**The growth and immune functions improvement inthe *Oncorhynchus mykiss* Juvenile with dietary supplementation of iron oxide nanoparticles**

**Abstract:**

The increasing trend in the use of nanotechnology illustrates the need to understand the possibility of application and toxicity of nanoparticles in different aspects such as aquaculture. In the present study, the effect of Iron oxide (Fe3O4) nanoparticles (Fe-NPS) was investigated on Rainbow trout juvenile (10 ± 0.5 g) growth performance, intestine bacteria flora, and some blood and immune parameters. Fish were fed a diet supplemented with 0, 50,100, and 200 mg Fe-NPS per 1 Kg of food as T0, T1, T2, and T3 respectively for 60 days from April to May. Finally, fish final weight and length were measured to determine growth parameters, blood samples were collected to examine hematology and immunology parameters, also, total bacteria count and frequency of Lactobacillus sp. were determined in the fish intestine. Results showed that adding 0.5 mg Kg-1 Fe-NPS (T1) led to better growth performance and immune function with less negative effects. The most weight gains and specific growth rate and the less feed conversation ratio (p<0.05) were observed in a fish-fed diet supplemented with 50 mg Kg-1 Fe-NPS (T1). The most concentration of Total Protein, Albumin, C3, C4, Neutrophil, IgM, and Reactive Oxygen, and the fewer glucose levels were observed in T1. Also, no significant differences were observed in the concentration of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Creatine phosphokinase (CPK), and Blood urea nitrogen (BUN) between groups (p>0.05). The total count of bacteria and Acid lactic bacteria decreased with the dose increase of Fe-NPS. Fe-NPS at the dose of 50 mg Kg-1 could improve the growth and immune performance of rainbow trout juvenile

**Keywords**: Fe-NPS, Rainbow trout, Hematology, bacterial flora, Immunology.

**Introduction:**

Nanotechnology and nanoparticles are increasingly recognized for their potential applications in various aspects of human and animal welfare (Mohanty*et al*, 2017). Nanoparticles, by definition, are structures that have one dimension in the 1-100 nm range (Ahmed *et al*, 2010). Nanotechnology involves the application of materials at the nanoscale to new products or processes (Mohanty *et al*, 2017). The potential benefits of nanotechnology in aquaculture are considerable. According to some studies, nanoparticles of elements like selenium, iron, etc. sources supplemented in diet could improve the growth of fish (Can *et al*, 2011). Nano-Fe is a form of nanoparticles with great interest to use as a food additive in fish food but little is known about its effects on fish. Iron (Fe) is an indispensable element for the functioning of organs and tissues of higher animals, including fish, because of its vital role in physiological processes such as oxygen transport, cellular respiration, and lipid oxidation reactions (Behera *et al*., 2014). Scientists from the Russian Academy of Sciences have reported that young carp and sturgeon exhibited a faster rate of growth (30% and 24% respectively) when they were fed nanoparticles of iron (Rather *et al*, 2011). Behera *et al.* (2014) evaluated the application of different iron sources, including iron nanoparticles (nano-Fe) and ferrous sulfate (FeSO4∙7H2O), as feed additives in diets for, rohu, *Labeo rohita* H and demonstrated that different iron sources (Nano-Fe and ferrous sulfate) supplemented in the basal diet could improve the final weight and antioxidant enzymatic activities, and induce hematological and immunological parameters of Indian major carps. However, the use of nanotechnologies and nanomaterial requires careful assessment of avoiding unexpected toxicities and their adverse biological interactions. While technological research is already well established, with many transfers to industrial production, research on the safe use of nanoparticles e.g. Fe-NPS in fish is in risk assessment and has lagged significantly. For the use of nanoparticles, the standard information requirements are insufficient to assess hazards and exposure. Among a series of Nano-metals that have been widely studied like copper, zinc, titanium (Retchkiman-Schabes *et al*., 2006), and alginate (Ahmad *et al*., 2005), in the case of Fe-NPS very few experiments are reported. Therefore, in this study, we evaluated the effect of Fe-NPS as a food additive on growth performance, intestine bacteria, and some biochemical and immunological blood parameters in rainbow trout (*Oncorhynchus* *mykiss*) Juvenile to determine the appropriate dose of Fe-NPS for rainbow trout juvenile and examine the potential positive or negative effects of Fe-NPS.

**Materials and methods:**

Fishpreparation

Healthy Juvenile rainbow trout (n=720) with an average weight of 10 ± 0.5 g, were obtained from a local fish farm and acclimated for two weeks in a separate concrete pond filled with flowing running water of a wall. Water factors included: dissolved oxygen: 7±0.5 mgL−1, pH: 7.5±0.03, total ammonia: 0.1±0.05 mg L−1, nitrate: 2.2±0.2 mg L-1, nitrite: 0.02±0.01 mg L-1 and temperature, 17.2±0.01ºC.

Treatmentsdesign

Trouts were randomly distributed into 12 experimental separate raceway ponds (200×200×100 cm3) with three replicates. Fish were fed a control diet (without Fe-NPS) and three different doses of Fe-NPS (50, 100, and 200 mg Fe-NPS per kg of food) as T0, T1, T2, and T3 respectively.

FoodandFeeding

To prepare the diets, a commercial diet (with the proximate composition of 47-50% protein; 18-20% lipid; 7-9% ash; 1-1.5% phosphorus, and 1-1.5% fiber, Biomar) was grounded and mixed with the Iron oxide nanoparticles (US Research Nanomaterials, Inc., USA) at various doses. Water was used to mix the food and Fe-NPS, then the mixture was plated again and allowed to be dried for 18 h at 45 °C by air circulation and stored at 4 °C until it was used. The control diet was prepared by adding only water (Aktar et al, 2018). The approximate chemical composition of the formulated diet was determined according to standard methodology (AOAC, 2005). Biomar ingredients included fish meal, soybean meal, wheat meal, fish oil, soybean oil, wheat gluten, vitamin, and mineral premix, 12 mg manganese oxide, 75 mg ZnO, 1.8 mg iodine, and 4.4 mg copper sulfate. The control group (T0) was fed with the basal diet without Fe-NPS, while fish in T1, T2, and T3 were fed with diets supplemented with 50, 100, and 200 mg Fe-NPS/Kg respectively. Fish were fed 3 times a day at 3% of body weight for 60 days. The pond was cleaned after each feeding and fish behavior was monitored during each feeding event.

Growthperformance

To analyze the growth indices, all fish were individually weighed at the start of the experiment, and 2 months thereafter by a digital scale (bearing: 0.01 g) after they were anesthetized. Fish were starved 1 day before biometry. Based on the results of the biometry, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) were calculated according to the following formulas (Handy et al. 1999):

WG = Wt – W0 (g)

SGR = (Ln Wt - Ln W0) × 100/t

FCR = dry feed fed in (g)/ wet weight gain in (g)

SR = (Nt / N0) ×100

Here Wt and W0 are final and initial body weights (g) respectively, t is experimental days, N0 is the initial number of fish and Nt is the final number of fish.

Bloodsamplingandmeasurements

Hematology and blood plasma analysis was performed as described in Smith et al. (2007). Three fish were randomly collected from each tank on day 60. Blood was collected via the caudal vein into heparinized and non-heparinized syringes. Non-heparinized samples were placed in the refrigerator for two hour and then centrifuged (13,000 rpm for 2 min, Micro Centaur MSE, 4○C). Serum was collected with a micropipette and stored at −20 C° until analysis. Measured biochemical parameters were included AST, ALT, ALP, CPK, BUN, Glucose, Albumin, Total protein, C3, C4, IgM and reactive oxygen that were measured with commercial clinical investigation kits (CUSABIO-CSB-E12045Fh-China) using auto-analyzer (Eurolyser, Belgium) (Binaii et al, 2014; Torfi Moazenzadeh et al., 2015; Mohiseni *et al*., 2016). Also, Tissue homogenates were analyzed at the end of the experiment after 60 days to determine reactive oxygen as described in Smith et al. (2007). Heparinized samples were used for counting neutrophils (Smith et al, 2007).

Bacterialcountsof theintestine

Bacterial counts were done on day 60. Five ﬁsh from each pond were randomly selected and sacriﬁced by immersion in a solution containing clove powder (200mg L-1). After disinfecting externally with 70% ethanol, ﬁsh were eviscerated aseptically and the entire intestine was removed. Intestinal contents were collected by carefully scraping with a sterile scalpel and weighted. 1 g of each intestine was homogenized separately in 0.9 ccs of physiology serum (Brunt and Austin, 2005). Samples were diluted using sterile physiology serum to 106 CFU mL-1 .10 µL were added to plates containing tryptic soy agar (TSA, Merck, Germany) for counting the total viable bacteria (Merriﬁeld et al, 2010). Also, 100 µL were added to plates containing MRS agar media (Merck, Germany) for counting Acid lactic bacteria. Bacteria were counted after incubating aerobically at 36°C for 48 h (Nikoskelainen et al, 2003; Capkin and Altinok, 2009).

StatisticalAnalysis

Data were analyzed using SPSS (version 18) software. Considering the Shapiro-Wilk test and certificating normal distribution of data, one-way ANOVA and Duncan were used for comparing means between different treatments at the significance level of 95%. Graphs were drawn using Excel software. Results showed with mean ± standard deviation and when p<0.05, differences were significant (Yousefian et al., 2013).

**Results:**

Growthperformance

Growth parameters and survival rate of rainbow trout-fed the diet supplemented with different doses of Fe-NPS for 60 days are shown in table 1. The most WG and SGR and the less FCR were observed in T1 (50 mg Kg-1 Fe-NPS) in comparison to other groups, while no significant differences were observed in growth parameters between T2 (100 mg kg-1 Fe-NPS), T3 (200 mg kg-1 Fe-NPS) and control group. No significant differences were observed in SR between groups.

Table 1. Growth performances (Mean ± SD) of rainbow trout juvenile fed diet supplemented with Fe-NPS during 60 days.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatments  Parameters | T0 | T1 | T2 | T3 |
| Initial weight (g) | 10.5±0.6a | 10.2±0.5a | 10.4±0.5a | 10.6±0.3a |
| Final weight (g) | 52.1±4.8a | 59.1±5.9b | 54.6±4.3a | 53.3±5.4a |
| Weight gain (g) | 41.4±4.1a | 48.7±5.7b | 44.4±3.8ab | 42.8±4.8a |
| Specific growth rate (%d-1) | 2.6±0.06a | 2.9± 0.06b | 2.7± 0.04a | 2.7± 0.05a |
| Feed conversion ratio (%) | 1.8± 0.06a | 1.5± 0.07b | 1.9± 0.05a | 2± 0.05a |
| Survival rate (%) | 97.8 | 99.4 | 98.9 | 98.3 |

Biochemicalandimmunologicalbloodparameters

Biochemical and immunological blood parameters of rainbow trout juvenile fed diet supplemented with different doses of Fe-NPS are presented in table 2. The most concentration of C3 and IgM were observed in T1 and the less were in T0 (p< 0.05). The highest levels of C4, Neutrophil, reactive oxygen, TP, and Albumin were in T1 with no significant differences with T2 (p>0.05). Also, the most glucose levels were observed in T0 and T3 with a significant difference with T1 (p<0.05). No significant differences were observed in AST, ALP, ALT, CPK, and BUN levels between groups (p>0.05).

Table 2. Biochemical and immunological factors of rainbow trout feed diet supplemented with Fe-NPS during 60 days

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatments  Parameters | T0 | T1 | T2 | T3 |
| C3 (mg/dl) | 20.4 ± 0.8 a | 28.4 ±1.6 c | 25.2 ±1.1 b | 24 ± 1.4 b |
| C4 (mg/dl) | 14.3 ± 0.9 a | 23.2 ± 1.1 c | 21.9 ± 1.4 c | 17.3 ±1.3 b |
| IgM (mg/dl) | 44.06 ± 2.1 a | 55.66 ± 2.4 c | 49.56 ± 3.1 b | 48.06 ± 2.7 b |
| Neutrophil (cells×103 mm3) | 3.1±1.2 a | 7.6±0.6 b | 6.2±1.1 b | 5.7±2.1 ab |
| Reactive Oxygen (µg/g) | 0.15±0.01 a | 0.32±0.0 c | 0.35±0.02 c | 0.19±0.02 a |
| Glucose (g/dl) | 5.7 ± 0.4 b | 3.5 ± 0.3 a | 4.9 ± 0.2 ab | 5.9 ± 0.2 b |
| Total protein (g/dl) | 4.7 ± 0.2 a | 7.6 ± 0.3 b | 6.8 ± 0.4 b | 5.2 ± 0.2 a |
| Albumin (g/dl) | 0.4 ± 0.01 a | 0.7 ± 0.02 b | 0.5 ± 0.01 b | 0.3 ± 0.01 a |
| ALT (U/L) | 14.3 ± 4.5 | 16.4 ± 2.3 | 18.7 ± 3.6 | 21.3 ± 3.9 |
| AST (U/L) | 50.3 ± 10.4 | 50.7 ± 10.9 | 41.8 ± 6.8 | 35.4 ± 9.7 |
| ALP (U/L) | 15.6 ± 2.6 | 16.1 ± 3.5 | 17.2 ± 3.6 | 19.8 ± 3.5 |
| CPK (IU/L) | 29.7 ± 4.3 | 27.6 ± 4.3 | 34.8 ± 5.2 | 31.8 ± 4.4 |
| BUN (mg/dl) | 2.1 ± 0.3 | 2.3 ± 0.3 | 2.7 ± 0.4 | 2.9 ± 0.4 |

Data in each row are the mean ± SD. Data in the same row with different superscripts are significantly different (P < 0.05).

Intestinalbacterialflora

A sharp decrease in the number of Lactobacillus bacteria and an increase in the number of Aeromonas hydrophila (Pathogenic bacteria) were observed with a dose increase of Fe-NPS. So that the less of the first and the most of the second-mentioned bacteria were observed in T3. Other bacteria count decreased followed by Fe-NPS doses increase.

Table 3. Bacterial total count and the number of Lactobacillus sp isolated from the intestine of rainbow trout Juvenile, 60 days after feeding with different doses of Fe-NPS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatments  Bacteria | T0 | T1 | T2 | T3 |
| Total count of bacteria (Log cfc/g) | 9.12±0.04c | 8.21±0.08bc | 7.98±0.1b | 5.36±0.07a |
| *Lactobacillus* sp (Log cfc/g) | 7.03±0.3c | 6.7±0.03bc | 4.6±0.2b | 2.7±0.02a |

Data in each row are the mean ± SD. Data in the same row with different superscripts are significantly different (P < 0.05).

**Discussion**

Iron because of its vital role in physiological processes is an essential element for the functioning of organs and tissues of higher animals, including fish, (Hilty et al., 2011). Iron oxide nanoparticle (Fe2O3 NPS) is of great interest due to their unique physicochemical properties. It has great potential in biomedical applications, as food additives, antimicrobial additives, drug carriers, etc., (Huber, 2005). Our results showed that 50 mg kg-1 of Fe-NPS will improve growth performance and immune function of Rainbow trout juvenile with no adverse effect but higher doses of Fe-NPS are not useful.

 The most SGR and WG and the less FCR were observed in T1 in comparison to other treatments (p<0.05). Similar to our finding inclusion of Fe-NPS as a feed supplement improved the growth performance of Labeo rohita (Behera et al., 2014), Clarias batrachus (Akter et al., 2018), and Clarias gariepinus (Onuegbu Chris et al., 2018).It seems that a low dose of Fe-NPS is useful for the improvement of the growth performance of Rainbow trout juvenile but the higher dose had no positive effect on growth performance.

No significant differences were observed in the survival rate of rainbow trout juveniles. The same results were reported in Sturgeon (Prochorov et al., 2011), Labeo rohita (Behera et al., 2014), and Clarias gariepinus (Onuegbu Chris et al., 2018). Although the use of 50 mg kg-1 of Fe-NPS led to better growth performance with a high survival rate, according to our results the higher dose of Fe-NPS causes an inappropriate physiological condition in rainbow trout and perhaps fish could not tolerate a higher dose of Fe-NPS in longer time (more than 60 days) and probably survival rate will decrease.

According to our experiment, all immune systems had a response to Fe-NPS, and C4, C3, and IgM levels increased in the blood of rainbow trout. The highest levels of C3, C4, IgM, Total protein, Albumin, and Neutrophil were observed in T1. Also, the highest level of reactive oxygen was in T2 and then in T1 (p>0.05). The less of all mentioned immune parameters were observed in T0 (p< 0.05). It shows the effect of Fe-NPS in stimulating immune function in Rainbow trout especially at the dose of 50 mg kg-1. Also, higher doses (100 and 200 mg kg-1) cause stress in fish based on the increases observed in glucose (p< 0.05), ALT, and ALP and decrease in AST (p> 0.05).

Neutrophils play a crucial role in fish immune responses (Anisworth, 1992). Similar to our finding, an enhancement was reported in Nile tilapia (Oreochromis niloticus) neutrophil percentage after feeding with Fe-NPS (El-shenawy et al., 2019). Productions of oxygen free radicals by neutrophils via respiratory burst are important events in bactericidal pathways in fish (Sharp et al., 1993) and superoxide anion is the first product to be released from the respiratory burst; therefore, measurement of O2 is an accurate method of measuring this activity (Secombes, 1990). Similar to our finding, an increase in respiratory burst activity followed by adding iron oxide nanoparticles to the Indian major carp (Labeo rohita) diet was reported (Behera et al., 2014).

Total proteins in the plasma include albumin and globulin. An increase in the levels of serum protein, albumin, and globulins in fish is thought to be associated with a stronger innate immune response (Wiegertjes et al., 1996). An increase in protein level could be due to the increased protein synthesis in the liver; an important function of serum proteins in the maintenance of osmotic balance between blood and tissue spaces (Awad et al., 2019). Similar to our finding, an increase in total protein and albumin was recorded in other fish species such as (L. rohita) (Behera et al., 2014), Begrudge catfish (Clarias batrachus) (Akter et al., 2018), Catfish (C. gariepinus) (Onuegbu Chris et al., 2018) and Nile tilapia (Oreochromis niloticus) (El-shenawy et al., 2019). In addition, it was reported that iron oxide nanoparticles can compromise subsequent antigen-specific immune reactions, including immunoglobulin productions and T cell responses (Shen et al., 2011). In our research, IgM levels like TP were improved by dietary supplementation of Fe-NPS in a dose of 50 mgKg-1, resulting in an improvement in fish immunity. The immunoglobulin M (IgM) is a tetrameric protein with four sites for antigen recognition well known in fish (Biller-Takahashi and Urbinati, 2014). It is the principal immunoglobulin that can evoke an effective specific humoral response in the teleost (Racine and Winslow, 2009). On the other hand, C3 and C4 are two components of the innate system that adding Fe-NPS into the diet of rainbow trout juveniles increased C3 and C4. The complement system is widely used as an immune status indicator due to its contribution to host protection (Awad et al., 2019). In the present study, alteration in measured immune parameters showed that Fe-NPS stimulate rainbow trout juvenile immune system and the most effective dose is 50 mg kg-1.

No significant differences were observed in liver enzyme levels (AST, ALP, and ALT) (p>0.05). However, with a dose increase of Fe-NPS, an increase in ALT and ALP and a decrease in AST were observed. An increase in liver enzyme levels due to the use of the Nano-Fe diet and cooper was recorded in C. batrachus and C. gariepinus respectively (Akter et al., 2018; Zaghloul et al., 2006). In another research, AST and ALT levels showed a disturbance in O. niloticus fed a diet supplemented with different doses of Fe-NPS (El-Shenawy et al., .2019). Changes in liver enzyme activity indicate liver damages that can be altered by infection, toxin, or any type of injury (Pascual et al., 2003). AST, ALP, and ALT could be used as sensitive biomarkers in ecotoxicology, they provided an early warning of potentially hazardous alterations in contaminated aquatic organisms (Levesque et al., 2002; Nel et al., 2009). Based on our results, Although no significant differences were observed in liver enzymes in different treatments it seems that lower doses of Fe-NPS did not cause liver damage and higher dose would be toxic in Rainbow trout juveniles (p>0.05) (Table 2).

In addition, BUN and CPK levels increased with the dose increase of Fe-NPS, but no significant differences were observed in the control and Fe-NPS groups. The increases in serum BUN concentrations have frequently been used in fish as an indicator of gill and kidney dysfunction (Adham *et al*., 2002; Yang and Chen, 2003). CPK is used to diagnose and monitor liver, kidney, and heart disease (Mohiseni *et al.*, 2016), although it can be elevated during stress (Huang *et al*., 2010). Alterations in the activity of CPK are a sensitive biomarker for damage to cell membranes (Gholizadeh Zare Tavana *et al*., 2018). Similar to our finding, high BUN has been reported in fish exposed to metals, pesticides, and nitrate (Adham *et al*., 2002; Chen *et al*., 2004; Mutlu *et al*., 2015). Also, the increases in serum CPK concentrations were reported in common carp (*Cyprinus carpio*) exposed to titanium dioxide nanoparticles (Banaee *et al*., 2019), Cadmium, and Lead (Mohiseni *et al*., 2016). Although no significant differences were observed in AST, ALT, ALP, BUN, and CPK enzymes, their higher levels in higher doses of Fe-NPS show the potential toxic effect of a higher dose of Fe-NPS on rainbow trout juveniles. The glucose level in T1 was significantly lower than in T0 and T3 (p<0.05), but it was not significantly different from T2 (p>0.05). Iron deficiency (in T0) does not meet the fish's needs and excess Fe-NPS (in T2 and T3) will be harmful and both conditions can cause stress and possible toxicity. Therefore, in fish treated with the appropriate dose of Fe-NPS (T1), the lowest glucose levels were observed. Su Kyoung and colleagues (2008), stated blood glucose level increases with sustained stress. Fish immediate responses under stress conditions are recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNS) and the release of stress hormones, cortisol, and catecholamine (adrenalin and nor-adrenalin) into the bloodstream (Randall and Perry, 1992). Secondary responses occur as a consequence of the released stress hormone, causing changes in the blood and tissue chemistry e.g. an increase in plasma glucose (Begg and Pankhurst, 2004). This entire metabolic pathway produces a burst of energy to prepare the fish for an emergency (Rottman et al., 1992). Therefore, supplementing the rainbow trout juvenile food whit an appropriate dose of Fe-NPS (50 mg kg-1) helps to fish welfare.

In this research, the total count and the number of lactic acid bacteria decreased with the dose increase of Fe-NPS but no significant differences were observed in T1 and control groups. According to different researches, it seems that bacterial flora changes are due to the direct effect of Fe-NPS on intestine bacteria flora or indirectly due to biochemical and immunological changes due to Fe-NPS consumption in the fish diet that affects fish health conditions. Results of bacterial flora of fish intestine in the different groups show the toxic effect of the high dose of Fe-NPS. Adams et al. (2006) reported that ZnO is toxic for Bacillus subtilis. Experiments on embryonic zebrafish demonstrated similar results (Zhu et al., 2008). Adams et al. (2006) found that bacterial growth inhibition induced by nano-ZnO is due to some undetermined mechanisms that are responsible for toxicity. A similar opinion was proposed by Zhu et al (2008). Therefore, it seems that a higher dose of Fe-NPS is toxic for rainbow trout juveniles but adding 50 mg kg-1 Fe-NPS has no unsuitable effects. On the other hand, the size of nanoparticles is a considerable factor. Panacek et al. (2006) found high antimicrobial and bactericidal activity of silver nanoparticles on Gram-positive and Gram-negative bacteria. They reported the antibacterial activity of silver nanoparticles is size-dependent, the nanoparticles of size 25 nm possessed the highest antibacterial activity. In our study, the size of Fe-NPS was in the range of 40-60 nm. Oberdorster et al., 2005 stated that when the size of particle decreases and the proportion of the surface area increase to the nanoscale particle it tends to show abnormal biological activity, particularly biochemical toxicity compared to micron-sized one. Mohammadi and Tukmechi, (2015) studied the effects of iron nanoparticles in combination with Lactobacillus casei on probiotic counts in rainbow trout (Oncorhynchus mykiss) intestine. Intestine bacterial counts increased by using Iron nanoparticles in combination with Lactobacillus casei but no L. casei were found in the control group (no L. casei and no Fe-NPS) and groups that fed by Fe-NPS without probiotic (L. casei). Also, they did not observe significant differences in lactic acid bacteria number in the control group and fish that received Fe-NPS in food which is similar to our finding for low used dose (T1). There are different ways of affecting metal oxide-NPS on fish species (useful or harmful), but it seems if there are some inappropriate effects, there are also methods to help cope with them. Therefore, we suggest examining the combined effect of the low dose of Nano-particle with some probiotics, antioxidants, or other additives to increase the advantages of Nano metals.

We conclude that Fe-NPS appropriate dose could improve growth and immune performance but an inappropriate dose will induce stress and damage to the rainbow trout juvenile organs. Therefore, it is suggested that further evaluation should be made concerning the benefits and risk assessment of the use of Fe-NPS on aquatic life.

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