate zinc level is crucial for supporting the overall health and disease resistance of fish (Awad et al., 2019). Therefore, adequate zinc intake is vital for overall health, and its deficiency can lead to various health issues (Wel-

ker et al., 2016). Tan et al. (2011) and Huang et al. (2018) found that zinc has the capability to improve growth, increase nutrient deposition, and enhance the activities of intestinal enzymes in Jian carp (*Cyprinus carpio* var. Jian), and juvenile grouper (*Epinephelus coioides*), respectively. Furthermore, the findings of Zheng et al. (2010) indicated that Zn supplementation could reactivate developmental pathways in the gill, prompting stem cell differentiation and reflecting gill remodeling in response to environmental changes. However, Fountoulaki et al. (2010) found that varying dietary zinc levels had no significant impact on the growth performance, immunological indices, or the wound-healing process in sea bass juveniles (*Dicentrarchus labrax*) (Table 4).

The disadvantage of zinc (Zn) in dietary supplements

One potential disadvantage of including zinc (Zn) in dietary supplements for fish is the risk of excess accumulation. Although zinc is an essential micronutrient, an excessive amount can lead to toxicity. High levels of dietary zinc may negatively impact fish health and physiology, affecting functions such as metabolism, growth, and immune response (Do Carmo e Sá et al., 2004). Vijayarajam et al. (2023) studied how chemical and green zinc nanoparticles affected serum biochemical levels, growth, and immunity in goldfish (*Carassius auratus*). Their findings revealed that higher doses of green-synthesized zinc nanoparticles showed reduced toxicity compared to the chemical form. Ghafarifarsani et al. (2023) confirmed green-synthesized zinc nanoparticles could be beneficial nutrient supplements for aquatic animals.

Conclusion

Various metallic elements play multiple roles in the body, impacting the health and physiology of fish. These elements are absorbed by fish from both their food and water sources. However, when administered orally in elevated quantities, these elements can yield inconsistent effects on fish. A comprehensive review of 50 articles revealed that metal elements, beyond their well-established functions in enhancing growth performance, influencing blood biochemical parameters, and contributing to the structure of vital body enzymes, also play a crucial role in catalyzing essential reactions. Notably, they exert fortifying effects on the immune system, enhancing resistance against diseases. Furthermore, the physiological response of fish to metal elements is influenced by factors such as species, gender, individual characteristics, and environmental conditions. This underscores the importance of understanding the non-toxic levels of various metal elements in both the diet and the environment, particularly customized for different fish species. Given these discoveries, it is crucial to determine the ideal levels of these elements to safeguard the health of fish. Finally, this knowledge contributes to the formulation of well-rounded diets that incorporate immune-boosting elements in precisely balanced proportions, thereby promoting overall fish health.

Table 4. The effects of zinc on immune system

Species	Diet	Administration route	Effects	References
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Fish (23.1 ± 0.4 g) were fed with a fishmeal- or plant-based diet supplemented with various levels of zinc (0, 15, 30, 60 or 120 mg kg ⁻¹) for 12 weeks.	Oral	In plant-based diet, growth increased significantly up to 30 mg kg ⁻¹ zinc after which growth was not affected. Versus the plant-based diet containing no zinc exhibited severe growth retardation, and in fish fed the 0 and 15 mg kg ⁻¹ zinc diets, cataracts were present.	(Welker et al., 2016)
Jian carp (<i>C. carpio</i>)	Diets supplemented with increasing levels (15.3, 26.9, 40.8, 58.2, 68.9 and 92.5 mg Zn kg ⁻¹) of zinc lactate were fed to Jian carp.	Oral	Per cent weight gain (PWG), feed efficiency (FE), protein efficiency ratio (PER) and lipid productive value (LPV) increased (P<0.05), and plateaued thereafter (P>0.05). Feed intake (FI) was similar to that observed for PWG. Intestinosomatic index (ISI), relative gut length (RGL), hepatopancreas protein content (HPC), intestine protein content (IPC), trypsin, chymotrypsin, lipase, amylase, alkaline phosphatase (AKP), Na ⁺ , K ⁺ -ATPase and γ -glutamyl transpeptidase (γ -GT) activities were all higher by dietary zinc (P<0.05)	(Tan et al., 2011)
Juvenile grouper (<i>Epinephelus coioides</i>)	Based on the zinc free feeds, dietary zinc levels of 0, 10, 20, 40, 80, and 160 mg/kg were added from zinc sulphate (ZnSO ₄), tetrabasic zinc chloride (TBZC), and 2-hydroxy-4-(methylthio) butyrate zinc (Zn-HMB).	Oral	The weight gain rate (WGR) and specific growth rate (SGR) were significantly higher than the control. The alkaline phosphatase (AKP) in serum, CAT, SOD, copper-zinc superoxide dismutase (Cu-Zn SOD) in liver and zinc concentrations in whole body and vertebrae increased significantly.	(Huang et al., 2018)
Zebrafish (<i>Danio rerio</i>)	Fish were maintained with either zinc supplemented water (4.0 μ M) and diet (2023 mg zinc kg ⁻¹) or water and diet containing Zn ²⁺ at 0.25 μ M and 233 mg zinc kg ⁻¹ , respectively.	Oral and added to water	The results suggest that zinc supplementation reactivated developmental pathways in the gill and stimulated stem cell differentiation, a response likely reflecting gill remodeling in response to its altered environment.	(Zheng et al., 2010)
European sea bass (<i>Dicentrarchus labrax</i>)	Fish weighing 10 g were fed five diets containing organic zinc at 30, 70, 110 and 150 mg kg ⁻¹ diet and one inorganic zinc source (zinc oxide) at 150 mg kg ⁻¹ diet for a period of 12 weeks.	Oral	No significant (P>0.05) differences were found in the growth performance parameters (final weight, specific growth rate), immunological indices tested (respiratory burst activity in whole blood) and the wound-healing process. Zinc accumulation in the skin, vertebrae and liver increased significantly (P<0.01) with increased dietary zinc levels but not in muscle. The highest zinc concentrations were obtained in skin tissue, followed by vertebrae, liver and muscle tissue.	(Fountoulaki et al., 2010)
Goldfish (<i>Carassius auratus</i>)	Controls (without ZnO-NPs (0 mg/L)), T1 (0.9 mg/L ZnO-NPs), T2 (1.9 mg/L ZnO-NPs), T3 (0.9 mg/L GZnO-NPs), T4 (1.9 mg/L GZnO-NPs).	Added to water	The quantity of hemoglobin increased, but the values of MCV and MCH decreased somewhat. Total protein and albumin decreased in T2 and triglyceride, cholesterol, glucose, cortisol, creatinine, and urea increased, while in T3 and T4 groups, changes in biochemical parameters were evaluated as positive. Mucosal and serum immunological parameters in the T2 group showed a significant decrease compared to other groups. In zinc nanoparticles, with increasing dose, oxidative damage is aggravated, so in the T2 group, a decrease in antioxidant enzymes and an increase in MDA were seen. In this regard, the concentration of liver enzymes AST and ALT	(Ghafarifarسانی et al., 2023)

Authors' contribution

Data curation: Bagheri and Gholamhosseini. Investigation: Banaee and Hoseinifar. Writing review and editing: Bagheri.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability statement

The data that support the findings of this study are available from Shiraz University but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Shiraz University.

Ethical approval

This article does not contain any studies with human participants by any of the authors. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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