



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(12): 377-383
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www.thepharmajournal.com
Received: 04-10-2023
Accepted: 19-11-2023

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Effect of supplementation of zinc nanoparticles on growth, feed utilisation, survival and carcass composition in Nile tilapia (*Oreochromis niloticus*) fry

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Abstract

The present study was carried out to evaluate the effect of Supplementation of Zinc Nanoparticles on Growth, Feed Utilisation and Survival on Nile Tilapia (*Oreochromis niloticus*) fry. The experiment was carried out for 60 days. The growth parameters were measured at fortnightly (15 days). Total four experimental diets were formulated with 40% protein. One was control diet and in other three zinc nanoparticle was incorporated in different concentration of 10 mg/kg, 20 mg/kg and 30 mg/kg. The result of the present study showed that up to 20 mg/kg (T2 treatment) the growth of tilapia fry has shown to increase. Whereas in T3 treatment (30 mg/kg) it has not shown increase in comparison to T2 treatment (20 mg/kg). Survival did not show any significant difference among the treatments. In the case of carcass composition, incorporation of zinc nanoparticle did not show any significant difference within treatment. Hence, it can be inferred that providing a supplement up to 20 mg/kg can have a beneficial impact on the growth of Nile tilapia (*Oreochromis niloticus*) fry.

Keywords: Nile tilapia, *Oreochromis niloticus*, zinc nanoparticle, fry

Introduction

In 2018, global fish production reached its pinnacle at approximately 179 million tonnes, with aquaculture accounting for 48% of this total, and 52% when excluding non-food purposes like fishmeal and fish oil (Anon, 2021) [1]. The total value of fisheries and aquaculture production in 2018 was estimated at USD 401 billion, with aquaculture contributing USD 250 billion to this sum. Capture fishery production had remained relatively stable since the late 1980s, making aquaculture the driving force behind the impressive growth in the availability of fish for human consumption. Over the past 25 years, the aquaculture industry has experienced an average annual growth rate of 8%. From 1961 to 2015, global food fish consumption increased by 3.2% annually, surpassing both population growth (1.6%) and meat consumption from terrestrial animals (2.8%). On a per capita basis, food fish consumption rose from 9.0 kg in 1961 to 20.2 kg in 2015, marking a consistent 1.5% yearly growth (Anon, 2021) [1]. Preliminary estimates for 2016 and 2017 suggest further increases, emphasizing the pivotal role of fish in providing nearly 20% of the average per capita animal protein intake for around 3.2 billion people. With the world population expected to reach 9 billion, aquaculture, as the fastest-growing food sector, bears the responsibility of meeting the growing demand for food (Anon, 2021) [1].

Nutrition assumes a vital role in the growth and sustenance of animals. Organisms with superior nutrition not only exhibit higher quality but also experience better growth. This is a fundamental factor contributing to the substantial cost of feed, which constitutes a significant portion, ranging from 30% to 70%, of the operational expenses in aquaculture, depending on the species (Rumsey, 1993) [24]. As the aquaculture industry expands, there is an associated surge in the demand for aqua feed. The principal challenge in the development of aquaculture is the consistent, efficient, and cost-effective provision of feed.

Tilapia, a term encompassing several fish species, is a significant player in the world of aquaculture and food production. These versatile fish belong to the Cichlidae family and are cultivated for their meat in nearly 100 countries, establishing them as a cornerstone of modern aquaculture practices (Fitzsimmons, 1997, 2000) [9, 10]. What makes Tilapia particularly attractive for farming is their omnivorous nature, meaning they can thrive on a diverse diet, often lower down the food chain.

The primary genus of Tilapia commonly raised in aquaculture is *Oreochromis*, which includes varieties like Nile Tilapia (*Oreochromis niloticus*), Mozambique Tilapia (*Oreochromis mossambicus*), and Blue Tilapia (*Oreochromis aureus* and *Oreochromis urolepis hornorum*) (Fitzsimmons, 1997) [9]. One standout member of the Tilapia family is Nile Tilapia (*Oreochromis niloticus*), celebrated for its suitability in intensive and super-intensive farming systems due to its rapid growth rate and resilience in challenging environmental conditions (Dawood *et al.*, 2021) [5]. This species thrives mainly in freshwater environments and is farmed in numerous countries across the globe. However, it's worth noting that these waters can sometimes be exposed to a range of pollutants and toxins, making the management of Nile Tilapia farms a task of both promise and caution (Dawood *et al.*, 2021) [5].

Nanotechnology has huge application in aquaculture fields like nanomaterials, nanosensors, DNA nanovaccines, Gene delivery and smart drug deliver. Currently over 300 nanoparticle products are available. Nanotechnology involves the application of materials at nano scale to new products or processes. It is a rapidly growing industry currently. There are several definitions of nano materials, but it is generally agreed that they have at least one dimension <100 nm (Masciaglioli and Zhang, 2003, Roco, 2003) [17, 23] or have a primary size in the 1–100 nm range (Schenir, 2007) [25]. For fish health in aquaculture, nano technological applications include antibacterial surfaces in the aquaculture system, nano delivery of veterinary products in fish food using porous nanostructures, and nano sensors for detecting pathogens in the water. The nanoparticles (NPs) are being used in different forms and shapes such as nanospheres (Donbrow, 1991) [6], nanocapsule (Torchilin, 2006) [29], carbon nanotubes (Reilly, 2007) [22], dendrimers (Aulenta *et al.*, 2003; Gillies and Frechet, 2005; Wu *et al.*, 2015) [4, 11, 30]. Undoubtedly nutraceuticals are known to play a significant role in scaling up growth and immunological parameters in fish. It was found that adding 1 mg of nano Selenium (Se) per kg of diet showed significant improvement in common carp (*Cyprinus carpio*); growth and antioxidant defence system as compared to the control ones (Ashouri *et al.*, 2015) [3]. Also, selenium (Se), zinc (Zn) and manganese (Mn) NP supplementation in early weaning diets improved stress resistance and bone mineralization of gilthead seabream (*Sparus aurata*) (Izquierdo *et al.*, 2017) [12]. Diet supplemented with iron NPs and *Lactobacillus casei* as a probiotic significantly improved growth parameters in rainbow trout (Mohammadi and Tukmechi, 2015) [18]. Zinc (Zn) is an essential trace mineral that is required for growth and metabolism of all vertebrates including fish. It is needed in more than 1000 structural, catalytic and regulatory proteins, which are important for growth, development and physiology of animals (Eide, 2006; Maret and Krezel, 2007) [7, 16]. Among the nanoscale metal oxides, ZnO nanoparticles have the third highest global production after TiO₂ and SiO₂ nanoparticles (Piccinno *et al.*, 2012) [20]. The retardation of bone growth due to deficiency of

Zn also proves its importance in the growth and mineralization of bone tissues (Liang *et al.*, 2012) [15].

Because of all the reasons above, present research was carried out to evaluate the supplementation of zinc nanoparticle in the diet of Nile tilapia fry. This study is trying to understand how nano particles of zinc, called zinc nanoparticles, specifically in the form of zinc oxide, affect the growth, feed performance, and survival of fry of *Oreochromis niloticus*. Using this one can substitute fish meal in the diet of *O. niloticus* fry.

Materials and Methods

Experimental setup

The experiment took place at the Inland Fisheries Research Station, JAU, Junagadh, and spanned a duration of 60 days. For the experiment, rectangular plastic aquariums measuring 2 × 1 × 1 feet were used. A total of 12 such aquariums were filled with 30 liters of filtered and disinfected freshwater. The experimental fish chosen for the study were Nile tilapia (*Oreochromis niloticus*) fry, which were obtained from a commercial hatchery. They were carefully transported in oxygenated polythene bags and introduced to the Inland Fisheries Research Station (IFRS), College of Fisheries Science, Junagadh. Here, the fry were acclimatized in circular FRP tanks with proper aeration and feeding. The experiment was conducted during the year 2020-21, and the experimental design involved randomly selecting the *O. niloticus* fry and distributing them across three different experimental groups with ten treatments in three replications. This distribution followed a completely randomized design (CRD), and the overall experiment lasted for a duration of 60 days.

Experimental feed: The fry of *O. niloticus* has protein requirement of 40% in their diet (Rathore and Yusufzai, 2018) [21]; considering the protein requirement at this (40%) constant, a total of four experimental diets were prepared zinc nanoparticles (in the form of ZnO) nanoparticle supplementation each with three treatments (10 mg/kg, 20 mg/kg and 30 mg/kg) and one common control for all three (Table- 1). Experimental diets were formulated following Pearson Square Method (Pearson and Tauber, 1984) [19].

Each tank was equipped with a plastic cover to prevent fish from leaping out. Prior to commencing the experiment, the fish were fed a control diet for a period of 10 days. No deliberate efforts were made to manipulate or regulate the environmental conditions, which remained consistent throughout the entire experiment. Fish weight measurements were taken at 15-day intervals to monitor growth, following an overnight fasting period. Water quality parameters were assessed on a weekly basis throughout the experimental duration.

To maintain a hygienic environment, the experimental tanks were manually cleaned, and siphoning was performed daily to eliminate excess feed pellets and residual fecal matter. The removed water was replaced with an equal volume of clean water. This maintenance routine was diligently carried out over the 60-day experimental period.

Table 1: Composition of experimental diet prepared by using ZnO as zinc nanoparticle

	Diet prepared with 40% protein			
	T0 (control)	T1 (10 mg/kg zinc)	T2 (20 mg/kg zinc)	T3 (30 mg/kg zinc)
Fish meal	74	74	74	74
Tapioca Powder	10	10	10	10
Wheat Flour	10	10	10	10
Plant oil	2	2	2	2
Fish oil	2	2	2	2
Vitamin and Mineral	2	2	2	2
Zinc nanoparticles	0	10 mg/kg	20 mg/kg	30 mg/kg

Growth parameters

Sampling was done at 15 days interval to examine the growth of experimental fish. Fishes were starved overnight before taking the weight. The weighing was done using an electronic balance.

Percentage weight gain

The weight gain (%) was estimated according to El-Rhman *et al.* (2009) [8]. The percentage weight gain was calculated using the following formula:

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Specific Growth Rate (SGR)

SGR (specific growth rate) as a percentage was calculated according to El-Rhman *et al.* (2009) [8] using the formula given below.

$$\text{SGR} = \frac{\ln W_2 - \ln W_1}{T} \times 100$$

Where,

T₁ number of days in the experiment and

W₂ = Final body weight of *O. niloticus*,

W₁ = Initial body weight of *O. niloticus*

Food conversion ratio (FCR)

FCR is the amount of food intake divided by wet weight gain. The FCR (Food Conversion Ratio) was calculated as per the formula of El-Rhman *et al.* (2009) [8].

$$\text{FCR} = \frac{\text{Amount of feed intake (g)}}{\text{Wet weight gain (g)}}$$

Protein Efficiency Ratio (PER)

Protein efficiency ratio is a measurement of utilization of protein. It was calculated as per El-Rhman *et al.* (2009) [8].

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Survival (%)

The survival of the experimental fish was estimated using the formula given below. It was calculated as per El-Rhman *et al.* (2009) [8].

$$\text{Survival (\%)} = \frac{\text{No. of fish survived after experiment}}{\text{No. of fish stocked}} \times 100$$

Proximate analysis of diets and carcass

The proximate composition of diets and carcass was determined by following the standard methods (AOAC, 2019) [2].

Moisture

The moisture content of diets was determined by taking a known weight of the sample in petri-dish and drying in a hot air oven at 100-105 °C till a constant weight was achieved. The difference in weight of sample gave the moisture content, which was calculated by using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample} - \text{Dried weight of the sample}}{\text{Wet weight of the sample}} \times 100$$

Crude protein (CP)

The nitrogen content of the sample was estimated constitutively by the Micro-Kjeldahl method using titration as a means for determining nitrogen percentage. The crude protein percentage was obtained by multiplying nitrogen percentage by a factor of 6.25.

$$\text{Crude protein (\%)} = N_2 (\%) \times 6.25$$

Lipids

The lipid was estimated by Soxhlet apparatus using petroleum ether (Boiling point 40-60 °C) as the solvent. The calculation was made as follows.

$$\text{Total lipid (\%)} = \frac{\text{Weight of the ether extract}}{\text{Weight of the sample}} \times 100$$

Ash

Ash content was estimated by taking a known weight of the sample in a silica crucible and placing it in a muffle furnace at 600 °C for 6 hours. The calculation was done as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) considering zinc nanoparticle incorporations as variables. All means were compared by the Duncan's Multiple Range Test using SPSS version 22.

Results and Discussions

Proximate composition of diet

The level of protein was kept at 40% among all treatment diets. Proximate composition of diets are shown in Table 2.

The nutritional composition of the experimental diets in the table exhibits a range of values for different components. The crude protein content in these diets falls within a range of 40.04% to 40.96%. The lipid content, representing fats, varies from 10.22% to 10.78%. The ash content, varies from 12.50% to 13.78%. Moisture content, ranges from 10.58% to 12.42%.

Table 2: Composition and proximate analysis of the experimental diets (ZnO)

	T0 (Control)	T1 (10 mg/kg)	T2 (20 mg/kg)	T3 (30 mg/kg)
Crude protein (%)	40.04	40.25	40.53	40.96
Lipid (%)	10.22	10.78	10.61	10.72
Ash (%)	12.50	13.62	13.32	13.78
Moisture (%)	10.58	10.77	11.48	12.42

Weight gain

The average wet weight (g) of *O. niloticus* observed at periodical intervals is shown in Table 3 and graphically presented in Fig. 1. The highest final wet weight (g) was

observed in T2 (5.840 ± 0.040 g) and lowest in T3 (4.637 ± 0.030 g) treatment. The treatments differed significantly from each other for wet weight ($p < 0.05$). The results shows that the supplementation of zinc nanoparticle @20 mg/kg exhibits the best growth performance.

At the end of 15 day of experiment, it was observed that treatment T2 (1.783 ± 0.008 g) showed highest growth, whereas treatment T1 (1.697 ± 0.008 g) exhibited lowest growth. At the end of 30 day of experiment, treatment T2 (2.717 ± 0.008 g) had shown highest growth. Whereas treatments T0 (2.470 ± 0.010 g), T1 (2.517 ± 0.010 g) and T3 (2.497 ± 0.020 g) has shown to be non-significant. At the end of 45 day of experiment, highest growth was observed in treatment T2 (3.947 ± 0.020 g) while lowest growth was observed in treatment T0 (3.477 ± 0.010 g), all the treatments differ significantly from each other. At the end of the experiment (60 days), treatment T2 (5.840 ± 0.040 g) has shown highest growth, whereas treatment T3 (4.637 ± 0.030) showed lowest growth. It was also observed that treatment T0 (4.690 ± 0.010) and T3 (4.637 ± 0.030) were at par.

Table 3: Effect of different treatments of zinc nanoparticle (ZnO) diets on weight gain (g) (Mean \pm SE) in *O. niloticus* fry during the culture period

Treatments	0 days	15 days	30 days	45 days	60 days
T0 (control)	1.233 ± 0.008^a	1.727 ± 0.008^a	2.470 ± 0.010^a	3.477 ± 0.010^a	4.690 ± 0.010^a
T1 (10 mg/kg)	1.220 ± 0.005^a	1.697 ± 0.008^a	2.517 ± 0.010^a	3.613 ± 0.010^c	5.077 ± 0.020^{ab}
T2 (20 mg/kg)	1.223 ± 0.008^a	1.783 ± 0.008^b	2.717 ± 0.008^b	3.947 ± 0.020^d	5.840 ± 0.040^b
T3 (30 mg/kg)	1.237 ± 0.008^a	1.760 ± 0.020^b	2.497 ± 0.020^a	3.537 ± 0.008^b	4.637 ± 0.030^{ab}

Values are expressed as mean \pm SE. a,b,c values in a column with different superscript differ significantly ($p < 0.05$).

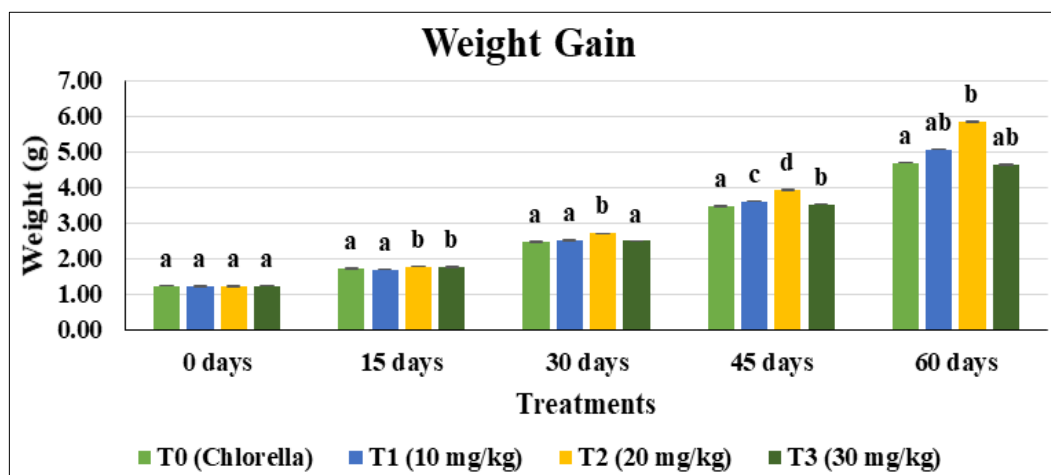


Fig 1: Effect of different treatments of zinc nanoparticle (ZnO) diets on weight gain (g) (Mean \pm SE) in *O. niloticus* fry during the culture period

Growth parameters

At the end of 60 days of experiment, weight gain (%) of *O. niloticus* observed at periodical intervals is shown in Table 3 and graphically presented in Fig 2. The highest final weight gain (%) was observed in T2 (477.46 ± 16.760 %) and lowest in T3 (375.00 ± 10.560 %) treatment. The treatments differed significantly from each other for weight gain (%). The results shows that the supplementation of zinc nanoparticle @20 mg/kg exhibits the higher growth performance. The specific growth rate (SGR) of *O. niloticus* fry in different treatment is given in Table 4 and graphically presented in Fig 3. Highest SGR was observed in T2 treatment (1.13 ± 0.008) followed by T1 (1.03 ± 0.005), T0 (0.97 ± 0.003) and T3 (0.96 ± 0.009) treatments. Significant difference in SGR was observed among the treatments ($p < 0.05$). However differences in SGR of treatments T0 (0.97 ± 0.003), T1 (1.03 ± 0.005) and T3

(0.96 ± 0.009) were found to be non-significant ($p > 0.05$). Food conversion ratio was observed highest in fishes fed with T3 diet (1.99 ± 0.010) followed by 1.93 ± 0.009 , 1.76 ± 0.040 and 1.57 ± 0.010 in T0, T1 and T2 treatments respectively (Table 4 & Fig 4). Lowest FCR was observed in T2 treatment (1.57 ± 0.01). Protein efficiency ratio was highest ($p < 0.05$) in fishes fed with T2 diet (1.59 ± 0.030) followed by 1.42 ± 0.010 , 1.29 ± 0.006 and 1.26 ± 0.010 in T1, T0 and T3 treatments respectively (Table 5 & Fig 4). Fishes fed @20 mg/kg zinc nanoparticles supplement showed the highest PER. There was no significant difference observed between the treatments. The probable reason behind increase in growth with increase in incorporation of zinc nanoparticle is that zinc is a major component in many biological processes. So because of that zinc in nanoparticle form makes it easy to absorb and be more effective.

In the present study, zinc nanoparticle was supplemented in the diet of Nile tilapia (*O. niloticus*) fry for 60 days. The result of the present study showed that up to 20 mg/kg (T2 treatment) incorporation of zinc nanoparticle has shown to increase the growth. But in 30 mg/kg (T3 treatment) showed a decrease in growth however, it was better than control group (T0 treatment).

The results we obtained in this study are in agreement with the research conducted by Kishawy *et al.* in 2020 [13]. Their study involved the supplementation of various zinc forms in the diets of Nile tilapia, and they found that fish receiving nano-ZnO at a dosage of 20 mg/kg exhibited better growth performance when compared to fish on alternative diets. Specifically, the nano-ZnO treatment resulted in higher growth rates, increased specific growth rates, and improved protein efficiency, all achieved with a lower food conversion ratio. This underscores the beneficial impact of incorporating zinc nanoparticles on growth performance, which may be attributed to zinc's involvement in a multitude of vital biological processes. Moreover, similar outcomes were observed by Kumar *et al.* in 2018 [14], who formulated two diets with zinc nanoparticles at 10 mg/kg and 20 mg/kg, alongside a control group for *Pangasianodon hypophthalmus*. Their study demonstrated that fish fed with zinc nanoparticles exhibited enhanced growth performance and higher survival rates in comparison to the control group, where zinc nanoparticles were not incorporated into the diet. Additionally, the nanoparticle-fed fish displayed improved disease resistance and reduced oxidative stress levels.

In summary, these consistent findings highlight the positive influence of incorporating zinc nanoparticles in fish diets, which can lead to improved growth, specific growth rates, protein efficiency, and disease resistance. Nevertheless, further research is warranted to provide a more comprehensive understanding of the underlying mechanisms driving these outcomes."

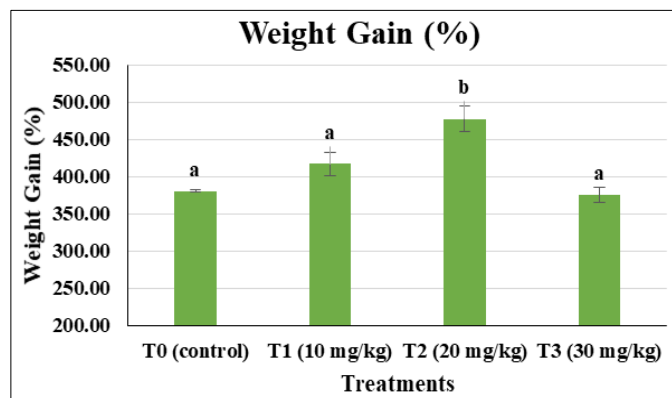


Fig 2: Effect of different treatments of zinc nanoparticle (ZnO) diets on weight gain (%) (Mean ± SE) in *O. niloticus* fry during the culture period

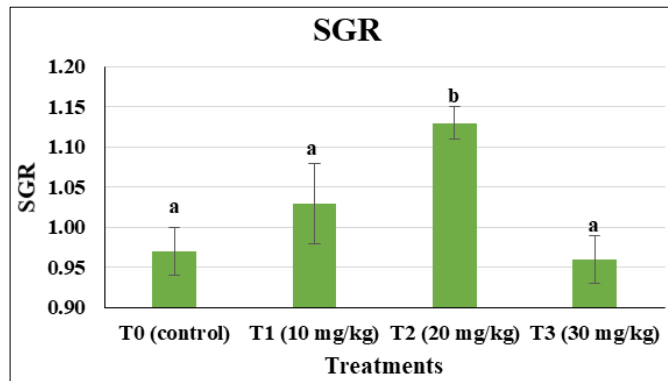


Fig 3: Effect of different treatments of zinc nanoparticle (ZnO) diets on specific growth rate (SGR) (Mean ± SE) in *O. niloticus* fry during the culture period

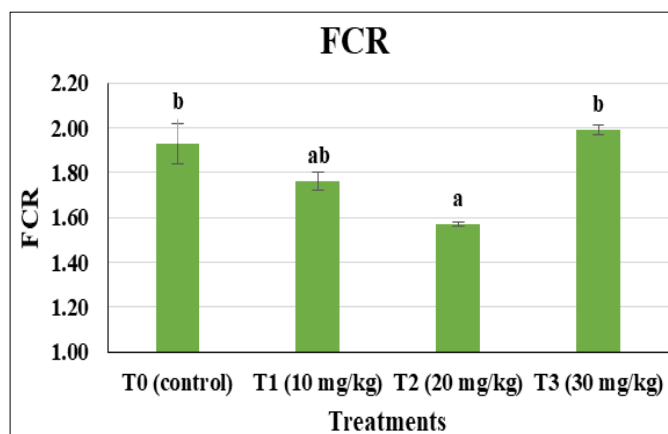


Fig 4: Effect of different treatments of zinc nanoparticle (ZnO) diets on food conversion ratio (FCR) (Mean ± SE) in *O. niloticus* fry during the culture period.

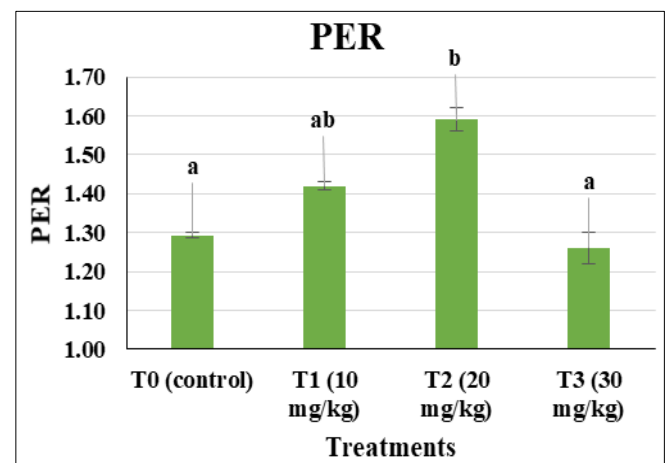


Fig 5: Effect of different treatments of zinc nanoparticle (ZnO) diets on protein conversion ratio (PER) (Mean ± SE) in *O. niloticus* fry during the culture period.

Table 4: Growth parameters observed at the end of experiment of Nile tilapia fry

Treatments	Weight gain (%)	SGR	FCR	PER	Survival (%)
T0 (control)	380.30 ± 1.790 ^a	0.97 ± 0.003 ^a	1.93 ± 0.009 ^b	1.29 ± 0.006 ^a	100.00 ± 0.00 ^a
T1 (10 mg/kg)	416.81 ± 15.110 ^a	1.03 ± 0.005 ^a	1.76 ± 0.040 ^{ab}	1.42 ± 0.010 ^{ab}	98.33 ± 1.66 ^a
T2 (20 mg/kg)	477.46 ± 16.760 ^b	1.13 ± 0.008 ^b	1.57 ± 0.010 ^a	1.59 ± 0.030 ^b	100.00 ± 0.00 ^a
T3 (30 mg/kg)	375.00 ± 10.560 ^a	0.96 ± 0.009 ^a	1.99 ± 0.010 ^b	1.26 ± 0.010 ^a	96.66 ± 3.33 ^a

Values are expressed as mean ± SE. a,b,c values in a column with different superscript differ significantly ($p < 0.05$)

Biochemical Composition

At the end of the feeding period, random samples of fish from

each tank were collected for subsequent biochemical analysis. The data from the proximate analysis of the fish whole body

after the experiment is given in Table 5.

Highest crude protein was found in T3 ($18.83 \pm 0.020\%$) followed by T2 ($18.51 \pm 0.090\%$) treatment. Result indicated that increasing zinc supplement has resulted in increased protein content in body of Nile tilapia. Lipid content in the fish body also did not differ within treatments. It ranged from 2.4 to 2.9%. However, lipid content of fishes with zinc nano particle had higher lipid deposition in their body compare to control group fish. However, it did not show significant variation among the treatments. Ash content in the fish body also did not differ significantly among the treatments. It ranged from 1.80 to 1.95%. Whereas in case of moisture content in the fish body was low in zinc fed fishes compare to control. It ranged from 76 to 79%. It did differ within treatment.

Table 5: Proximate analysis of fish carcass (% of wet weight)

Experimental fish	Crude protein%	Lipid%	Ash%	Moisture%
T0 (control)	17.46 ± 0.040^a	2.48 ± 0.050^a	1.88 ± 0.003^a	78.18 ± 0.070^a
T1 (10 mg/kg)	18.26 ± 0.040^a	2.73 ± 0.020^a	1.93 ± 0.008^a	77.08 ± 0.050^a
T2 (20 mg/kg)	18.51 ± 0.090^a	2.72 ± 0.008^a	1.92 ± 0.003^a	76.85 ± 0.100^a
T3 (30 mg/kg)	18.83 ± 0.020^a	2.82 ± 0.030^a	1.91 ± 0.010^a	76.43 ± 0.010^a

Conclusion

Tilapia is the third most cultured fish in the world, because it is tolerant to a variety of aquatic environments; it can be farmed in wide range of salinity and also in pond or cage systems. Major tilapia production is mainly due to Nile tilapia (*Oreochromis niloticus*) which accounts for roughly 75% of farmed tilapia. Global tilapia production has exceeded 5 million ton per year since 2014 with a steady growth rate of 5-8% per annum. In late 2017, the production of tilapia in India was roughly 18, 000 tonnes. In this experiment, zinc in the form of zinc oxide was selected as the nanoparticle supplement. Four experimental diets were prepared with zinc nanoparticle supplementation at 0 mg/kg (T0), 10 mg/kg (T1), 20 mg/kg (T2) and 30 mg/kg (T3). Growth performance, feed utilisation and carcass composition were measured in Nile tilapia (*O. niloticus*) at the end of 60 days. In terms of growth performance, fishes fed T2 (20 mg/kg) diet showed better performance. It has exhibited highest weight gain, SGR and PER, along with lowest FCR. It can be said that in terms of growth, zinc nanoparticle at 20 mg/kg (T2) showed the overall better performance. The survival difference was non-significant among the treatment. Similar results were also obtained in carcass composition. Therefore to increase growth performance zinc nanoparticle can be incorporated up to 20 g/kg.

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A research was conducted by Thangapandiyan and Monika in 2020 [28] where zinc nanoparticles were incorporated in *L. rohita* diets. Zinc nanoparticles were incorporated in three variation of 5, 7.5 and 10 mg/kg. The results of their study showed increase level in protein and lipid level *L. rohita*. Which is in contradiction of the result of the present study. Where incorporation of zinc nanoparticle did not show any effect on biochemical characteristics. Probable reason behind that is difference in incorporation levels of zinc nanoparticle in the diet. Similar result was also observed when nano Zinc oxide was incorporated in the diet of Nile tilapia (Tawfik *et al.* 2017) [27]. Nano zinc was incorporated in different concentrations (15, 30, 45 and 60 mg/kg). Result exhibited increase in protein in Nile tilapia. Which is in contrast with the findings of the present study.

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