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Effect of Iron Nanoparticles on the Development of Fish Farm Feeds

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ABSTRACT

Two hundred and seventy monosex males Nile Tilapia *(Oreochromis niloticus)* with an average body weight of 16.65±0.2 g/fish were used to investigate the effect of dietary replacement of inorganic iron by different levels of Fe-NPs on growth performance, body composition and health status of *A. hydrophila* challenged Nile tilapia fish. Fish maintained on 30% protein level, Six different experimental diets supplemented with 0.0 (T_1), 85.0 mg inorganic Fe/kg (T_2) and 85.0, 63.75, 42.5 and 21.25mg Fe-NPs/kg (T_3-T_5) were fed to triplicates groups of fish. It was found that the final body weight, weight gain, SGR, FCR and PER were significantly improved in the fish fed on the iron-supplemented diets compared to those fed on the iron-deficient diet (T_1). Blood pictures and serum biochemical were significantly increased before and after *A. hydrophila* infection in the iron-supplemented groups compared to the iron-deficient diet group. The dietary administration of iron NPs improved the antioxidant enzymatic activities (CAT and TAC) irrespective of the different iron sources in the basal diet. Moreover, it was observed that the diets containing 63.75 and 42.5 mg/kg of Fe-NPs exhibited the best survival rate compared to the other groups.

1. INTRODUCTION:

Iron (Fe) is an essential element for body functions such as oxygen transport and lipid oxidation and its presence constitutes a vital defense line to protect fish against infectious diseases (Hilty et al., 2011). In addition, it is considered a vital element for immunity improvement (Hube, 2005). On the other hand, iron deficiency in fish leads to immune suppression, impairs growth performance and deteriorates feed conversion. However, iron excess can result in fish toxicity (Andersen et al. 1996; Andersen et al. 1997 and Chu et al. 2007). Feed is regarded as the main source of iron for fish owing to low concentration of soluble iron in water and limited iron absorption through gills (NRC, 2011 and Bury et al. 2003).

Different iron chemical forms can be used in fish diets to meet the nutritional requirements. However, the available data determining the suitable concentrations of iron and its bioavailability from different sources are still limited and need more investigations. Nanotechnology plays an important role in aquaculture industries through using new tools such as the rapid diagnosis of diseases which will enhance the ability of cultivable organisms to uptake drugs like hormones, vaccines and essential nutrients (Rather et al. 2011). Metallic nanoparticles (NPs) proved to have a positive effect on aquaculture development. For example, iron NPs improved carp growth and increased iron concentration in their muscles (Behera et al. 2014). As well, different Se sources (nano and organic) in carp diets improved fish growth and increased Se concentration in muscles (Zhou et al. 2009). In a recent

study, nano Zn improved growth and increased Zn concentration in the muscles of Nile Tilapia (Tawfik et al. 2017).

Chemically, iron oxide (Fe_2O_3) and nano-iron oxide (n-Fe_2O_3) have the same formula which suggests similar iron to oxygen ratio. However, at the nano scale, atoms are arranged with a wider energy level confinement and smaller size Fe, leading to more reactive atoms as the surface is increased (Zhon 2004). Fe_2O_3 NPs have attracted significant attention and research interest compared to other iron forms, attributable to their high availability through intestinal mucosa (Stephen 2007). Normally, iron is accompanied by protein in different body tissues (Barton and Edwards 2000), consequently, muscle meat and seafood are dependable dietary sources of minerals (Gibson 1990). Meat oxidation leads to loss of its nutritional value. Therefore, antioxidants must be added to animal and fish feed such as iron to prevent such oxidative process and keep meat quality (Min et al. 2008).

Scarce literature has compared between the effects of inorganic and nano iron supplemented diets on Nile Tilapia fish. Therefore, the objective of the present study was to examine the effect of inorganic iron replacement with different levels of Fe-NPs on the growth performance, immune response, some blood serum biochemical changes, body chemical composition and iron concentration in the gills and dorsal muscle of *A. hydrophila* challenged Nile Tilapia.

2. MATERIALS AND METHODS

2.1.Experimental Fish:

To achieve the purpose of the present study, a total of 270 monosex males Nile tilapia *(Oreochromis niloticus)* were obtained from a private local farm in Kafrelsheikh, Egypt, with an average body weight of 16.65±0.2 g/fish. Fish were transported in a well-aerated tank to the laboratory of Animal Health Research Institute at Kafrelsheikh and then kept in glass aquaria. These aquaria were supplied with chlorine-free tap water**.** The aquaria were continuously aerated by an electric pump and were held at 28±2ºC. Half of the water was changed daily. Fish were acclimated for two weeks. During the acclimation period, fish were fed on the basal diet only.

2.2. Experimental Design and Procedure:

The formulation of 30% crude protein and 3371 Kcal DE/kg basal diet for Nile tilapia is shown in table 1. Six treatments were used. In treatment 1 (T_1) only the basal diet without iron supplement (Fe-deficient group), treatment 2 (T_2) 85mg inorganic iron/kg diet (optimum Fe level according to NRC 2011) were added to the basal diet, while treatments 3-6 (T_3-T_6) 85, 63.75, 42.5 or 21.25 mg of Fe-NPS/kg were added to the basal diet respectively and that Fe-NPs concentrations represents 100%, 75%, 50% and 25% of Nile tilapia Fe requirement according to the NRC recommendations.

Table 1. Physical and Chemical Composition of the Basal Diet

Physical Composition		**Chemical Composition**	
Ingredients	**%**	**Items**	**%**
Yellow corn	17.85	Moisture	12.43
Soybean meal 44%	44.0	Crude protein	30.3
Fish Meal 59%	12.0	Crude fat	5.12
wheat flour	15.0	Ash	6.88
wheat grain	5.0	CHO	45.27
Soybean oil	2.5	Calcium	1.07
DCP	1.0	Total phosphorus	0.89
DL-methionine	0.2	DE***	3371.07 Kcal/Kg
Salt	0.25	P/E ratio****	
Vitamins mixture*	0.1		89.88
Mineral mixture**	0.1		
Carboxy methyl cellulose	2.0		

*Vitamins mixture- each one Kg contains: vitamin A 12000000 IU, vitamin D3 2200000 IU, vitamin E 10 g, vitamin K3 2 g, vitamin B1 1 g, vitamin B2 5 g, vitamin B6 1.5 g, vitamin B12 0.01 g, vitamin C 250 g, Niacin 30 g, Biotin 0.050 g, Folic acid 1 g and Pantothenic acid 10 g and carrier to 1000 g.

**Mineral mixture (iron free): Manganese 60g, Copper 4 g, Zinc 50g, Iodine 1g, Cobalt 0.1g, Selenium 0.1g, calcium carbonate (CaCO3) carrier to 3000g.

***Digestible energy: Protein (4.49 Kca/g), Ether extract (8.5 kcal/g), Carbohydrate (3.48 Kcal/g) (Shiau and Haung 1990).

****P/E ratio = mg of protein/Kcal of DE (NRC 2011).

Table 2. Experimental Design Outline

Treatment No.	Experimental Diet	Inorganic Iron Concentration*	Nano-iron Concentration**
1	Basal diet	---	--
2	"""	85ppm	--
3	"""	--	85ppm
4	"""	--	63.75ppm
5	"""	--	42.5ppm
6	"""	--	21.25ppm

***Inorganic iron**: supplemented and calculated from iron oxide (Fe_2O_3), produced by El-Gomhoria Co., Egypt.
****Nano iron**: supplemented and calculated from iron oxide nanoparticles (Fe_2O_3 NPs), produced by Mknano Co. "M K Impex Corp, Canada" with 30nm.

The feed ingredients were thoroughly mixed, moisten with warm water (400 ml/kg) and then cold pressed and extruded to produce 2mm pellets. The diets were dried in an air convection oven set at 45^0c. After drying, the diets were stored in airtight bags prior to use.

Fish were randomly allotted into 6 equal groups (45 fishes per group) of 3 replicates (in aquaria measuring 40 x 40 x 80 cm) containing 15 fishes each. Table 2 shows the applied experimental design. Fish were fed to apparent visual satiation; by hand twice a day at 9:00 and 14:00. Extreme care was taken to assure that all supplied feed was consumed. Fish in each group were weighed at the beginning (W0) and then biweekly weighed for a successive period of 8 weeks.

Weight gain was calculated as follows:

Weight gain = (Final body weight- Initial body weight).

Gain% = (Total gain/Initial Wt.) X100

Specific Growth Rate (SGR) was calculated as follows:

SGR = (lnWf - lnWi x100)/t. InWf = the natural logarithm of the final weight

InWi = the natural logarithm of the initial weight. t = time (days) between lnWf and lnWi

Feed Conversion Ratio (FCR) was calculated by dividing total feed intake per aquarium by the total body weight gain per the same aquarium.

Protein Efficiency Ratio (PER) was calculated as follows:

PER = Weight gain/Protein intake

Nutrient Retention Efficiency% = 100 X {Nutrient gain in fish body (g)/Nutrient intake (g)}

2.3. Hemato-immunological Parameters:

Blood samples were collected without anticoagulant from six fishes of each group (two of each replicate) at the end of the growth trial and one week after infection. Samples sera were separated by centrifugation and stored at -20°C until analysis. Serum total protein, albumin, globulin, ALT, AST, catalase (CAT) and total antioxidant capacity (TAC) were estimated using commercial kits produced byBio Diagnostic (Diagnostic and Research Reagents). Another sample of the blood was taken on anticoagulant (sodium citrate). Blood smears were prepared for the determination of differential leukocyte counts (Schalm 1986). Additional smears were prepared for phagocytic index and activity calculation (Kawahara et al. 1991).

2.4. Samples Collection and Chemical Analysis:

Feed samples were collected and a total of 42 fishes were sampled for proximate analysis. Six fishes at the start of the experiment and 6 fishes from each group at the end of the experiment (2 fishes from each replicate) were collected for whole body composition. All feed and fish samples were stored at -4°C until analysis. Dry matter (DM) contents of feed samples were determined by oven-drying overnightat 105°C (AOAC 2000). Ash contents of feed samples were determined by incineration overnight at 550°C. Crude protein (CP) in feed samples was determined using the Kjeldahl method as described by Randhir and Pradhan (1981). Ether extract (EE) was determined according to the technique of (Bligh and Dyer 1959) modified by Hanson and Olly (1963). Calcium and phosphorus in feed and fish samples were determined according to (Slavin1982) and (Geriche and Kurmies 1952) respectively.

2.5. Iron Determination:

Tissue (gill and muscle) samples were dried. One gram of dried tissue samples were separately digested with 10 ml of followed by 3 ml $HClO_4$, diluted with HCl then stored until analysis. Iron concentrations in tissues were determined using the flame atomic absorption spectrophotometer (Model AA-6300 Shimadzu, Japan) and standard solutions to develop concentration curves according to procedures described by (AOAC 2000).

2.6. The Challenge Test:

At the end of growth performance trial, 144 fishes (8 fishes for each aquarium; each group 24 fishes) were

challenged with the pathogen *Aeromonas hydrophila* (which was kindly obtained from the Dept. of Bacteriology, Animal Health Research Institute, Kafrelsheikh, Egypt) at 0.2 ml/ fish dose of 24 hrs. The bacterial broth (3 x 10^8 cells/ml) was injected intraperitoneal (IP) according to the technique of Schaperclaus et al. (1992). Fishes were kept under observation for two weeks where the clinical signs and mortality rates were recorded according to (Amos 1985).

2.7. Statistical Analysis:

The data obtained were statistically analyzed by one-way ANOVA to study the effect of different treatments on the studied variables using the statistical analysis system (SAS 2004).

3. RESULTS

3.1. Growth Performance

Table 3 shows that there was no significant difference between the experimental groups in body weight at the start of the experiment. At the end of the experiment (after 8-week culture), it was observed that inorganic iron supplementation (T_2) non significantly ($P \geq 0.05$) increased final body weight of Nile tilapia by about 4.5% compared to the iron-deficient treatment (T_1). Similarly, the supplementation of 85, 63.75, 42.5 and 21.25 mg of Fe-NPs (instead of the inorganic source) non significantly ($P \geq 0.05$) improved final body weight of Nile tilapia by about 1.0%, 10.6%, 6.1% and 4.8% respectively, compared to optimum inorganic iron supplemented group (T_2).

Moreover, inorganic-Fe supplemented diet (T_2) significantly ($P < 0.0$) improved the total body gain, average daily gain and weight gain while, non-significantly ($P \geq 0.05$) improved FCR and PER values compared to the iron-deficient treatment (T_1). Replacement of inorganic iron (T_2) by 85mg of Fe-NPs/kg diet (T_3) non significantly reduced total body gain, average daily gain and weight gain, FCR and PER values while replacement by lowers levels of Fe-NPs (T_4-T_6) non significantly improved the mentioned parameters. The best growth performance and feed efficiency parameters was obtained by 63.75mg of Fe-NPs supplementation (T_4).

Table 3. Growth Performance and Feed Efficiency Parameters of Nile Tilapia Fed on the Iron- deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels

Parameters	Iron Supplement Sources and Levels					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Initial body weight (g/fish)	16.91 ±0.84^{a}	16.34 ±0.52^{a}	16.81 ±0.62^{a}	16.34 ±0.59^{a}	17.06 ±0.69^{a}	16.41 ±0.62^{a}
Final body weight (g/fish)	27.60 ±1.23^{b}	28.83 ±1.08^{ab}	29.13 ±1.13^{ab}	31.88 ±1.12^{a}	30.58 ±1.28^{ab}	30.21 ±1.30^{ab}
Total body gain (g/fish)	9.24 ±0.74^{c}	11.35 ±0.60^{b}	11.17 ±0.61^{b}	14.88 0.60^{a}	12.46 ±0.67^{b}	12.67 0.84^{b}
Average daily gain (g/fish)	0.17 ±0.01^{c}	0.20 ±0.01^{b}	0.20 ±0.01^{b}	0.27 ±0.01^{a}	0.22 ±0.01^{b}	0.23 ±0.01^{b}
Weight gain %	51.53 ±3.72^{d}	64.48 ±1.89^{bc}	62.23 ±2.44^{c}	88.97 ±3.90^{a}	68.75 ±2.48^{bc}	72.61 ±4.09^{b}
SGR%	89.30 ±3.90^{d}	101.78 ±6.66^{bc}	98.21 ±6.23^{c}	119.43 ±7.45^{a}	103.57 ±6.09^{bc}	110.71 ±8.05^{b}
Total feed intake (g/fish)	24.30	30.50	34.60	28.80	31.90	32.70
Feed conversion ratio	3.02 ±0.22^{ab}	2.82 ±0.12^{ab}	3.29 ±0.16^{a}	2.06 ±0.13^{c}	2.74 ±0.14^{b}	2.88 ±0.22^{ab}
Protein efficiency ratio	1.27 ±0.10^{b}	1.24 ±0.07^{b}	1.08 ±0.06^{b}	1.72 ±0.07^{a}	1.30 ±0.07^{b}	1.29 ±0.09^{b}

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

3.2. Iron Concentration in Gills and Muscles:

Inorganic iron supplementation (T_2) non significantly ($P \geq 0.05$) increased dorsal muscle iron content (table 4) compared to iron-deficient diet (T_1), while supplementation of 85mg Fe-NPs/kg diet (T3) significantly ($P < 0.05$) increased dorsal muscle iron content (table 4) compared to inorganic iron supplemented group (T_2). Moreover, reducing Fe-NPs supplementation from 85 mg to 63.75, 42.5 and 21.25 mg/kg diet linearly reduced iron concentration in

muscles. Replacement of inorganic iron by different levels of nano sources increased iron concentration in muscles of Nile tilapia fish which indicates that Fe-NPs incorporation in diets were highly absorbed through the gastrointestinal tract compared to the inorganic source. Additionally, it was observed that inorganic iron or Fe-NPs at 85mg/kg diet (T_2 and T_3) supplementation non significantly ($P \geq 0.05$) increased dorsal muscle moisture by about 0.2% and 4.6% respectively compared to iron deficient treatment (T_1). However, reducing iron NPs supplementation from 85 mg to 63.75, 42.5 and 21.25 mg/kg linearly reduced dorsal muscle moisture. On the other hand, iron levels and sources had no significant effect on gills moisture and iron concentrations in Nile tilapia fish.

Table 4. Moisture and Iron Content of Dorsal Muscle and Gills of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels

Parameters	Iron Supplement Sources and Levels					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Moisture %						
Dorsal muscle	76.78 ±1.16^{ab}	76.93 ±0.19^{ab}	80.35 ±2.48^{a}	77.56 ±0.21^{ab}	75.99 ±0.68^{b}	75.82 ±0.09^{b}
Gills	77.08 ±1.04^{a}	74.81 ±2.81^{a}	75.92 ±0.24^{a}	76.56 ±1.05^{a}	74.23 ±2.25^{a}	74.80 ±1.67^{a}
Iron content (mg/100g fresh tissues)						
Dorsal muscle	0.73 ±0.45^{c}	1.75 ±0.72^{bc}	5.23 ±0.23^{a}	2.79 ±0.66^{ab}	0.99 ±0.23^{bc}	0.67 ±0.32^{c}
Gills	3.03 ±0.86^{a}	3.33 ±0.40^{a}	2.51 ±0.83^{a}	2.49 ±0.46^{a}	3.66 0.92^{a}	2.79 0.59^{a}

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Table 5. *Fresh Body Chemical Composition of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels*

Items (%)	Initial Body Composition	Final Body Composition					
		Iron Supplement Sources and Levels					
		(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Dry matter (DM)	26.80± 0.45	28.49 ±0.18^{a}	30.51 ±0.42^{a}	31.06 ±0.54^{a}	30.01 ±0.72^{a}	31.00 ±0.83^{a}	29.50 ±0.01^{a}
Moisture	73.20± 1.23	71.51± 0.39^{a}	69.49± 1.19^{a}	68.94± 1.39^{a}	69.99± 1.87^{a}	69.00± 2.07^{a}	70.50± 0.12^{a}
Crude protein (CP)	14.62± 0.65	11.61 ±0.42^{b}	14.93 ±0.47^{a}	14.50 ±0.30^{a}	14.51 ±0.88^{a}	15.68 ±1.11^{a}	14.15 ±0.54^{a}
Ether extract (EE)	6.79± 0.26	9.47 ±0.37^{b}	11.06 ±1.54^{a}	12.63 ±0.31^{a}	12.31 ±0.61^{a}	10.85 ±0.18^{ab}	9.95 ±0.01^{b}
Ash	4.37± 0.12	4.50 ±0.26^{a}	3.97 ±0.64^{a}	3.62 ±0.18^{a}	2.50 ±0.28^{a}	3.70 ±0.45^{a}	4.39 ±0.63^{a}
Carbohydrate (CHO)	1.02± 0.07	2.91 ±0.13^{a}	0.55 ±0.01^{b}	0.31 ±0.03^{b}	0.69 ±0.17^{b}	0.77 ±0.36^{b}	1.01 ±0.09^{b}
Calcium (Ca)	0.57± 0.08	1.12 ±0.07^{a}	1.07 ±0.05^{a}	1.09 ±0.13^{a}	1.29 ±0.10^{a}	1.19 ±0.15^{a}	1.22 ±0.10^{a}
Phosphorus (P)	0.32± 0.02	0.39 ±0.04^{a}	0.36 ±0.03^{a}	0.38 ±0.03^{a}	0.42 ±0.05^{a}	0.48 ±0.01^{a}	0.45 ±0.09^{a}

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

3.3. Body Composition of Fish before the Experimental Infection:

Table 5 shows that both iron sources and levels of Nile tilapia diet non significantly ($P \geq 0.05$) increased body moisture% compared to iron-deficient treatment (T_1). On the other hand, inorganic iron supplementation (T_2) significantly increased body protein and lipids while non significantly decreased body ash and CHO compared to iron-deficient treatment (T_1). Moreover, replacement of inorganic iron by different levels of Fe-

NPs had no significant effect on body protein, lipids, ash and CHO contents of Nile tilapia fish. There was no significant difference in body ash, Ca or P content in this trial.

3.4. Nutrient Retention:

Table 6 shows that the diet containing 85 mg of inorganic iron supplementation (T_2) significantly increased CP and EE retention whereas it did not significantly affect DM retention compared to the iron-deficient diet (T_1). On the other hand, the replacement of inorganic ironby 85, 63.75 and 42.5 mg Fe-NPs/kg (T_3-T_5) increased DM, CP and EE retention whereas the diet supplemented with 21.5 mg/kg reduced the aforementioned parameters compared to the basal diet supplemented by 85 mg inorganic iron/Kg. Moreover, it was observed that the highest nutrient retention was obtained by the Nile Tilapia group fed on the basal diet supplemented by 42.5 mg nano iron/Kg (T_5).

3.5. Hematological Parameters:

Hematological analysis (table 7) showed an increase in Hb%, RBCs and WBCs counts before and after *A. hydrophila* infection of Nile tilapia fish fed on the basal diet supplemented by 85 mg inorganic iron/kg (T_2) or other diets supplemented by different levels of Fe-NPs (T_3-T_6) compared to the fish group fed on the iron-deficient diet (T_1). Moreover, the 100% replacement of the inorganic iron by the nano source did not significantly ($P \geq 0.05$) reduce Hb, RBCs and WBCs counts whereas the lower levels of Fe-NPs did not significantly improve the above mentioned parameters. The highest values were observed in the Nile Tilapia group fed on the basal diet with 63.75 mg of nano-iron/kg (T_4) compared to the inorganic ironsupplemented group (T_2).

3.6. Protein Profile:

Inorganic iron supplementation or its substitution by lower levels of Fe-NPs in Nile tilapia diets non significantly ($P \geq 0.05$) improved blood serum total protein and globulin concentrations before and after *A. hydrophila* challenge (See Table 8) compared to the iron-deficient diet (T_1). best serum globulin concentration before and after A. hydrophila infection of Nile tilapia fish was obtained by 63.75 or 42.5mg of Fe-NPs/kg diet

Table 6. Nutrient Retention Efficiency of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels

Items	Initial Body Composition	Final Body Composition					
		Iron Supplement Sources and Levels					
		(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
DM (g/fish body)	4.48	7.87 ±0.02^{c}	8.78 ±0.12^{ab}	9.02 ±0.16^{bc}	9.57 ±0.23^{a}	9.49 ±0.25^{ab}	8.91 ±0.03^{bc}
DM retention efficiency%	--	15.62 ±0.23^{b}	15.79 ±0.44^{b}	14.68 ±0.50^{b}	19.79 ±0.88^{a}	17.55 ±0.88^{a}	15.16 ±0.06^{b}
CP (g/fish body)	2.43	3.20 ±0.12^{c}	4.30 ±0.14^{b}	4.22 ±0.02^{b}	4.64 ±0.27^{ab}	4.80 ±0.34^{a}	4.27 ±0.16^{b}
CP retention efficiency%	--	10.51 ±1.59^{c}	20.42 ±1.46^{ab}	17.05 ±0.82^{b}	25.30 ±3.10^{a}	24.52 ±3.51^{a}	18.63 ±1.66^{b}
EE (g/fish body)	1.11	2.33 ±0.10^{b}	3.33 ±0.44^{a}	3.67 ±0.09^{a}	3.93 ±0.19^{a}	3.32 ±0.06^{a}	3.04 ±0.02^{a}
EE retention efficiency%	--	98.81 ±8.18^{c}	141.87 ±28.48^{b}	145.57 ±5.21^{b}	191.32 ±13.17^{a}	135.39 ±3.47^{b}	113.23 ±0.11^{c}
Ash (g/fish body)	0.70	1.25 ±0.07^{a}	1.15 ±0.19^{a}	1.05 ±0..05^{a}	1.09 ±0.08^{a}	1.13 ±0.14^{a}	1.32 ±0.19^{a}
Ash retention efficiency%	--	32.25 ±4.05^{a}	21.22 ±8.85^{b}	14.57 ±2.17^{c}	14.81 ±4.46^{c}	19.73 ±6.32^{b}	28.48 ±8.48^{a}

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Table 7. Hematological Parameters of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets supplemented with Different Iron Sources and Levels

Parameters	Iron Supplement Sources and Levels					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Before *A. hydrophila* Challenge:						
Hemoglobin (g/dl)	7.70 ±0.10^b	9.00 ±0.10^a	9.00 ±0.20^a	9.25 ±0.25^a	9.15 ±0.25^a	9.05 ±0.15^a
RBC (X10^6 cells/µl)	1.50 ±0.10^a	2.00 ±0.10^a	2.00 ±0.20^a	2.20 ±0.20^a	2.10 ±0.20^a	2.10 ±0.20^a
WBCs (X10^3/µl)	29.33 ±6.32^a	31.50 ±1.54^a	29.53 ±2.13^a	37.82 ±4.80^a	33.09 ±5.52^a	40.32 ±3.86^a
After *A. hydrophila* Challenge:						
Hemoglobin (g/dl)	7.35 ±0.55^b	9.20 ±0.10^{ab}	8.20 ±0.90^{ab}	9.5 ±0.55^a	9.10 ±0.70^{ab}	9.50 ±0.10^a
RBC (X10^6 cells/µl)	1.45 ±0.05^b	2.15 ±0.05^a	1.90 ±0.20^b	2.30 ±0.15^a	2.10 ±0.20^a	2.25 ±0.15^a
WBCs (X10^3/µl)	28.73 ±0.34^a	40.31 ±1.33^a	30.87 ±0.80^a	40.80 ±1.86^a	39.31 17.86^a	34.32 ±6.31^a

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Table 8. Blood Serum Protein Profile of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels

Parameters	**Iron Supplement Sources and Levels**					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Before *A. hydrophila* Challenge:						
Total protein (g/dl)	5.98 ±0.04^b	6.24 ±0.08^a	6.10 ±0.05^{ab}	6.20 ±0.08^a	6.26 ±0.01^a	6.10 ±0.02^{ab}
Albumin (g/dl)	5.16 ±0.07^a	5.13 ±0.06^a	4.99 ±0.03^a	5.08 ±0.11^a	4.98 ±0.02^a	5.04 ±0.02^a
Globulin (g/dl)	0.81 ±0.10^b	1.11 ±0.13^{ab}	1.15 ±0.07^{ab}	1.12 ±0.15^{ab}	1.28 ±0.02^a	1.07 ±0.04^{ab}
After *A. hydrophila* Challenge:						
Total protein (g/dl)	6.08 ±0.11^a	6.27 ±0.01^a	6.15 ±0.19^a	6.23 ±0.11^a	6.14 ±0.15^a	6.08 ±0.09^a
Albumin (g/dl)	4.91 ±0.01^a	4.98 ±0.01^a	4.87 ±0.11^a	4.77 0.10^a	4.81 ±0.12^a	4.95 ±0.05^a
Globulin (g/dl)	1.18 ±0.11^a	1.29 ±0.02^a	1.28 ±0.08^a	1.46 ±0.21^a	1.33 ±0.26^a	1.13 ±0.04^a

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

3.7. Liver Function and Antioxidant Enzymatic Activities:

The potential impact of iron oxide and Fe-NPs on liver enzymes (AST and ALT) and antioxidant enzymatic activities (CAT and TAC) was investigated in blood serum at the end of growth trial; before *A. hydrophila* infection and one week after infection. In the present study, the diet containing 85 mg of inorganic iron/kg (T_2) significantly ($P< 0.05$) increased blood serum AST and ALT activities (See Table 9) of Nile tilapia before and after *A. hydrophila* challenge compared to the iron-deficient diet (T_1). However, the inorganic iron substitution by different levels of Fe-NPs reduced blood serum AST and ALT activities to normal levels. On the other hand, the inorganic iron supplemented diet (T_2) had no significant effect on blood serum CAT activity compared to the iron-deficient diet (T_1) whereas its replacement by different levels of Fe-NPs (T_3-T_6) increased blood serum CAT activity before and after *A. hydrophila* infection. However, the groups fed on diets containing both iron sources and levels supplementation had an increase in blood serum TAC activity compared to the group fed on the iron-deficient diet (T_1).

Table 9. Blood Serum Liver Enzymes and Some Antioxidant Enzymatic Activities of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels

Parameters	Iron Supplement Sources and Levels					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Before A. hydrophila Challenge:						
AST (Unit/L)	125.00 ±30.14^{a}	140.00 ±17.32^{a}	117.50 ±22.5^{a}	116.67 ±3.33^{a}	97.50 ±2.50^{b}	117.60 ±2.50^{a}
ALT (Unit/L)	47.33 ±5.33^{ab}	66.67 ±12.02^{a}	49.00 ±9.00^{ab}	41.33 ±3.18^{ab}	31.50 ±1.50^{b}	31.00 ±1.00^{b}
CAT (U/ml)	82.87 ±59.72^{b}	87.00 ±22.25^{b}	193.70 ±1.50^{a}	145.25 ±59.59^{a}	147.40 ±65.00^{a}	92.35 ±8.85^{b}
TAC (mM/ml)	0.44 ±0.03^{b}	0.65 ±0.08a	0.79 ±0.06^{a}	0.80 ±0.05^{a}	0.66 ±0.04^{a}	0.67 ±0.05^{a}
After A. hydrophila Challenge:						
AST (Unit/L)	70.00 ±5.00^{bc}	159.50 ±19.50^{a}	70.00 ±5.00^{bc}	44.00 ±1.00^{c}	48.00 ±6.00^{c}	87.00 ±8.00^{b}
ALT (Unit/L)	22.50 ±2.50^{b}	43.50 ±1.50^{a}	17.50 ±2.50^{b}	18.50 ±1.50^{b}	28.50 ±5.50^{b}	28.50 ±3.50^{b}
CAT (U/ml)	98.00 ±9.78^{a}	97.10 ±21.00^{a}	129.34 ±27.09^{a}	118.34 ±11.67^{a}	93.00 ±16.54^{a}	89.00 4.65^{a}
TAC (mM/ml)	0.59 ±0.05^{b}	0.78 ±0.06^{a}	0.87 ±0.08^{a}	0.84 ±0.05^{a}	0.68 ±0.04^{a}	0.69 ±0.03^{a}

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Table 10. Phagocytosis and Differential Leukocyte Counts of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets Supplemented with Different Iron Sources and Levels

Parameters	Iron Supplement Sources and Levels					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Before A. hydrophila Challenge:						
Phagocytic activity	55.07±1.19^{a}	59.08±4.18^{a}	56.43±5.91^{a}	64.30±3.98^{a}	56.43±2.99^{a}	56.32±1.78^{a}
Phagocytic index	1.69±0.06^{a}	1.72±0.08^{a}	1.65±0.12^{a}	1.66±0.12^{a}	1.72±0.07^{a}	1.59±0..09^{a}
Neutrophil%	50.40±2.07^{b}	59.35±2.65^{a}	58.05±1.99^{a}	63.55±3.09^{a}	59.50±2.18^{a}	57.35±3.02^{a}
Lymphocyte%	31.10±1.09^{a}	30.10±1.32^{a}	28.55±2.01^{a}	29.40±1.43^{a}	28.60±1.29^{a}	32.70±2.87^{a}
Monocyte%	8.15±1.56^{a}	5.80±0.76^{b}	5.90±0.69^{b}	2.95±0.59^{c}	5.15±1.09^{a}	5.25±0.86^{b}
Eosinophil%	4.40±0.34^{a}	1.30±0.12^{b}	2.75±0.42^{ab}	1.80±0.14^{b}	2.15±0.19^{b}	1.65±0.11^{b}
Basophil%	5.95±0.67^{a}	3.45±0.43^{b}	4.75±0.45^{ab}	2.30±0.12^{b}	4.60±0.56^{ab}	3.05±0.22^{b}
After A. hydrophila Challenge:						
Phagocytic activity	61.67±7.27^{a}	74.67±2.88^{a}	66.38±3.63^{a}	79.24±1.47^{a}	68.59±4.38^{a}	66.10±3.16^{a}
Phagocytic index	1.23±0.07^{b}	1.25±0.03^{b}	1.24±0.02^{b}	1.64±0.12^{a}	1.31±0.07^{b}	1.23±0.11^{b}
Neutrophil%	49.22±2.56^{b}	54.49±3.05^{a}	52.87±2.39^{ab}	56.50±2.65^{a}	57.09±3.08^{a}	50.98±2.76^{a} ^{b}
Lymphocyte%	32.78±1.75^{a}	32.19±1.76^{a}	30.99±2.89^{a}	30.23±1.98^{a}	29.67±1.76^{a}	32.98±2.54^{a}
Monocyte%	6.56±1.09^{a}	6,44±0.96^{a}	5.09±0.54^{a}	5.95±0.89^{a}	5.76±1.36^{a}	5.09±0.97^{b}
Eosinophil%	5.67±0.53^{a}	1.87±0.52^{b}	4.75±0.87^{a}	4.78±0.64^{a}	3.89±0.32^{b}	4.98±0.45^{a}
Basophil%	5.77±0.66^{a}	5.01±0.67^{a}	6.30±0.95^{a}	2.34±0.22^{b}	3.59±0.34^{ab}	5.97±0.78^{a}

Values are means ± standard error. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

3.8. Immune Response:

Phagocytic activity and index were not significantly ($P \geq 0.05$) improved in both the inorganic and nano iron supplementation groups (See Table 10) before and after *A. hydrophila* infection of Nile tilapia fish compared to the group fed on the iron-deficient diet (T_1). However, the diet containing the 85 mg of Fe-NPs supplementation (T_3) non significantly reduced phagocytic activity and index before and after infection compared to the diet supplemented with inorganic iron (T_2). The highest response was obtained by the fish group fed on the basal diet supplemented with 63.75 mg of Fe-NPs/kg (T_4). Moreover, it was found that the iron supplementation improved the neutrophil of Nile tilapia before and after *A. hydrophila* infection when compared to the iron-deficient diet. The highest value

was obtained by the groups fed on 63.75 and 42.5mg of Fe-NPs supplemented diets. In contrast, such diets containing different iron sources and levels had no clear effect on the lymphocyte, monocyte, basophil and esoniophil of Nile tilapia when compared to the iron-deficient diet.

3.9. Health Condition and Survival Rate:

Table 11 shows that the lowest survival rate (58.3%) belonged to the fish group fed on the iron-deficient diet (T_1) and that iron supplementation improved the survival rate of Nile tilapia. Moreover, it was observed that the 63.75 and 42.5 mg of Fe-NPs/kg diets (T_4 and T_5) exhibited the best survival rate compared to the other groups. Mortality was noted in fishes fed on the iron-deficient diet which was decreased by the iron-supplemented diets. The majority of fishes died without exhibiting any clinical signs. Others showed petechial hemorrhages widely distributed on different parts of the external body surface. They also showed loss of scales and skin ulceration. Internally, ascites, hemorrhagic liver, enlarged spleen and gall bladder were commonly detected (See Fig. 1).

Table 11. Survival and Mortality Rates of Nile Tilapia Fed on the Iron-deficient Diet and Other Diets supplemented with Different Iron Sources and Levels

Items	Iron Supplement Sources and Levels					
	(T_1) Without Supplement	(T_2) Inorganic (85mg/Kg)	(T_3) NPs (85 mg/Kg)	(T_4) NPs (63.75 mg/Kg)	(T_5) NPs (42.5 mg/Kg)	(T_6) NPS (21.25 mg/Kg)
Total No.	24	23	23	25	25	23
Dead No.	10	6	6	4	3	8
Survival%	58.3	73.9	73.9	84.0	8.0	65.2
Mortality%	41.7	26.1	26.11	16.0	12.0	34.8

Fig. 1. Enlarged spleen and gall bladder (A), ascites (B), loss of scales (C), skin ulcer (D) and protruded eye (E) of Nile Tilapia fish after *A. hydrophila* challenge.

4. DISCUSSION

In the present study 63.75mg Fe-NPs/kg diet (T_4) supplementation produced the maximum growth and better feed efficiency of Nile tilapia fish, however both iron sources and levels supplementation improved fish growth performance compared with iron-deficient group (T_1) and supported by Roeder and Roeder (1968). Very little information is known about iron metabolism in fishes and to the researchers' knowledge; this is the first article investigating the nutritional and immunological value of Fe-NPs in Nile tilapia fish. Results of growth performance in the present study are in agreement with those obtained by Srinivasan et al. (2016) who indicated that WG and PER were found to be increased in prawns fed on 10 and 20 mg kg^{-1} Fe_2O_3 NPs supplemented diets with the best performance in the 20 mg kg^{-1} Fe_2O_3 NPs ($P < 0.05$) group when compared to the control one. However, these parameters were reduced in other concentrations (30-50 mg kg^{-1} of Fe_2O_3 NPs) with the lowest values (below the control level) in the 50 mg kg^{-1} of Fe_2O_3 NPs supplemented diet. In addition, Behera et al. (2014) found that the iron-supplemented diets significantly improved the growth performance of carp compared to the iron-deficient diet. Moreover, optimum levels of zinc and its nano source have produced better growth performance in Nile tilapia (Tawfik et al. 2017).They indicated that the iron-supplemented diet could improve the final weight of Indian major carp, *Labeorohita* H and that fishes showed growth retardation when given a purified diet without iron supplementation (Gatlin and Wilson 1986).

Iron deficiency may distress up to 40% of the global population WHO (2007) and can adversely affect health in terms of reducing cognitive function and physical work capacity and eventually economic productivity (Graham et al. 2012). Around 50% of the prevalence of iron deficiency is due to inadequate dietary Fe intake or poor absorption of Fe from the diet. Therefore, the identification of foods of high iron content and high bioavailability is of significant importance. Mammal, bird, and fish muscle tissues (meat) are considered good sources of Fe for their high total Fe concentration. Iron is an essential element for humans as it participates in a wide variety of metabolic processes. The concentration of iron in body tissues must be tightly regulated because in excessive amounts it can lead to tissue damage, where it can form free radicals (Abbaspour et al. 2014). Besides, iron is a coenzyme and is necessary for the synthesis of hemoglobin (Yacoub 2007) but very high intake of these elements can cause adverse health problems.

The obtained result of iron concentration in muscles was supported by Behera et al. (2014) who reported that iron content in the muscles of fish fed on diets containing nano iron was increased compared to those fed on free-iron diet or diet supplemented by inorganic iron. Furthermore, Srinivasan et al. (2016) found that the content of Fe was significantly elevated in the carcass of experimental prawns when compared to the control ones. As well, Ates et al. (2017) concluded that Fe levels in muscle tissues of Nile tilapia fish exposed to 0.5 and 1.0 mg/L suspensions of γ-Fe_2O_3 NPs increased significantly in 30 days ($P< 0.05$) and continued to increase with more exposure (e.g., 60 days).

Fe concentration (52.3 mg/kg) in the Fe-NPs supplementation (85mg/kg) group was higher than the international standards (43 mg/kg) adopted by FAO/WHO (2011, and the level adopted by the Egyptian Organization of Standardization (30 mg/kg) [38]. However, iron concentrations (27.9mg/kg, 9.9mg/kg and 6.7mg/kg) in the Fe-NPs supplementation groups (63.75, 42.5 and 21.25 mg/kg) were lower than the international standards (43 mg/kg) adopted by FAO/WHO (2011), and the level adopted by the Egyptian Organization of Standardization (30 mg/kg) (EOS 1993).

The most common pathological event appeared in humans due to excessive intake of iron ions is Parkinson's disease caused by the deposition of iron oxide (Candelaria et al. 2006). Other than aiding neurological depositions, such ions have been credited with enhancing oxidative damage which is a key component of chronic inflammatory disease (Umanzor et al. 2006). Also, extreme iron intake can enhance cancer initiation (Elst et al. 2007). Conversely, iron and iron associated protein in the fish carcass was considered a critical nutrient for human health; consequently, muscle protein and seafood are dependable dietary sources of minerals (Gibson 1990). Iron source and levels had no significant effect on iron accumulation in Nile tilapia gills. Generally, there is a lack of information on possible effect of different iron sources and levels (inorganic or nano) on gills health. However, it well known that gills is the main site of iron toxicity and several authors reported that deposition of iron on gill epithelium leading to gill clogging and damage (Brenner et al. 1976; Dalzell and Macfarlane 1999). The present data indicated that inorganic or nano iron had no detrimental effect on gill

epithelial tissues of Nile tilapia fish (Carriquiriborde et al. 2004).

Fish is an essential source of most minerals which the body needs to perform its functions properly. Although fish contains less iron than the amount found in red meat, iron in white fish is easily absorbed and is therefore a valuable source of dietary iron (Abelti 2017). Fish proteins have high biological values because they are characterized by the presence of essential amino acids in good proportions (Toppe et al. 2007). Fish is also one of the richest sources of ω3 polyunsaturated fatty acids (Ackman 1989). Such polyunsaturated fatty acids have been found to play a vital role in developing human nutrition (Tanaka et al. 1998). They have curative and preventive effects on many human diseases such as cardiovascular diseases, cancers, rheumatoid arthritis, and inflammation (Raatz et al. 2013).

In the present study, there was no significant difference among the groups in body ash, Ca or P content that could be related to the supplementation of any forms of these minerals and consequently the carcass mineral contents may depend on their dietary concentrations. However, the absorption was continuous in the groups fed on all iron sources and levels (85 mg of inorganic Fe_2O_3 and 85-21.25 mg kg^{-1} of Fe-NPs).It was also reported that the Nile Tilapia fed on the iron supplemented feed has synthesized and stored protein and lipid. The present data are supported by those obtained by Behera et al. (2014) who indicated that iron supplementation in *Labeorohita H* diet increased body protein, EE and ash percentage. Also, Srinivasan et al. (2016) found that body total protein, amino acids, carbohydrate, lipid and ash percentage were increased in PL fed on 10 and 20 mg kg^{-1} Fe_2O_3 NPs supplemented diets with the maximum performance in the 20 mg kg^{-1} Fe_2O_3 NPs group ($P< 0.05$) when compared to the control one.

Fish has received increasing attention as a potential source of animal protein and essential nutrients for human diets. Fish meat contains significantly low lipids and higher water contents than beef or chicken and is favored over other white or red meats (Nestel 2000). The nutritional value of fish meat comprises the contents of moisture, dry matter, protein, lipids, vitamins and minerals. The human body usually contains a small amount of these minerals and the deficiency in these principal nutritional elements induces a lot of malfunctioning; as it reduces productivity and causes diseases (Mills 1986). The high CP and EE retention in the fish groups fed on diets containing 63.75 and 42.5 mg of nano-particles Fe_2O_3/kg instead of inorganic source might be attributable to the improved growth rate. This result is supported by that of El-Serafy et al. (2013) who reported that there was an increase in muscle total protein content in the Nile Tilapia groups fed on Cu and Cd supplemented diets compared to the control group.

Normally, blood picture reflects the health condition of fish and is considered a good indicator of iron supplementation in fish diets (Chu et al. 2007). In the present study, the higher values of RBCs, WBCs counts and hemoglobin was found in the 63.75 mg of Fe-NPs supplemented diet unlike the other diets of different sources and levels. Similarly, fish receiving a diet containing a nano-disperse form of iron had a significant increase in the erythropoiesis and hemoglobin levels and a drastic decrease in the mortality rate, without any sign of polychromatic anemia (Behera et al. 2014, Prochorov et al. 2011).

Lower hematological parameters of Tilapia fish with higher nano iron supplementation reflect the physiological suffering of the organism under stress conditions (Shaw et al. 2012, Kaya et al. 2014). In accordance, α-Fe-NPs induced significant hemolytic disturbances on Indian major carp, *Labeorohita*, at 500 mg/L levels (Remya et al. 2015) and on Nile Tilapia (Ates et al. 2017). This result reflecting the increased oxygen demand or reduced gas-exchange might be caused by the heavy deposition of NPs on the gills under elevated Fe-NPs conditions.

The data of protein profile in the present study are consistent with those obtained by Ates et al. (2017) who reported that the results of total serum protein, albumin, globulin and albumin/globulin ratio showed no significant differences among all Nile Tilapia's treated groups(whether fed on a diet containing nano or inorganic iron) and the control group.

Minerals are involved in the regulation of protein synthesis in animals (Furriel et al. 2000, Cortinhas et al. 2010). Dietary supplementation of minerals such as Zn, Cu, Fe, Ca, Mg, Na and K can improve the synthesis of protein in fishes (El-Saidy and Gaber 2004, Li et al. 2010). In the present study, it was recorded that there was an increase in serum total protein concentration in the supplemented diets with different iron sources and levels. Moreover, the improvement of serum globulin concentration due to iron supplement indicates that iron supplementation is particularly necessary for Nile Tilapia fish as an immune stimulator. Several reports have shown that a deficiency in iron might contribute to several immune

deficiency syndromes such as impaired T cell functioning and atrophy in lymph organs in both humans and animals (Farthing 1989).

Disturbances in AST and ALT activities are indicators of toxic conditions when these enzymes penetrate to the blood due to cytolyses of the liver (Kaya et al. 2014). Iron has a number of biological functions in animals including fish. It is well known that dietary iron modulates the antioxidant defense system such as glutathione peroxidase (GSH), TAC and CAT by preventing free radical production and lipid peroxidation (MDA). In the present study, it was found that iron supplementation in the basal diet could improve the TAC activity irrespective of different iron sources. This result is in agreement withBehera et al. (2014). Moreover, Muralisankar et al. (2014, 2016) reported that nano Zn and Cu supplementation improved the antioxidant capacity of M. rosenbergii PL.

The improvement in phagocytosis and neutrophil before and after *A. hydrophila* infection with iron supplementation indicates that iron plays an important role in the immune response of fish. These results are consistent with those of Farthing (1989) who found that a deficiency in iron might contribute to several immune deficiency syndromes such as impaired T cell functioning and atrophy in lymph organs in both humans and animals. In addition, Behera et al. (2014) reported that there was an improvement in the immune response of the fish groups fed on inorganic and nano iron supplemented diets compared to the fish group fed on the iron-deficient diet.

Moreover, the supplementation of low levels of Fe-NPs (75% and 50%) acted as immune stimulants more than the other levels of supplementation did. Some studies reported that when NPs enter the body, they can interact with the immune cells and trigger an inflammatory response, which is accompanied by the secretion of signaling molecules (cytokines, chemokines) that provide communication between the immune cells and coordinate molecular events (Tawfik et al. 2017).

The obtained results concerning the health condition and survival rate are compatible with those of Prochorov et al. (2011) who concluded that the nano-dispersed form of iron in feed drastically reduced the mortality rate in commercial fish farms of carp and sturgeon. As well, Srinivasan et al. (2016) indicated that the survival rate increased in PL fed on 10 and 20 mg kg $^{-1}$ Fe_2O_3 NPs supplemented diets with the maximum performance in the 20 mg kg^{-1} Fe_2O_3 NPs group ($P< 0.05$) when compared to the control one. However, it was decreased (below the control level) in the group fed on the 50 mg kg^{-1} of Fe_2O_3 NPs supplemented diet in prawns. The mortality of iron-deficient diet group might be attributable to lower RBCs counts, anemia. As previously mentioned, fish receiving a diet containing a nano-disperse form of iron witnessed a significant increase in the hemoglobin levels and a radical decrease in the mortality rate (Prochorov et al. 2011).

CONCLUSION:

It can be concluded that dietary iron sources (iron oxide or Fe-NPs) supplementation improved growth performance and feed efficiency parameters of Nile tilapia fish. Dietary iron supplementation of both sources increased muscle iron content, body protein and lipids contents, improved RBCs or WBCs counts, Hb% and increased serum total protein and globulin concentrations of Nile tilapia fish. Replacement of inorganic iron by different levels of Fe-NPs improve liver and antioxidant enzymes activities before and after A. hydrophila infection of Nile tilapia fish. Dietary iron supplementation of both sources improved phagocytosis and reduced mortality of Nile tilapia fish. Moreover, 63.75 mg of Fe-NPs more effective than iron oxide and other levels of Fe-NPs in improvement of growth, Hb%, meat quality and immune response of Nile tilapia fish.

Declaration of Conflicting Interests:

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REFERENCES:

Abbaspour, N., Hurrell, R., Kelishadi, R., 2014. Review on iron and its importance for human health. J. Res. Med. Sci. the official J. Isfahan University of Medical Sci. 19: 164.

Abelti, A. L., 2017. Minerals Content and Fatty Acids Profile of Nile Tilapia (*Oreochromis niloticus*) Fillet from Lake Zeway: Effect of Endogenous Factors. J. Nutr. Food Sci. 7:574.

Ackman, R. G., 1989. Nutritional composition of fats in sea foods, Prog. Food Nutr. Sci. 13: 161–241.

Amos, H. K., 1985. Procedures for the detection and identification of certain fish pathogens, 3rd ed. Fish Health Section, Am. Fisheries Soc. Corvallis, Oregan. 6-21.

Andersen, F., Lorentzen, M., Waagbo, R. Maage, A., 1997. Bioavailability and interactions with other micronutrients

of three dietary iron sources in Atlantic salmon, Salmo salar, smolts,Aquacult. Nutr. 3: 239–346

Andersen, F., Maage, F., Julshamn, M., 1996. An estimation of dietary requirements of Atlantic salmon, Salmo salar L., parr. Aquacult. Nutr. 2: 41–47.

AOAC (Association of Official Analytical Chemists) 2000. Official Methods of Analysis of Official Analytical Chemists International, 16th edn. Association of Official Analytical Chemists, Arlington, VA.

Ates, M., Demir, V., Arslan, Z., Kaya, H., Yılmaz, S., Camas, M., 2017. Exposure of tilapia (*Oreochromis niloticus*) to iron oxide nanoparticles: Effects of particle morphology on accumulation, elimination, hematology and immune responses, Aquat. Toxicol. 177: 22–32.

Barton, J. C., Edwards, C. Q. 2000. Hemochromatosis: Genetics, Pathophysiology, Diagnosis and Treatment, Cambridge University Press, New York, 616.

Behera, T., Swain, P. S., Rangacharulu, P. V., and Samanta, M., 2014. Nano-Fe as feed additive improves the hematological and immunological parameters of fish, Labeorohita H, Applied Nanoscience. 4: 687–694.

Bligh, E.G., Dyer, W.J. 1959. Fat extraction (cited by Pearson's chemical analysis of foods). 8th ed.

Brenner, F. J., Corbett, S. & Shertzer, R. 1976. Effect of ferric hydroxide suspensions on blood chemistry in the common shiner, Notropuscornutus. Transactions of the American Fisheries Society, 105: 450–455.

Bury, N. R., Walker, P. A., Glover, C. N. 2003. Nutritive metal uptake in teleost fish, J. Exp. Biol. 206: 11–23.

Candelaria, M., Garcia-Arias, A., Cetina, L., Dueñas-Gonzalez, A. 2006. Radio sensitizers in cervical cancer: Cisplatin and beyond, Radiat. Oncol.1: 15-32.

Carriquiriborde, P., Handy, R. D., Davies, S. J. 2004. Physiological modulation of iron metabolism in rainbow trout (*Oncorhynchus mykiss*) fed low and high iron diets. J. Exp. Biol. 207: 75-86.

Chu, J. M., Chen, S. M., Huang, C. H. 2007. Effect of dietary iron concentrations on growth, hematological parameters, and lipid peroxidation of soft-shelled turtles, Pelodiscussinensis, Aquacult. 269: 532–537.

Cortinhas, C. S., Botaro, B. G., Sucupira, C. A., Renno, F. P., Santos,M. V. 2010. Antioxidant enzymes and somatic cell count in dairy cows fed with organic source of zinc, copper and selenium, Livestock Sci. 127 :84-87.

Dalzell, D. J. B., Macfarlane, N. A. A. 1999. The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate. J. Fish Biol. 55(2): 301-315.

El-Saidy, D. M., Gaber, M. M. 2004. Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish meal in Nile tilapia, *Oreochromis niloticus* (L.) diets, Aquaculture Research, 35, 859-865.

El-Serafy, S. S., Zowail, M. E., Abdel-Hameid, H., Nassr-Allah, H., Awwad, M. H., Omar, E. H. 2013. Effect of diet borne Cu and Cd on body indices of Nile tilapia (*Oreochromis niloticus*) with emphasis on protein pattern, Turk. J. Fish. Aquat. Sci. 13: 593-602.

Elst, P., Ahankour F., Tjalma W. 2007. Management of recurrent cervical cancer, Eur. J. Gynaecol. Oncol. 28: 435-41.

EOS, 1993. Egyptian standard, maximum levels for heavy metal concentrations in food, 546-815.

FAO/WHO, 2011. Joint FAO/WHO food standards programme codex committee on contaminants in foods, fifth. Session. 64-89.

Farthing, M. J., 1989. Iron and Immunity, ActaPaediatr Sc. Suppl. 361: 44–52.

Furriel, M. C., Namara, J. C., Leone, F. A., 2000. Characterization of (Na+, K+)-ATPase in gill microsomes from the freshwater shrimp Macrobrachiumolfersii, Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 126: 303-315.

Gatlin, D. M., Wilson, R. P., 1986. Characterization of iron deficiency and the dietary iron requirement of fingerling channel catfish, Aquacult. 52: 191–198.

Gericke, H. U., Kurmies, B., 1952. Die colorimetrischePhosphorsäurebestimmungmit Ammonium-Vanadat-Molybdat und ihreAnwendung in der Pflanzenanalyse, ZeitschriftfürPflanzenernährungDungung und Bodenkunde. 104: 235 – 247.

Gibson, R. S., 1990. Principles of Nutritional Assessment, Oxford University Press, New York.

Graham, R. D., Knez, M., Welch, R. M., 2012. How much nutritional iron deficiency in humans globally is due to an underlying zinc deficiency? In D. L sparks (Ed.), Advances in agronomy, 115: 1–40.

Hanson, S. W. F., Olly, J., 1963. The Bligh and Dyer method for lipid extraction to tissue homogenates. Biochem. J. 83:101-105.

Hilty, F. M., Knijnenburg, J. T., Teleki, K. F., Hurre, R. F., Pratsinis, S. E. & Zimmermann, M. B., 2011. Incorporation of Mg and Ca into nano structured Fe2O3 improves Fe solubility in dilute acid and sensory characteristics in foods. J. Food Sci. 76: 1-10.

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

Kawahara, E., Ueda, T., Nomura, S., 1991. In vitro phagocytic activity of white-spotted char blood cells after injection with Aeromonassalmonicida extracellular products, Fish Pathol. 26: 213-214.

Kaya, H., Çelik, E. S., Yılmaz, S., Tulgar, A., Akbulut, M., Demir, N., 2014. Hematological, serum biochemical, and immunological responses in common carp (Cyprinuscarpio) exposed to phosalone, Comparative Clinical Pathol. 24: 497–507.

Li, W. W., Gong, Y. N., Jin, X. K., He, L., Jiang, H., Ren, F., 2010. The effect of dietary zinc supplementation on the growth hepatopancreas fatty acid composition and gene expression in the Chinese mitten crab Eriocheirsinensis (H Milne-Edwards) (*Decapoda, Grapsidae*), Aquacult. Res. 41: 828-837.

Mills, C. F., 1986. The mineral nutrition of livestock. E. J. Underwood, (Ed.), (pp. 9). Common Wealth Agricultural Bureaux,.

Min,B., Nam, K. C., Cordray, J., Ahn, D. U., 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats, J. Food Sci. 73: 439–C446.

Muralisankar, T., Bhavan, P. S., Radhakrishnan, S., Seenivasan, C., Manickam, N., Srinivasan, V., 2014. Dietary supplementation of zinc nanoparticles and its influence on biology, physiology and immune responses of the freshwater prawn, Macrobrachiumrosenbergii, Biol. Trace Elem. Res. 160: 56-66.

Muralisankar, T., Bhavan, P. S., Radhakrishnan, S., Seenivasan, C., Srinivasan, V., Santhanam, P., 2015. Effects of dietary zinc on the growth, digestive enzyme activities, muscle biochemical compositions, and antioxidant status of the giant freshwater prawn Macrobrachiumrosenbergii, Aquaculture. 448: 98-104.

Nestel, P. J. N., 2000. Fish oil and cardiovascular disease: lipids and arterial function, Am. J. Clin. Nutr. 71: 228-231.

NRC, National Research Council, 2011. Nutrient requirements of fish. Washington, DC: National Academy Press.114 pp.

Prochorov, A. M., Pavlov, G. V., Godwin, A. C., Okpattah, K. A. V., 2011. The effect of nano-disperse iron on the biological parameters of fish. In: An abstract in 10th Foresight Conference on Molecular Nanotechnology.

Raatz, S. K., Silverstein, L., Jahns, M. J., Picklo, S. R., 2013. Issues of fish consumption for cardiovascular disease risk reduction, Nutrients. 5: 1081–1097.

Randhir, S., Pradhan, K., 1981. Forage evaluation. First published, Printox, New Dalhi, Dhawan printing works.

Rather, M. A., Sharma, R., Aklakur, M., Ahamad, S., Kumar, N., Khan, M. &Ramya, V. L., 2011. Nanotechnology: Anoval tool for aquaculture and fisheries development. Aprospectiv mini-review, Fisheries Aquacult. J. 16: 1-5.

Remya, A. S., Ramesh, M., Saravanan, M., Poopal, R. M., Bharathi, S., Nataraj, D. 2015. Iron oxide nanoparticles to an Indian major carp, Labeorohita: Impacts on hematology, iono regulation and gill Na+/K+ ATPase activity, J. King Saud University Sci. 27: 151–160.

Roeder, M., Roeder, R. H., 1968. Effect of iron on the growth rate of fishes, J. Nutri. 90: 86–90.

SAS, 2004. SAS User's Guide, Statistics. SAS Institute Inc., Cary, NC.

Schalm J. N. C., 1986. Veterinary Haematology, 4th edn. (Lea and Febiger, USA)

Schaperclaus, W., Kulow, H., Schreckenbach, K., Balkema, A. A., 1992. Fish diseases Rotterdam, the Netherlands. 2456.

Shaw, B. J., Al-Bairuty, G., Handy, R. D., 2012. Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (*Oncorhynchus mykiss*): physiology and accumulation, Aquatic. Toxicol. 116-117: 90–101.

Shiau, S. Y., Haung, S. I., 1990. Optimal dietary protein level for hybrid tilapia ((*Oreochromis niloticus* X O. aureus) reared in seawater. Aquaculture, 81: 119-127.

Slavin, W., 1982. Atomic absorption spectroscopy. The present and future. Anal. Chem., 54 (6), pp 685A–694A.

Srinivasan, V., Saravana, P., Rajkumar, G., Satgurunathan, T., Muralisankar, T., 2016. Effects of dietary iron oxide nanoparticles on the growth performance, biochemical constituents and physiological stress responses of the giant freshwater prawn Macrobrachiumrosenbergii post-larvae, I.J.F.A.S. 4: 170-182.

Stephen, D., 2007. Nano iron could boost human bioavailability, Retrieved from http://www.nutraingredients.com

Tanaka, T., Hattori, T., Maki, K., Kaoru, H., Satouchi, K., 1998. Methylene-interrupted double bond in polyunsaturated fatty acid is an essential structure for metabolism by the fatty acid chain elongation system of rat liver, Biochim. Biophys. Acta. 1393: 299–306.

Tawfik, M. M., Moustafa, M. M., Abumourad, I. M. K., El-Meliegy, E. M.,Refai, M. K., 2017. Evaluation of Nano Zinc Oxide feed additive on tilapia Growth and Immunity. 15th International Conference on Environmental Science and Technology, Rhodes, Greece, Sept. (2017).

Toppe, J., Albrektsen, S., Hope, B., Aksnes, A., 2007. Chemical composition, mineral content and amino acid and lipid profiles in bones from various fish species, Comp. Biochem. Physiol. B. 146: 395–401.

Umanzor, J., Aguiluz, M., Pineda, C., Andrade, S., Erazo, M., Flores, C., 2006. Concurrent cisplatin/gemcitabine chemotherapy along with radiotherapy in locally advanced cervical carcinoma: a phase II trial, Gynecologic oncology. 100: 70-75.

WHO, 2007. Assessing the iron status of populations: Including literature reviews Geneva, Switzerland: World Health Organization. 108.

Yacoub, A. M., 2007. Study on Some Heavy Metals Accumulated in Some Organs of Three River Nile Fishes from Cairo and Kalubia Governorates. African J. Biol. Sci. 3; 9-21.

Zhong, L.W., 2004. Zinc oxide nanostructures: growth, properties and applications, J. Phys. Condens. Matter. 16: R829-R858.

Zhou, X., Wang, Y., Gu, Q., Li, W., 2009. Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance muscle composition and glutathione peroxidase enzyme activity of Crucian carp (*Carassiusauratus Gibeli*o). Aquaculture. 291: 78-81.