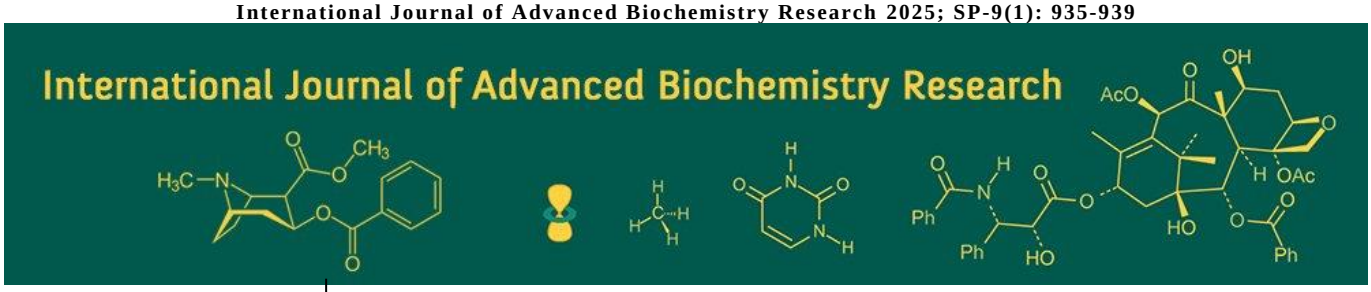

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Exploring the effects of iron nanoparticles in aquaculture: Review

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Abstract
Iron (Fe) nanoparticles have emerged as a promising supplement in aquaculture due to their enhanced bioavailability, solubility, and unique properties. Their application spans growth enhancement, immune modulation, and antioxidant benefits in aquatic species. Iron nanoparticles, particularly iron oxide forms like Fe_2O_3, exhibit remarkable potential in improving fish growth performance, feed efficiency, and haematological parameters. Studies reveal that nano-Fe enhances specific growth rates and nutritional uptake in species such as *Labeo rohita*, *Clarias batrachus*, and *Oreochromis mossambicus*, outperforming traditional iron forms. Additionally, these nanoparticles positively impact blood biochemistry and antioxidant enzyme activities, reducing oxidative stress and promoting overall health. However, excessive iron levels can be toxic, leading to oxidative damage, impaired growth, and histopathological alterations in vital tissues like the liver and kidney. Toxicity studies on various fish species, including zebrafish and rainbow trout, underscore the importance of optimizing dietary Fe nanoparticle dosages to balance growth benefits and avoid adverse effects. Further exploration into the molecular mechanisms of Fe metabolism, immune-related gene regulation, and long-term impacts is warranted. The integration of iron nanoparticles in aquaculture diets holds substantial promise for improving fish health and farm profitability, provided their application adheres to species-specific dosage guidelines to minimize toxicity risks.

Keywords: Iron nanoparticles, aquaculture, growth performance, oxidative stress, toxicity

Introduction
Iron nanoparticles (FeNPs) have gained significant attention due to their remarkable properties, such as high solubility, bioavailability, and unique magnetic and catalytic characteristics. These attributes make them highly effective in diverse applications, from biomedicine to aquaculture. As the demand for improved animal nutrition and environmental remediation grows, FeNPs are increasingly utilized as dietary supplements in aquatic animal feeds and as agents for water treatment. Their ability to enhance iron bioavailability and support essential biological processes highlights their importance in advancing aquaculture productivity and sustainability.

In fish, iron is a vital micronutrient involved in numerous metabolic and physiological processes, including oxygen transport, gene regulation, and energy metabolism. While the gastrointestinal tract is the primary site of iron absorption, the bioavailability of iron varies significantly depending on its chemical form and delivery method. Research has demonstrated that FeNPs exhibit enhanced bioavailability compared to traditional iron compounds, enabling better absorption and utilization in fish diets. This advancement addresses challenges such as low bioavailability and undesirable sensory changes in food associated with other forms of iron.

Despite their benefits, the use of FeNPs must be carefully managed due to potential toxicity risks at high concentrations. Excessive exposure can lead to oxidative stress, tissue damage, and adverse effects on growth and reproduction in aquatic organisms. Studies have documented the delicate balance required to optimize iron supplementation levels to enhance fish growth and immune function while minimizing harmful effects. This duality underscores the importance of continued research to determine safe and effective dosages for different species.

This review aims to explore the multifaceted roles of FeNPs in aquaculture, emphasizing their effects on fish growth, health, and environmental implications.

By examining recent studies, the review highlights the potential benefits and challenges associated with incorporating FeNPs into fish diets, offering insights for developing sustainable and efficient aquaculture practices. Understanding these dynamics is crucial for harnessing the full potential of FeNPs while safeguarding the health of aquatic ecosystems and organisms.

Metabolism of Iron in Fish

Iron (Fe) supplementation plays a vital role in enhancing animal growth, particularly in aquaculture, where deficiencies can severely hinder development. Research highlights the critical importance of Fe in aquatic animal diets, showcasing its role in bypassing deficiencies and promoting overall growth [1-4]. Recent advancements have explored the application of nanoform Fe supplements due to their high solubility and bioavailability. The nanoform can exist in various forms, including metal, metal oxide, and other compounds, each showing promise in enhancing the growth and health of aquatic species [5-12].

Iron oxide nanoparticles, particularly magnetite (Fe_2O_3), exhibit unique catalytic and magnetic properties, making them valuable not only for dietary supplementation but also for applications in biomedicine, bioengineering, and environmental remediation [13-16]. These nanoparticles are extensively used in human food products like iron-fortified cereals and beverages [17], and they have demonstrated benefits in maintaining the freshness of meat and enhancing the nutritional value of farm animals by delaying oxidation in muscle tissues [18]. Additionally, they have shown potential in water treatment processes, further broadening their utility [16, 19].

Despite the growing body of research, significant gaps remain in understanding iron absorption and metabolism in fish. Mechanisms of Fe absorption, storage, and excretion in fish are hypothesized to resemble those in other vertebrates [20]. Iron is pivotal for several metabolic processes, including oxygen transport, gene regulation, and cellular differentiation, and is a component of both heme and non-heme proteins involved in energy metabolism (e.g., NADH dehydrogenase, succinate dehydrogenase, and xanthine oxidase). However, the reactive properties of Fe^{2+} can also pose risks, generating hydroxyl and lipid radicals that damage membranes, proteins, and nucleic acids. Additionally, iron plays a crucial role in immune function and infection resistance [21]. While fish gills contribute minimally to Fe uptake, the gastrointestinal tract remains the primary absorption site, with dietary intake serving as the primary Fe source [22, 23].

Addressing the challenges of Fe bioavailability in fish diets is critical for improving aquaculture profitability. Factors such as Fe's chemical structure, gut conditions, and dietary components influence absorption. While forms like ferrous fumarate, ferrous sulfate, and heme iron demonstrate higher bioavailability, others, such as ferric phosphate nanoparticles, show limited nutritional value [24]. Nanoform Fe supplements have garnered attention for their superior bioavailability due to their small size, with multiple studies confirming their benefits for fish growth and health [5, 7, 11, 12]. Determining optimal dietary concentrations and bioavailable forms of Fe, ranging from 30-170 mg kg^{-1} depending on species, is essential for sustainable aquaculture practices [25].

Toxicity of iron in fish

Iron, a vital nutrient for biological processes, becomes toxic when present in excess. Iron nanoparticles have been studied extensively due to their ability to penetrate water systems and accumulate in living organisms, often causing harmful effects. Chen *et al.* observed that exposure of to nano iron resulted in significant mortality, hypoxia, and reactive oxygen species (ROS) production over seven days [26]. Similarly, studies on zebrafish embryos revealed that Fe_2O_3 nanoparticle concentrations exceeding 10 mg L^{-1} led to embryo mortality, delayed hatching, and deformities, with mortality rates reaching 75% and 45% at 50 and 100 mg L^{-1}, respectively [27]. Additional studies on *L. rohita* confirmed the toxicity of iron nanoparticles, with lethal concentrations leading to mortality and excessive nanoparticle accumulation [28, 29]. Dietary exposure also showed adverse effects, such as reduced growth, increased mortality, and histopathological liver damage, indicating a need for careful regulation [30].

Conversely, controlled usage of iron nanoparticles has demonstrated potential benefits in aquaculture. Prochorov *et al.* reported reduced mortality rates in carp and sturgeons with nano-dispersed iron in feed [31]. Stellate sturgeon survival rates improved significantly with 25-100 mg kg^{-1} iron nanoparticle-enriched diets [10]. Some evidence showed enhanced survival of *L. rohita* fingerlings with specific iron nanoparticle supplements [5, 11]. However, some studies highlighted growth retardation or toxicity at higher doses, emphasizing the importance of finding an optimal balance [6, 7]. These findings underscore the dual role of iron nanoparticles as both a beneficial and potentially harmful supplement, necessitating careful dose management to maximize benefits while minimizing risks.

Effect of iron on the growth of fish

Iron plays a pivotal role in fish growth and overall health, as its dietary supplementation significantly enhances growth metrics across species. Roeder & Roeder demonstrated that ferrous sulfate in water stimulates fish growth, a finding further supported by Gatlin & Wilson, who noted notable weight gain in *L. rohita* with iron-supplemented diets [1, 32]. Optimal dietary iron levels, such as the 30 mg kg^{-1} supplementation studied by Lim *et al.* in juvenile channel catfish (*Ictalurus punctatus*), improved growth, feed efficiency, immune response, and haematological parameters [2]. Similarly, Chu *et al.* observed increased weight and protein efficiency in soft-shelled turtles fed with 50-450 mg kg^{-1} ferric citrate, highlighting iron's critical role in aquatic nutrition [4].

Recent studies on iron nanoparticles reinforce their potential to boost fish growth and feed efficiency. Some reported significant weight and length gains in *L. rohita* fingerlings with nano-iron supplementation. Positive outcomes were also noted in koi carp (*Cyprinus carpio*), African catfish (*Clarias gariepinus*), and goldfish (*Carassius auratus*), where iron nanoparticles improved growth performance, feed conversion ratios (FCR), and specific growth rates (SGR) [5, 7, 11, 33]. Researcher highlighted similar growth improvements in Nile tilapia (*Oreochromis mossambicus*) with varying dietary Fe-nanoparticles (SGR) [34]. Additionally, synergistic effects of iron nanoparticles with probiotics, ascorbic acid, or other minerals, demonstrated enhanced growth and feeding indices in trout and catfish [12, 35, 36]. These findings underscore the transformative potential

of iron nanoparticle incorporation in aquaculture to optimize growth and health outcomes.

Effect of iron on blood and immunity of fish

Iron supplementation, particularly in nanoparticle form, plays a significant role in modulating the haematological and biochemical parameters in fish, serving as a robust indicator of their physiological and pathological status. Studies demonstrated improved erythropoiesis, haemoglobin levels, and reduced mortality in fish fed with nano-iron diets. Similar findings revealed higher red blood cell counts and haemoglobin levels in *L. rohita* fingerlings fed iron oxide nanoparticle-enriched diets [5, 11, 31]. In koi carp, progressive blood parameter improvements were observed with increasing dietary iron nanoparticle concentrations [33]. Additionally, evidence reported that combining iron and copper nanoparticles in trout diets enhanced RBC counts, hematocrit levels, and antioxidant enzyme activities, while reducing oxidative stress markers like malondialdehyde (MDA) [12].

Biochemical analyses further elucidate the effects of iron supplementation. Researcher noted dose-dependent increases in total protein, cholesterol, and triglycerides in *Clarias batrachus* with iron nanoparticle-enriched diets up to 40 mg kg^{-1}, though higher doses (50 mg kg^{-1}) led to significant reductions and signs of liver stress [6]. Other observed elevated serum protein, albumin, globulin, and lipid levels, coupled with reduced glucose, in snow trout fed with Fe and Cu nanoparticulate diets [12]. Some also reported enhanced digestive enzyme activity in *L. rohita* with iron oxide nanoparticle supplementation, indicating improved metabolic efficiency [11]. However, some even cautioned against excessive waterborne iron nanoparticles, which could induce oxidative damage, emphasizing the need for controlled iron levels [37]. These studies underline the dual nature of iron—its potential to enhance fish health and performance when optimally managed, and its risk of toxicity at excessive concentrations.

Effect of iron on body composition of fish

Dietary supplementation of iron nanoparticles has demonstrated significant effects on the biochemical composition, histopathology, and gene expression in fish. Concentration-dependent increments in iron levels enhance protein synthesis and storage, as evidenced in *Clarias batrachus*, where peak iron accumulation was observed in serum at 50 mg kg^{-1} Fe-nanoparticles [6]. Similarly, reported increased protein, carbohydrate, and lipid levels in *Labeo rohita* and *Cyprinus carpio*, respectively, when fed diets with iron nanoparticles [11, 33]. Proximate composition showed higher iron content in muscle tissues of *L. rohita* fed with nano-Fe compared to inorganic $FeSO_4$ diets, with noting reduced protein, carbohydrate, and lipid levels in tissues with increasing Fe_3O_4 nanoparticle concentrations, emphasizing the sensitivity of fish tissues to stress [5, 28].

Iron nanoparticles also induce significant histopathological changes, particularly under waterborne exposure. Evidence found irreversible liver damage in *Oreochromis mossambicus* after exposure to 15 mg L^{-1} iron oxide nanoparticles, including necrosis and vacuolization [38]. Similarly, documented hypertrophy, cytoplasmic vacuolization, and sinusoidal expansion in rainbow trout exposed to α-Fe_2O_3 and γ-Fe_2O_3 nanoparticles [39]. Others observed liver anomalies such as vacuolization, portal infiltration, and haemorrhage in *L. rohita* exposed to sub-lethal concentrations of ferrous iron [40]. Dietary studies also revealed histopathological liver lesions, including lipidosis and hyperemia, in *C. auratus* fed with 0.5-1 g kg^{-1} Fe_2O_3, highlighting potential risks at higher nanoparticle concentrations [7].

Limited studies have explored the influence of iron nanoparticles on growth and immune-related gene expression. Reports investigated the mRNA levels of Ghrelin (GHR) and Insulin Growth Factor-1 (IGF-1) in *C. auratus* fed dietary Fe_2O_3, finding upregulation of growth-related genes in the liver and intestine at 0.2 and 1 g kg^{-1} concentrations [7]. Results also observed downregulation of immune-related genes (HSP 70 and GRP 78) during severe iron oxide nanoparticle toxicity in marine organisms, such as sea urchins [41].

Conclusion

In conclusion, iron nanoparticles offer a promising avenue for improving fish growth, health, and aquaculture productivity due to their high bioavailability and unique properties. Their inclusion in diets has shown benefits in growth performance, haematological parameters, and biochemical indicators, alongside antioxidant enhancement and muscle composition improvements. However, careful consideration of optimal dosages is imperative to avoid toxic effects and histopathological damage. The dual role of iron as both a nutrient and a potential toxicant underscores the need for further research into its metabolism, absorption mechanisms, and the safe incorporation of its nanoparticle forms in aquaculture practices. Establishing guidelines for dosage, bioavailability, and environmental safety will be vital to harnessing the full potential of iron nanoparticles in aquaculture.

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