Review Article

Impact of Nanoparticles on Haemato-physiological Status of Fish – Mini Review

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ABSTRACT

Nanotechnology, particularly the use of nanoparticles (NPs), is highlighted as a promising technique to address these challenges. Nanoparticles, due to their small size (1-100 nm), exhibit high surface area and increased chemical reaction efficiency. The review notes that nanotechnology has been successfully applied in various fields, such as medicine, textiles, sports, cosmetics, and engineering. It emphasized that mineral nanoparticles, including zinc, copper, iron, selenium, and silver, play crucial roles as micronutrients in fish health. The use of nanoparticle supplements demonstrates positive effects on growth, haematological parameters, immune responses, and antioxidant enzyme activities in various fish species. The review suggests that nanotechnology has the potential to significantly contribute to improving overall fish health and aquaculture system performance.

Keywords: Nanotechnology, fish, aquaculture, mineral nanoparticles.

INTRODUCTION

In the contemporary global scenario, the demand for protein consumption is on the rise, propelled by the expanding world economy and human population. Fish, being an excellent source of easily digestible animal protein, is capable of meeting this growing demand. Apart from being rich in protein, fish provide essential polyunsaturated omega-3 fatty acids, fat-soluble vitamins (A, D, E, K), and various micronutrients such as phosphorus and selenium[1]. The consumption of fish is associated with multiple health benefits, including the reduction of saturated fat and cholesterol in the diet. Currently, around 16.6% of fish is utilized as food globally, with the global capture fish production increasing from 69 to 93 million tons between 1997 and 2020[2]. Over the past few decades, world aquaculture production has surged from 5 million to 63 million tons. Despite this rapid development, the fish industry faces uncertainties related to sustainability and the management of aquaculture water environments. Several underdeveloped sectors include drug use, water quality management, disease detection and treatment, productivity improvement, fish breeding, and proper harvesting[3]. Challenges also persist in areas like fish epigenetics, nutrigenomics connection, drug and nutrient delivery for rapid growth, reduced culture time, and effective vaccines for aquaculture, all of which are works in progress. The need of the hour is for the scientific community to comprehend, develop new strategies, and employ techniques to upgrade the aquaculture environment. Among the recent advancements in science and technology, nanotechnology emerges as a promising and rapid technique to address the underdeveloped areas of fishery science.

Nanoparticles (NPs) have found widespread applications in various fields such as medical science, textiles, sports, cosmetics, weapon production, construction, and engineering. Nanoparticles, characterized by their tiny size (1-100nm), possess a high surface area per unit volume, enhancing their efficiency in chemical reactions[4]. In 2018, it is being reported by Chris et al, nanoparticles are able to sustain at high pressure and temperature. Because of its typical size it can penetrate any biological system of living organism and work more quickly than other macromolecules[5]. From the last few decades, nanotechnology is a highly evolving and innovation techniques used in various field physics, chemistry, biology, food packing, and so on. It was being reported in 2015 by shaalan et.al., that it has a potential efficiency in case of aquaculture industry in therapeutics, antimicrobial activity, mineral supplementation, detection of different fish pathogen. Growth is an important parameter in case of healthy fish production, stock size maintains, mortality of aquaculture population in aquaculture and fishery science. Nanoparticle use as drug delivery, gene delivery, vaccine production, fish harvesting and various aquaculture system[6]. Therefore, nanotechnology can applied to enhance fish growth, and overall fish health maintenance purpose.

The various types of nanoparticles include metal-based nanoparticles (Zn NPs, Cu NPs, Se NPs, Ag NPs), carbon-based nanoparticles (graphene, carbon nanotube), and nanocomposites (bio-organic complexes). Nanoparticles can be synthesized through methods such as bottom-up and top-down approaches using chemical or green synthesis[31,32].

Impact of different nanoparticles in fish growth, immunity and antioxidant enzyme response:

***Zinc nanoparticle**:*

Zinc is the second abundant metal in living organism which is essential for fish nutrition due to its metabolic pathway. It is responsible for growth as well as muscle and bone

development in plant and animal.it helps to build innate immunity is found almost all the body tissue mostly in muscles and bones[7] in human, animal. Organic and inorganic zinc use in fish diet to maintain zinc requirement in fish. It is reported that dietary zinc in organic source, mineral source and Nanoparticle source at 50mg/kg diet concentration are used as supplement in rainbow trout gives a good result in growth performance like weight gain (WG), final body weight (FBW) etc[8]. From the study twafik et al., it was being reported that nZNO supplement in 30mg/kg diet in Nile tilapia improve weight gain (WG) and specific growth rate (SGR). nZNO at 30mg/kg dose enhance the immunity i.e. Total protein, IgM and conventional Zno enhance the IL-1 beta gene expression at 60mg/kg diet dose[9]. According to the report by awad et.al. that nZnO at 30mg/kg diet supplementation to tilapia fish (*Oreochromis niloticus*) enhance serum total protein, IgM level. nZnO at 30mg/kg diet able to decreased lipid peroxidation (MDA) level and enhance antioxidant enzymes superoxide dismutase (SOD and catalase (CAT) activity at the same dose[10]. Swain et.al., report that nZnO supplementation of 10mg/kg diet dose to rohu fish (*Labeo rohita*) effect on serum stress enzymes i.e. ALP, LDH decreased on the other hand SOD level increased. It's also increased myeloperoxidase, respiratory burst, haemolysis, and (*Aeromonas hydrophila*) bacterial agglutination capacity than control one[11]. An experiment conducted by Wang et.al., in the year 2017, nZnO supplementation at to Marine medaka and red drum was reported that nZnO is less accumulated in liver both at 80mg/kg and 300mg/kg diet dose. nZnO at 300mg/kg diet is significantly higher than $ZnCl_2$ At 80mg/kg dose of nZnO treatment give a good result on antioxidative responses like 1.5 fold GSH concentration increased and 0.5 fold MDA level decreased in fish. On the other hand, this report is found that the bioavailability of nZnO at 300mg/kg diet is more than $ZnCl_2$[12]. In 2018, in an experiment nano zinc use as feed supplement of 1,2g/kg diet in African catfish fingerlings and by Onuegbu et.al, it was reported that nano zinc has higher bioavailability than normal zinc and it help to increased weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ration (PER) level. Blood parameter like RBC, WBC, haemoglobin, hematocrit level are also increased at 1.2g/kg diet dose intake of nano zinc supplement[13]. Zuberi et.al. reported that by inorganic zinc (ZnO), zinc sulphate ($ZnSo_4$), zinc nanoparticle (ZnO-NP) supplementation at 30mg/kg and 60mg/kg diet to juvenile grass carp and it is observed that 30mg/kg diet concentration of nano zinc give good result in weight gain (WG%) and also increased haematological parameter like RBC, haemoglobin, MCV, MCHC, MCH level and decreased the haematocrit value in fish blood[14].

Selenium nanoparticle

One of the most important trace element selenium is an essential nutrient. See available mostly in two form: inorganic (selenate and selenite) and organic selenomethionine and selenocysteine[15]. Selenium plays a crucial role in case of metabolic pathway, reproduction for animal health. Se act as an antioxidant to protect cell from oxidative stress with glutathione peroxidases (GPx) enzyme[16]. It is being reported by Ashouri et.al. (2015), that nano-Se at different concentration (0.5,1 and 2mg/kg feed diet) supplemented to common carp and the growth parameter like final weight and weight gain are increased at 1mg/kg diet. At 2mg nano se /kg diet showed the best accumulation in liver and muscle tissue and liver GPx and superoxide dismutase (SOD) increased at 1,2mg nano se/kg group. High catalase (CAT) and low MDA activity shows at 2mg/kg group. Total protein, globulin, high density lipid (HDL), AST, ALT level increased and LDL decreased in blood at 2mg nano se/kg diet[17]. Supplementation of nano-se with 0.5,1 and 2 mg/kg diet to Red sea bream (*Pagrus major*) was reported that at 1 mg/kg diet, the final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER) value are increasing also liver and muscle bioaccumulation have been shown at 1,2mg.kg diet dose. Hematocrit value (HCT) increased and triglyceride (TG) decreased and biological antioxidant potential (BAP) is upregulated, reactive oxygen metabolites (d-ROMs) is downregulated in case of 0.5,2 mg/kg diet group that mean with nano se fish can tolerate more oxidative stress[18]. From the study it was shown that the synergistic effect of nano Se (1mg/kg diet) and vitamin E (100mg/kg diet) supplementation in Nile tilapia (*Oreochromis niloticus*) enhance the growth performance like weight gain (WG), specific growth rate (SGR) and lower feed conversion ratio (FCR) value. Total protein, lysozyme activity, phagocytic index increased and immune stimulator gene of liver and spleen like TNF-α, IL-1β upregulated with nano-se /vit E [19]. Liu et al, 2016 has done a brief study on different type of selenium i.e. sodium selenite (Na_2SeO_3) 0.2mg/kg diet, selenium nanoparticle (Nano-Se) at 0.2mg/kg diet, selenium yeast (Se-yeast) at 0.1,0.2,0.4,0.8mg/kg diet concentration on blunt snout bream and report that se-yeast (0.2mg/kg diet) is highly efficient in growth performance like weight gain (WG), lower FCR both in se-yeast. se np. Highest se accumulation in liver by Na_2SeO_3 (0.2mg/kg diet). se-yeast showing different result in catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA) activity (0.4mg/kg diet) and glutathione reductase (GR) activity decreased at (0.2mg,0.4 mg/kg diet). No significant changes shows in total essential amino acid (TEAA) and total amino acid (TAA) with se-yeast. Fish muscle colour effected by nano-se and se-yeast (0.2mg/kg diet)[20]. Naderi et.al.,2017, used a combination of vitamin E and nano-se supplementation with different concentration 500mg/kg, 1mg/kg diet respectively, on rainbow trout (*Oncorhynchus mykiss*) and high growth performance have seen with the combination group (vit E and nano se) in final weight (FW), weight gain (WG), specific growth rate (SGR), feed intake (FI), lower feed conversion ratio (FCR) value. With supplementation of combined dose immune parameter like lysozyme activity, serum osmolarity, albumin, HDL and lower globulin, LDL level have seen in the above mention fish model[21]. Zhou et.al., 2009, studied on crucian carp

(*Carassius auratus gibelio*) by application of two different selenium sources (selenium nanoparticle and selenomithionine) at 0.5mg/kg diet concentration and reported that significantly higher result in relative gain rate (RGR) and final weight in compare with control in both groups. Nano se has higher bioaccumulation rate in muscle tissue, glutathione peroxidase (GSH-Px) level is higher in nano se than other groups[22]. A study of synergistic effect of Selenium NP and vitamin C (L-ascorbyl -2-polyphosphate) supplementation at (0.68mg Nano Se/kg diet), (100,200,300mg APP/kg diet) on mahseer fish (Tor putitora) by khan et,al., 2017, report that weight gain percentage, feed conversion efficiency, specific growth rate percentage are increased and feed conversion ratio is decreased. Beside this haematological parameter like RBC count, Haemoglobin level (Hb), haematocrit percentage (HCT%), lysozyme activity, serum growth hormone level are also increased by combine supplementation of Nano-Se and vit C at 0.68mg/kg diet,300mg/kg diet respectively[23]. Saffari et.al, 2016, used three sources of inorganic, organic, nano selenium like sodium selenite (Na_2SeO_3), selenomethionine (SeMet), nanoselenium at 0.7mg/kg diet concentration on common carp (*Cyprinus caprio*) and report that by nano-Se supplementation can improve final weight (FW), specific growth rate (SGR), feed conversion ratio (FCR) values. nano se and semet both group showing higher accumulation of se in fish liver and muscle tissue. Beside this antioxidant activity like Glutathione peroxidase (GSH-Px). superoxide dismutase (SOD), catalase (CAT) enzyme level are increased and malondialdehyde (MDA), aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) activity are decreased and no difference observed in alkaline phosphatase (ALP) by nano-Se and semet supplementation[24].

Iron nanoparticle: Iron is an essential component for almost all the cell of every living organism[25]. Iron exists as heme compound i.e. haemoglobin and myoglobin and non heme compound i.e. ferritin, transferring in any living system. Iron helps in cellular respiration in oxidative phosphorylation and oxygen transport from lung cell to other body cell[24,25]. For a detail study by application of macro and nano iron oxide (Fe_2O_3) in gold fish (*Carassius auratus*) at 0,0.2,0.5,1g/kg diet concentration and it was being reported by Akbary et.al., 2019, that final body weight (FBW), weight gain(WG), specific growth rate(SGR), feed conversion ratio (FCR),CF are showing higher value by both nano and macro iron oxide(Fe_2O_3) supplimentation. The biochemical parameter like alkaline phosphatase (ALP) activity increased but no significant different is showing for glucose, triglyceride and total protein, aspartate aminotransferase (AST), alanine transaminase (ALT) level. Ghrelin and IGF-1 mRNA level expression are increased in both Fe AND nFe diet group that mean food intake capacity is increased in those group of fish[26]. Behera et.al., 2014, used two iron sources like nano Fe and ferrous sulphate at 0.5mg/kg feed concentration on fish (*Labeo rohita* H.) and report that the growth parameter like relative weight gain, final weight can be improved by nano Fe supplementation. Iron accumulation is higher in muscle by nano Fe. Haemoglobin and RBC count as well as Respiratory burst activity and serum bactericidal activity are also increased by nano Fe treated group. Antioxidant enzyme activity like SOD, GSH activity increased by both treatment group. But no significant difference has been shown in between WBCs content, total serum protein, albumin, globulin and albumin/globulin ratio, alternative complement activity etc in both treatment group in fish[27]. Remya et.al.,2015, studied that by iron oxide (Fe_2O_3) NP supplementation at 2,10,100,250,500,1000ppm to Indian major carp (*Labeo rohita*) and report that haematological parameter like haemoglobin (Hb), haematocrit (Hct), RBC count are increased and WBC count, MCV, MCH, are decreased. But No significant changes present in MCHC value. Elevation of potassium level and depletion of chloride level have been shown by nano Fe at 500ppm dose. Gill sodium/potassium-ATPase activity is increased by nano Fe application[28]. Saravanan et.al., 2015, studied that iron oxide NPs can enhance the different performances in Indian major carp *Labeo rohita* at 1,25mg/L concentration and report that Hb, Hct, MCV and MCH count are decreased and WBC and MCHC count are increased and no changes is found in RBC count. plasma glucose level is increased at 25mg/L nano Fe concentration but plasma protein level, sodium, potassium and chloride levels are decreased. The inhibition gill Na/K ATPase activity is inhibited by nano Fe accumulation in gill of the fish *L. rohita* [29]. From a study on dietary nano and macro iron oxide (Fe2O3) at 0.2,0.4,0.8,1.2,1.6g/kg diet concentration on African catfish (*Clarias gariepinus*) fingerlings by onuegbu et.al, 2018, and the observations are percent weight gain is increased at 0.4g/kg feed by both groups also weight gain (WG), percent weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR) are increased by inorganic fe addition rather than nano Fe. haematological parameter like RBC count is increased but WBC count, hb count are low in nano Fe treatment group. Beside this liver Fe accumulation is showing highest in with inorganic Fe supplementation in rohu fish[30].

Copper nanoparticle:

One of the most efficient trace mineral exists in animal, human body is present in all most all body tissue is copper. It is act as a cofactor in iron metabolism, red blood cell formation as well as important for cardiovascular integrity, maintain immune system[31]. In case of premix copper use in an amount in animal feed[32]. copper sulphate used as fish feed to enhance hormone like FSH, as well as haematological and biochemical parameter in fish[34]. Beside organic and inorganic sources of copper, copper nanoparticle are also used in fish feed. Gupta et.al, 2016, used nano copper (Cu-Nps) at 20,100µg/L doses on common carp (*Cyprinus carpio*) and report that body weight and kidney, liver, gills tissue growth are increased. Antioxidant enzyme SOD, CAT, GSH level is elevated by both Cu-NP treatment groups. Higher dose of Cu-np can increase the degeneration tubules in glomerulus Bowmans

capsule, Lower dose of Cu-NP sometimes responsible for oedema of gill epithelium. Copper accumulated in liver occurred by both doses and utilise in metabolism in fish development. Some liver protein are examined like: Selenide, water dikinase 1 (UR1) which is unregulated and ferritin heavy chain (DR1), rho guanine nucleotide exchange factor 17-like (DR2), Cytoglobin-1 (DR3) and Diphosphomevalonate decarboxylase (DR4) are down regulated, which is very much important for detail toxicological study of Cu-NP[33]. Basuini et.al., 2016, used two sources of copper i.e. copper nanoparticles (Cu-NPs) or copper sulphate (CuSO4) at 2,4,6,8 mg nano cu/kg diet, 4 mg CuSO4 /kg diet dose in red sea bream (*Pagrus major*) and showed that all growth parameter like final body weight (Fn wt), weight gain (WG) and specific growth rate (SGR), Feed intake (FI) are increased at 2,4mg/kg Cu-np diet and feed efficiency ratio (FER), protein efficiency ratio (PER), protein gain (PG) and protein retention (PR) are increased in both groups at same dose. Digestive enzyme protease activity (PA) is increased at 2 mg/ kg Cu-NPs diet. But no significant differences are showing in CF, HSI and VSI value in fish. whole body Cu accumulation showed highest at 8 mg Cu-NPs/ kg diet. Blood glucose level is increased with CuSO4 at 4 mg/ kg diet. Oxidative enzymes like reactive oxygen molecules (d-ROMs) is decreased with 2,4mg/kg nano-Cu and 4 mg/kg CuSO4 diet also Biological antioxidant potential (BAP) are showing higher value with 2 mg/ kg Cu-NPs diet group. Immunological activity like Lysozyme activity, Serum bactericidal activity (BA), total serum protein are enhanced at 2 mg /kg nano Cu diet (41).A combine effect on nano Cu and vitamin C on Red sea bream at 2 mg/kg dry diet and 800,1000,1200mg/kg vitamin C diet concentration studied by baisuni et.al, 2017, and observed that final body weight (FBW), weight gain (WG), specific growth rate (SGR), protein gain(PG) and protein retention (PR), protein conversion ratio (PER), feed conversion ratio (FER), feed intake (FI) values are higher in both vit C and nano Cu 2/800,2/1000,2/1200mg/kg diet dose. Beside this, whole body protein and lipid content are increased with all doses of vit C. With nano Cu found that highest cu accumulate in liver lowest in gill and muscle. Immune response is also enhanced like lysozomal activity and bacteriocidal activity with (2nano cu/1000mg VC /kg diet dose) but not significant changes are found in blood parameter in fish[35].

Silver nanoparticle:

Silver used as mainly in wound dressing perposes, cream, antibiotic coating on medical device[36]. Nanosilver play a crucial role in bacteriostatic and bacteriocidal activity for different human bacteria[37]. Here it was enlighten that nano silver have an important role in fish immunity performance. Vali et.al.,2020, applied of nanosilver (Ag-NP) and waterborne silver nitrate (Ag–NO3) LC50 at 12.5%, 25%, and 50% dose on common carp (*Cyprinus carpio*) and report that bioaccumulation in gill and liver is much more by 50% dose of Ag-np than other groups. In case of haematological parameter low HCT, neutrophil level shown by 25,50% dose of Ag-np and MCHC highest in 50% dose, whereas WBC level is higher at 12.5,25 % dose. On the other hand, hemoglobin levels, mean cell hemoglobin (MCH) and mean cell volume (MCV), albumin, globulin level, are shown no significant difference in any groups. Immune parameter like lysozomal activity, total immunoglobulins (Ig) are decreased at 25,50% nano silver dose. Level of Total protein, SOD, CAT enzymes level are increased at 12.5% dose and glucose, cortisol are increased at 25,50% dose[38].

CONCLUSION

Mineral nutrient like zinc, copper, iron, selenium, silver all are very much important micronutrient for every living organism animal as well as fish. Those have different metabolic activity as well as they are playing a crucial role in haematological level alteration in between the system. Mineral nanoparticle uses as poultry feed to enhance growth performance and, immunity response[39]. In case of aquatic animal Zn,Fe,Cu,Se in nano form showing a very much satisfactory result in growth performance like FBW,WG,FCR, as well as haematological performance like RBC, WBC, Hb, HCT, values are increased. Beside this, those mineral nanoparticle have a good antioxidant activity like SOD,CAT,GSH-Px value are increased in nano treated fish group. So undoubtly nano mineral can use to enhance overall fish health and that will be much more beneficial for maintain a good health aquaculture system. However, there is a huge difference between nanotechnology application in the area of aquaculture research field. By this review, we want to enlighten that nanotechnology as well as nanoparticle have huge potential which can use in fish industry to get a better performance.

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REFERENCES

1.Fish as food. In *Wikipedia, The Free Encyclopedia*. Retrieved 19:12, February 8, 2024, from https://en.wikipedia.org/w/index.php?title=Fish_as_food&oldid=1200065512

2.FAO Fisheries & Aquaculture - Global Statistical Collections [Internet]. www.fao.org. Available from: http://www.fao.org/fishery/statistics/en

3.Aklakur M, Asharf Rather M, Kumar N. Nanodelivery: an emerging avenue for nutraceuticals and drug delivery. Critical reviews in food science and nutrition. 2016 Oct 25;56(14):2352-61.

4. Wikipedia contributors. (2023, November 27). Applications of nanotechnology. In *Wikipedia, The Free Encyclopedia*. Retrieved 19:56, February 8, 2024,

from https://en.wikipedia.org/w/index.php?title=Applications_of_nanotechnology&oldid=1187039291

5. Chris UO, Singh NB, Agarwal A. Nanoparticles as feed supplement on Growth behaviour of Cultured Catfish (*Clarias gariepinus*) fingerlings. Materials Today: Proceedings. 2018 Jan 1;5(3):9076-81.

6. Shaalan M, Saleh M, El-Mahdy M, El-Matbouli M. Recent progress in applications of nanoparticles in fish medicine: a review. Nanomedicine: Nanotechnology, Biology and Medicine. 2016 Apr 1;12(3):701-10.

7. Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME. Zinc and human health: an update. Archives of toxicology. 2012 Apr;86:521-34.

8. Shahpar Z, Johari SA. Effects of dietary organic, inorganic, and nanoparticulate zinc on rainbow trout, Oncorhynchus mykiss larvae. Biological Trace Element Research. 2019 Aug 15;190:535-40.

9. Tawfik M, Moustafa M, Abumourad IM, El-Meliegy E, Refai M. Evaluation of nano zinc oxide feed additive on tilapia growth and immunity. In 15th international conference on environmental science and technology, Rhodes, Greece 2017 Aug (Vol. 1342, No. 1, pp. 1-9).

10. Awad A, Zaglool AW, Ahmed SA, Khalil SR. Transcriptomic profile change, immunological response and disease resistance of *Oreochromis niloticus* fed with conventional and Nano-Zinc oxide dietary supplements. Fish & Shellfish Immunology. 2019 Oct 1;93:336-43.

11. Swain P, Das R, Das A, Padhi SK, Das KC, Mishra SS. Effects of dietary zinc oxide and selenium nanoparticles on growth performance, immune responses and enzyme activity in rohu, Labeo rohita (Hamilton). Aquaculture nutrition. 2019 Apr;25(2):486-94.

12. Wang J, Wang A, Wang WX. Evaluation of nano-ZnOs as a novel Zn source for marine fish: importance of digestive physiology. Nanotoxicology. 2017 Sep 14;11(8):1026-39.

13. Onuegbu CU, Aggarwal A, Singh NB. ZnO nanoparticles as feed supplement on growth performance of cultured African catfish fingerlings. 2020.

14. Faiz H, Zuberi A, Nazir S, Rauf M, Younus N. Zinc oxide, zinc sulfate and zinc oxide nanoparticles as source of dietary zinc: comparative effects on growth and hematological indices of juvenile grass carp (*Ctenopharyngodon idella*). International Journal of Agriculture and Biology. 2015 Jun 1;17(3):18-22.

15. https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional, accessed on 07 Jan 2024.

16. Margaret P. Rayman" The Importance of Selenium to Human Health"

17. Saffari S, Keyvanshokooh S, Zakeri M, Johari SA, Pasha-Zanoosi H, Mozanzadeh MT. Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (*Cyprinus carpio*). Fish Physiology and Biochemistry. 2018 Aug;44:1087-97.

18. Dawood MA, Koshio S, Zaineldin AI, Van Doan H, Ahmed HA, Elsabagh M, Abdel-Daim MM. An evaluation of dietary selenium nanoparticles for red sea bream (Pagrus major) aquaculture: growth, tissue bioaccumulation, and antioxidative responses. Environmental Science and Pollution Research. 2019 Oct;26:30876-84.

19. Dawood MA, Zommara M, Eweedah NM, Helal AI. Synergistic effects of selenium nanoparticles and vitamin E on growth, immune-related gene expression, and regulation of antioxidant status of Nile tilapia (*Oreochromis niloticus*). Biological trace element research. 2020 Jun;195:624-35.

20. Liu GX, Jiang GZ, Lu KL, Li XF, Zhou M, Zhang DD, Liu WB. Effects of dietary selenium on the growth, selenium status, antioxidant activities, muscle composition and meat quality of blunt snout bream, *Megalobrama amblycephala*. Aquaculture Nutrition. 2017 Aug;23(4):777-87.

21. Naderi M, Keyvanshokooh S, Salati AP, Ghaedi A. Combined or individual effects of dietary vitamin E and selenium nanoparticles on humoral immune status and serum parameters of rainbow trout (Oncorhynchus mykiss) under high stocking density. Aquaculture. 2017 May 1;474:40-7.

22. Zhou X, Wang Y, Gu Q, Li W. Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus* gibelio). Aquaculture. 2009 Jun 3;291(1-2):78-81.

23. Khan KU, Zuberi A, Nazir S, Ullah I, Jamil Z, Sarwar H. Synergistic effects of dietary nano selenium and vitamin C on growth, feeding, and physiological parameters of mahseer fish (*Tor putitora*). Aquaculture Reports. 2017 Feb 1;5:70-5.

24. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2014 Feb;19(2):164-9.

25. https://en.wikipedia.org/wiki/Iron_deficiency

26. Akbary P, Jahanbakhshi A. Nano and macro iron oxide (Fe2O3) as feed additives: effects on growth, biochemical, activity of hepatic enzymes, liver histopathology and appetite-related gene transcript in goldfish (Carassius auratus). Aquaculture. 2019 Aug 15;510:191-7.

27. Behera T, Swain P, Rangacharulu PV, Samanta M. Nano-Fe as feed additive improves the hematological and immunological parameters of fish, Labeo rohita H. Applied Nanoscience. 2014 Aug;4:687-94.

28. Remya AS, Ramesh M, Saravanan M, Poopal RK, Bharathi S, Nataraj D. Iron oxide nanoparticles to an Indian major carp, Labeo rohita: Impacts on hematology, iono regulation and gill Na+/K+ ATPase activity. Journal of King Saud University-Science. 2015 Apr 1;27(2):151-60.

29. Saravanan M, Suganya R, Ramesh M, Poopal RK, Gopalan N, Ponpandian N. Iron oxide nanoparticles induced alterations in haematological, biochemical and ionoregulatory responses of an Indian major carp Labeo rohita. Journal of Nanoparticle Research. 2015 Jun;17:1-2.

30. Uzo-God OC, Agarwal A, Singh NB. Effects of dietary nano and macro iron oxide (Fe_2O_3) on the growth, biochemical, and hematological profiles of African catfish (*Clarias gariepinus*) fingerlings. Journal of Applied Aquaculture. 2019 Apr 3;31(2):153-71.

31. https://en.wikipedia.org/wiki/Nanoparticle

32. COPPER IN NUTRITION https://www.micro.net/siteassets/research/poultry/2.6-feed-info-7-22-03.pdf

33. Gupta YR, Sellegounder D, Kannan M, Deepa S, Senthilkumaran B, Basavaraju Y. Effect of copper nanoparticles exposure in the physiology of the common carp (*Cyprinus carpio*): Biochemical, histological and proteomic approaches. Aquaculture and Fisheries. 2016 Dec 1;1:15-23.

34. AM Shokr E. Effect of copper on hematological, biochemical changes and reproductive hormones of the nile tilapia oreochromis niloticus. Egyptian Journal of Aquatic Biology and Fisheries. 2020 Mar 1;24(2):1-8.

35. El Basuini MF, El-Hais AM, Dawood MA, Abou-Zeid AS, El-Damrawy SZ, Khalafalla MS, Koshio S, Ishikawa M, Dossou SJ. Effects of dietary copper nanoparticles and vitamin C supplementations on growth performance, immune response and stress resistance of red sea bream, Pagrus major. Aquaculture Nutrition. 2017 Dec;23(6):1329-40.

36. https://en.wikipedia.org/wiki/Medical_uses_of_silver

37. Bąkowski M, Kiczorowska B, Samolińska W, Klebaniuk R, Lipiec A. Silver and zinc nanoparticles in animal nutrition–a review. Annals of Animal Science. 2018 Oct 1;18(4):879-98.

38. Vali S, Mohammadi G, Tavabe KR, Moghadas F, Naserabad SS. The effects of silver nanoparticles (Ag-NPs) sublethal concentrations on common carp (Cyprinus carpio): Bioaccumulation, hematology, serum biochemistry and immunology, antioxidant enzymes, and skin mucosal responses. Ecotoxicology and environmental safety. 2020 May 1;194:110353.

39. Mahmoud H ED, Ijiri D, Ebeid TA, Ohtsuka A. Effects of dietary nano-selenium supplementation on growth performance, antioxidative status, and immunity in broiler chickens under thermoneutral and high ambient temperature conditions. The Journal of Poultry Science. 2016;53(4):274-83.