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Original Article

Applying Nano-technology in tilapia Nutrition: influence of Iron and Zinc nanoparticles as dietary supplementary on biological performance and body composition of *Oreochromis niloticus* fry

Mohammed S Abd-Elhamed[1], Sobhy M Allm[2], Kamal El-Deeb[2], Atalla A Metwalli[1], Hamed H E Saleh[1], Mohamed F Abdel-Aziz[1]

1, Aquaculture division, National institute of Oceanography and fisheries (NIOF) Egypt

2, Animal production dep., Faculty of Agriculture, Fayoum University, Egypt

ABSTRACT

A 102 day feeding trial were conducted to compare the effect of three levels of iron (nFe2O3) and zinc (nZnO) nanoparticles with the same levels of Fe (Fe2O3) and Zinc (ZnO) bulk particles as feeding additive on growth performance, feed efficiency, chemical body composition and tissue accumulations of Nile tilapia *Oreaochromis niloticus* fry. Fry with an initial weight of 1.66 g were reared in cement pond (0.8m3). The basal diet was formulated to be containing 30% CP and mixed with the mineral additives according to the tested treatments. The fish were fed twice daily at (8, am) and (4, pm), with a rate of 5% of biomass. The obtained results showed a significant differences($P \leq 0.05$) between treatments in growth parameters, feed efficiency , body chemical composition and tissue accumulation from Fe and Zn. Fry fed a diet containing (20 mg Nano iron oxide + 20 mg Nano zinc oxide) achieved the best values of each growth performance and feed conversion ratio (FCR). On the other hand, the addition of nanoparticles (Fe+Zn) in supplemented feed resulted a high accumulation in fry whole body compared with the bulk form of (Fe+Zn).

Key words: Nile tilapia, Iron, Zinc, Nano-particles, Growth performance

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Correspondence:
Hamed H E Saleh
Aquaculture division, National institute of Oceanography and fisheries (NIOF) Egypt
Phone: +2 01022697780
E-mail: hhsaleh90@gmail.com

1. INTRODUCTION

Balanced aqua-feed is the key factor for increasing the fish production. Fishes need optimal amounts of nutritional components. The raw ingredients of plants or animals in the basal diets are insufficient to provide fish feed with suitable amounts of minerals. As it is known, minerals should be incorporated in aqua-feed with optimal doses, which, differ according to the basal diets' species, age, size, and composition (Abd-Elhamed et al., 2019 and Dawood et al., 2021).

Minerals are needed in lower levels than other ration required nutrients (e.g., protein, carbohydrates, and fat) (Prabhu et al., 2014). Nanotechnology has a tremendous potential to revolutionize aquaculture and fisheries.

Nanotechnology tools are like nano materials, nanosensors, nanovaccines, gene delivery and smart drug delivery etc. have the potential to solve many puzzles related to animal health, production, reproduction, prevention and treatment of diseases

Nano minerals are characterized by higher surface area affinity, higher solubility, thermal resistance, low toxicity, slow excretion rate, and sustained release (Diallo and Brinker, 2011). Accordingly, Nano minerals can beneficially affect animals' metabolic, physiological, and biological functions (Khan et al., 2017) For example. Iron and Zinc is an important trace mineral in fish nutrition as it plays a key role in various metabolic pathways like prostaglandin metabolism and structural role in nucleoproteins (Chanda et al., 2015).

Zinc is an integral part of about 20 metalloenzymes such as alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase. As well as, Iron is necessitated by most of living organisms including fish because it is required for doing the metabolic processes including oxygen transport, drug metabolism, steroid synthesis, DNA synthesis, ATP production.

Therefore, this work was aimed to estimate the different levels of nanoparticles or bulk of Zinc and Iron as supplementary feed on growth performance, feed conversion efficiency and whole body composition of Nile tilapia *Oreochromis niloticus* fry.

2. MATERIALS AND METHODS

This study was conducted in the fish feeding laboratory, Fayoum Research Station, National Institute of Oceanography and Fisheries (NIOF), Fayoum Governorate, Egypt.

Nile tilapias were obtained from a commercial hatchery. Experiment was carried out to evaluate the effect of addition Nano-Iron and zinc to tilapia diet compared with normal iron and zinc on growth performance, feed utilization and chemical composition of Nile tilapia *Oreochromis niloticus* fry.

2.1.Experimental design

This trial was contained seven groups: the first treatment (G1) fry fed on the control diet. In G2, G3 and G4 fry fed at three levels of normal iron and zinc as (20, 40 and 60 mg/ kg, respectively). In G5, G6 and G7 fry fed at three levels of Nano- iron and zinc as (20, 40 and 60 mg/kg, respectively).

Fry were acclimated for a week, them distributed in experimental units. The average initial weight of fry was 1.66±1.25 g. Fry was reared in 14 cement ponds (duplicated), the pond size was 1m3 and the water volume in each pond was 0.8m3.

All ponds were aerated continuously using an air blower. Fry was stocked as 20 fry in each pond, then its fed twice daily. The water exchange rate was 30% of total e water volume every three days. The trial was continued for a period of 102 days.

2.2.Diets preparation

Seven experimental diets were used. The first was considered as a control diet (G10 and the others diets was formulated to contain (20 mg iron oxide + 20mg zinc oxide, G2), (40mg iron oxide + 40mg zinc oxide, G3), (60 mg iron oxide + 60mg zinc oxide, G4) ,(20 mg nano iron oxide + 20 mg nano zinc oxide, G5), (40 mgnano iron oxide + 40 mg Nano zinc oxide, G6) and (60 mg nano iron +60 mg nano zinc, G7). All diets were formulated by hand and formulated to be contain30% crude protein.

The mineral additives were mixed with starch. The diets ingredient and their chemical composition are shown in Table (1).

Table 1. The basal diet used in the experimental feeding.

Ingredients	g/100g
Fish meal 72%	14
Soybean meal	39
Yellow corn	40
Starch	1.5
Fish oil	5
Vit.Mix	0.5g
Total	100
chemical analysis % on Dry matter basis	
Moisture (M)	10.2
Dry matter (DM)	89.8
Crude protein (CP)	30.71
Ether extract (EE)	12.96
Total carbohydrate*	46.13
Ash	10
Fe	245 ppm
Zn	18.7ppm
Gross energy (GE, Kcal/g)**	4.91

*, Total carbohydrate was calculated by difference.

**, Calculated according to NRC (1993).

2.3.Running water system

The system contained one water pump and two large tanks (10000 liters/tank) which used to storage the water two days before using in water exchange, this aim to devoid the negative effect of water chloride.

2.4.Water quality parameters

Water temperature, pH was measured daily and dissolved oxygen (DO) was measured every week by a thermometer, Orion digital pH and oxygen meter (Cole Parmer model 5946), respectively.

2.5.Measurements of growth performance

These parameters were calculated according to the following equations:

Total gain TG, g = Final weight (W_2) – initial weight (W_1).

Average daily gain ADG, g/day = average weight gain, g / experimental period, day.

SGR, % /day = $[(\ln W_2 - \ln W_1)/t] \times 100$.

whereas ln: is the natural log. and t: is the time in days.

Survival rate SR% = (Number of fish at the end/ Number of fish at the start) × 100.

Feed intake FI = gram/fish feed intake during the trial period/ the final number of fish for this trial,

Feed conversion ratio FCR = feed intake, g / weight gain, g.

Protein efficiency productive PER= Weight gain, g/ Protein intake, g..

Protein productive value PPV, % = (Retained protein, g/ Protein intake, g) × 100.

Energy efficiency ratio EER = Weight gain, g/ Energy intake, Kcal.

Energy productive value EPV, % = (Retained Energy, Kcal/ Energy intake, Kcal) × 100

2.6.***Chemical analysis of feeds and fish***.

Initial fish sample were collected by scarfided three fish in each treatment before experimental start and the same final samples were used at the end of trial. Samples were frozen and stored untile composition analysis. Chemical analyses of the experimental diet and whole body were performed by the standard methods of AOAC (2005). Gross energy (GE) was estimated for formulated diets by using these values, 5.64, 9.44 and 4.11 Kcal/g for CP, EE and carbohydrates, respectively (NRC, 1993), where for fish 5.5 and 9.5 Kcal/g for protein and fat, respectively (Viola et al., 1981)

2.7.Statistical analysis

The analyses of variance and LSD of Duncan Waller were used to compare means of treatments . Data was analyzed using SPSS (2019) Inc. Released 2019 for Windows, Version 25.0 at the level of significance of 0.05.

3. RESULTS AND DISCUSSION

3.1. water quality

Means of water parameters in the present trial were within the recommended ranges for the tilapia cultured (El-Sayed, 2006). Whereas, these means were 23^{o}C, 8.04 and 7.1mg/l for temperature, pH and DO, respectively.

3.2. Growth performance

The results of the growth parameters were displayed in Table (2). The results revealed that there were insignificant differences between the groups of (G2, G4, and G7) in final weight, total weight gain, average daily gain, specific growth rate, and relative growth rate. While these treatments significantly differed with G5 and control.

The best parameters were obtained with G5, while control was the worst. In view of these results, it can be indicated that G5 had the highest W_2, TG, ADG, SGR, and RGR followed by other treatments, while the control group was the lowest. Fish fed the Nano Fe-Zn supplemented with the concentration of (20+20mg/kg diet) was the best compared with other treatments in all growth parameters.

In the same trend, there are insignificant differences between G2, G3 and G4, however, G4, G3 and G2 were better than control in growth parameters values, respectively.

Table 2. Growth performance parameters.

Items	W_2, g	T.G, g	ADG, g	SGR, %/day$^-$	SR,%
Control	12.25^d	10.59^d	0.104^d	1.95^d	87.50^b
G2	13.70^c	12.04^c	0.117^c	2.06^c	92.50^{ab}
G3	14.24^{bc}	12.58^{bc}	0.123^{bc}	$2.10b^c$	87.50^b
G4	13.89^c	12.23^{bc}	0.119^{bc}	2.08^c	87.50^b
G5	18.48^a	16.82^a	0.165^a	2.36^a	90.00^b
G6	14.82^b	13.16^b	0.128^b	$2.14b^c$	90.00^b
G7	13.59^c	11.93^c	0.116^c	2.06^c	97.50^a

Values in the same column with different superscripts are significantly different ($P<0.05$).

The basal diet contained 245mg/Kg Fe and 18.5 mg/kg Zn, which was less than the diet of G3 which contained by 265mg/kg and 38.5mg/kg as the best treatment of bulk form Fe and Zn supplementation.

Iron plays a vital role in the synthesis of organs and tissues of higher fish such as the physiological processes of cellular respiration, oxygen transport and lipid peroxidation (Crichton, 1991).

In addition, Zinc metal is considered as one of the vital elements that has an important role in homeostasis maintenance. Thus, any alteration in its concentration may lead to several harmful impacts. Zinc metal is necessary for regular growth, reproduction, and other physiological activities, but it may show toxicological impacts on aquatic organisms at high concentrations (Fahmy et al., 2014).

There are many of interpretations which cleared that, the nanoparticles have a vital role in fish nutrition, in particular iron and zinc Nanoparticles, such as Huber, (2005) who illustrated that Fe_2O_3 nanoparticles boost bioavailability than other forms of iron nanoparticles fishes. Also, nanoparticles have a higher influence on the activity of digestive enzymes in *Mondono rosenbergii* PL, leading the maximum digestion of the feed offered. The increased activities of digestive enzymes led to enhance the feed consumption and feed conversion, then improving in survival and growth of *M. rosenbergii* PL (Srinivasan et al., 2016).

The small particle size of nZnO increases absorption rate, bioavailability, and catalytic activities as reported by Alishahi et al., (2011).

Also, it may be attributed to stimulate of DNA and RNA synthesis and growth hormone protein synthesis (Siklar et al., 2003). This agreed with Hina et al., (2015) showed that, Zinc oxide nanoparticles promoted the growth performances of juvenile *C. idellain* more than other inorganic conventional forms.

Over and above, nanoparticles have the ability on the immune boost this was supported by Luo et al., (2015) who stated that nanoparticles can stimulate innate and adaptive immune response depending on their physicochemical properties.

In the present study, it could be noted that control diet followed by G7 (60mg nFe_2O_3+60 nZnO mg/kg diet) had the lowest growth parameters. This may be due to the less dose used in nFe and nZn in G7, which don't sufficient for tilapia fry requirements. Whereas, fish fed Fe and Zn bulk form was better in growth than G7.

So its probably the high amount of nFe and nZn are the reason for lower growth aswas reported by (Uzo-God et al., 2018a) and they interpreted that, Nano-sized Fe_2O_3 has a much higher surface area compared to macro Fe_2O_3. This covers the surface of the control diet and allows only a very small amount to pass. Hence fish are unable to get sufficient nutrients available in the control diet. However, in the case of macro Fe2O3, the particle size is much higher than that of nFe_2O_3; hence the covering of Fe_2O_3 over the diet is not very compact, and the fish get a sufficient amount of basal feed nutrients along with iron.

Moreover, high supplementation of Zn may have negative effect rather than the status of other elements such as Fe (Heijerik et al., 2002), also may be led to lose palatability thereby causing eating less, the higher level of nZnO result in cytotoxicity and cell death as mentioned by Uzo-God et al., (2018b).

In excess of, the toxicological impacts of NPs are strongly associated with many unique properties such as small size, high surface area, hydrophobicity, surface modification, and high reactivity (Garcia et al., 2014).

It can be concluded that G5 (20mg nFe_2O_3+20 nZnO) followed by G6 (40mg nFe+40mg nZn) as a feed additive to the basal diet (contained 245mg/kg Fe and 18.5mg/kg) was the optimum supplementation for fry tilapia growth and survival rate. The current study examined in equal amount of Fe and Zn as an additive for each treatment but there may be differences from the results in the case of testing concentrations of iron higher or lower than zinc concentrations and or testing of each element individually as previous studies have done. This was in agreement with Srinivasan et al., (2016) who stated that 20 mg kg-1 Fe_2O_3 NPs has the potency to produce the maximum enhancement in survival and growth of *M. rosenbergii* PL.

The obtained results were supported by ETC (2003) who observed that the growth of sturgeon and young carp increased from 24% to 30 % in consequence of using Nano Fe as compared to bulk form. Whereas, juvenile Grass carp fed 30 mg nZnO/kg as feed additive achieved better growth than 60 mg and this agrees with our results.

In another study, 54 mg /kg of Nano Fe as a supplemented diet had the best final weight of *Labeo rohita* fish comparable to 55mg /kg Fe sulfate and diet control (Faiz et al., 2015).

3.3.Feed efficiency

As shown in Table (3), the highest FI was achieved with G5 followed by G6 and G3. The best FCR was achieved with G5 and the worst FCR was with control. Also, there was insignificance different between G2, G3, G4, G6 and G7 in PER and EER.

On the other hand, G5 had the highest PER, PPV, EER and EPV.

Feed efficiency parameters illustrated that G5 was the best in all parameters when compared to the other treatments. It can be revealed that the dietary 20mg Fe + 20 mg Zn/Kg supplementation in nanoparticle form was the optimum dose for the best growth and feed efficiency.

Considering of nanoparticle form of Fe, Zn have a higher efficiency compared to another inorganic form of Zn. The nano form of particles has higher intestinal absorption, bioavailability and catalytic activities (Dube et al., 2010). Therefore, it might possible that the conversion of ZnO in nano form increases the efficiency of Zn by enhancing its absorption and bioavailability in the gastrointestinal tract (Faize et al., 2015). This was agreed with Uzo-God et al., (2018b), which showed that feed conversion ratio was more improved with fish fed nZnO supplemented diet than those fed ZnO, they added, nZnO being much smaller can penetrate the cell while bulk ZnO are bigger in size face difficulty in passing the cells.

Table 3. Feed efficiency parameters of Zn and Fe in fry.

Items	FI, g/fish	FCR*	PER, g	PPV, %	EER, g/Kcl	EPV, %
Control	27.28^{bcd}	2.57^{a}	1.31^{c}	70.79^{e}	0.08^{c}	51.49^{c}
G2	25.52^{cd}	2.12^{bc}	1.57^{b}	84.96^{bc}	0.09^{b}	62.48^{b}
G3	28.43^{ab}	2.26^{ab}	1.47^{bc}	79.50^{c}	0.09^{bc}	58.08^{bc}
G4	28.03^{abc}	2.29^{ab}	1.45^{bc}	77.29d	0.08^{bc}	58.36^{bc}
G5	30.23^{a}	1.80^{d}	1.84^{a}	105.00^{a}	0.11^{a}	70.25^{a}
G6	29.44^{ab}	2.24^{ab}	1.49^{bc}	85.24^{bc}	0.09^{bc}	56.09^{bc}
G7	25.20^{d}	2.11^{bc}	1.63^{b}	88.40^{b}	0.09^{b}	62.67^{b}

Values in the same column with different superscripts are significantly different ($P<0.05$)
*The lower value is the highest performance

In the same trend, 40mg/Kg Fe_2O_3 nanoparticles supplemented diet had a high effect on the activities of digestion enzymes.

Increasing the digestion of feed intake results in high PPV and feed efficiency. Many researchers suggested that nFe_2O_3 has more effect on metabolism rates and influence on amino acids synthesis, this lead to better growth and FCR. Furthermore, Tawfik et al., (2017) mentioned, the highest feed efficiency was achieved with fish fed nZnO. On the other trend, Uzo-God et al., (2018a) contrast with our results and reported that FCR significantly better improved with fish fed Fe_2O_3 supplemented diet than those fed nFe_2O_3.

3.4. Whole body chemical composition

As shown in Table (4) there were significant differences between the treatments in Dry Matter DM, fat EE, ash, and gross energy GE.

Fish fed diets containing the bulk Fe+Zn had higher EE content than those fed nanoparticles diets supplementation. G4 had the highest EE (36.63%) and GE (6.44kcal/g) and achieved the lowest CP (53.71%). Fish fed diets containing nanoparticles had higher protein content and ash than those fed diets containing bulk Fe and Zn. Whereas G5 had the highest in CP (56.93%) and G6 had the highest in ash (11.94%).

This may be due to nano-Fe is more effectiveness as a cofactor for many enzymes in practical protease enzymes feeding to an increase in retained protein. Moreover, nanoparticles are more solubility, assimilation, and availability of iron and Zn in the nanoparticles compared to the macro forms and control as decided (Muralisankar et al., 2016).

Our results disagreed with Uzo-God et al., (2018a) who reported that control had the highest CP content followed by Fe_2O_3 and nFe_2O3.

Table 4 Whole body chemical composition of Nile tilapia fry

Items	DM, %	CP,%	EE, %	Ash, %	Kcal/g (GE)
Start	**22.196**	**58.612**	**24.47**	**17.463**	**5.54**
Control	**29.27^{b}**	**54.68^{b}**	**35.42^{c}**	**10.13^{g}**	**6.36**
G2	**30.25^{a}**	**54.39^{b}**	**35.72^{b}**	**10.36^{f}**	**6.38**
G3	**29.05^{c}**	**54.26^{b}**	**35.24^{c}**	**10.54^{e}**	**5.82**
G4	**29.27^{b}**	**53.71^{c}**	**36.63^{a}**	**10.95^{d}**	**6.44**
G5	**27.89^{d}**	**56.93^{a}**	**31.69^{d}**	**11.07^{c}**	**6.14**
G6	**27.76^{f}**	**56.34^{a}**	**31.51^{e}**	**11.94^{a}**	**6.10**
G7	**30.26^{a}**	**54.56^{b}**	**33.45^{f}**	**11.75^{b}**	**6.17**

Values in the same column with different superscripts are significantly different ($P<0.05$)

3.5. Accumulation of Fe and Zn in the whole body

From Table (5) it can be observed that control had the lowest concentrate of Fe and Zn in whole body, which is natural.

This may be attributed to the ease with Fe nano which be taken up by the body and higher absorption and availability.

On the contrary, conventional Fe as mentation by Feng et al., (2009). Also, this was agreed with Behera et al., (2014) who cleared that iron nanoparticles appeared to be more effective than ferrous sulfate in increasing muscle iron and hemoglobin content. On the other hand, fish fed bulk Fe and Zn, their livers and gills contained Fe concentrates more than those fed Fe and Zn in nanoparticles.

Table 5. Fish content of iron and zinc elements

Items	Iron (mg/kg)	Zinc (mg/Kg)
Control	**145^{ab}**	**43.4^{h}**
G2	**192^{ab}**	**49.5^{d}**
G3	**111^{b}**	**54.3^{c}**
G4	**259^{ab}**	**59.9^{b}**
G5	**122^{b}**	**69.1^{a}**
G6	**321^{a}**	**44.5^{g}**
G7	**285^{ab}**	**46.0^{f}**

Values in the same column with different superscripts are significantly different (*P<0.05*)

Fish body content of Zn significantly differed between the treatments as shown in Table (5). Body content of Zn was higher in fish fed bulk Fe+Zn than those fed nanoparticles supplementation while the control had the lowest. This was supported by Tawfik et al. (2017) who reported that Zinc concentration in whole body showed higher values in all fish groups compared with the control.

Whole body content of Fe and Zn at the end of experimental period showed that groups fed with Fe and Zn bulk had lower level of Fe content than Nano Fe and Zn supplemented diet while control had the lowest, whereas, G6 recorded (321 mg/Kg) followed by G7, G4, G3, G5, G2 and control (145 mg/kg).

Regarding the Whole body content of Zn, fish fed nano Fe and Zn had the highest concentration of Zn followed by bulk Fe and Zn and control. This was in agreement with Uzo-God et al., (2018b). Likewise, this result was in partial agreement with Abdel-Khalek et al., (2015) who concluded that zinc nanoparticles (Zn NPs) had more efficiency to penetrate the studied tissues such as the liver, kidneys, gills, skin, and muscle.

4. CONCLUSION

From the results of the present study, the addition of nano-iron oxide and zinc nano-oxide compared to ordinary iron oxide and zinc oxide had a significant effect on the manifestations of growth as represented in the final weight and total increase rate of growth parameters. The addition of the two elements in the the forms of nano image were better than the normal image and the least was recorded with the control.

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