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Effect of Different Concentrations of Zinc Oxide Nanoparticles on Growth and Hematology of Catla *Catla*

Alfa Sherin[1], Nimisha P[2]

Department of Zoology, PSMO College, Tirurangadi 676306, Kerala, India

Abstract: ***Zinc is essential for aquatic biota including fishes at lower concentration, but when it reaches higher concentrations it becomes toxic. The objectives of the present work were related to the impact of different quantities of zinc oxide nanoparticles on the growth and haematology of Catlacatla. Different quantities of zinc nanoparticles such as 0.3mg/l, 0.6 mg/l, 0.9 mg/l and 1.2 mg/l were prepared by using a fish meal. Feed utilization, length weight studies, survival rate and haematological parameters were estimated after 45 days of feeding. This study provides that 1.2mg/l of zinc oxide nanoparticles incorporated feed was suitable for the growth and haematological parameters of Catlacatla and it would be used in the feed of fishes as micronutrients.Freshwater is the main habitat of this fish, lakes, ponds, rivers, streams, swamps, wetland pools came under the freshwater ecosystem. Several fishes like Rohu, Catla, Shrimps, etc. are found in such habitat. In this study zinc oxide nanoparticles are given to the catla fish in different doses with their normal fish feed. The feeding to fish was carried out every day in two equal installments. The study period was 6 weeks. In each week weight of the fishes were record. After 6th week length and weight of the fishes were measured and compared.***

Keywords: Nanoparticles, growth, haematology, mortality, *Catlacatla*

1. Introduction

Aquaculture is described as the farming of aquatic organisms with fish as the principal form which implied some sort of interventions in the rearing process to enhance production process such as regular stocking, feeding and protection from diseases and predators.It involves cultivating fresh water and salt water populations under controlled conditions, and can be contrasted with commercial fishing, which is the harvesting of wild fish (Ramya,2019).Raising of fish commercially occurs through aquaculture specifically in tanks, fish ponds or ocean enclosures. It is usually used for food worldwide. There are some commonlyusedcultivating fishes are there, carp, Catla fish and salmon. Fish play an important role in supplementing humannutrition, such as supplying essential nutrients including vitamin D, iodine, calcium and animal protein. Tacon and Marcmetian, (2013) were provide essential fatty acids such as omega-3 and -6 fatty acids needed by humans for good health, immunity enhancement and prevention of cancer and heart diseases(FAO,2017).In fish production packing and transportation activities are inevitable, because the fry, fingerlings, juvenile and brood stock must be moved from one region or farm to another, either within the same are, state,country,or even across a country to another.

In this study Catla fish fingerlings are used. The economically significant freshwater fish Catla a member of the *Cyprinidae f*amily of carps, is also known as the large South Asian carp. Although it has been brought to other parts of South Asia and is widely farmed, it is native to rivers and lakes in northern India, Bangladesh, Myanmar, Nepal, and Pakistan.Catla is a fish with large and broad head, a large protruding lower jaw, and upturned mouth, but the fingerlings are nearly 1kg in weight and their size is also miniatures. It is a surface and midwater feeder. Fingerlings consume some planktonic algae, vegetable debris along with larger size zooplankton. Adults feed on zooplankton using large gill, but young ones on both zooplankton and phytoplankton. Catla attains sexual maturity at an average age of two years and an average weight of 2 kg. It is one of the most important aqua cultured freshwater species in South Asia. It is grown in polyculture ponds with other carp-like fish, particularly with the Rohu*(Labeorohita*) and mrigal carp. In 2012, there were around 2.8 million tonnes recorded annually, a significant increase from the reported quantities throughout the 2000s.Locally and regionally, fresh Catla is offered for sale and consumed. It is moved using ice. The weight of fish that people desire is about 1-2 kg.

The necessary amount appears to be the same for all fish species, typically falling between 1 and 2 mg/kg. According to growth and feed performance, protein retention, antioxidant and immunological characteristics, and a recent study on fingerling *Catlacatla* fed a semisynthetic diet, a requirement of 0.4 mg/kg was necessary, while 0.6 mg/kg was required for liver folate saturation (Khan and Khan, 2020). Having high-quality fish seed is one of the most essential requirements for a fruitful fish culture. Fishermen and farmers frequently encounter difficulties in obtaining fish because the spawn they gather from natural water is not pure or of the necessary grade. Mixed seed sources from different species make it very difficult to separate pure seed from unwanted wild species or to find pure seed. One of the most crucial conditions for effective fish farming is the availability of the necessary number of fish seed of the desired species. The widely cultured Indian major carps in inland waters like catla (Catlacatla), Rohu (*Labeorohita*), Mrigal (*Cirrhinusmrigala),* and calbasu (*Labeocalbasu*), and Chinese carps like silver carp (*Hypophthalmichthys molitrix*), big head carp(*Aristichthysnobilis*), grass carp (*Ctenopharyngodonidella*), black carp (*Mylopharyngodonpiceus*), and mud carp (*Cirrhinusmolitorella*), normally do not breed in confined waters. Although they do reach sexual maturity there, they only have babies in the flooded, shallow areas along the rivers' courses during the monsoon season, which is their natural home.

The fish farmers are fortunate that the intervention is necessary at this late stage, when the required environmental cues are missing and a synthetic approach is required to speed up the process. This has been achieved using the induced breeding method. In general, induced breeding refers to a process in which fish, who do not reproduce in still bodies of water, will do so while being affected by hormones or stimulants injected into their bodies.Brachionus sp., *Moinamicrura*, *Daphnia carinata*, *Diaptomus* sp., and Mesocyclops sp. are the most often utilized live feed organisms to promote growth and survival of Catla larvae under tank culture conditions. In modern aquaculture, fish feed is an integral part of a source of nutrients and energy for the growth, reproduction and health of fish. After being consumed as a natural food supplement, artificial fish food is a sort of substance that promotes fish or prawn reproduction, growth, and health regulation.

The main issue with catfish farming is the lack of reasonably priced, high-quality feed. Fish have typically been fed by being given food in the form of food pellets. This pellet is primarily designed to meet the daily nutritional needs of fish, which include ingredients like lipids, proteins, carbs, minerals, and vitamins. nanoparticles (109–107 m) have numerous technical and biological advantages. Their compact size and high specific surface area provide them special characteristics. The global fishing business is recognized to include fish farming as a significant and vital component (Singh andAgarwal,2018. It has been discovered that nanoparticles will improve aquafeeds by boosting the proportion of fish meal components that move past the gut tissue and into the fish, as opposed to going through the fish digestive system untouched. Dietary minerals with a nanoscale size may enter cells more easily than minerals with a greater size. This quickens the process of their incorporation into the fish. The addition of mineral elements to fish diets on a regular basis at the nanoscale may also have a significant impact on the fishes' general health and growth. Numerous nanomaterials as supplements for fish feed have been discussed in relation to growth performance. The numberof nanomaterials as supplement for fish feed on growth performance have been discussed. These are the main reason for choosing nanoparticles as feed supplement to fingerlings to detect how fast their growth and development to occur. Also zinc oxide is one of the principal growths enhancing nanoparticles which I can see most of the experiment and studies. These are the other factors which attracted me to do this project.

Freshwater is the main habitat of this fish, lakes, ponds, rivers, streams, swamps, wetlandpools came under the freshwater ecosystem. Several fishes like Rohu,Catla, Shrimps, etc. are found in such habitat. In this study zinc oxide nanoparticles are given to the Catla fish in different doses with their normal fish feed. The feeding to fish was carried out every day in two equal installments. The study period was 6 weeks. In each week weight of the fishes were record. After 6^{th} week length and weight of the fishes were measured and compared.

Like zinc oxide there are some other minerals are there which are inevitable for their growth and development, that are copper, manganese, iron, calcium, phosphorus, potassium, magnesium, sodium, Sulphur, cobalt, iodine and selenium.Zinc oxide is an inorganic compound with the formula ZnO.ZnO is a white powder that is widely utilized as an additive in a wide range of materials and goods, including foods, lubricants, paints, ointments, adhesives, sealants, pigments, batteries, ferrites, ceramics, glass, cement, lubricants, and paints. Most animals, plants, and microbes require zinc, a trace metal that is necessary.

Zinc is required for the function of over 300 enzymes and 1000 transcription factors. Zinc interacts with a wide range of organic ligands, and has roles in the metabolism of RNA and DNA, signal transduction and gene expression and also regulates apoptosis. Zinc oxide naturally occurs as the mineral zincite. Zinc oxide mostly produced synthetically. It is also known as zinc white, philosopher's wool, calamine, flowers of zinc and Chinese white. As a feed supplement, zinc oxide performs better than normal fish feed in Catla fish. Micron-sized particles known as nanoparticles have at least one dimension that is smaller than 1000 nm. Due to this, handling these particles in a biological system is particularly appealing. Nanoparticles are very suitable in sensing and detection of biological structures and systems. Metal nanoparticles come in a variety of forms, like nanocrystals, and sizes, from 2 nm to 1000 nm. Nanoparticles serve as a sort of link between bulk materials and atomic or molecular structures, which makes them of significant importance to science. Man-made nanomaterials have drawn a lot of attention because they have a positive effect on numerous economic sectors, including consumer goods, pharmaceuticals, cosmetics, transportation, energy, and agriculture, among others. Increasing numbers of them are being produced for a variety of industrial uses.

Zinc oxide nanoparticle (ZnO) used for this study got from the Cochin University of science and technology (CUSAT) Ernakulam. The zinc oxide nanoparticle in different doses to the Catla fish fingerlings with their normal fish feed. These particles are having larger surface area and catalytic activity. They are being researched to eliminate harmful germs from packaging and UV-protective materials like textiles (Saraf, 2013).ZnO nanoparticles are utilized as sunscreen because they absorb UV light while remaining transparent to visible light (Lopes *et al.*, 2014).It has been demonstrated in animal tests that the blood-brain barrier, individual cells, and nuclei can all be penetrated by the nanoparticles, which are incredibly small and can move throughout the body. They are difficult to detect since tissues can absorb them easily due to their size (Ramya,2019). Due to their great biocompatibility, affordability, and low toxicity, ZnO nanoparticles have emerged as one of the most widely used metal oxide nanoparticles in biological applications over the past two decades. No nanoparticles have demonstrated a potential in biomedicine, particularly in the fields of anticancer and antibacterial fields (Wang *et al.,* 2011), which are involved with their potent ability to cause excessive reactive oxygen species (ROS) production, release zinc ions, and induce cell apoptosis. Zinc is also well known to maintain the structural integrity of insulin. So, ZnO nanoparticles also have been effectively developed for antidiabetic treatment. Nano-ZnO, with small particle size, makes zinc more easily to be absorbed by the body. Thus, Nano-ZnO is commonly used as a food additive.

The zine oxide nanoparticle gives to the Catla fish fingerlings as a feed supplement in different doses makes better results in their growth (length and weight), haematological parameters such as leucocyte, erythrocyte, haemoglobin, haematocrit etc. And also, there may a great positive change in the biochemical components such as carbohydrate, lipids and protein. At a very Higher amount of zinc nanoparticles to the fish fingerlings may cause cell damage or the reduction of weight and length. Growth performance of cultured *Catlacatla* fingerlings also done by the feed supplementation of macro and nano copper oxide (CuO) in other studies. There are other studies that use the same nanoparticles as feed supplements with different fish types, such as iron nanoparticles feed supplementation studies with another fish *Clariasbatrachus*(Linnaeus, 1758), to achieve maximum benefit. Iron nanoparticle should ensure growth, immunity, and sound health of fish to achieve maximum benefit. The critical trace elements zinc (Zn), copper (Cu), and iron (Fe) are found in fish and are important for the biological processes of bone development, hemoglobin synthesis, nervous system maintenance, and as primary component of enzymes like cytochrome oxidase, which is involved in oxidation reduction reactions. Due to their antibacterial and other qualities, copper and zinc nanoparticles are just two of the other nanoparticles that have recently found usage in biological research and engineering. The impact of zinc oxide nanoparticles on onion seed germination and mouse spermatogenesis, including improvements in sperm count and motility as well as a decrease in the percentage of defective sperm, has also been researched. Some fish benefit from dietary copper in terms of development performance and immunological processes, whilst others can die from copper deficiency or excess. High concentrations of NanoZineOxide particles significantly reduced haematological parameters in juvenile grass carp (*Ctenopharyngodonidella)*, but enhanced RBC count and corpuscular haemoglobin concentration at lower supplementation rates.

2. Objective of the study

- To observe and study the effect of Zinc oxide Nano particles as feed supplement on the growth performance of *Catlacatla* (Catla fish)
- To measure the weight and length of the *Catlacatla* (Catla fish) fed with nZnO particles after the experimental period. Also, to check which concentration of the formulation is more effective and which is least effective.
- To study how ZnO Nano particles food supplement effects the heamatological parameters such as WBC, RBC count, heamoglobin of the *Catlacatla*
- To estimate the protein content of the fish consumed with each concentration of nZnO and compared to the control.

3. Scope of the study

The major challenge facing fish farming is availability of cheap but high-quality feed. In order to improve the production and selling in aquaculture, it has become essential for farmers to produce good quality and muscle mass fishes. Zinc oxide (ZnO) nanoparticles have been a subject of interest in various fields, including aquaculture, due to their unique properties and potential applications. Since zinc is a trace element, it is not harmful to nature and society. Moreover, it has a lot of helpfulness. Fish farming has improved a lot in their economy with the use of nanoparticles as feed supplement. This is because of the wide variety of benefits of nanoparticles as shown below. According to several research, adding ZnO nanoparticles to fish diets may improve culturable fishes' abilities to better health. They were believed to boost the immune system and increase the fish's resistance to diseases and infections. It was shown that ZnO nanoparticles had antioxidant characteristics, which may benefit fish with oxidative stress. For fish to remain healthy and perform better overall, oxidative stress must be reduced. Zinc is a mineral that is crucial for the growth and health of fish. The potential of ZnO nanoparticles in fish feed to decrease water pollution brought on by an excess of nutrients in aquaculture systems was also investigated.

4. Materials and Methods

Test animal –*Catlacatla*fingerlings

Catla (*Catlacatla*) fingerlings average weight 4.00g and average length 4cm (picture.1) were purchased from a local fish farm-Aqua fish Aquarium, Kottakkal, Kerala. Fingerlings were reared and acclimated for 45 days in a plastic container with 5 liters of freshwater. Fish were given commercially available fish pellets, while pH, temperature, and dissolved oxygen levels in the water were continuously measured at regular intervals. Also, water in the tub were changed regularly. TheCatla fish has a major use for their muscle mass in the market. (Plate 1)

Plate1: *Catlacatla* fingerlings

Zinc Oxide Nanoparticles (nZnO) were purchased from Cochin university of science and technology (CUSAT), Ernakulam's. Other materials required for the experiment are water and vegetable oil purchased from local market, Malappuram. (Plate:2)

Plate 2: Zinc oxide nanoparticles

Culture system

Aquaculture system was constructed by using 5L capacity plastic tub. A total of 5 plastic containers were taken for the experiment to accommodate different concentrations of nZnOfeeded fish fingerlings and other one for the control. In each plastic tub equal number of (5) fish fingerlings are taken.

Diet formulations

Different weights (0.3g, 0.6g, 0.9g, 1.2g, 1.5g) of zinc oxide nanoparticle (nZnO) were carefully measured out and combined with vegetable oil in plastic bowls (picture.2&3). Grinded fish pellet was added into each of those bowls and mixed thoroughly to form the maximum absorbance of the nanoparticles into the fish pellets. Grinded fish pellet alone also treated with vegetable oil to form the control diet. These were kept in open bowls at normal room temperature for two days, so that the oil could be absorbed into the feed. The diet was then placed in a dry, cool place for usage. (Plate:3, Plate:4).

Plate 3: Basal fish feed

Plate 4: Basal feed mixed with zinc oxide nanoparticle

Fish feeding and management of cultural system

The feeding to fish was carried out every day in two equal instalments. That was at 7:30am and 6:30pm. At every morning and evening 1gram of feed which is sorted out in to different bowls were fed on to them. Daily feed consumptions for each group of fish were recorded (almost 1gm at a time). The average daily food consumption is therefore 2 gm. In the evenings of everyday before i fed them, water was drained from each plastic container and replace with freshwater.

Length weight measurement

Initial length and weight of the catla fishes of each concentrations are measured before the acclimatization. After 6 week of the experimental period change in their length and weight also detected . At the end ofeach week, weight of the fishes was taken by using weighing machine and also at the end of experimental period that is after 6-week, fish weight and length of all the levels such as control, were recorded. Fish length taken by using the graph paper, Petri dish and scale.Put the fish on the petridish and place it above the graph paper. So that easily measured the length of it by placing scale on the side of graph paper, then count the number of squares including the fish.

Haematological parameters

At the conclusion of the trial, specific hematological parameters were found in all treated fish groups. Leucocytes (WBC) and total erythrocyte (RBC) counts were calculated using diluting fluids. In Neubauer's counting chamber, the cell count was seen. The level of blood haemoglobin (Hb) was also measured. Another important estimation that is proteinestimation (Plate 5).

Statistical analysis:
Results of length and weight of Catla fish after the experimental period were analyzed by multiple bar diagram.

Protein estimation
At the end of experimental period, the protein content of the fish consumed with each concentration of nZnO was assessed and compared to the control test (Plate 6).

Plate 6: Protein estimation

5. Results and Discussion

Length and Weight changes in *Catlacatla*(Catla fish) fingerlings in the presence of different concentration of supplements (0.3g, 0.6g, 0.9g, 1.2g, 1.5g) of nano zinc oxide were measured weekly up to 6 weeks. After 6 weeks (end of the experiment), the weight and length increase followed the sequence given below. The length weight increase in control sample was comparatively low than that of different feed supplements.

Control <nZnO

Results have shown that nZnO supplemented feed was more effective in weight increase as compared to control supplemented feed (fish pellet) when measured after 6 weeks. The weight increased till 1.2g concentration and after that (1.5g concentration) the weight decreased. Also, the length increased till 1.2g concentration and decreased from 1.5g concentration. Initial length weight of *Catlacatla* and after the administration of nZnO particles at different concentrations were represented in Table 1 and Figure 1 respectively.

After experimental period the control fish weight gain is 1.7g from the initial weight of 1g and length raised to 4.6cm from the initial length of 4cm.

After six weeks that is the end of the experiment, all blood parameters (RBCs, WBCs, haemoglobin) were showed (Table.2) and following sequence was followed.

Control <nZnO

Analysis of haematological parameters showed that fish fed with nZnOdiets had higher levels of all blood parameters studied as compared to that of control. In 1.2g of n ZnO the weight and length gain is better. So, the haematological parameters calculated on the basis of 1.2g of nZnO (Table.2)

Table 1: Initial and final weight and length gain by the administration of ZnO nanoparticles

NZnO (MG/L)	Initial weight (g)	Final weight(g)	Initial length(cm)	Final Length (cm)
0.3	1	2 g	4	6cm
0.6	1	2.06g	4	7cm
0.9	1	2.17g	4	7.5cm
1.2	1	3.13g	4	8cm
1.5	1	2.53g	4	6.8cm
Control	1	1.7g	4	4.6cm

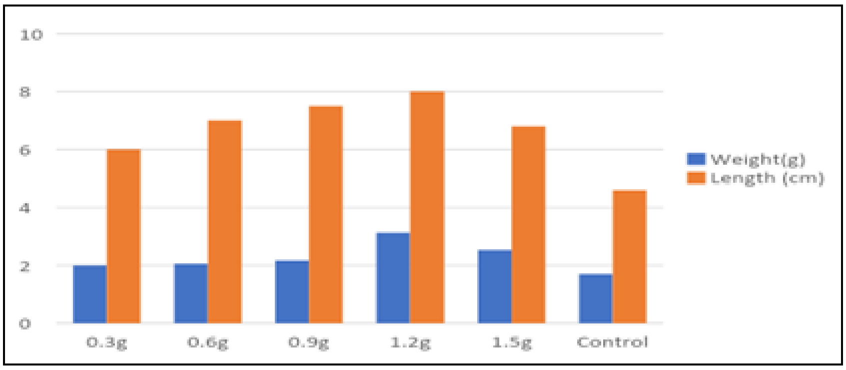

Figure 1: Weight and length gain by the administration of ZnO nanoparticles

Table 2: Hematological parameters in presence of nZnO v/s control

Feed	RBC (million/cumm)	WBC (cumm)	Hb (g/dl^{-3})
Control (1.2g)	5.48	12800	13%
nZnO (1.2g)	7.84	61300	15.4%

The presence of ZnO in feed in the Nanoform improved feed palatability thereby causing fish to take more feed than those fed control diets. Again, the small size of nZnO might have facilitated the passage of particles across tissues and cell membrane leading to improved gastrointestinal tract absorption. nZnO particle in cells would possibly mean higher stimulation of the synthesis of DNA, RNA and proteins leads to increase of body cells of the fish. nZnO can enter cells easily. Since it is considerably smaller than macro ZnO, which has trouble doing so because of its size. As a result, the effect of nZnO is more effective.

The supplemental levels up to 1.2gnZnO concentration feed showed an increase in the growth of the fish, *Catlacatla* and after that there was a decrease indicating that the fish could tolerate dietary Zn up to this level only. It is possible that above this concentration of Zn, feed starts to lose palatability and other sensory appeals thereby causing fish to feed less. Cytotoxicity and cell death resulting from higher levels of nZnO consumption may also lead to weight loss.

According to research on the role of zinc in animal growth, development, andphysiology, some fish species, including common, can tolerate high dietary Zn intake levels of 1.7 and 1.9gm per day, respectively, without experiencing any negative effects on their survival and growth. Onuegbu *et al.*, (2018) were reported that growth and health in African catfish (*Clariasgariepinus)* fingerlings can be enhanced through dietary Zn supplementation with macro and NanoZincoxide. It further revealed thatNano form of Zinc oxide is more effective than the bulk form not only in impacting on the growth andhealthofthe fish but also in bringing about economy of material, as less quantity of the nanoforms used to achieve same or even greater effects than that of bulk form. Feed consumption and feed conversion efficiency of Mrigal were higher in nZnO supplied fish. Higher specific growth rate of *Labeorohita* fingerlings fed a diet containing 20 mg ZnO-NP per kg. The assimilation and metabolism of Mrigal were increased with an increase in ZnO nanoparticles in the feed. The hematological analysis acts as a rapid and economical method for assessing growth on fishes.

Control <nZnO

It is the sequence ofbloodparameters after45 days of experimental period.The hematological parameters such as hematocrit, Hb, RBC, and WBC are used to assess the functional status of the oxygen-carrying capacity of the bloodstream. AccordiHemoglobin, RBC, Hematocrit, MCV, MCH, MCHC of Mrigal progressively increased and WBC and platelets decreased with an increase in the quantity of Zinc Oxide nanoparticles. Ashouri*et al.*, (2015) were discovered that a high quantity of selenium nanoparticles added to the meal of African catfish (*Clariusgariepinus*) increased blood parameters as compared to the control.Also, another fact that Faiz *et al.*, (2015) were suggested that high concentrations of nZnO dramatically reduce haematological parameters in young grass carp *(Ctenopharyngodonidella), while* at lower supplementation rates, they enhanced the RBC count and corpuscular haemoglobin concentration (MCHC). Protein content of each concentrations were assessed by Lowry method. In this considerable amount of increase has detected up to 1.2g of nZnO consumption that is 1.44mg/ml. After this protein content was decreased (1.5g) i.e. 1.37. Ghazi *et al.*, (2021) were conducted an experiment on synergistic effects of selenium (Se-NP) and zinc oxide (ZnO-NP) nanoparticles on Nile tilapia (Oreochromis niloticus). Fish fed with Se/Zn-NP had a higher hemoglobin, red blood cells, and globulin ($P<0.05$).In conclusion, dietary supplementation with Se-NP and Zn-NP induces synergistic effects on Nile tilapia that improve growth performance, blood health, and intestinal histomorphology. Sarkar *et al.*, (2021) were reported Nanotechnology has emerged as an innovative and effective tool in the field of fish nutrition, biotechnology, genetics, reproduction, and sustaining environmental quality etc. The application of nano-elements enriched feed has intentionally enhanced the fish growth. Emerging nano particles of mineral elements are currently applied in aquatic system to increase large scale production only limited information currently available on the toxic effect of NanoZnO in fish revealed. The possible toxic mechanisms of NanoZnO in fish are quite less understood. Zinc nanoparticles of higher doses have been studied for their potential impacts on aquatic organisms, including fish. In order to prevent its effect and enhance fish health, Nigella sativa and its oil have been recommended for aquaculture.

6. Conclusion

It is acknowledged that adding zinc sources, such as nano zinc oxide, to baseline feed at various concentrations (0.3g, 0.6g, 0.9g, l.2 g, and 1.5g) after six weeks can significantly increase the final weight and length of *Catlacatla* fish fingerlings. Additionally, there is a change in the haematological parameters such as RBCs, WBCs and haemoglobin composition up to an optimum concentration, that is control <nZnO (maximum rate at 1.2g). Also, Nano zinc oxide will increase the protein content of the fish up to a level which shows their maximum growth at its fingerlings stage. A greater surface area of nZnO will increase water (moisture) absorption.This study has shown that zinc oxide in the Nano form have higher bioavailability and could be more readily absorbed in the gastrointestinal tract of the Catla fingerlings. The presence of ZnO in feed in the Nano form improved feed palatability thereby causing fish to take more feed than control diets. Again the small size of nZnO might have facilitated the passage of particles across tissues and cell membrane leading to improved gastrointestinal tract absorption and bioavailability of Zn.

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