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# The Effect of Copper Mineral, Copper Sulfate and Copper Nanoparticles on Fish

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## Abstract

Copper is a mineral element that is widely found in nature and is very important for humans and aquatic animals as an essential element for the growth and development of fish and also in the activity of biochemical enzymes and in processes such as blood production, immune system, The production of energy molecules and salt water balance plays an important role in the fish body. Its deficiency in water can lead to growth and health problems in fish. Its compounds, such as copper sulfate and copper nanoparticles, can have various effects on fish. Copper sulfate is usually used as a source of copper in fish feed. Its consumption in nutrition can improve the growth of fish, strengthen the structure of bones and maintain their immune system. However, too much copper sulfate can be toxic and cause problems such as skin irritation, discoloration, scratched gills and even fish death.

**Keywords:** Aquatic Animals, Copper, Compounds, Growth, Nanoparticles, Sulfate

## 1. Introduction

Following the increase in population and meeting the growing need for human food, the use of aquatic resources has increased as a source of protein needs. In the meantime, fish have a significant role in providing the nutritional needs of humans and the health of their heart, body and mind with a significant amount of vitamins, minerals and fatty acids (Gheysari, 2016). Today, attention has been paid to the production and breeding of freshwater species, and based on the forecasts made by FAO, it seems that approximately 60% of the share of aquaculture products will be included (FAO, 2016). The cost of feeding in aquaculture usually includes more than 60% of the total costs. Therefore, to achieve successful aquaculture, it is necessary to use the appropriate food ration in such a way that all the nutritional needs of the fish are provided in different conditions of the breeding environment. For this purpose, in addition to being fully familiar with the needs of different types of aquatic species, fish breeding specialists should also be aware of food analysis in order to be able to adjust rations according to the needs of aquatic animals. In recent decades, keeping aquarium fish has become one of the most popular pastimes of mankind (Wu *et al.*, 2010). In this regard, aquaculture has turned to new technologies in order to increase the growth and efficiency of fish feeding, as well as to find natural and

inorganic antibacterial methods and compounds to control disease-causing agents. Among these, nanoparticle technology has received special attention as one of the most effective antibacterial methods and compounds, and has created a suitable opportunity for the development of the aquaculture industry, which should be considered with its advantages and disadvantages (Kim *et al.*, 2007). Minerals are among the most important components of the aquatic diet, which are separated from other nutrients by their main components and inorganic nature, and many of these elements are important due to the existence of structural and metabolic functions in the body of organisms (Davis and Gatlin, 1996). Despite conducting research activities in the field of aquatic nutrient requirements since the 1950s, research on mineral substances began in the mid-1970s and has been progressing slowly until today (Prabhu *et al.*, 2016). Copper element is considered as a necessary mineral in the nutrition of aquatic animals and the dependence of the physiological reactions of aquatic growth on the activity of copper-containing enzymes necessitates the continuous need for copper in the diet. According to the presented studies, organic copper or copper in the form of nanoparticles has more biosupply compared to mineral copper and if the particle size of large copper molecules is reduced to the size of nano particles, small molecules can be easily absorbed from the intestine and as a result copper is digestible. It increases in the digestive system. Also, unlike the antibiotics and chemicals used that cause sensitivity in the patient's fluid or resistance caused by mutation in the pathogenic agent, it seems that nanoparticles are stable antimicrobial agents that do not cause resistance in pathogenic agents. On the other hand, it should be noted that different types of nanoparticles, such as copper nanoparticles, act as stimulants or inhibitors of growth and immunity based on their amounts in biological processes; therefore, special attention should be paid to the amount of their use in the diet of different aquatic animals.

## **2. The importance of growth and nutrition in fish**

Growth is seen as an important goal in aquaculture in the form of significant changes in characteristics such as length, weight and even energy levels of fish. Living organisms in biological processes, for biochemical interactions that lead to tissue construction and to maintain water and salt balance. Movement of food along the digestive system, digestion and absorption of food, air breathing, reproduction, movement and to maintain their health, they need energy (Silva and Anderson, 1995). Creating energy through the oxidation of various fats, the glycogen reserves in the body, the breakdown of protein compounds into smaller components, and the oxidation of organic compounds in the diet takes place after the consumption of food and the processes of digestion and absorption of food (Kaushik and Seiliez, 2010). Many factors are effective in the process of growth and energy acquisition, one of the most important of which is feed and nutrition management (Caruso *et al.*, 2009). Many physicochemical factors such as temperature, oxygen level, light, ammonia nitrogen and salinity along with biological factors such as gender, species and density of farmed fish and even the amount and type of fish activity by affecting the appetite and eating of fish, indirectly influence the growth. They put (Cuenca *et al.*, 1985). Studying the nutritional needs of different aquatic species leads to the improvement of the formulation of food rations with the optimal balance of nutrients to improve the growth rate of fish, increase their health and quality, and finally, success in intensive breeding. In the past decades, many studies have been carried out in order to achieve the proper formulation of food rations with the aim of producing high quality commercial food rations (Aprodu *et al.*, 2012). Since the use of formula food is for 72.3% of the species that are cultivated in fresh water (FAO, 2012). Therefore, farmed fish in closed circuit or super dense systems should be fed with a high quality and complete diet in terms of nutrients in order to achieve rapid growth of aquatic animals (Nasopoulou *et al.*, 2014). Therefore, it is necessary to understand the appropriate information in the field of biological and nutritional needs of the breeding species, which is an important matter, to make a balanced and optimal formulation of the food ration with proper quality control and also to provide all the nutritional needs of the fish (Aprodu *et al.*, 2012).

## **3. Importance of minerals in fish diet**

Minerals are among the most important components of the aquatic diet, which are separated from other nutrients by their main components and inorganic nature, and many of these elements are important due to the existence of structural and metabolic functions in the body of organisms (Davis and Gatlin, 1996). The role of minerals in the body can be divided into structural, physiological, catalytic and regulatory functions; Minerals form the structural components of organs and tissues of the body and can also play a role as a part of the membrane in the

stability of the structure of molecules and control the reproduction and differentiation of cells. These substances are important in body fluids and tissues as electrolytes to maintain osmotic pressure, acid-base balance, membrane permeability and nerve message transmission. In addition, they act as catalysts in enzymes and endocrine systems and play a role in the structure of metalloenzymes, hormones and activators (coenzymes) (Prabhu *et al.*, 2014). The role of minerals in nutrition, physiology and life of many living organisms has been studied. However, access to information on the mineral requirements of fish and their effects on the biochemical composition and function of the body, enzymatic reactions and non-enzymatic structural units is limited. Despite conducting research activities in the field of aquatic nutrient requirements since the 1950s, research on mineral substances began in the mid-1970s and has been progressing slowly until today (Prabhu *et al.*, 2016). In addition to the limited access to information on the minerals needed by fish compared to other food groups, there is little information about the mineral requirements of aquatic species compared to land animals, and the reason for this is the absorption of these elements by fish through water and It is food (Stickney, 2000). In general, the assessment of the mineral elements needs of fish is much more complicated than that of terrestrial organisms due to interactions with the aquatic environment (Kaushik and Seiliez, 2010). Biological parameters such as the species of the farmed fish, the stages of life or sexual maturity in which it is located, its habit, level and nutritional status, signs of lack of elements in the body, diet compositions and some parameters of the breeding environment such as the concentration of substances in the water, salinity, Temperature, type of rearing system and other factors such as body mineral content, ion stability and regulation, material concentration in tissue, plasma, activity of different enzymes, excretory secretions and even gene expression related to different mineral elements can be useful in determining mineral levels. to occur (Jobling, 2012).

#### **4. Importance of copper mineral in fish diet**

Copper is a rare vital element in the diet of animals, however, it cannot be stored in the body. In addition, animal feed ingredients are copper-free; Therefore, commercial food should provide the necessary amount of copper in a biologically energetic form, which depends on the physical and chemical properties of the supplemental form that is added to the diet. Copper, as an essential mineral in aquatic nutrition, is an important compound in enzyme systems that plays a vital role in oxidation-reduction activities. Copper is present in the combination of cytochrome oxidase, tyrosinase, superoxide dismutase, amine oxidase, lysyl oxidase and ceruloplasmin enzymes. It also seems that the presence of copper element is necessary for the formation of melanin and skin pigments, the formation of bones and connective tissue. It is a part of the heme part of the hemocyanin of crustaceans, a cofactor in tyrosinase and acidocubic oxidase (Lall and Milley, 2008). The copper mineral element or its compounds act as growth and safety stimulators or inhibitors based on their amounts in biological processes. It has been reported that using a suitable source or a form of mineral material that has more biosupply can be a way The appropriate solution to reduce the consumption of rare minerals in their food and nutritional ration should be more. The use of compounds containing copper in the appropriate amount and in the amount needed by aquatic species in the diet increases the digestibility and absorption of copper in the body, reduces its accumulation in aquatic waste and environmental pollution, and leaves favorable antimicrobial effects in the digestive system. The presence of sufficient amounts of copper element in the body causes the exocytosis of the lysosome of the liver cells, the release of the copper in the liver into the bile and the stimulation and increase of bile glucosidase secretions. Finally, it leads to facilitating and improving the digestion of carbohydrates, increasing apparent metabolizable energy, improving feed efficiency, and finally increasing growth performance (Akinsanmi and Igbasan, 2012).

#### **5. Different effect of copper mineral on fish**

Food ration is the main source of copper supply for optimal aquatic growth. Copper is absorbed through the intestinal epithelium (anterior half of the intestine) and gill appendages (Taylor *et al.*, 2007). The dependence of the physiological reactions of aquatic growth on the activity of copper-containing enzymes necessitates the continuous need for copper in the diet. Aquatic nutritional changes somehow cause changes in the ability to consume copper and more fluctuations in delivering copper to tissues. Following the decrease in the amount of dietary copper in the body, first the copper reserves in the body decrease, and as a result, the emptying phase, and then the deficiency phase occurs, in which only the main copper-dependent reactions are maintained, while the copper is released from the reserves along with absorbed copper. It does not seem to be enough to stabilize

the serum copper concentration in the normal range. After this stage, a state of disorder occurs when one or more of the physiological reactions related to copper are damaged, and in the last stage, a disease occurs, which is the clinical manifestation of the disorders. The lack of this element in the aquatic body causes disruption in the collagen bond and bone structure and increases bone fragility (Papagiannis, 2004; Eisler, 1998). The amount of water need for copper depends on the physiological conditions, the type of species, the copper content of the water and the amount of zinc, cadmium, iron and molybdenum ions in the food, which are all copper metabolic antagonists (Kamunde *et al.*, 2002). Unwanted excretion of copper is through urinary secretion, feces and skin, because when dietary copper is low, some copper is excreted despite the body's need. When the absorption of copper is more than needed, its excretion is necessary for copper balance in the body. It has been proven that many of the changes caused by copper deficiency are due to the decrease in copper-dependent metalloenzymes. The physiological state of the cells during the emptying phase affects the intensity of the effects of these defects, biochemical defects produce different effects in specific cellular lesions, which are different effects due to cellular kinetics. Also, copper deficiency may increase if there are functional defects in other tissues (Arthington, 1996; Tabinda *et al.*, 2010). Of course, it should be noted that although this element supports the physiological processes of aquatic animals, it has an inhibitory and toxic effect in concentrations higher than the aquatic requirement (Rainbow and Furness, 1990). Also, excessive amount of copper in aquatic feed can have negative effects on intestinal morphology and increase the rate of apoptosis and cell division in intestinal cells. Copper, in the amount of 3 to 11 mg per kilogram of diet, is a necessary element in the feed of most aquatic species. It should be noted that the amount of copper needed is different among different species and it may even be different in different stages of the same species. Using 3 milligrams of copper per kilogram of food ration increases the growth of common carp and rainbow trout. In an experiment, rainbow trout fish were fed a diet with low copper (0/8 micrograms per gram of fish weight) and also in water with low copper 0/73. Micrograms per liter were placed; which caused a sharp decrease in growth in the period of 50 days. The dietary requirement of copper in catfish does not exceed 1/5 mg and shrimps do not exceed 25 mg per kilogram of food ration. The nutritional toxicity threshold of copper element for channel catfish in a daily dose higher than 1 mg per kilogram of body weight, for Atlantic salmon depending on the life stage is 1 to 11 mg per kilogram of body weight per day and for colored trout. It is 44 milligrams per kilogram of body weight per day. Also, Mohsenii *et al.*, 2012, concluded in their studies that the need of baby elephant fish for copper element in the diet is 10-13 mg per kilogram of diet. Adding 8 and 16 milligrams of copper per kilogram of food ration, which is 2 and 3 times the sufficiency level of tilapia fish, respectively (Shiau and Ning, 2003). (And channel catfish) (Gatlin and Wilson, 1986). It caused a decrease in the growth of these fish. Lack of copper strongly changed the development of zebra fish embryos (Lundebye *et al.*, 1999).

## 6. Effects of copper sulfate on fish

Copper sulfate is a non-mineral compound whose chemical composition consists of copper, sulfur and oxygen. Its chemical formula is  $\text{CuSO}_4$  and it is also known as blue cut. This substance is widely used as a disinfectant compound to prevent fins from rotting. Copper sulfate, which is the most common form of this material, is light blue in color and is produced industrially through the electrolysis of copper metal with concentrated sulfuric acid or copper oxide with dilute sulfuric acid. Copper sulfate, an odorless crystalline substance, light blue in color, is toxic and can destroy pathogens such as bacteria, fungi, etc. Skin damage, as well as preventing the entry of external parasites into aquariums are used in freshwater fish. The toxicity of copper sulfate depends on the amount of copper in it and causes kidney necrosis, destruction of hematopoietic tissue, increase in liver fat, and inhibition of some digestive enzymes in fish. Therefore, the amount used should be adjusted according to the hardness of the water based on 0.25-3 ppm of copper ions. Although the use of copper sulfate for edible fish has been declared illegal, it significantly destroys the external parasites of fish. When copper sulfate is used to treat valuable fish, the amount of copper can be determined using laboratory diagnostic kits, and then the permissible dose for daily consumption can be determined in this way. Copper sulfate is one of the most widely used substances in tropical fish farms to control the growth and development of phytoplankton and aquatic plants (Havens, 1994). High hardness of the water causes the effectiveness of this drug to decrease, but it is not allowed to use it in water with low hardness (especially less than 50 mg/liter) due to poisoning. One of the most important cases of poisoning of this substance, which is what we usually face is hepatotoxicity and liver poisoning, which in many cases causes liver damage in fish without the fish being killed (Mazandarani *et al.*, 2015).

## 7. The effect of copper nanoparticles on fishes

The current growth of the nanotechnology industry and the increase in the production of engineered nanoparticles due to their special characteristics have caused them to occupy a special place in the world economy. Due to their unique physicochemical properties, nanoparticles can be used in many biological and environmental studies and therefore have attracted the attention of scientists and researchers. In order to meet these needs, the science of toxicology of nanomaterials will play a very important role in the development and expansion of sustainable and safe nanotechnology. The average size of nano particles is in the range of 1 to 100 nm. By changing the size of the particles from micrometer to nanometer, which is equal to  $10^{-9}$  or one billionth of a meter, due to the increase in the ratio of the surface to the volume of the particles, all the physical and chemical properties change and the reactivity of the particle increases greatly (Liu, 2006). Copper nanoparticles are used as a biocide in the formulation of biological anti-adhesion paint in the hull of ships and submarines, in docks and also in some fishing tools. Other important applications of these materials are as antimicrobials, insecticides, and sensors (Lei R *et al.*, 2008). Due to the fact that with the increase in the growth rate of farmed fish, the duration of keeping and also its costs are reduced, so the main issue in aquaculture is the growth of the farmed species. Among the limiting factors for fish growth, nutritional factors are very important. Some of these factors are related to the rare or essential elements in the diet, which, if they are provided optimally, will create proper growth. Based on the presented studies, organic copper or copper in the form of nanoparticles has more bioavailability compared to mineral copper. If the particle size of large copper molecules is reduced to the size of nano particles, small molecules can be easily absorbed from the intestine and as a result the digestibility of copper in the digestive system increases. Reducing the size of the particles increases the reaction surface per unit volume and greatly reduces the effect of barriers to the penetration of particles into the body (Dang *et al.*, 2009; Wang *et al.*, 2011). Unlike the antibiotics and chemicals used that cause sensitivity in the patient's water or cause resistance due to mutation in the pathogenic agent, it seems that nanoparticles are stable antimicrobial agents that do not cause resistance in pathogenic agents (Gogoi *et al.*, 2006). Also, by connecting to the membrane of microorganisms, it prolongs the phase of the growth cycle and the germination time of microorganisms becomes longer (Zhang *et al.*, 2007). One of the reasons that nanoparticles are more effective than other antibiotics is because they have a much greater contact surface with pathogenic microorganisms. Nanoparticles penetrate into the microorganism's membrane by binding to it; They react with proteins containing sulphurous amino acids and phosphorous compounds such as Deoxy Ribonucleic Acid (DNA) and cover the DNA by forming a low weight molecule in the center of the bacteria. Also, studies have shown that the ions of nanoparticles, when they penetrate into the bacterial cell, make the DNA molecules compact and dense, and as a result, destroy its ability to be transcribed (Morones *et al.*, 2005). Therefore, if the size of the molecules of mineral elements such as copper reaches the billionth of a meter, copper nanoparticles are actually produced, and with this work, its specific surface area increases significantly. Also, the ratio of external surface atoms to internal atoms increases rapidly with the reduction of particle size, and copper nanoparticles will have a larger external surface and a higher surface activity compared to the usual copper element (Hajipour *et al.*, 2012). Because metal oxide nanomaterials have extensive cell-killing activity against bacteria, fungi, and viruses, and copper oxide is one of the most important metal oxides due to its remarkable properties, including antimicrobial properties; Therefore, if Oxidms is attached to nanoparticles, its bactericidal ability increases (Li *et al.*, 2011; Wu *et al.*, 2012).

## 8. Conclusion

Today, attention to the production and breeding of freshwater species has increased and forecasts have been made, to achieve successful aquaculture, the use of appropriate food rations. In addition to being fully familiar with the needs of different types of aquatic species, fish farming specialists should also be aware of food analysis. Minerals are among the most important components of aquatic diets, which are separated from other nutrients by their main components and inorganic nature, and many of these elements are important due to the existence of structural and metabolic functions in the body of organisms. In addition to the limited access to information on the minerals needed by fish compared to other food groups, there is little information about the mineral requirements of aquatic species compared to land animals, and the reason for this is the absorption of these elements by fish through water and It is food. Copper element is considered as an essential mineral in the nutrition of aquatic animals, and the dependence of the physiological reactions of aquatic growth on the activity of copper-containing enzymes necessitates the continuous need for copper in the diet. It seems that the presence

of copper element is necessary for the formation of melanin pigment and skin pigments, the formation of bones and connective tissue, and excessive amount of copper in aquatic feed can have negative effects on intestinal morphology and increase the rate of apoptosis and cell division in intestinal cells according to the presented studies, organic copper or copper in the form of nanoparticles has more biosupply compared to mineral copper and if the particle size of large copper molecules is reduced to the size of nano particles, small molecules can be easily absorbed from the intestine and as a result copper is digestible. It increases in the digestive system.

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