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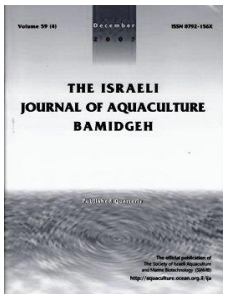

Effects of Different Iron Sources on the Growth and Iron Concentrations of Tissues, Organs, and Blood of Genetically Improved Farmed Tilapia (*Oreochromis niloticus*)

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Keywords: iron source; *Oreochromis niloticus*; iron concentration; $FeCl_2$; Fe.

Abstract

A 70-day feeding trial was conducted to elucidate the effects of different iron (Fe) sources on growth and Fe concentrations in the tissues, organs, and blood of genetically improved farmed tilapia (*Oreochromis niloticus*). Five experimental diets (45.0% crude protein, 3.7% crude lipid) with the same Fe concentration (100 mg/kg of feed) were formulated from different Fe sources: ferrous sulfate ($FeSO_4$), ferric chloride ($FeCl_3$), ferrous chloride ($FeCl_2$), ferrous citrate ($C_6H_5FeO_7$), and iron porphyrin (Fe-porphyrin). The results showed that the percent weight gain (%WG) of fish in the $FeCl_2$ group was the greatest among all five groups ($p < 0.05$). With %WG as the main indicator, $FeCl_2$ was the most suitable source of dietary Fe for *O. niloticus*. In the $FeCl_2$ group, the content of Fe in the spleen was significantly greater than that in the $C_6H_5FeO_7$ group on day 28, and that of the $FeSO_4$ group on day 56. The Fe concentrations in the liver, spleen, gill, vertebra, blood, whole intestine, and dorsal white muscle of *O. niloticus* were not significantly affected by different dietary sources of Fe on day 70. There were only slight dynamic changes to the Fe content of tissues and organs during the experimental period. The Fe contents in different tissues and organs were as follows: spleen > mid-hind intestine > liver > gill, muscle, foregut > spine. The spleen had the highest Fe content in all groups on day 70. These results suggest that $FeCl_2$ is the best source of dietary Fe for *O. niloticus* with %WG as the main indicator.

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Introduction

Fe is an essential mineral in living organisms and is necessary for several biological processes, including oxygen transport, cellular respiration, and lipid oxidation (Lee et al., 1981). Fish obtain Fe from both water and from their diet. However, the Fe concentration in natural water is low, therefore diet is the main source of Fe for fish (Andersen et al., 1997). Organic forms of mineral elements have greater bioavailability than inorganic forms. Studies of *Salmo salar* L. have shown that utilization of erythritol is significantly greater than that of elemental Fe and ferrous sulfate (Andersen et al., 1997). In addition, a study of *Cyprinus carpio* revealed that ferrous fumarate supplementation improved growth and disease resistance, compared to ferrous sulfate (Jie et al., 2010). Fe concentrations in the organs and tissues of the Gibel carp (*Carassius auratus gibelio*) fed a diet supplemented with $FeSO_4 \cdot 7\ H_2O$ were, in ascending order, as follows: spleen > kidney > heart > intestine > vertebrate > muscle > gill (Xiao 2007).

Common Fe additives to feed include $FeSO_4$, $C_6H_5FeO_7$, ferrous fumarate, ferrous lactate, ferrous gluconate, ferrous succinate, ferrous malate, ferrous ascorbate, and Fe amino acid chelates. The relative biological potency of these Fe sources differ as that of inorganic Fe is relatively low, while that of Fe amino acid chelate is relatively high (Tang 2003). Due to differences in the chemical structures of various Fe sources, research objectives, diets, and environmental factors, the most suitable type of Fe additive for a specific animal remains uncertain.

Reportedly, the growth potential of genetically improved farmed tilapia (*Oreochromis niloticus*) is significantly greater than that of Nile tilapia (Hussain et al., 2000) and, thus *O.niloticus* was chosen as the animal model for this study, which aimed to investigate the effects of different sources of Fe on growth and Fe concentrations in the tissues, organs, and blood.

Materials and Methods

Genetically improved farmed tilapia *O. niloticus,* were obtained from a tilapia hatchery in Yingshan, Hubei Province, China and acclimated to the experimental conditions for 3 weeks prior to starting the feeding trial. During the acclimation period, the fish were fed to satiation twice daily (09:00 and 15:00) with one of five experimental diets. As presented in Table 1, the five experimental diets (45.0% crude protein, 3.7% crude lipid) were formulated to contain the same Fe level (100 mg/kg of dry diet) from different sources ($FeSO_4$, $FeCl_3$, $FeCl_2$, $C_6H_5FeO_7$, and Fe-porphyrin). The feed size was 1 mm, and was dried at a low temperature, and then stored at 4°C for further use.

Table 1. Formulations and chemical compositions of experimental diets (g/100 g of dry matter).

Ingredients	*Diet*				
	$FeSO_4$	*$FeCl_3$*	*$FeCl_2$*	*$C_6H_5FeO_7$*	*Fe-porphyrin*
Casein	47.3	47.3	47.3	47.3	47.3
Dextrin	33.5	33.5	33.5	33.5	33.5
Fish oil	4.0	4.0	4.0	4.0	4.0
Soybean oil	4.0	4.0	4.0	4.0	4.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)_2$	2.5	2.5	2.5	2.5	2.5
Mineral premix1	2.0	2.0	2.0	2.0	2.0
Vitamin premix2	1.0	1.0	1.0	1.0	1.0
Cellulose	5.2	5.2	5.2	5.2	5.2
Chemical composition(g 100g-1 in dry matter)					
Crude protein (%)	45.21	46.11	45.63	46.30	45.56
Water (%)	8.42	9.67	8.95	9.75	9.24
Crude lipid (%)	3.61	3.71	3.81	3.56	4.09
Crude ash (%)	8.26	8.56	8.73	8.65	8.71

[1]Per kilogram of vitamin premix containing (g/kg mixture): vitamin B1, 5 g; vitamin B_2, 5 g; pantothenic acid calcium, 10 g; nick acid, 6.05 g; vitamin D,120000 IU; vitamin B6, 0.825 g; folic acid, 0.041 g; vitamin C 2.025 g; vitamin E, 3.35 g; vitamin K, 4 g; vitamin A, 1333333 IU; and alpha cellulose powder, 963.306 g.

[2]Per kilogram of mineral premix containing (g/kg mixture): $MgSO_4 \cdot 7\ H_2O$, 6.85 g ; NaCl, 2.175 g ; $AlCl_3 \cdot 6\ H_2O$, 0.0075 g ; KI, 0.0075 g ; KCl, 3.75 g ; $CuSO_4 \cdot 5\ H_2O$, 0.2924 g ; $MnSO_4 \cdot H_2O$, 0.04 g ; $CoCl_2 \cdot 6\ H_2O$, 0.05 g ; $ZnSO_4 \cdot 7\ H_2O$, 0.15 g; and cellulose powder, 986.6776 g.

The trial was conducted in a circulating system consisting of 15 cylindrical plastic tanks (diameter, 80 cm; height, 60 cm; water volume, 300 L). At the beginning of the trial, the fish were fasted for 24 h. Then, a total of 525 fish of similar size (initial body weight, 6.79 ± 0.5 g) were randomly selected, weighed, and stocked in 15 tanks and fed five different diets; there were therefore three tanks per diet. These were five groups, each group with three replicates. Each tank of each replicate held 35 fish. During the experiment, aeration was provided to each tank to maintain a dissolved oxygen level of 7.5-7.8 mg/L. Water temperature was maintained at 28–30°C and recorded daily. pH was maintained at about 7.5, while ammonia-N content was monitored once a week and kept at less than 0.02 mg/L with residual chloride of less than 0.01 mg/L. Fish were hand-fed to apparent satiation twice daily (09:00 and 16:00). The amount of food supplied was recorded daily.

All fish in each tank were sampled after 1 day of food deprivation and then on days 28, 56, and 70. Ten random fish from each tank were weighed and 1-mL blood samples were collected immediately from the caudal vein using disposable medical syringes and stored at -80°C until analysis. Then, the sampled fish were dissected immediately and the liver, spleen, gill, vertebra, foregut, midgut, hindgut, and dorsal white muscle (from posterior edge of operculum to end of dorsal-fin base above the lateral line) were collected and frozen in liquid nitrogen and stored at -80°C for subsequent analysis. The percent weight gain (%WG) was calculated according to formula (1), while the specific growth ratio (SGR; %/d) was calculated according to formula (2):

(1) %WG = 100 × [(final body weight (g) - initial body weight (g)] / initial body weight (g)

(2) SGR (%/d) = 100 × ln[final body weight (g) / initial body weight (g)] / experiment days

Tissue samples were dried in an oven at 105°C until the weights were consistent. Dried tissue samples were made into ash by incineration at 550°C for 7 h in a muffle furnace. Then, the ash was dissolved in 5 mL of hydrogen chloride solution and the resulting solution was combined with 20 mL of ultrapure water. The obtained solutions were stored at -20°C until analysis (Xu 2017).

Blood samples (200 µL) were digested with 2 mL of nitric acid (guaranteed reagent (GR)), 1 mL of hydrogen peroxide (GR), and 1 mL of cell digestion solution in a Mars 5 microwave accelerated reaction system (CEM Corporation, Matthews, NC, USA). Then, the volume of the resulting solution was increased to 25 mL with the addition of ultrapure water and stored at -20°C until analysis (Huang 2006).

The Fe content was determined by flame atomic absorption spectrophotometry (TAS-990; Beijing Purkinje General Instrument Co., Ltd., Beijing, china) (Xu 2017).

Statistical analysis of the resulting data was performed using SPSS Statistics for Windows, version 17.0 (SPSS, Inc., Chicago, IL, USA). Variables among treatments were compared using one-way analysis. Significant differences ($p < 0.05$) were reanalyzed using Duncan's shortest significant ranges test.

Results

After being fed for 70 days with diets containing different Fe sources, the %WG of each fish was calculated. As shown in Fig. 1, the %WG of *O. niloticus* fed with the $FeCl_2$ diet was greater than that of the other four groups and was significantly greater than that of the $FeSO_4$ treatment group ($p < 0.05$), but not significantly different from that of the fish fed diets supplemented with $FeCl_3$, $C_6H_5FeO_7$, or Fe-porphyrin ($p > 0.05$).

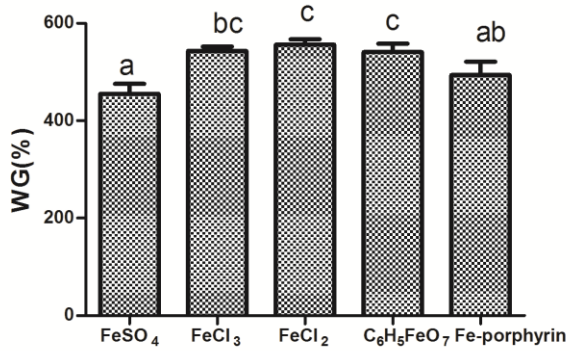

Fig. 1. Effects of different Fe sources on %WG of *O. niloticus*

Note: Different lowercase letters indicate significant differences ($p < 0.05$)

The Fe contents in the tissues and organs of *O. niloticus* on day 28 day are presented in Table 2. The Fe concentrations in the liver tissues of *O. niloticus* were not significantly affected by dietary Fe from different sources ($p > 0.05$). The Fe content in the spleen tissues of the $FeCl_2$ treatment group was significantly greater than that of the $C_6H_5FeO_7$-supplemented group ($p < 0.05$). The Fe concentration in the vertebra of the $FeCl_2$ treatment group was significantly greater than that of the $FeCl_3$-supplemented group ($p < 0.05$). The Fe content in the foregut of the $FeCl_2$ treatment group was significantly greater than that of the groups supplemented with $FeCl_3$ and Fe-porphyrin. The Fe content in the hindgut of the $FeCl_2$ treatment group was significantly greater than that of the groups supplemented with $FeCl_3$ and Fe-porphyrin ($p < 0.05$).

Table 2. Fe concentrations (mg/g of dry tissue weight) in tissues and organs of *O. niloticus* on day 28

Parameters	*Diet*				
	$FeSO_4$	*$FeCl_3$*	*$FeCl_2$*	*$C_6H_5FeO_7$*	*Fe-porphyrin*
Liver	2.45±0.17	1.77±0.11	1.82±0.22	2.36±0.27	1.77±0.25
Spleen	11.68±0.61^{a,b}	11.64±0.66^{a,b}	10.51±0.73^{a}	13.56±0.52^{b}	11.74±0.83^{a,b}
Gill	1.40±0.18	1.45±0.07	1.37±0.09	1.35±0.12	1.34±0.16
Muscle	1.54±0.15	1.51±0.11	1.84±0.19	1.69±0.19	1.53±0.13
Vertebra	1.47±0.23^{a,b}	1.20±0.04^{b}	1.49±0.11^{a}	1.78±0.20^{a,b}	1.62±0.14^{a,b}
Foregut	1.97±0.20^{a,b}	2.23±0.25^{a}	1.61±0.06^{b}	1.98±0.06^{a,b}	1.50±0.16^{b}
Midgut	6.34±0.70^{a}	5.19±0.71^{a,b}	4.87±0.31^{a,b}	5.99±1.01^{a}	3.42±0.64^{b}
Hindgut	5.23±0.73^{a,b}	4.58±0.60^{a}	6.88±0.36^{b}	5.03±0.45^{a,b}	4.27±0.67^{a}

Each parameter is presented as the mean ± standard error. Superscript letters indicate significant differences ($p < 0.05$). The lack of a superscript letter indicates no significant differences among diets ($p > 0.05$).

The Fe concentrations of the tissues and organs of *O. niloticus* on day 56 are presented in Table 3. The Fe concentration in the spleen tissues of the $FeSO_4$ treatment group was significantly lower than that of the other groups ($p < 0.05$). The Fe content in the gill tissues of the $FeCl_3$ treatment group was significantly lower than that of the $FeSO_4$-supplemented group ($p < 0.05$). The Fe concentration in the liver, vertebra, whole intestine, and dorsal white muscle of *O. niloticus* were not significantly affected by dietary Fe from different sources ($p > 0.05$).

Table 3. Fe concentrations (mg/g of dry tissue weight) in tissues and organs of *O. niloticus* on day 56

Parameters	*Diet*				
	$FeSO_4$	*$FeCl_3$*	*$FeCl_2$*	*$C_6H_5FeO_7$*	*Fe-porphyrin*
Liver	2.83±0.23	2.27±0.27	2.56±0.28	2.21±0.18	2.48±0.10
Spleen	7.60±0.54^{a}	10.67±0.38^{b}	11.82±0.95^{b}	10.96±0.69^{b}	10.64±0.89^{b}
Gill	1.21±0.08^{b}	0.99±0.05^{a}	1.06±0.08^{a,b}	1.03±0.04^{a,b}	1.04±0.04^{a,b}
Muscle	1.39±0.14	1.71±0.21	1.79±0.14	1.83±0.22	1.50±0.15
Vertebra	0.74±0.07	0.73±0.03	0.68±0.03	0.70±0.05	0.74±0.05
Foregut	2.20±0.37	2.20±0.28	1.46±0.18	2.28±0.28	1.58±0.23
Midgut	7.80±0.62	8.05±0.47	6.21±0.51	7.33±1.04	7.01±0.75
Hindgut	7.62±0.43	6.61±0.60	7.43±0.51	6.88±0.64	6.34±0.75

Each parameter is presented as the mean ± standard error. Superscript letters indicate significant differences ($p < 0.05$). The lack of a superscript letter indicates no significant differences among diets ($p > 0.05$).

The Fe content in the tissues and organs of *O. niloticus* on day 70 are presented in Table 4. The Fe concentration in the gill tissues of the $FeCl_3$ treatment group was

significantly lower than that of the $FeCl_2$-supplemented group ($p < 0.05$). The Fe content in the hindgut of the $FeSO_4$ treatment group was significantly lower than that of the $FeCl_2$-supplemented group ($p < 0.05$). The Fe concentration in the liver, spleen, vertebra, foregut, midgut, hindgut, and dorsal white muscle of *O. niloticus* were not significantly affected by dietary Fe from different sources ($p > 0.05$).

Table 4. Fe concentrations (mg/g of dry tissue weight) in tissues and organs of *O. niloticus* on day 70

Parameters	*Diet*				
	$FeSO_4$	*$FeCl_3$*	*$FeCl_2$*	*$C_6H_5FeO_7$*	*Fe-porphyrin*
Liver	3.15±0.52	2.50±0.29	2.59±0.42	2.47±0.46	2.53±0.28
Spleen	10.89±0.93	11.58±1.04	10.10±0.77	10.52±0.30	11.86±0.90
Gill	$1.23±0.10^{a,b}$	$1.04±0.08^{a}$	$1.32±0.05^{b}$	$1.13±0.10^{a,b}$	$1.16±0.07^{a,b}$
Muscle	1.41±0.10	1.40±0.07	1.46±0.04	1.53±0.11	1.35±0.07
Vertebra	1.21±0.12	1.05±0.05	1.02±0.04	1.04±0.05	1.14±0.10
Foregut	1.77±0.34	1.72±0.18	1.71±0.24	1.54±0.10	1.64±0.25
Midgut	6.33±0.67	6.01±0.64	6.29±0.64	5.83±0.38	4.59±0.58
Hindgut	$5.07±0.27^{b}$	$7.52±0.95^{a,b}$	$8.30±0.49^{a}$	$7.18±1.09^{a,b}$	$6.51±1.10^{a,b}$

Each parameter is presented as the mean ± standard error. Superscript letters indicate significant differences ($p < 0.05$). The lack of a superscript letter indicates no significant differences among diets ($p > 0.05$).

As shown in Tables 2-4, the Fe content was maintained at about 10–11 mg/g in the spleen, 5-7 mg/g in the mid- and hindgut, 1.8-2.5 mg/g in the liver, and 1-2 mg/g in the gill, muscle, and foregut. The Fe content of the spine was the most stable and lowest (0.7–1 mg/g).

The Fe contents in the blood of *O. niloticus* are presented in Table 5. The Fe concentration in the blood of *O. niloticus* was not significantly affected by dietary Fe from different sources on day 28 ($p > 0.05$), while concentrations among the other four groups were similar on day 56 ($p > 0.05$). The content of Fe in the blood of the $FeCl_3$ treatment group was significantly greater than that of the $FeSO_4$-supplemented group on day 56 ($p < 0.05$). The Fe concentration in the blood of *O. niloticus* was not significantly affected by dietary Fe from different sources on day 70 ($p > 0.05$).

Table 5. Fe concentrations (μg/mL) in blood of *O. niloticus*

Day	*$FeSO_4$*	*Diet*			
		$FeCl_3$	*$FeCl_2$*	*$C_6H_5FeO_7$*	*Fe-porphyrin*
28	1.42±0.10	1.65±0.12	1.55±0.13	1.61±.09	1.58±0.20
56	1.15±0.05b	$1.88±0.19^{a}$	$1.72±0.22^{a,b}$	$1.49±0.23^{a,b}$	$1.67±0.30^{a,b}$
70	1.95±0.24	1.90±0.16	2.11±0.20	2.20±0.31	2.06±0.41

Each parameter is presented as the mean ± standard error. Superscript letters indicate significant differences ($p < 0.05$). The lack of a superscript letter indicates no significant differences among diets ($p > 0.05$).

The Fe concentration in the blood of the $FeCl_3$, $FeCl_2$, and Fe-porphyrin treatment groups increased with an increase in feeding time. The Fe content in the blood of the treatment groups on day 70 day was greater than that on days 56 and 28.

Discussion

Previous studies have shown that dietary Fe should first be reduced to Fe^{2+} before it can be absorbed and utilized by the body (Ammerman and Luo, 1996). After the 70-day feeding experiment, the %WG of *O. niloticus* fed with the $FeCl_2$-supplemented diet was greater than other four Fe diets and was significantly greater than that of the $FeSO_4$-supplemented diet ($p < 0.05$), although there were no significant differences when compared with the $FeCl_3$, $C_6H_5FeO_7$, and Fe-porphyrin treatment groups ($p > 0.05$). This finding indicates that $FeCl_2$ is more easily digested by *O. niloticus* than $FeCl_3$, which may be related to the type of fish, the composition and palatability of the feed, the size of the experimental fish, the number of feedings, the experimental conditions, certain species-specific effects of Fe on growth, and difference in digestion and absorption of Fe among species (Feng et al., 2012).

The %WG of *O. niloticus* (Shiau et al., 2003), *Pelodiscus sinensis* (Chu et al., 2009), *Lateolabrax japonicus*, and *Pseudosciaena crocea* were used to evaluate Fe requirements. In the present study, $FeCl_2$ was a better source of dietary Fe to promote growth of *O. niloticus*.

Growth is an effective, but inadequate indicator to assess the dietary intake of trace elements in fish and terrestrial animals (Baker 1986; Cowey 1992) mainly because growth is not specific (Tacon 1992). The contents of trace elements in animal body tissues can be used as important indicators to assess demand (Maage et al, 1993; King, 1990; Wekell et al, 1986; Dato et al, 1996). Previous studies have demonstrated that different organs and tissues of fish had different concentrations of trace elements, which were affected by the source of dietary Fe (Xiao, 2009). The present study determined the Fe concentrations in eight organs and tissues of *O. niloticus*, which could reflect the necessity of Fe for important physiological functions in specific organs and tissues of fish. It also provides some basic data of the mechanism of action to further explore the distribution of Fe at the cellular and molecular levels.

In the present study, Fe content was greater in the spleen than in the gill, muscles, foregut, and vertebra, as Fe is mainly used for oxygen transport by hemoglobin, which is mainly formed in the lymphatic medulla of the spleen in fish (Jacobs 1985). The intestine is the main channel for the environmental uptake of Fe (Watanabe et al, 1997), which was reflected by the greater Fe content in the mid- and hindgut of *O. niloticus*.

The demand for Fe in fish can be estimated by the Fe content of the liver (Shiau et al., 2003). In the present study, because the Fe content in the liver of *O. niloticus* was lower than that in the spleen, the overall Fe requirement of *O. niloticus* could not be determined, demonstrating that the liver Fe content is not an effective indicator of the nutritional requirements of *O. niloticus.* Previous studies have reported that Fe concentrations in the organs and tissues of *Epinephelus coioides* were, in ascending order, as follows: spleen (600~700 μg/ kg of dry weight) > kidney > heart > liver > intestine > muscle > eye > bone > brain (Xu 2007). In the present study, Fe contents in the organs and tissues were, in ascending order, as follows: spleen (13.56 mg/g of dry weight) > mid-hind intestine > liver > gill, muscle, foregut > spine. Hence, Fe content in the spleen could be used as an effective indicator of the nutritional requirements of *O. niloticus.* The results showed that Fe concentration in the spleen of *O. niloticus* differed with the various sources of Fe, which may be related to factors such as the species and size of the fish. In addition, differences in the valences and dosages of Fe in feed is also correlated to differences in Fe requirements. The requirement for elemental Fe by *Salvelinus fontinalis* is greater than that of ferrous sulfate and the demand for ferrous sulfate is greater than that of heme Fe (Andersen et al., 1997). The demand for ferric acid by *Chrysophrys majoris* is greater than that of ferrous chloride and ferric chloride (Sakamoto et al., 1979). *O. niloticus* requires more ferric citrate than ferrous sulfate (Shiau et al., 2003). The results of the present study showed that there were distinct distribution patterns of Fe in the organs and tissues of *O. niloticus*, which may be directly related to the important physiological functions of specific organs and tissues. The Fe concentration in the spleen probably has a significant effect on growth performance.

In the present study, the Fe content of organs and tissues showed no abnormally large fluctuations in the three samplings with the passage of time, thereby demonstrating that the Fe content in the organs and tissues of *O. niloticus* did not change much during the growth period.

In previous studies, changes to the hemoglobin content in piglets were studied with three different Fe sources (Fe lysine, Fe glycinate, and $FeSO_4$). The results showed that the two kinds of Fe amino acid chelate of lysine promoted a significant increase ($p < 0.05$) in hemoglobin levels of piglets (Hua et al, 2006), indicating that differences in Fe preparations are very likely to affect the blood Fe content of animals. In the present study, the fish acquired Fe from five different dietary sources and blood samples were collected at three times (days 28, 56, and 70). However, there was no significant effect on Fe content derived from different dietary Fe sources ($p > 0.05$). The result showed that the Fe concentration in blood of *O. niloticus* was not significantly affected by dietary Fe from different sources.

In conclusion, the results of the present study suggest that $FeCl_2$ was the best source of dietary Fe for *O. niloticus* when considering %WG as the main indicator. The

distribution of Fe, in ascending order, was as follows: spleen (13.56 mg/g of dry weight) > mid- to hindgut > liver > gill, muscle, foregut > spine. These findings demonstrate that the Fe content in the spleen is an effective indicator of the nutritional requirements of *O. niloticus.*

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