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# Best Choice for Using the Nanoparticles Additives to Enhance the Growth of the Cultured Nile Tilapia *Oreochromis niloticus*

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**ABSTRACT**

Minerals deficiency in aquatic animals disturbs the biological manners and physiological functions. Wheras, nanoparticles play an important role in aquaculture, as they serve as essential minerals to increase fish growth and supplementation in feeds since many practical feedstuffs contain low levels of these microminerals. Dietary supplementation of nanoparticles produces greater survival, growth, antioxidant levels and immunity of aquatic organisms including fish. In this study, the diet of the Nile tilapia *Oreochromis niloticus* was supplemented with different levels of selenium nanoparticles (SeNPs), magnesium (MgNPs), and a mix of both. Moreover, the growth, survival and body vitality were evaluated after 50 days of feeding. During the experiment, the fish were fed diet of 30% crude protein twice a day. SeNPs, MgNPs, and a mix of both were added to the control diet at different levels of 0.0 (control). The diet consisted of different concentrations: 1 (T1 Se, T1 Mg & T1 mix), 3 (T2 Se, T2 Mg & T2 mix), 5 (T3 Se, T3 Mg & T3 mix), 6 (T4 Se, T4 Mg & T4 mix), and 7mg/ kg (T5 Se, T5 Mg & T5 mix) diet to feed the Nile tilapia fingerlings whose average initial weight was 4.0± 1.0g. After a 2-week acclimation period, fish were randomly distributed into 16 glass tanks, each with a volume of 40 L, at a density of 25 fish per tank. The results indicate that the growth indices, feed efficiency and survival rate were significantly enhanced ($P < 0.05$) by incorporating a mix of Se-Mg NPs at a concentration of 6mg/ kg diet. In conclusion, the supplementation of both SeNPs and Mg NPs together in the diet of *O. niloticus* showed a higher growth rate than those which fed by each NPs separately. These findings have beneficial implications for the performance of the fish farming industry.

## INTRODUCTION

The fisheries and aquaculture industries are one of the most important sources of animal protein for human feeding **(Maulu *et al.*, 2021; Eissa *et al.*, 2022)**. The Nile tilapia *Oreochromis niloticus* is one of the highest cultured fish in Egypt and worldwide **(Eissa *et al.*, 2022; Khalil *et al.*, 2022)**. It is among the most important farmed freshwater fish species due to its nutritional value, fast growth, and resistance to opposed environmental conditions. Tilapia production is one of the world's fastest-expanding aquaculture sector **(Khalil *et al.*, 2021; Hendam *et al.*, 2023)**. Egypt produced 15.4% of the world's tilapia production, totalling 6,510,700 ton in 2017 **(El Basuini *et al.*, 2021)**. Due to the increasing demand for animal protein, intense agricultural actions are required to obtain the anticipated level of production **(Mansour *et al.*, 2021)**. As aquaculture is subject to exotic, endemic pathogens (mainly parasites and bacteria), climate changes and environmental disturbances, evolving resistant tilapia that can combat infectious diseases and attain better growth rates is of significant importance **(Kord *et al.*, 2021)**. The conception of well-designed diets is currently being engaged in the food industry for the production of animals since diets must meet nutritional necessities, as well as support the health of cultivated animals such as fish **(El Basuini *et al.*, 2021)**. A nutrient-rich aqua feed should contain minerals and vitamins, as well as protein, lipids, and carbohydrates **(Van Doan *et al.*, 2020)**. Trace minerals are also associated to numerous physiological, metabolic and functional features of an organism's body **(Khan *et al.*, 2017)**. Immuno-nutrition involves the use of fish additives and/or essential trace elements to enhance their growth, antioxidant defences, and immunological responses **(Eissa *et al.*, 2023)**.

Nanotechnology delivers exceptional ways to raise the safety, absorption and bioavailability of chemicals in aquaculture due to their small size and large surface area **(Khalil *et al.*, 2023)**. Moreover, it provides major opportunity for the economy and sustainable development of aquatic resources in many countries. Although the application of nanotechnology is at a very early stage in aquaculture, it may have the potential to solve most of the problems in aquaculture and fisheries with better practical innovation at different levels. Nanotechnology is a rapidly progressing science of small bodies with increased reactivity and solubility and represents a new enabling technology suitable for biomedical, pharmaceutical, and food system applications **(TatliSeven *et al.*, 2018)**. Furthermore, it provides a rapid disease detection and an improved fish drug absorption. In aquaculture, it is used in fish biotechnology, genetics, and reproduction. Additionally, nanomaterials may inhibit and treat fish diseases **(Rather *et al.*, 2011; Korni & Khalil, 2017)**. The application of nanotechnology is an emerging but very promising technological advancement in the aquaculture industry. In particular, the application of nanoparticles and emulsion-based systems have been reported in the production of aquafeeds **(Shakibaie *et al.*, 2015; Abdel-Tawwab *et al.*, 2019)**. Numerous studies have shown that nanoparticles, such as Fe, FeO, CuO, Zn, ZnO, Se, Mn, and Mg enhance antioxidant activity by raising antioxidant enzyme levels and reducing oxidative stress **(Talas *et al.*, 2008; Dawood *et al.*, 2019; Khalil *et al.*, 2019; Rajan *et al.*, 2021)**.

The present study was conducted to evaluate the impact of adding some nanoparticles to the Nile tilapia diet on its growth and survival. It also showed valuable insights by adding these particles to enhance the fertility and growth of the cultured tilapia.

## MATERIALS AND METHODS

### Experiemental fish

The Nile tilapia fingerlings were bought from the Tilapia Fish Hatchery of the Central Laboratory for Aquaculture Research (CLAR) in Abbasa. They were safely transported to the laboratory in plastic bags, half filled with hatchery water and well-oxygenated. The fish were acclimatized in Fish Population Dynamics Laboratory, National Institute of Oceanography and Fisheries, Suez Branch in glass tanks (70×40×40cm) for two weeks. During the acclimatization period, specimens were fed with control feed three times daily, and two third of aquarium's water was renewed daily in the early morning.

### Feeding experiement

In the present study, 16 groups of *O. niloticus* fingerlings (3.5± 0.15cm length; 4.0± 1.0g weight) were assigned for this experiment for 50 days. One group served as control and was fed with '0' concentration of Se, Mg NPs supplemented diet. The remaining 15 groups were fed with 1, 3, 5, 6 and 7mg/ kg supplemented diets, respectively. Each group consisted of 25 fingerlings in an aquarium maintained with 40L of ground water. The water medium was changed every 24h by a siphoning method, with a minimal disturbance to the fish and an adequate aeration. The experimental tilapia were fed with 7% of body weight twice per day. During the feeding trial, the unconsumed feed and feces were removed on a daily basis while renewing the aquarium water.

### Water quality parameters

The water quality parameters, such as temperature (25- 26$^{o}$C), pH (7.5- 7.7), total dissolved solids (TDS), turbidity, alkalinity, determination of chloride, sulfate, nitrite, magnesium, and calcium were monitored frequently using standard methods **(Uddin *et al.*, 2014)**.

### Fish performance

Five fish per aquarium were collected, washed, and weighed for the final body composition at 25°C. Performance traits, including weight gain (WG), percentage specific growth rate (SGR) per day, total feed intake (TFI), feed conversion ratio (FCR), and percentage survival rate (SR), were calculated according to the following equations:

WG (g) = $W_2 - W_1$

SGR% (Specific growth rate) = 100 ($LnW_2 - LnW_1$) / T

TFI (g feed/fish) = The total amounts of fish diets / fish number throughout the experimental period

FCR (feed conversion ratio) = feed given (dry weight)/total wet weight gain.

SR(% ) = 100*Fish numbers at the end of the experiment / Fish number at the beginning of the experiment

Where, $W_1$ is the initial weight, $W_2$ is the final weight, T is the number of days that were recorded during the feeding duration (50 days).

## RESULTS AND DISCUSSION

In recent years, nanotechnology can be used for improving the biochemical and physiological responses (including growth performance, enzyme activity, and immunity) of fish **(Khan *et al.*, 2016; Akbary & Jahanbakhshi, 2019)**. The growth performance of aquatic animals depends on several factors, such as well management, water quality, vaccination, and temperature **(Dawood, 2021)**. Moreover, nutritionally balanced aquafeed is another vital factor associated with improving the feed digestibility and thereby the health condition and growth performance of finfish species **(Mugwanya *et al.*, 2021)**. Optimum feed formulations should contain both macro and microelements to fulfill the basic requirements of finfish species. Over or low levels of these elements cause impaired metabolic and physiological functions and led to malnutritional features. Tilapia culture is one of the fastest- growing aquaculture commodities in the world's food production industry. Egypt is the third largest tilapia producer, after China and Indonesia. Egypt produced 15.4% of the 6,510,700 tons of global tilapia production in 2017 **(FAO, 2018)**. Demand for animal protein is increasing, therefore intensive cultivation activities are needed to reach the targeted production level.

## 1. Water quality indices

All estimated water quality parameters were within the normal range. All these parameters (temperature $T^0C$, salinity ppt, pH, DO mg/ L, $NH_4$, $NO_2$ and $NO_3$) were kept in the normal and constant values in different treatments. The values were: T between 25 and $26^0C$, salinity between 1.9 and 2ppt, pH from 7.5 to 7.7, DO around 5.9mg/ L, $NH_4$ was 0.3, $NO_2$ was 0.02, and $NO_3$ was 0.2.

## 2. Growth performance and nutrient utilization

The growth performance and nutrient consumption of the Nile tilapia fed diets fortified with selenium nanoparticles, magnesium nanoparticles and a mix of both for 50 days are presented in Tables (1, 2, 3). There were significant differences in the final body weight, weight gain, SGR, and FCR ($P< 0.05$) when compared to the fish fed basal diet, while no significant difference ($P> 0.05$) was noticed in the survival of the fish. The FCR of the fish fed Se NPs, Mg NPs and Se-Mg NPs based diets decreased with the increase in the inclusion levels.

Selenium is an essential trace element in human and animal nutrition and it is closely associated with health **(Luo *et al.*, 2010)**. It can protect cells from $H_2O_2$ and reactive oxygen species (ROS)-induced damage, detoxify heavy metals, regulate immune and reproductive systems, and bolster resistance against pathogenic bacteria. An appropriate amount of selenium in fish feed can promote growth and improve the feed conversion rate and body protein content in fish **(Rayman, 2000; Su, 2008)**. However, the toxic threshold for inorganic selenium is very narrow **(Kieliszek & Blazejak, 2016; Qiu *et al.*, 2018)**.

The present results revealed that there is no significant difference among the different treatments and the control in respect to the initial weight, while there is a significant difference ($P<0.05$) in the final weight of T3, T4 and T5 compared with the control (Table 1). Both of FCR and survival rate were not impacted by the different treatments after 50 days experiement.

**Table 1.** Growth indices with selenium supplementation (mean ± SD)

| Parameter | Control | T1: 1 mg/Kg | T2: 3 mg/Kg | T3: 5 mg/Kg | T4: 6 mg/Kg | T5: 7 mg/Kg |
|---|---|---|---|---|---|---|
| **Initial weight g** | 4.0±1.0 g | 4.3±1.1 g | 4.1±0.92 g | 4.0±1.0 g | **4.0±1.0 g** | 4.0±1.0 g |
| **Final weight g** | 23.5±1.7 g | 26.3±1.5 g | 30.5±1.5 g | 33.9±1.6 g | **35.4±1.7 g** | 26.2±1.5 g |
| **Weight gain g** | 19.5 | 22.0 | 26.4 | 29.9 | **31.4** | 22.2 |
| **Feed intake g/day** | 7.0 | 7.5 | 7.2 | 7.0 | **7.0** | 7.0 |
| **FCR g/g** | 17.9 | 17.0 | 13.6 | 11.7 | **11.1** | 15.8 |
| **SR%** | 100 | 100 | 100 | 100 | **100** | 100 |
| **SGR** | 3.54 | 3.62 | 4.01 | 4.27 | **4.36** | 3.76 |

The biochemical role of magnesium in intermediary metabolism, neuromuscular transmission, bone and scale mineralization, osmoregulation and respiratory adaptation of freshwater fishes has been documented **(Lall, 1989)**. Magnesium deficiency symptoms have also been identified for a number of fish species; these include anorexia, sluggishness, reduced growth, reduced bone magnesium concentrations and high mortality. In the present study, the growth performance and feed utilization in terms of final weight (g), weight gain (g), SGR (% /day), FCR and survival rate of the tilapia fingerlings recorded were significantly higher ($P < 0.05$) in T3, followed by T4, then T2, T1, and T5 diets than in the control group (Table 2). The survival rate was not significant ($P > 0.05$) in different treatments with Mg nanoparticles, but with the high Mg-concentrations, some fish suffered from abnormal symptoms, and one fish died in T4, while two fish died in T5. Generally, a dietary Mg content of 5mg $kg^{-1}$ was adequate for the optimum performance of the Nile tilapia species (Table 2).

In the previous studies, the optimal magnesium concentration for the Nile tilapia cultures varies depending on the specific conditions. In freshwater, the optimum dietary magnesium requirement for tilapia is around 0.2g Mg $kg^{-1}$ diet **(Yu-Hung *et al.*, 2013)**. However, in seawater, dietary magnesium beyond 0.025g Mg $kg^{-1}$ is required for tilapia **(Dabrowska *et al.*, 1989)**. Another study found that a dietary magnesium content of 0.59- 0.77g $kg^{-1}$ was adequate for the optimum performance of tilapia **(Dabrowska *et al.*, 1989)**. Additionally, the form of magnesium supplementation can also affect the growth. Tilapia fed a diet supplemented with magnesium acetate showed the highest growth rate, followed by magnesium oxide and magnesium sulfate **(Dato-Gajegas *et al.*, 1996)**. Therefore, the optimal magnesium concentration for the Nile tilapia cultures depends on the water environment (freshwater or seawater) and the form of magnesium supplementation. Hence, further experiements based on the water environment and the form of magnesium were needed to detect the best conditions to apply this supplementation to achieve the highest growth rate without any deformation or mortalities in the cultured fish.

**Table 2.** Growth indices with magnesium supplementation (mean ± SD)

| Parameter | Control | T1: 1 mg/Kg | T2: 3 mg/Kg | T3: 5 mg/Kg | T4: 6 mg/Kg | T5: 7 mg/Kg |
|---|---|---|---|---|---|---|
| **Initial weight g** | 4.0±1.0 g | 3.9±0.95 g | 3.9±1.0 g | **4.3±1.1 g** | 4.1±1.2 g | 4.2±1.1 g |
| **Final weight g** | 23.5±1.7 g | 23.9±1.5 g | 25.5±1.4 g | **31.5±1.6 g** | 29.6±1.5 g | 24.1±1.3 g |
| **Weight gain g** | 19.5 | 20.0 | 21.6 | **27.2** | 25.5 | 19.9 |
| **Feed intake/day g** | 7.0 | 6.8 | 6.8 | **7.5** | 7.2 | 7.3 |
| **FCR g/g** | 17.9 | 17.0 | 15.7 | **13.8** | 14.1 | 18.3 |
| **SR%** | 100 | 100 | 100 | **100** | 99 | 97 |
| **SGR** | 3.54 | 3.62 | 3.75 | **3.98** | 3.95 | 3.49 |

To assess the efficacy of each mineral used individually or mixed together, a mix of both Se-Mg NPs (Table 3) with different concentratios was utilized in 5 treatments. The results were fruitful and the growth rates were higher than the usage of each mineral separately, and T4 yielded the most favorable outcomes (Table 3).

**Table 1.** Growth indices with selenium+magnesium supplementation (mean ± SD)

| Parameter | Control | T1: 1 mg/Kg | T2: 3 mg/Kg | T3: 5 mg/Kg | T4: 6 mg/Kg | T5: 7 mg/Kg |
|---|---|---|---|---|---|---|
| **Initial weight g** | 4.0±1.0 g | 4.1±0.9 g | 4.1±1.1 g | 4.2±1.1 g | **4.0±1.0 g** | 4.3±1.11 g |
| **Final weight g** | 23.5±1.7 g | 27.2±1.7 g | 31.1±1.5 g | 38.6±1.7 g | **49.8±2.1 g** | 39.1±1.9 g |
| **Weight gain g** | 19.5 | 23.1 | 27.0 | 34.4 | **45.8** | 34.8 |
| **Feed intake/day g** | 7.0 | 7.2 | 7.2 | 7.3 | **7.0** | 7.5 |
| **FCR g/g** | 17.9 | 15.6 | 13.3 | 10.6 | **7.6** | 10.8 |
| **SR%** | 100 | 100 | 100 | 100 | **100** | 100 |
| **SGR** | 3.54 | 3.78 | 4.05 | 4.44 | **5.04** | 4.41 |

## CONCLUSION

In conclusion, dietary selenium and magnesium supplementation enhances the growth performance of the Nile tilapia fingerlings. Based on the growth performance, the optimal dietary Se level for the Nile tilapia fingerlings was found to be 6mg $Kg^{-1}$, while the optimal Mg level was 5mg $kg^{-1}$. These levels were determined for fish fed with varying dietary selenium and magnesium supplementation. The mix of the two NPs greatly accelerate the fish growth, moreover there is a significant difference between the growth indices in the different steps of the experiement (control, SeNPs, and Mg NPs). Therefore, the use of the mix is regarded the best choice to enhance the growth of the Nile tilapia in the fish farms. Moreover, more detailed studies about the best percentages of the mix and about the another nanoparticles should be conducted to get the highest benefit during the culture periods and growth rates.

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