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A comparative study of Nano-iron and zinc as feed additive on growth performance, feed efficiency and chemical body composition of Nile tilapia fingerlings (*Oreochromis nilotiucs*)

Mohamed S. M. Abdel-Hammed[1], Sobhy M. Allam[2], Attalla A. Metwally[1], Kamal A. El-Deeb[2] and Mohamed F. Abdel-Aziz[1]

1- Aquaculture Division, National Institute of Oceanography and Fisheries, Egypt
2- Animal production Dep. Faculty of Agriculture Fayoum University, Egypt
Corresponding author: madaalembrator@yahoo.com

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ABSTRACT

An experiment was conducted to compare the effect of three levels of nanoparticles iron (nFe_2O_3) and zinc (nZnO) with the same levels of bulk Fe (Fe_2O_3) and Zinc (ZnO) as feed additive on growth performance, feed efficiency, chemical body composition and tissue accumulations of Fe and Zn of Nile tilapia fingerlings (17.73 g). A basal diet (30.71%CP) was used with all treatments. This experiment consisted of seven. The fish reared in fiberglass tanks (1.5m3). The fish fed twice daily 8: am and 4: pm, a rate of 5% of the biomass. The experimental period was 80 days. Statistical analysis showed significant differences between the treatments in growth parameters, feed efficiency parameters, body chemical composition, and tissue accumulation of Fe and Zn. T6 (40 mg Nano iron oxide + 40 mg Nano zinc oxide) achieved the best growth and feed conversion ratio (FCR). This experiment cleared that, nanoparticles Fe+Zn resulted in increasing the muscle content of Fe, while the liver and gills content of Fe was increased with bulk Fe+Zn. Also, bulk Fe+Zn led to an increase in the muscles, gills content of Zn in comparison with the liver content of Zn.

INTRODUCTION

Tilapia is one of the most widely used fish cultivated all over the world and in Egypt in particular. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2009), and this contribution has been exponentially growing in recent years. The low trophic level and the omnivorous food habits of tilapia make them a relatively inexpensive fish to feed, unlike other finfish, such as salmon, which rely on high protein and lipid diets based on more expensive protein sources like a fish meal (Obirikorang *et al.,* 2016).

Fish feed is an essential component of the inputs in any fish farm. Minerals are required in fewer amounts than other ration required nutrients. The role of trace elements in biological systems has been described in fish. The fish requirements for the normal life processes like skeletal formation, biologically important compounds such as hormones and enzymes (Watanabe *et al*., 1997). Many of the factors are that affect the dietary level of mineral and trace elements in fish can be in one or more a combination of; the biological factors such as species, life stage, sex, trophic level, feeding habits, and nutritional status of the fish, dietary factors such as diet composition, availability and nutrient interaction and environmental factors such as aqueous mineral concentration, salinity and temperature of the rearing system (Prabhu *et al.,* 2014). From is considered as one of the most essential microelements. Its effect is mainly on the functioning of the immune system and defense against various infections (Huber, 2005) Iron is necessitated by most of living organisms including fish because it is required for execution of metabolic processes including oxygen transport, drug metabolism, steroid synthesis, DNA synthesis, ATP production, electron transport (Crichton, 1991 and Hilty *et al.*, 2010a). However, Iron deficiency causes immune suppression, growth depression, changes in hematological parameters, susceptibility to diseases and poor food conversion.

Besides Zinc is no less important than iron as it plays a key role in various metabolic pathways like prostaglandin metabolism and structural role in nucleoproteins (Chanda *et al.*, 2015). Zinc (Zn) is an essential trace mineral required for growth and metabolism of all vertebrates including fish. It is a specific cofactor of many enzymes, where it is involved in different metabolic pathways (Eckerich *et al*., 2001). The development of nanotechnology produces many nanoparticles (NPs) that are important in medicine, agriculture and industry (Płaza *et al.,* 2014). Nanoparticles are defined as any particle with at least one dimension less than100 nm, and consequently, their properties are altered compared to their bulk counterparts (Auffan *et al.,* 2009). Also, Nanoparticles have enormous potential in controlling the pathogens in aquaculture. Swain *et al.*, (2014) showed that, different metal and metal oxide nanoparticles were screened for their antimicrobial activities against a wide range of bacterial and fungal agents including certain freshwater cyanobacteria. More recently, nan particulate versions of these metal oxides have been manufactured and introduced in commercial products such as cosmetics and sunscreens (TiO_2, Fe_2O_3 and ZnO) (Nowack and Bucheli, 2007).

This study aimed to apply of Nano science in aquaculture, through comparing and showing the differences between diets containing levels of Nano-iron-zinc with diets containing levels of ordinary iron and zinc. And their influence on growth performance, feed efficiency, body chemical composition of Nile tilapia fingerlings.

MATERIALS AND METHODS

The present study was carried out in Shakshouk Fish Research Station, Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF), Egypt. Nile tilapias were obtained from private hatchery fish beside Qaroun are Feeding experiment was conducted to evaluate the effect of the addition of Nano-Iron and zinc in tilapia diet in comparison with ordinary iron and zinc on growth performance, feed utilization and accumulation of iron and zinc in fish body. This study included fingerlings which were acclimated for a week after which they were them distributed in experimental units.

This trial started in date 1/10/2017 and continued for 80 days. It consisted of seven treatments: the first treatment (T1) fingerlings fed on control diet. In T2, T3 and T4 fingerlings fed at three levels of Nano iron and zinc in diet (20, 40 and 60 mg/kg diet respectively). In T5, T6 and T7 fingerlings fed at three levels of ordinary iron and zinc in diet (20, 40 and 60 mg/kg diet respectively). Fingerlings reared in 14 fiberglass tanks (duplicated), the size of the tank was $2m^3$, with water volume of $1.5m^3$. Initial body weight of fingerlings was 17.73 g and it was stocked at 20 fingerlings per tank. The water exchange rate was 30% of water volume in the tank. The feeding twice aday at 8 am and 4 pm.

Diets preparation

One experimental diet was used in this study. Different levels of Iron and Zinc ordinary or Nano were added to this diet according to different treatments except T1 (control), With T2, T3 and T4 it was formulated of the addition of (20 mg iron oxide + 20 mg zinc oxide/ 1000 g), (40 mg iron oxide + 40 mg zinc oxide/1000 g) and (60 mg iron +60 mg zinc/1000 g), while (20mg Nano iron oxide + 20 mg Nano zinc oxide), (40 mg Nano iron oxide + 40 mg Nano zinc oxide), (60 mg Nano iron oxide + 60 mg Nano zinc oxide) with T5, T6, and T7 respectively.

The diets were formulated to contain 30% crude protein. The mineral additives were mixed with starch. The diet ingredient and its chemical composition are shown in Tables 1, 2.

Table 1: the basal diet was used in the 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).

Table 2: The supplementation of Iron and Zinc in all experiment treatments (mg/Kg diet)

Treatments	Control	T2	T3	T4	T5	T6	T7
Iron and zinc source	-	Ordinary Fe and Zn	Ordinary Fe and Zn	Ordinary Fe and Zn	Nano- Fe and Zn	Nano- Fe and Zn	Nano- Fe and Zn
Addition/Kg	-	20mg+20mg	40 mg +40 mg	60 mg +60 mg	20 mg +20 mg	40 mg +40 mg	60 mg +60 mg

Characterization of Zinc Oxide and iron oxide Nanoparticles:

The calculated crystallite size of nZnO ranged from 31-77 nm in diameter as revealed from The XRD. The calculated crystallite size of nFe2o3 ranged from 24-75 nm in diameter as revealed from The XRD. Figs. 1, 2

Figs. 1, 2: Characterization of ZnO and Fe_2O_3 nanoparticles; measurement XRD.

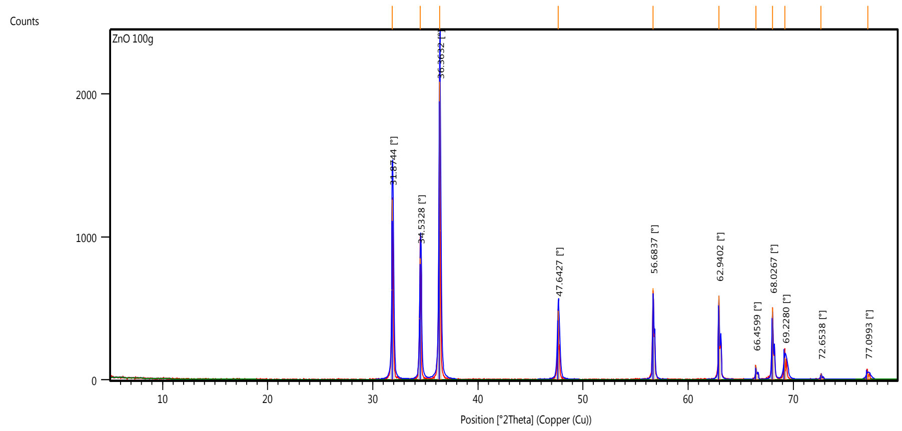

Fig. 1:

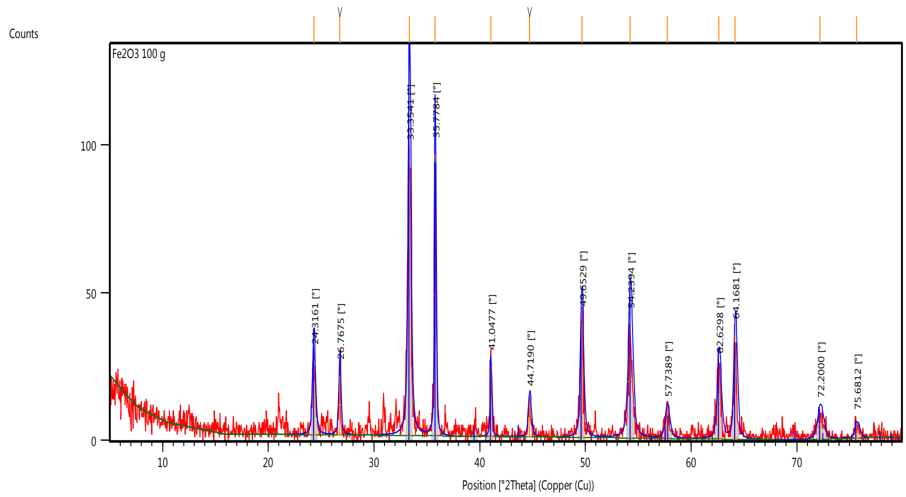

Fig. 2: Nanoparticles Fe_2O_3 and ZnO were obtained from the Graduate School of Advanced Sciences, Beni Suef University, Egypt.

The system of running water and aeration in experimental tanks

The system contained a water pump, sand filter unit and two large tanks (10000 liters/tank) used to stor water for two days before using to change water, This aim to avoid the negative effect of water chloride. While, the aeration system contained an air pump connected to a network of plastic pipes. These pipes transport air to each experimental tank. The air was controlled by the taps and the air diffusers was used to distribute the air in all tank trends.

Water quality parameters

The water quality parameters were recorded and showed in Table (3). The temperature degree, pH was measured daily and dissolved oxygen (DO) was measured every week. By thermometer, Orion digital pH meter and oxygen meter (Cole Parmer model 5946), respectively.

Table 3: the means and SE (±) of water quality parameters

Water quality parameters	
Temperature °C	21.95±0.51
pH	8.12±0.05
DO mg/l	7.38±0.15

Measurements of growth performance

Total weight gain (TG), average daily gain (ADG), Relative growth rate (RGR), specific growth rate (SGR), condition factor (CF) and survival rate (SR).

These parameters were calculated according the following equations:

TG, g = final weight (W_f) – initial weight (W_i), ADG, g/day = average weight gain, g / experimental period, day, RGR, % = [(W_f – W_i) / W_i] × 100, SGR, % /day = [(ln W_f-ln W_i)/t] × 100 whereas ln: is the natural log. and t: is the time in days, ($CF_{,\ g/cm^3}$) = (W_f/ L^3_f) × 100 whereas L_f: is the final length of fish in cm, SR% = (Number of fish at end/ Number of fish at start) × 100

Measurements of feed utilization efficiency

Feed intake g/ fish (FI), feed conversation ratio (FCR), protein efficiency ratio (PER), apparent protein utilization (APU), energy efficiency ratio (EER) and energy productive value (EPV) are the measure used for feed utilization efficiency.

These parameters were calculated according the following equations:

FI, g/fish feed intake during the trial period/ the final number of fish for this trial, FCR = feed intake, g / weight gain, g., PER= Weight gain, g/ Protein intake, g., APU, % = (Retained protein, g/ Protein intake, g) × 100, EER = Weight gain, g/ Energy intake, Kcal, EPV, % = (Retained Energy, Kcal/ Energy intake, Kcal) × 100,

Chemical analysis of feeds and whole fish body

Chemical analysis of diet and whole body fish samples were carried out as described by (A. O. A. C, 2005). Gross energy (GE) for formulated diets was estimated with the factors 5.64, 9.44 and 4.11 Kcal/g for CP, EE and carbohydrates, respectively (NRC, 1993), for fish and 5.5 and 9.5 Kcal/g for protein and fat respectively (Viola *et al.*, 1981).

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) at a 95% confidence limit, using (SPSS, 2007) software, version 1622. Duncan’s Multiple Range 23 test was used to compare means when F-values from the ANOVA were significant ($p<0.05$).

RESULTS AND DISCUSSION

Water quality

The recorded parameters in table (3) were within the optimum range for Nile tilapia rearing according to (El-Sayed, 2006).

Growth performance

Effect of supplementing Nano - iron oxide and Nano- zinc oxide in diet comparison ordinary iron oxide and zinc oxide on growth performance of *O. niloticus* fingerlings.

Growth parameters results were presented in table (4) There were not significant differences between the treatments (control, T2, T3, T4, and T5) in final weight, total weight gain, average daily gain, specific growth rate and relative growth rate. But these treatments significantly differed with T6 and T7. Whereas, E was the best in these parameters while, T7 was the worst. In view of these results it can be noted that, T6 had the highest FW, TG, ADG, SGR and RGR while T7 and control gave the lowest. Fish fed the Nano Fe-Zn supplemented in concentrate (40 +40 mg/kg diet) was the best compard with the other treatments in all growth parameters as well as CF, % and SR, %.

Table 4: growth performance parameters.

Treatments	Wi	F_W(g)	L_f	T.G (g)	ADG (g)	SGR (%) day^{-1}	RGR(g)	CF, %	SR (%)
Control	17.73	31.19^{ab}	12.26^{b}	13.46^{ab}	0.169^{ab}	0.705^{ab}	75.91^{ab}	1.69	90.00^{ab}
T2	17.73	31.780^{ab}	12.50^{ab}	14.05^{ab}	0.175^{ab}	0.725^{ab}	79.20^{ab}	1.62	87.50^{bc}
T3	17.73	32.38^{ab}	12.57^{ab}	14.65^{ab}	0.183^{ab}	0.745^{ab}	82.65^{ab}	1.62	92.50^{ab}
T4	17.73	33.16^{ab}	12.18^{ab}	15.43^{ab}	0.193^{ab}	0.785^{ab}	87.28^{ab}	1.72	80.00^{c}
T5	17.73	31.30^{ab}	12.50^{ab}	13.57^{ab}	0.169^{ab}	0.710^{ab}	76.50^{ab}	1.60	90.00^{ab}
T6	17.73	38.87^{a}	13.06^{a}	21.14^{a}	0.264^{a}	0.980^{a}	119.23^{a}	1.74	97.50^{a}
T7	17.73	29.315^{b}	11.89^{b}	11.58^{b}	0.144^{b}	0.630^{b}	65.33^{b}	1.74	85.00^{bc}

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

There are not significant differences between control, T2, T3 and T4, however, T4, T3 and T2 were better than control in growth parameters values respectively. The basal diet contained 245mg/Kg Fe and 18.5 mg/kg Zn, hence the best concentration of Fe and Zn are 305mg/kg and 78.5mg/kg diet as realized with T4 which was the best treatment of bulk form Fe and Zn supplementation.

It is known that, Iron plays an important role in the work of the organs and tissues of higher fish such as the physiological processes of oxygen transport, cellular respiration, and lipid peroxidation also, its role in steroid synthesis, DNA synthesis, drug metabolism, and electron transfer (Lee *et al.,* 1981and Crichton, 1991). Besides, Zinc is no less important than Iron, it plays a key role in various metabolic pathways like prostaglandin metabolism and structural role in nucleoproteins (Chanda *et al.*, 2015). Moreover, Zinc is an integral part of about 20 metalloenzymes such as alkaline phosphatase, alcohol dehydrogenase and carbonic anhydrase. It also serves as a specific cofactor of several enzymes and acts as a catalyst for regulating the activity of several Zn-dependent enzymes. Recent research on zinc-gene interactions has revealed the basic role of zinc in controlling growth (Chesters, 1991). Hence, offering the element for the fish at suitable form and amount is essential to the highest growth and the best health. Many of previous researches which tested the chemical forms of elements confirmed that Nano forms have been reviewed to have different effects from enhancing growth and immunity through antioxidant effect to their use in less amount than its bulk counterparts which enhances ration criteria (Rather *et al.*, 2011 and Rajendran, 2013). As well as, metal oxide nanoparticles were screened for their antimicrobial activities against a wide range of bacterial and fungal agents including certain freshwater cyanobacteria (Swain et al., 2014). In the same trend, Faiz *et al.* (2015) found that, Juvenile Grass carp fed zinc oxide nanoparticles supplemented diet recorded the highest value of weight gain in contrast with diets supplemented with zinc in sulfate and oxide. Furthermore, Nano-Fe appeared to be more effective than ferrous sulfate in improving antioxidant enzymatic activities irrespective of different iron sources in the basal diet (Behera, 2014).

Srinivasan *et al.* (2016) reported 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, growth of sturgeon and young carp increased from 24% to 30 in consequence of using Nano Fe as compared to bulk form. In relation to nano-zn, Tawfik *et al.* (2017) showed that, Nano zinc supplementation increased the growth more than the ordinary Zn oxide Uzo-god *et al.* (2018 a) demonstrated, the basal diet supplemented with nZnO achieved higher weight gain than other was supplemented ZnO. However, the results are in conflict with Uzo-god *et al.* (2018b) who reported that, all growth parameters, weight gain (WG), percent weight gain (%WG), specific growth rate (SGR), as well as feed conversion ratio (FCR) were significantly ($P < 0.05$) better improved in fish fed the Fe_2O_3-supplemented diet compared to the nFe_2O_3.

There are many of interpretations which cleared the nanoparticles role in fish nutrition, in particular iron and zinc Nano particles, such as Huber (2005) reported that, Fe_2O_3 NPs boost bioavailability than other forms of iron nanoparticles fishes, this was in agreement with the nano form of iron oxide (Fe_2O_3) wich is highly bioavailable (96 % similar with $FeSO_4$) in rats without tissue accumulation (Hilty *et al.*, 2010 a, b). Also, NPs has maximum influence on the activity of digestive enzymes in M. rosenbergii PL, led to the maximum digestion of the food offered. The enhanced activities of digestive enzymes recorded in test prawns led to enhanced food consumption and food conversion, which in turn ultimately led to better survival and growth of *M. rosenbergii PL* (Srinivasan *et al.* (2016). Likewise, Behera (2014) suggested that, nano-Fe could have a special metabolism pathway and deposition mechanism in fish. A reduction of macromolecule to nanoscale changed their properties and increased their application (Rather *et al.,* 2011). The small particles size of nZnO increase absorption rate, bioavailability and catalytic activities as reported by Alishahi *et al.* (2011). Also, it may be attributed to somatic growth by stimulation of DNA and RNA synthesis and growth hormone protein synthesis (Siklar *et al.,* 2003). In addition to the value of tilapia growth hormone was noticed to be increased more in serum in case of fish fed on the nZnO compared to conventional zinc oxide within the same concentration (Tawfik *et al.,* 2017), this agreed with Hina *et al.* (2015) reported that, nZnO promoted the growth performances of juvenile *C. idellain* more than other inorganic conventional forms. Over and above, nanoparticles have ability on the immune boost this was supported by Luo *et al.* (2015) who reported that, nanoparticles can stimulate innate and adaptive immune response depending on their physicochemical properties.

Regarding, the optimum amount of added element. From table (4) it could be noted that, T7 (60mg nFe_2O_3+60 nZnO mg/kg diet) had the lowest in growth parameters followed by T5 (20mg nFe_2O_3+20 nZnO mg/kg diet) and control. Meaning that the supplementation of nFe and nZn did not fit for tilapia requirements with T7 and T5. Whereas, fish fed Fe and Zn bulk form were better in growth than T7. So may be the high amount of nFe and nZn are the reason of lower growth as reported by (Uzo-God *et al.,* 2018b) 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 Fe_2O_3, 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 element 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 reported by Uzo-God *et al.* (2018a). 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). 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 to aquatic organisms at high concentrations (Fahmy *et al.,* 2014).

It can be concluded that, 40mg nFe+40mg nZn as feed additive to basal diet (contained 245mg/kg Fe and 18.5mg/kg) was the optimum supplementation for juvenile tilapia growth and survival rate. The present study examined in equal amount of Fe and Zn as 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. Whereas, juvenile Grass carp fed 30 mg nZno/kg as feed additive achived better growth than 60 mg and this agree with our results. In other study, 54 mg /kg of NFe as supplemented diet had the best final weight of *Labeo rohita* fish comparable to 55mg /kg Fe sulfate and diet control (Faiz *et al.,* 2015 and Behra *et al.,* 2014). Also, in another study evaluated the effect ZnO and nZnO as feed additive in tilapia diet, suggested that, 60mg and 40mg/kg of nZno had the best SGR.

Feed efficiency

Effect of supplementation Nano-iron oxide and Nano-zinc oxide in diet comparison ordinary iron oxide and zinc oxide on feed efficiency of *O. niloticus* fingerlings.

Table (5) shows the significant differences between the treatments in feed efficiency, the highest FI was achieved with T4 followed by T5 and T7while the lowest FI was achieved with T6. Also control, T2, T3, T5and T7 did not significantly differ in FI while, the best FCR was achieved with T6 and the worst FCR was with T7. Also, there was insignificance between control, T2, T3, T4, T5 in PER and EER and this treatment which differed with T6 and T7 whereas T6 had the highest PER, APU, EER and EPV vice versa obtained with T7.

Table 5: feed efficiency parameters

Treatment	FI, g/fish	FCR*	PER, g	APU, %	EER, g/Kcl	EPV,%
Control	58.64^{ab}	4.36^{ab}	0.747^{b}	43.11^{ab}	0.0467^{b}	27.06^{b}
T2	59.99^{ab}	4.37^{ab}	0.763^{b}	42.42^{ab}	0.0477^{b}	28.63^{b}
T3	56.41^{ab}	4.00^{ab}	0.838^{b}	47.52^{ab}	0.0524^{b}	31.47^{ab}
T4	69.23^{a}	4.59^{ab}	0.752^{b}	40.21^{b}	0.0470^{b}	27.84^{b}
T5	67.76^{ab}	5.22^{ab}	0.661^{b}	33.09^{b}	0.0413^{b}	24.51^{b}
T6	52.30^{b}	2.50^{b}	1.325^{a}	73.96^{a}	0.0828^{a}	48.32^{a}
T7	66.99^{ab}	5.87^{a}	0.559^{b}	30.40^{b}	0.0349^{b}	21.22^{b}

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

Feed efficiency parameters revealed that T6 was the best in all parameters compared with the other treatments. It can be said that, the dietary 40mg Fe +40 mg Zn/Kg supplementation in nanoparticles form was the optimum supplementation for the best growth and feed efficiency, considering of nanoparticle form of Fe, Zn have higher efficiency compared to other inorganic form of Zn. The Nano form of particles have higher intestinal absorption, bioavailability and catalytic activities (Dube *et al.*, 2010). Therefore, it might possible that conversion of ZnO in Nano form increase 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.* (2018a) 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. In the same trend, 40mg/KgFe_2O_3 nanoparticales supplemented diet had high effect on the activities digestion enzyemes. Led to the maximum digestion of feed intake resulted in high APU and feed efficiency. Many of researches suggested that, NFe_2O_3 has moer effect on metabolisim rates and influence on amino acids synthesis, this led to better growth and FCR. Furthermore, Tawfik *et al.* (2017) mentioned, the highest feed efficiency was achived with fish fed nZnO. On the othe hand, Uzo-God *et al.* (2018b) differed with our results and reported that FCR significantly better improved with fish fed Fe_2O_3 supplemented diet than those fed nFe_2O_3.

Whole body chemical composition

Effect of supplementation Nano-iron oxide and Nano-zinc oxide in diet compared to ordinary iron oxide and zinc oxide on body chemical composition of *O. niloticus* fingerlings.

Whole body chemical composition in at the start and the end of this study are shown in Table (6) there were significant differences between the treatments in DM, EE, ash and GE. T7 had the highest EE (32.47%) and GE (6.08 kcal/g) and achieved the highest DM (29.73) followed by fish fed Fe_2O_3+ZnO and nFe_2O_3+nZnO supplemented diets. This was in agreement with Uzo-God *et al.* (2018b) who explained that; higher mineral content and exposed surface area, therefore, imply higher water adsorption capacity. nFe_2O_3 and Fe_2O_3-supplemented diets contain more minerals than the control feed, while nFe2O3 contains much higher surface area than the same amount of Fe_2O_3.

Table 6: Whole body chemical composition of Nile tilapia juveniles at first and end of trial.

Treatment	Dry matter (DM)	Crude protein (CP)	Ether extract (EE)	Ash	Gross energy Kcal/g (GE)
Start	**27.14**	**54.57**	**32.54**	**12.48**	**6.09**
Control	29.73^{a}	50.90	30.50^{bc}	13.35^{b}	5.96^{b}
T2	29.71^{a}	55.00	31.90^{ab}	13.03^{c}	6.05^{ab}
T3	28.42^{ab}	55.44	31.62^{abc}	12.30^{d}	6.05^{ab}
T4	28.42^{ab}	54.03	32.00^{ab}	13.43^{b}	6.01^{ab}
T5	25.20^{c}	57.46	30.11c	12.10^{d}	6.02^{ab}
T6	25.97^{c}	55.53	30.72^{bc}	13.93^{a}	5.59^{b}
T7	26.75^{c}	54.46	32.47^{a}	13.41^{b}	6.08^{a}

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

CP did not significantly differ between the treatments however, fish fed Fe+Zn in nanoparticles had the highest CP followed by fish fed F_2O_3+ZnO –supplemented diet and control. This is attributed to Fe which works as cofactor for many enzymes in paticial protease enzymes feeding to and increase in Retained protein. This disagreed with Uzo-God *et al.* (2018b) who reported that, control had the highest CP followed by Fe_2O_3 and nFe_2O_3. T6 had the highest ash (13.93%). This may be due to 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).

Accumulation of Fe and Zn in tissues

From Table (7) it can be observed that, control had the lowest concentrate of Fe and Zn in muscles, gills and liver, which is natural. Also, the treatments did not significantly differ in gills and liver content of Fe, however Muscles content of Fe was higher with fish fed Fe+Zn nanoparticles as feed additive in general than those fed conventional Fe+Zn or control. T6 recorded high value (131.93mg/kg) followed by T7, T5, T4, T3 T2 and control (68.29 mg/kg). This may be attributed to the ease with which Fe nano be taken up by body and higher absorption and avilability on the contrary conventional Fe as mentation by Feng *et al.,* (2009). Also this was agree with Behera *et al.* (2014) who cleared that, nano-Fe 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.

In relation to Muscles, gills and liver content of Zn were significantly differed between the treatments as shown in Table (7). The muscles content of Zn was higher in fish muscle which fed bulk Fe+Zn than those fed nanoparticles supplementation while control had the lowest. This is supported by Tawfik *et al.* (2017) who reported that, Zinc concentration in muscles showed higher values in all fish groups fed with ZnO supplemented feed compared to the control.

Table 7: The content of fish muscles (M), gills (G) and liver (L) of elements iron and zinc (mg/kg) on basis dry matter.

Treatment	Iron (M)	Zinc (M)	Iron (G)	Zinc (G)	Iron (L)	Zinc (L)
Control	68.29^{b}	16.5^{f}	241.34^{c}	37.4^{f}	228.34^{c}	46.2^{e}
T2	72.25^{b}	24.7^{b}	403.08^{b}	48.0^{a}	235.20^{b}	57.4^{b}
T3	76.04^{b}	26.9^{a}	254.68^{c}	42.1^{c}	346.04^{b}	47.8^{d}
T4	108.68^{a}	21.6^{c}	509.00a	44.9^{b}	700.20^{a}	54.8^{c}
T5	125.23^{a}	17.3^{e}	264.08^{c}	39.9^{d}	322.22^{b}	62.3^{a}
T6	131.93^{a}	17.6^{e}	264.08^{c}	41.9^{c}	322.56^{b}	54.9^{c}
T7	125.78^{a}	20.4^{d}	394.42^{b}	38.8^{e}	370.96^{b}	54.4^{c}

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

Concerning, liver content of Fe and Zn at the end of experimental period. The treatments of Fe and Zn nanoparticles had lower level of Fe content in juveniles muscles than bulk Fe and Zn supplemented diet while control had the lowest. Whereas, T4 recorded (700.20 mg/Kg) followed by T7, T3, T2, T6, T5 and control (228.34 mg/kg).

In relation to liver content of Zn, fish fed Nano Fe and Zn had the highest concentration of Zn followed by bulk Fe and Zn and control. This is in agreement with Uzo-God *et al.* (2018a). Likewise this result was in partial agreement with Abdel-Khalek *et al.* (2011) who concluded that, zinc nanoparticles (Zn NPs) had more efficiency to penetrate the studied tissues such as the liver, kidneys, gills, skin, and muscle .It can be observed that the accumulation of Fe in liver was in the same trend of that in muscles. Moreover, increasing liver content of Fe and Zn in comparison with muscles content of Fe and zinc may be due to, the fact that several important chemicals in the body are stored in the liver from where they are released to other parts of the body as reported by Uzo-God *et al.* (2018a). In the same trend , the gills content of Fe increased with fish fed Fe+Zn bulk than those fish fed nFe+nZn as feed additive, while the gills content of Zn was also higher with fish fed Fe+Zn bulk. Perhaps the tissue is affected by the element form so we find that the iron and Zn both accumulated in the gills were bulk while the iron accumulation in the muscles was Nano, in the same trend was the Zn accumulation in the liver. In view of Fe and Zn concentrations in muscles, they both were lesser than their concentrations in liver and gills, this return to the muscles have low blood perfusion rates as mentioned (Di Giulio and Hinton, 2008). Furthermore, Aly (2016) measured heavy metal concentrates in muscles, gills and liver of Nile tilapia; the fish were collected from a fish farm located at Al-Abbassa, Sharkia governorate, Egypt. He found that, iron concentration in liver and gills were higher than that of muscles. He explained that, may be because liver and gills had a high tendency to accumulate high concentrations of heavy metals, while muscles tend to retain lower concentrations, whereas, iron concentrates in muscles, gills and liver were 299.3, 332.9 and 351.2 mg/kg(basis on dry weight) respectively, this was in agreement with our results. In the same trend, Khalil and Hussien (1996) who found that, heavy metals were significantly higher in fish viscera, including liver tissue, than in the edible muscle tissues. In view of the present study, the concentration of Fe in fish muscles (which fed on Nano or bulk iron) agree with El-Ghobashy *et al*. (2002) who decided that, iron concentrations are between 23.8- 167.3 ppm in tilapia muscles. Also, Ali (2007) said that, Fe concentrate is between 30.3-194 ppm in muscles and gills of tilapia spp. In addition to, our finding was in agreement with Aly (2016) who cleared that, Zn in tilapia increased order of muscles < gills< liver and Murugan *et al*. (2008) confirmed the highest concentration of Zn in liver of *C. punctatus*.

Generally in the present study, Fe and Zn concentration in tilapia muscles, gills and liver are lower than the levels that issued by some organizations as mentioned (Aly, 2016).

CONCLUSION

It can be concluded that, fish fed on 40mg Fe/kg+40mg Zn/kg (T6) in nanoparticles form as feed additive was the best in growth performance parameters, survival rate and feed conversion ratio. On the contrary, fish fed on 60mgNFe+60mg NZn supplemented diet (T7) was the worst. While did not differ between the others treatments in growth parameters and FCR (T4, T3, T2, T5 and control). Also this study cleared that, fish gills and Liver content of Fe at the end increased with bulk treatments in special (T4) while the muscles content of Fe increased with nanoparticles treatments. The muscles and gills content of Zn was increased with bulk treatments (T4, T3 and T2) but the Accumulation Zn in liver was adverse that. Finally it could be said that, the result revealed that the optimum amount of Fe and Zn supplemented feed have an important role to release the best growth and FCR. Hence, in the case of adding two equal quantities of Fe and Zn in the basal diet, the best growth of *tilapia juvenile* was achieved with nanoparticles at level 40mg Fe and 40mg Zn /kg basal diet while with bulk at level to 60mg Fe and 60mg Zn /kg.

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REFERENCES

Abdel-Khalek, A.A.; Kadry, M., Hamed, A. and Marie, M.A. (2015). Ecotoxicological impacts of zinc metal in comparison to its nanoparticles in Nile tilapia (*Oreochromis niloticus*). J Basic Appl. Zool., 72:113–125.

Alishahi, A.; Mirvaghefi, A., Tehrani, M. R., Farahmand, H., Shojaosadati, S. A., Dorkoosh, F. A. and Elsabee, M. Z. (2011). Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles. Food Chem., 126(3): 935-940.

Ali, M. M. (2007). An analysis of the impact of human activities on water quality and ecological responses in the Suez irrigation Canal. Management of Environmental Quality. An int. J., 15(3):377-401.

Aly, M. Y. M. (2012). Comparison of heavy metals levels in muscles, liver and gills of three fish species collected from agricultural drainage water AT El- Abbassa fish farm, Sharkia, Egypt. Egypt. J. Aquat. Biol. & Fish., 20(3): 103 – 112.

AOAC (2005). Official methods of analysis. Association of official analytical chemists. E.U.A. 14 a. Ed. Washington, DC: Association of Official Analytical Chemists Inc.

Auffan, M.; Rose, J., Bottero, J. Y., Lowry, G. V., Jolivet, J. P., and Wiesner, M. (2009). Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Natu. Nanotechnol., 4: 634–641

Behera, T.; Swain, P., Rangacharulu, P. V and Samanta, M. (2014). Nano-Fe as feed additive improves the hematological and immunological parameters of fish (*Labeo rohita* H). Appl. Nanosci., 4:687–94.

Chanda, S.; Paul, B.N., Ghosh, K. and Giri, S.S. (2015). Dietary essentiality of trace minerals in aquaculture: A review. Agri. Review. 36: 100-112.

Chesters, J. K. (1991). Trace element-gene interactions with particular reference to zinc. Proc. Nutr. Soc., 50:123-129.

Crichton, R. R. (1991). Inorganic biochemistry of iron metabolism. West Sussex: Ellis Horwood

Di Giulio, R.T. and Hinton, D. E (Eds.). (2008). The toxicology of fishes. CRC Press. 1096 P. https://doi.org/10.1201/9780203647295

Dube, A.; Nicolazzo, J. A. and Larson, I. (2010). Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+) - catechin and (-) - epigallocatechin gallate. Eur. J. Pharm. Sci., 41: 219–225.

Eckerich, C.; Fackelmayer, F.O and Knippers, R (2001). Zinc affects the conformation of nucleoprotein elements formed by replication protein A (RPA) and long natural DNA molecules. Biochim. Biophys. Acta, 1538: 67–75.

El-Ghobashy, H. A.; Zoghloul, K. H. and Metwally, A. A. (2002). Effect of some water pollutants on the Nile Tilapia (*Oreochromis niloticus*) collected from the River Nile and some Egyptian lakes. Egypt. J. Aquat. Biol. & Fish, 5(4): 251-279.

El-Sayed, A.-F. M. (2006). Tilapia culture. CAB Interna- tional, Wallingford, UK.

ETC (2003). Action Group on Erosion, Technology and Concentration. Down on the Farm: The Impact of Nano scale Technologies on Food and Agriculture. Available at http://www.etcgroup.org/en/materials/ publications.

Fahmy, S. R.; Abdel-Ghaffar, F., Bakry, F. A., and Sayed, D. A. (2014). Ecotoxicological effect of sublethal exposure to zinc oxide nanoparticles on freshwater snail Biomphalaria alexandrina. Archives of Envir. Contamination and Toxicol., 67:192–202.

Faiz, H.; Zuberi, A., Nazir, S., Rauf, M. and Younus, N. (2015). Zinc Oxide, Zinc Sulfate and Zinc Oxide Nanoparticles as Source of Dietary Zinc: Comparative Effects on Growth and Hematological Indices of Juvenile Grass Carp (*Ctenopharyn godonidella*). Int. J. Agri. Biol., 17(3): 568-574.

FAO, Food and Agriculture Organization of the United Nations. (2009). FAO yearbook.Fishery and aquaculture statistics. http://www. fao.org /fishery/ publications/ yearbooks/en.

Feng, M.;Wang, Z. S., Zhou, A. G., and Ai, D. W. (2009). The effects of different sizes of nanometer zinc oxide on the proliferation and cell integrity of mice duodenum-epithelial cells in primary culture. Pakistan J. Nut., 8(8): 1164-1166.

Garcia, A. J.; Rodriguez, S. N., Misra, S. K., Valsami, J. E., Croteau, M. N., Luoma, S. N. and Rainbow, P. S. (2014). Toxicity and accumulation of silver nanoparticles during development of the marine polychaete platynereis dumerilii. Sci. the Total Envir., 476: 688–695.

Heijerick, D.G.; De Schamphelaere , K.A.C. and Janssen, C.R. (2002). Predicting acute zinc toxicity for Daphnia magna as a function of key water chemistry characteristics: development and validation of a Biotic Ligand Model. Environ. Toxicol. Chem., 21: 1309–1315.

Hilty, F.M.; Knijnenburg, J.T.N., Teleki, K.F., Hurrell, R.F., Pratsinis, S.E., Zimmermann, M. B. (2011a). Incorporation of Mg and Ca into nanostructured Fe_2O_3 improves Fe solubility in dilute acid and sensory characteristics in foods. J. Food Sci., 76:1-10.

Hilty, F.M.; Arnold, M., Hilbe, M., Teleki, A., Knijnenburg, J.T., Ehrensperger, F., Hurrell, R.F., Pratsinis, S.E., Langhans, W. and Zimmermann, M.B. (2010b) Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nat. Nanotechnol., 5:374–380

Hina, M.I.; Dhanapal, S. and Sekar, D.S. (2015). Studies on antibacterial activity of some fungi collected from K.R.P Dam, Krishnagiri (TN). Int. J. Eng. Res. Manage., 1-2.

Huber, D.L. (2005). Synthesis, properties, and applications of iron nanoparticles. Small 1:482–501.

Khalil, M.T. and Hussein, A. H, (1996). Recycling and reuse of wastewater for fish farming: An experimental field study at Sewage Treatment Plant, Egypt. J. Egypt. Ger. Soc. Zool., 19(B): 59-79.

Lee, Y. H.; Layman, D. K. Bell, R. B. and Norton. H. W. (1981). Response of glutathione peroxidase and catalase to excess dietary iron in rats. J. Nutr., 111(2):195–202.

Luo, Y. H.; Chang, L. W. and Lin, P. (2015). Metal-based nanoparticles and the immune system: activation, inflammation, and potential applications. BioMed res. Int., 2015.

Muralisankar, T.; Bhavan, P. S., Radhakrishnan, S., Seenivasan, C. and Srinivasan, V. (2016). The effectsof copper nanoparticles supplementation on the giant freshwater prawn (*Macrobrachiu rosenbergii*) post larvae. J. Trace Elements in Med. Biol., 34:39–49.

Murugan, S.S.; Karuppasamy, R., Poongodi, K. and Puvaneswari, S. (2008). Bioaccumulation Pattern of Zinc in Freshwater Fish (*Channa punctatus* Bloch.) After Chronic Exposure. Turk. J. Fish. Aquat., Sci., 8: 55-59.

Nowack, B. and Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. Environ. Pollu., 150 (1): 5-22.

NRC (1993). National Research Council, Nutrient requirements of fish. National Academy Press, Washington D.C., USA.

Obirikorang, K. A.; Amisah, S., and Skov, P. V. (2016). Growth performance, feed utilization and sensory characteristics of Nile Tilapia (*Oreochromis niloticus*) fed diets with high inclusion levels of copra meal. J. Anim. Res. Nutr., 1(4):18.

Płaza, G. A.; Chojniak, J., and Banat, I. M. (2014). Biosurfactant mediated biosynthesis of selected metallic nanoparticles. Int. J. molecular sci., 15(8): 13720–13737.

Prabhu, A.J.P.; Schrama, J. W. and Kaushik, S. J. (2014). Mineral requirements of fish: a systematic review. Reviews in Aquacult. 6: 1-48.

Rajendran, D. (2013). Application of nano minerals in animal production system. Res. J. Biotechnol., 8(3):13.

Rather, M. A.; Sharma, R., Aklakur, M., Ahmad, S., Kumar, N., Khan, M. and Ramya, V. L. (2011). Nanotechnology: a novel tool for aquaculture and fisheries development. A prospective mini-review. Fish. Aquacult. J., 16:1-5.

Siklar, Z.; Tuna, C., Dallar, Y. and Tanyer, G. (2003). Zinc deficiency: a contributing factor of short stature in growth hormone deficient children. J. tropical pediatrics, 49(3): 187-188.

SPSS (2007). Statistical Package For Social Science (for Windows). Release 16 Copyright ©, SPSS Inc., Chicago, USA.

Srinivasan, V.; Bhavan, P. S., Rajkumar, G., Satgurunathan, T. and Muralisankar, T. (2016). Effects of dietary iron oxide nanoparticles on the growth performance, biochemical constituents and physiological stress responses of the giant freshwater prawn (*Macrobrachium rosenbergii*) post-larvae. Int. J. Fish. Aquatic Studies, 4(2): 170-82.

Swain, P.; Nayak, S. K., Sasmal, A., Behera, T., Barik, S. K., Swain, S. K., Mishra, S.S., Sen, A. K., Das, J.K., and Jayasankar, P. (2014). Antimicrobial activity of metal based nanoparticles against microbes associated with diseases in aquaculture. World J. Micro. Biotechnol., 30(9): 2491-2502.

Tawfik, M. M. M.; Moustafa, M. M., Abumourad, I.M.K., El-Meliegy, E. M. and Refai, M. K. (2017). Evaluation of Nano Zinc Oxide feed additive on tilapia Growth and Immunity. 15th Int. Con. Envi. Sci. &Technol. Rhodes, Greece, 31 August to 2 September 2017.

Uzo-God, O. C.; Agarwal, A. and Singh, N. B. (2018b). Effects of dietary nano and macro iron oxide (Fe_2O_3) on the growth, biochemical, and hematological profiles of African catfish (*Clarias gariepinus*) fingerlings. J. App. Aquacult., 31(2): 153-171.

Uzo-God, O. C.; Aggarwal, A. and Singh, N. B. (2018a). ZnO nanoparticles as feed supplement on growth performance of cultured African catfish fingerlings. J. Sci. Indus. Res., 77:213-218.

Viola, S.; Malady, S. and Rappaport, U. (1981). Partial and complete replacement of fish meal by soybean meal in feeds for Intensive culture of carp. Aquacult., 26: 223-236.

Watanabe, T.; Kiron, V., and Satoh, S. (1997). Trace minerals in fish nutrition. Aquacult., 151(1-4): 185-207.

ARABIC SUMMARY

دراسة لمقارنة مستويات مختلفة من عنصرى الحديد والزنك فى الصوره النانو بالصوره العادية كأضافات غذائية على مظاهر النمو ،الكفاءة الغذائية والتركيب الكيميائى لأصبعيات البطى النيلى

محمد سعيد محمد عبد الحميد[١] ، صبحى محمود علام[٢] ، عطاالله عبد التواب متولى[١] ، كمال الديب[٢]، محمد فتحى عيد عبد العزيز[١]

١- شعبة تربية الأحياء المائية، المعهد القومي لعلوم البحار والمصايد، مصر
٢- قسم الإنتاج الحيوانى، كلية الزراعة، جامعة الفيوم، مصر

دراسة إجريت لمقارنة تأثير ثلاثة مستويات من الحديد والزنك معا فى الصوره النانو ($nZnO+nFe_2O_3$) مع نفس المستويات من الحديد والزنك فى الصوره العادية (Fe_2O_3+ ZnO) كأضافات غذائية على أداء النمو والكفاءة الغذائية والتركيب الكيميائى وتراكمات الأنسجة من الحديد و الزنك لأصبعيات أسماك البلطي النيلي (وزن ابتدائى ١٧,٧٣جم). استخدمت عليقة أساسية (٣٠,٧١ ٪ بروتين) مع جميع المعاملات. تكونت هذه التجربة من سبعة معاملات، المعاملة الأولى كنترول وغُذيت الاسماك على العليقة الاساسية فقط، المعاملة الثانية أُضيف للعليقة الاساسية (٢٠ملجم حديد عادى مع ٢٠ملجم زنك عادى/ كجم علف) المعاملة الثالثة (٤٠ملجم حديد عادى مع ٤٠ملجم زنك عادى/كجم علف) المعاملة الرابعة (٦٠ملجم حديد عادى مع ٦٠ملجم زنك/كجم علف) المعاملات الخامسة والسادسة والسابعة أختبرت المستويات السابقة ولكن فى الصوره النانو. رُبيت الأسماك في تنكات فيبرجلاس (١,٥ م٣) بمعدل تسكين ٢٠سمكة/ تنك. غُذيت الأسماك مرتين يوميًا ٨صباحا و ٤مساءا ، بمعدل تغذية ٥٪ من وزن الجسم. و استمرت التجربة لمدة ٨٠ يوما. التحليل الإحصائي أظهر فروق معنوية بين المعاملات في مقاييس مظاهر النمو، الكفاءة التغذية ، التركيب الكيميائي للجسم ومحتوى أنسجة العضلات والخياشيم والكبد من الحديد والزنك. المعاملة السادسة (٤٠ملجم حديد نانو+٤٠ملجم زنك نانو/ كجم علف) حققت أفضل معدل نمو وتحويل غذائى على النقيض المعاملة السابعة (٦٠مجلم حديد نانو +٦٠ملجم زنك نانو/كجم علف) كانت الأقل فى مقاييس النمو ومعدل التحويل الغذائى. محتوى الجسم من البروتين فى نهاية التجربة لم يختلف معنويا بين المعاملات . كما أوضحت التجربة أن الصوره النانو للحديد والزنك أدت إلى زيادة محتوى العضلات من الحديد ، بينما الصوره العادية أدت الى زيادة محتوى الكبد والخياشيم من الحديد. أيضا الصوره النانو للحديد والزنك أدت إلى زيادة الزنك فى الكبد على العكس الصوره العاديه ادت الى زيادة محتوى الخياشيم والعضلات من الزنك.