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Investigation of the impact of iron oxide (Fe3O4) nanoparticles on the ultrastructure of the intestine and on the embryonic development of common carp (*Cyprinus carpio* Linnaeus, 1758) reared under aquaculture conditions

Chingiz Mammadov*, Rovshan Khalilov

Baku State University, 23 Academician Zahid Khalilov Str., AZ1148, Baku, Azerbaijan

***For correspondence:** m_chingiz@yahoo.com*

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The vast presence of metal nanoparticles within the global aquatic environment and the detrimental effect on human health have become issues of global concern. Therefore, the research focus of this project was to investigate the microscopic bioaccumulation and localization of magnetite (Fe3O4) nanoparticles within the cellular structures of *Cyprinus carpio* L. (Common Carp) in aqueous environments. A number of researchers have reported the accumulation of Fe3O4 nanoparticles by aquatic organisms, including fish and have also described the numerous pathological changes caused to the host organism by the presence of these particles. This study examined the bioaccumulation of iron oxide (Fe_3O_4) nanoparticles in the intestinal tissue of Cyprinus carpio L., which was raised in an aquaculture environment, as well as the effects of the presence of these nanoparticles on the early stages of embryonic development during artificial breeding. After being exposed to Fe3O4 nanoparticles (10 and 100 mg/10g of food) over 7 days in the current investigation using the Common Carp, it was noted that the intestinal tissue exhibited pronounced pathomorphological changes. These included: 1) loss of microvilli; 2) cytoplasmic edema; 3) damage to mitochondria; and 4) damage to vascular endothelium. At the lowest dose (10 mg) used in the study, clear indications of damage, such as villi breakdown in the intestine and pathology of cytoplasmic structure in enterocytes, were visible. Electron microscopy demonstrated the sequential entry and bioaccumulation of Fe3O4 nanoparticles through the enterocytes, beginning with the microvilli and progressing through various cellular organelles. The size of the nanoparticles found in the structural components of the fish intestine was consistent at up to 20 nm. The results demonstrate that Fe3O4 nanoparticles may accumulate in fish at all stages of breeding and can be used in practice in aquaculture. The use of nanoparticles as a result of studies on the effects of the nanoparticles on the embryonic development of fish resulted in an increase in the amount of viable free embryos and the number of fertilized eggs by approximately 12-14% when 0.001 grams of Fe_3O_4 was added to the sperm before it fertilized the eggs. These results can be important for determining the toxicity of the nanoparticles at different stages of fish reproduction and could have practical applications in aquaculture.

Keywords: *Common carp, Fe_3O_4 nanoparticles, small intestine, bioaccumulation, embryonic development, fertilization rate*

INTRODUCTION

With the large-scale increase in the production and application of nanomaterials over the last few years the risk of environmental pollution from these materials has grown. Due to this growing number of produced nanomaterials that end up in the water systems (lakes, rivers, seas) they are causing great effects on the ecosystems and its elements (Gupta et al., 2016). Nanoparticles have the potential to pollute the environment and the health of humans through contaminating the aquatic environment, which then accumulates within the bodies of living organism in the ecosystem, with examples being commercial fish (Sabeeh et al., 2021).

Therefore, based on the information provided, it is reasonable to consider these types of particles as fish biomodels, being consumed by humans, with an example being the Common Carp (Cyprinus Carpio Linnaeus, 1758). The Common Carp is an aquatic fish, with the advantages of having an easy way of reproducing commercially and being farmed in many countries around the world, including in Azerbaijan. These fish are also an excellent model for the study of animal ecology, developmental biology, and evolution (Bongers et al., 1998). More than 2,400 species are included in the Cyprinidae family (Nelson, 2006). Nevertheless, although there are a number of species included in the Cyprinidae family, it is the Common Carp that represents 14% (3.4-4.0 million tons) of all freshwater fish (commercial) produced through aquaculture (Mammadov et al., 2016; Fiorino, 2018). Aquaculture farms use spring, river, and basin waters as their water source. To some degree, these basins have been impacted by human activities, resulting in pollution of various levels. In

this regard, over the past several years, there has been increasing interest from many researchers concerning selecting fish as a biological, ecological, toxicological model (Burgos-Aceves, 2019).

Some experiments tested free-form nanoparticles or metal oxides in carp model fish (Lokka et al., 2013; D'Amico, 2005; Nelson, 2006; Gupta et al., 2016; Jha et al., 2022) and examined the absorption of these various nanoparticles into different organs of the host (stomach, intestines, liver, vascular-blood system), the toxicity of these nanoparticles and the morphological changes in carp fish caused by exposure to the nanoparticles under experimental conditions. Iron oxide has both catalytic and magnetic properties. Additionally, iron is a significant component in vertebrate animals and takes part in carrying oxygen and electrons (carriage of transport); in DNA synthesis; and in the development of the immune system (Abbaspour et al., 2014).

Although, as a result of an excess (beyond the normal limits), in an organism, it may be associated with some negative consequences (the weakening of motility and visual acuity, increasing hemoglobin, erythrocytes, hematocrit levels and decreasing the number of white blood cells, the damage to tissues) (Raji and Norouzi, 2013; Chen et al., 2013; Abbaspour, 2014; Valiyeva et al., 2022). In spite of that, little is known about how metal oxide nanoparticles affect fish (Karthikeyeni et al., 2018).

Currently, in Azerbaijan there are large scale scientific studies being conducted with respect to the synthesis of free nanoscale particles and their compounds, assessment of their biological properties and practical uses, and the accumulation of free nanoscale particles in a variety of components of an ecosystem (bacteria, soil, mollusks, fish, unicellular and multicellular organisms, water, etc.) as well as natural nanoparticles (ferritin) (Hajiyeva et al., 2019; Agayeva et al., 2020). Therefore, the primary goal of this research is to establish if magnetite nanoparticles (Fe_3O_4) can accumulate in the different parts of the small intestine and liver of the common carp (Cyprinus carpio Linnaeus, 1758) that have been raised under aquaculture conditions and to determine if pathological morphologic changes occur in the areas where the nanoparticles are accumulated by employing light and electron microscope techniques for visualization, and to assess how the exposure of the embryos of artificially bred carp to these nanoparticles affects development.

MATERIALS AND METHODS

In this study, 33 yearlings (0+) of common carp (*Cyprinus carpio* Linnaeus, 1758) were used as biomodels to investigate possible bioaccumulation of Fe_3O_4 nanoparticles in organs of organisms included in the food chain. These yearlings were raised at a fish farm in the Neftchala district of Azerbaijan, where they were initially fed granulated feed for sturgeons. In September 2022, the fry was transported to Baku, and experiments were conducted under laboratory conditions at the Department of Biophysics and Biochemistry. The experimental fish were divided into three groups of 11 individuals each and placed in three aquariums of equal volume (60 liters): I control group, II and III experimental groups. The average length (L) of the yearlings was 6.9 cm, and the average weight (P) was 4.9 g. In the experimental aquariums, constant conditions were maintained: water volume 30 liters, hydrochemical parameters temperature 22–24°C, oxygen content 8.2–8.6 mg/L, pH 7.4–7.6. Feeding ration 10 g of compound feed per day.

The experiment used Fe_3O_4 nanoparticles (98+%, 10–30 nm, product number: 3320DX), purchased from Skyspring Nanomaterials Inc., Houston, Texas, USA. Fish in the control group (I) received only compound feed, while fish in groups II and III were daily supplemented with 10 mg and 100 mg of Fe_3O_4 nanoparticles, respectively, along with feed. The duration of the experiment was 7 days, after which the internal organs (small intestine and liver) of yearlings from all three groups were extracted and fixed for further analysis. Fixation of extracted organs was carried out in a solution containing 2.5% glutaraldehyde, 2% paraformaldehyde, 4% sucrose, and 0.1% picric acid in phosphate buffer (pH = 7.4). After 24-hour fixation, samples were post-fixed in 1% osmium tetroxide solution in the same buffer for 2 hours. Then, Araldite-Epon blocks were prepared according to the standard electron microscopy method.

Using a Leica EM UC7 ultramicrotome (Leica, USA), semi-thin sections (1–2 μm) were obtained and stained with methylene blue, azure II, basic fuchsin, or toluidine blue. Sections were examined under a Primo Star microscope (Zeiss, Germany), and images were recorded with an EOS D650 digital camera (Canon, Japan). From the same blocks, ultrathin sections (50–70 nm) were also obtained and studied using a JEM-1400 transmission electron microscope (JEOL, Japan) at 80–120 kV, producing electronograms (Hajiyeva et al., 2023; Mammadov et al., 2024; Rzayev et al., 2022).

When analyzing electronograms of ultrathin unstained sections using the computer program “Intensity profile,” the horizontal axis displayed the length of the structure in nanometers, while the vertical axis showed grayscale levels. Image intensity depends on the number of shades of gray (from black to the lightest). The Veletta camera (Olympus, Germany) used in the study processes 14-bit information per pixel, allowing differentiation of 16,384 shades of gray, ensuring precise determination of nanoparticle localization in cells of living organisms.

Studies aimed at investigating the effects of iron oxide nanoparticles (Fe_3O_4) on embryonic development of common carp were initially conducted (Hajiyeva et al., 2022) in June–July 2021 at the “Samukh-fish” fish farm (Barda city, Azerbaijan Republic), but repeated experiments were carried out in June 2025 at LLC “Salyan Agropark Agribusiness” (Salyan city, Azerbaijan Republic). During the studies, mature males and females prepared for fish farming were preliminarily grouped according to morphological and physiological characteristics. Their length (L), weight (P), and Fulton’s condition factor (Pravdin, 1966; Hajiyev et al., 2024) were determined. In the latter case (2025), the male measured 35 cm in length and weighed 2.0 kg, while the female measured 76 cm and weighed 4.0 kg.

Mature eggs were obtained in vivo by the “stripping” method from sexually mature breeders and placed in plastic containers with smooth surfaces. First, Fe_3O_4 nanoparticles were added to 1 ml of milt obtained by stripping from mature males, and then this mixture was added to 20 g of carp eggs obtained in the same way. Fertilization of eggs was carried out by the “dry” method (Dettlaff et al., 1981; Agayeva et al., 2020). In the course of the study, Fe_3O_4 nanoparticles at various concentrations (0.0001 g, 0.001 g, and 0.05 g) were added both to gametes (sperm, eggs) before fertilization and to already fertilized eggs. In accordance with the objectives, the obtained results were compared with the control group.

RESULTS

Before examining how nanoparticles accumulate within the carp organism via bioaccumulation the authors initially used both light and electron microscopy to assess the regular structural characteristics of the small intestine and all of its layers for comparative purposes (the small intestine has three distinct anatomical parts or segments- the anterior, the middle, and the posterior; each part has a different morphology; and among the digestive system's organs the longest segment is the middle portion of the small intestine which contains the majority of the body's intestinal digestion and absorption of nutrients); therefore the authors chose the middle portion of the small intestine as the subject of this investigation.

Between the muscle layers, elements of the myenteric nerve plexus are visible. In the center of the Schwann cell (neurolemmocyte) lies an oval nucleus with euchromatin and a well-defined nuclear envelope. The cytoplasm contains the Golgi apparatus, lysosomes, mitochondria, endoplasmic reticulum, and other organelles. Surrounding the plasmalemma of the neurolemmocyte are numerous axons containing neurofilaments and neurotubules, as well as regions of mitochondria and cisternae of agranular endoplasmic reticulum. Beneath the muscle layer lies the submucosa (SM), where elements of connective tissue are visible blood vessels, lymphocytes, macrophages, and other cells. The final layer is the mucosa, which is divided into three parts: epithelium (Ep), lamina propria (LP), and muscularis mucosae. The epithelial layer consists of columnar cells enterocytes (En) and goblet cells (GC). On the apical surface of enterocytes are microvilli. In the studied middle section of the small intestine, goblet cells occur more frequently than in other sections, and their secretion (mucus) protects the microvilli from damage caused by bacteria and toxins.

After 7 days of administering iron nanoparticles at doses of 10 mg and 100 mg together with feed to experimental fish raised under aquaculture conditions, the small intestine was examined by light and electron microscopy and compared with the normal structure. First, the effect of the 10 mg dose of nanoparticles on various layers of the intestinal wall was studied. It was observed semi-thin (1 μm) sections of the intestinal mucosa, including the epithelium. Pathomorphological changes were noted in the apical regions of epithelial cells facing the intestinal lumen. The microvilli of enterocytes (MV) located on the lateral parts of intestinal folds were preserved, but in the central part of the intestinal lumen, their structure was disrupted they were scattered, and organelles of cells, including fat droplets (FD), entered the lumen. These processes are clearly visible in electronograms. In the area marked with snowflakes, the microvilli in the apical part of enterocytes were completely destroyed. Such changes impair the absorptive function of the intestine.

At higher magnification under the electron microscope, it was observed that the outer membranes of some organelles, including mitochondria (Mt), were damaged, the structure of cristae was destroyed, and edema was present in the cytoplasm (marked with an asterisk). Clear signs of cytoplasmic edema in enterocytes and disruption of the integrity of epithelial cells located at the intestinal lumen are visible, while cells farther from the lumen partially retained their structure. At the 10 mg dose, no serious changes were detected in other layers of the intestine.

Under exposure to a dose of 100 mg of Fe_3O_4 nanoparticles, more severe changes were observed in the structure of the small intestinal wall. Pathological alterations were noted in the serosal and muscular layers, as well as in neural elements, the submucosa, and blood vessels. In the electronogram, the serosal layer is completely destroyed, and in the muscular layer, vacuolization and edema are visible between muscle cells. In the cytoplasm of muscle cells, mitochondria are swollen and the structure of cristae is disrupted.

Damage to the integrity of neurolemmocyte membranes located between muscle layers leads to the formation of myelin-like bodies of various shapes, vacuolization of the cytoplasm, swelling of mitochondrial cristae, and thickening of the nuclear envelope of the neurolemmocyte. The integrity of the basal membrane of non-myelinated nerve fibers is disrupted. In axons, swelling of the granular endoplasmic reticulum is observed.

In the submucosa (SM), edematous fluid accumulates between connective tissue elements, and numerous macrophages are also observed. In this same layer, changes in the ultrastructure of blood vessels were detected. The endothelium lining the vessel lumen is deformed, forming finger-like protrusions into the lumen.

It is important to note that once nanoparticles enter erythrocytes, they begin to circulate throughout the organism via the vascular system. All observations were confirmed by electron micrographs and grayscale intensity profiles. Regardless of localization, grayscale values ranged between 5200–5400, indicating the presence of particles identical in composition Fe_3O_4 nanoparticles. The size of these particles was 10–20 nm. This proves that Fe_3O_4 nanoparticles with magnetic properties, when introduced into the carp intestine, travel from the microvilli to the erythrocytes and accumulate in various organelles.

As a result of studies conducted in 2025, it was established that when Fe_3O_4 nanoparticles (20–30 nm) were added to the sperm of common carp before egg fertilization in amounts of 0.0001 g, 0.001 g, and 0.05 g, the percentage of egg fertilization and the release of free embryos from the egg membrane were higher compared to other variants. Thus, at a concentration of Fe_3O_4 nanoparticles (20–30 nm) of 0.0001 g, egg fertilization was 66.6%; at 0.001 g 80.5%; and at 0.05 g 75.0% (Table 1).

DISCUSSION AND CONCLUSION

The study of the normal structure of the digestive organs, in particular the small intestine of the common carp, was carried out using histological and electron microscopic methods. As a result, it was established that the wall of the small intestine consists of four layers: serosa, muscular layer, submucosa, and mucosa. All of these layers were identified by different methods. These layers, in turn, are subdivided into components. For example, the mucosa is divided into the epithelium (enterocytes and goblet cells), lamina propria, and muscularis mucosae. A similar mucosal structure has been noted in other species of bony fish, such as *Catla catla*, *Anguilla anguilla*, *Clarius batrachus*, *Salmo salar*, *Oncorhynchus mykiss*, and *Serrasalmus nattereri*.

The muscular layer of the small intestine consists of circular and longitudinal muscle fibers, between which lies the myenteric nerve plexus an element of the enteric nervous system. During the study, neurolemmocytes (Schwann cells) and non-myelinated nerve fibers were detected, confirming similar findings by other researchers who studied the neural elements between the muscular layers of the carp intestine.

Table 1. Addition of iron oxide nanoparticles (Fe_3O_4) to the sperm of common carp before egg fertilization.

Fe_3O_4 nanoparticles (20-30 nm), amount (g)	Fish length L, cm	Fish weight P, g	Egg mass in experiment, g	Number of eggs per 1 gram, pcs.	Total number of eggs in experiment, pcs.	Free embryos output		Number of dead eggs	
						%	pcs.	%	pcs.
0,0001	76	4000	20	451	9020	66.6	6007	33.4	3013
0,001	76	4000	20	451	9020	80.5	7261	19.5	1759
0,05	76	4000	20	451	9020	75.0	6765	25.0	2255
Control	76	4000	20	451	9020	67.3	6070	32.7	2950

In addition to its detoxification function, the fish intestine also serves as an important indicator of the general condition of the organism and as a pathway for nanoparticle penetration. This makes it a convenient model for assessing the toxicity of various nanoparticles and the associated pathological changes. In other studies, Fe_3O_4 was shown to cause changes in the stomach, liver, skin, muscles, and scales. It has been established that iron nanoparticles can induce immunotoxicity, accumulating in the small intestine and liver. Depending on the dose and duration of exposure, pronounced pathological changes are observed: villus degeneration, disruption of wall integrity, reduction in villus number, thinning of the wall, epithelial edema, and rupture of intercellular junctions.

Despite prolonged administration of high doses, by the 21st day, the level of nanoparticles in the carp organism decreased significantly. Other authors have noted that disturbances in the mechanism of nanoparticle penetration into enterocytes (via endocytosis and subsequent exocytosis into the vascular system) are caused by the structural changes described above. In contrast, in the present study, at comparatively high doses (10 and 100 mg per 10 g of feed), alterations were already detected by day 7: destruction of enterocyte microvilli, release of fat droplets into the intestinal lumen, mitochondrial damage, and cytoplasmic edema. At the 100 mg dose, pathological processes affected all layers of the intestinal wall: complete destruction of the serosa, muscle vacuolization, intercellular edema, disruption of neural elements, submucosal swelling, endothelial deformation of blood vessels, and enterocyte destruction.

Based on literature analysis and our own observations, it can be stated that the severity of pathological changes in the host organism depends on both dose and duration of nanoparticle exposure. In particular, the study showed that changes in the nuclei of vascular erythrocytes varied depending on the Fe_3O_4 dose: in the lamina propria and vascular lumina of the submucosal layer, abnormalities in nuclear envelopes of erythrocytes, cytoplasmic vacuolization, and deformation of individual cells were recorded.

Accumulation of metallic nanoparticles in the organism depends on their size: 4 nm predominantly in the kidneys; 10–28 nm in the stomach wall and small intestine. Larger particles are retained in mucus secreted by goblet cells, without penetrating deeper into the tissue. According to transmission electron microscopy (TEM) data, nanoparticles sized 10–30 nm can pass through the lamina propria and reach connective tissues, endothelium, and erythrocytes, spreading to various organs. Although nanoparticles of 10–30 nm were used in this study, TEM visualized particles predominantly 10–20 nm in villi, enterocyte cytoplasm, organelles, endothelium, and erythrocytes, consistent with other results obtained in rainbow trout. Both literature and our data confirm that nanoparticle size plays a key role in cellular and tissue penetration.

Under aquaculture conditions, ultrastructural changes associated with the penetration and accumulation of iron oxide nanoparticles in the small intestine and liver of common carp were investigated. Exposure to different doses (10 and 100 mg) caused dose-dependent damage: from enterocyte destruction at the lower dose to total morphological alterations of all intestinal wall layers at the higher dose. Nanoparticles (especially those ≤20 nm) passed through microvilli, entered the cytoplasm, then penetrated the mucosa, lamina propria, endothelium, and erythrocytes spreading further to other organs (Hajiyeva et al., 2023; Mammadov et al., 2024).

Based on the results of studies on the effects of iron oxide (Fe_3O_4) nanoparticles on carp embryonic development, it was established that adding 0.001 g of iron nanoparticles to sperm before egg fertilization ensured the highest fertilization rate (80.5%). It is assumed that Fe_3O_4 nanoparticles (20–30 nm) at this concentration exert a catalytic effect on sperm acrosomes, enhance their energetic activity, and consequently increase sperm motility. The obtained results may be applied in the aquaculture of economically important fish species across various taxonomic groups, to reduce losses at embryonic developmental stages and increase overall productivity.

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ETHICAL CONSIDERATIONS

All experimental procedures involving live fish were conducted in strict accordance with internationally accepted guidelines for the care and use of aquatic organisms in scientific research. The study design complied with ethical principles aimed at minimizing stress, suffering, and the number of animals used. Handling, feeding, exposure to iron oxide (Fe_3O_4) nanoparticles, sampling, and euthanasia procedures were performed following standard aquaculture and laboratory animal welfare protocols. The experimental procedures were approved by the relevant institutional ethics committee of Baku State University, and all efforts were made to ensure humane treatment of the experimental animals throughout the study.

AUTHOR CONTRIBUTIONS

Chingiz Mammadov conceived and designed the study, supervised the experimental work, conducted the histological and ultrastructural analyses, and drafted the original manuscript.

Rovshan Khalilov contributed to the experimental setup, aquaculture maintenance, nanoparticle exposure protocols, embryonic development assessments, and data interpretation.

Both authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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ORCIDS:

Chingiz Mammadov: https://orcid.org/0000-0001-5785-2392

Rovshan Khalilov: https://orcid.org/0000-0002-8684-1390