The © Israeli Journal of Aquaculture

An interdisciplinary online
Open Access
scientific journal

Published by the

AquacultureHub

A non-profit organization 501c3

http://www.aquaculturehub.org

in partnership with the

University of Hawaii at Manoa Library

~

The Israeli Journal of Aquaculture (IJA) is an interdisciplinary journal that is dedicated to sharing new research and tested applications of aquaculture

~

The IJA is devoted to scholarly articles for improved aquaculture practices and related industries

~

The IJA is a peer-reviewed, open-access, electronic journal

ISSN 0792 - 156X

The Israeli Journal of Aquaculture - Bamidgeh, IJA.71.2019.1591, 9 pages

Dietary Iron Requirements of Adult GIFT Tilapia (*Oreochromis niloticus*)

Cheng X[1, 2], Tian J[2], Yao Y[2], Jiang M[2],Liu W[2], Wu F[2], Yu LJ[2], Lu X, Gao W[1]* & Wen H[2]*

[1] *Department of Fisheries, College of Animal Science, Yangtze University, Jingzhou 434024, China*

[2] *Key Lab of Freshwater Biodiversity Conservation of Ministry of Agriculture and Rural Affairs, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China*

Keywords: iron; fish; tilapia; hematopoietic function

Abstract

Dietary iron requirement of adult Nile tilapia (*Oreochromis niloticus*), genetically improved farmed tilapia (GIFT strain), was determined in this study through a 77 day feeding trial. Diets with six concentrations of iron (45.55 - the control group, 94.91, 193.62, 292.34, 391.05, and 489.77 mg iron/kg diet) from ferrous sulfate were formulated and hand-fed to adult GIFT (initial body weight 174.58±3.49g). The results indicated that no fish died in all groups. Weight gain ratio, feed efficiency rate, as well as specific growth rate decreased in relation to increasing dietary iron levels, and reached the lowest when supplemented with 489.77 mg iron/kg diet. Crude body fat content showed an increasing trend in relation to increasing dietary iron levels. The iron content in the body, vertebrae, and liver significantly increased with dietary iron levels which reached up to 391.05 mg iron/kg diet. The number of red blood cells, the hemoglobin content and the packed cell volume was highest in 94.91 mg iron/kg group. In conclusion, dietary iron made no obvious improvement on growth performance, but improved hematopoietic function of adult Nile tilapia, reared in freshwater. We found that no additional iron needs to be added to the adult Nile tilapia (174.6–558.1 g) feed based on the growth, whereas broken-line regression analysis were used to determine the optimum iron requirements for maximum hemoglobin levels, the dietary iron requirement for hemoglobin was 120.94 mg iron/kg diet with ferrous sulfate as a source of iron.

* Corresponding author.
Weihua GAO Tel.: +86-716-8062788 Fax: +86-716-8066332, e-mail: gaoweihua@yangtzeu.edu.cn
Hua WEN Tel.: +86-27-81780258 Fax: +86-27-81780258 Email address: wenhua@yfi.ac.cn

Introduction

Iron is an essential component of hemoglobin, myoglobin, cytochrome, peroxidase, and catalase, that is mainly involved in oxygen transport, transformation, and tissue respiration (Robbins and Pederson 1970, Shiau and Su 2003, Ye et al. 2010, Ling et al. 2010. Water-soluble iron can be absorbed by fish via the gill membrane and intestinal mucosa, and dietary iron is still considered to be the major source for fish because of low contents of soluble iron in farming water and a huge demand gap in intensive culture model (Shiau and Su 2003). If fish iron content intake is insufficient or poorly utilized, the fish may suffer from iron deficiency, which may slow down their growth and reduce their resistance to diseases (Shiau and Su 2003, Ye et al. 2010, Ling et al. 2010, Asuka et al. 2010). The iron requirement of many small sized fish has been determined. The dietary demand is estimated to be 150–160 mg Fe/kg ferric citrate as the main iron source or 85 mg Fe/kg with ferrous sulfate for juvenile tilapia (initial weight: 0.63 g) (Shiau and Su 2003), 202 mg/kg (ferrous chloride) for juvenile gibel carp (initial weight 2.12 g) (Pan et al. 2009), 30 mg/kg (iron methionine and iron sulfate) for channel catfish (initial weight 8.5 g) (Lim et al. 1996), 100 mg/kg (iron sulfate) for juvenile grouper (initial weight 21.0 g) (Ye et al. 2010), 60–100 mg/kg (ferrous sulfate) for Atlantic salmon (initial weight 5.0 g) (Andersen et al. 1996) and 147.4 mg/kg(ferrous sulfate) for Jian carp (initial weight 11.4 g)(Ling et al. 2010). Through the analysis and comparison of the above research results, we conclude that ferrous sulfate is the better iron source used in fish feed, and dietary iron requirement in different species fish is different.

Tilapia has become an important and rapid-growing aquaculture product in the world. According to reports from the Food and Agriculture Organization (FAO) in 2017, global tilapia output was 6.4 million tons, which was second only to that of carp (FAO, 2018). This rapid increase of global tilapia output is partly due to the rapid development of breeding technologies and the wide use of commercial pelleted feeds. The Genetically Improved Farmed Tilapia (GIFT) strain *Oreochromis niloticus*, a relatively new strain, chosen for over 14 years, are 9 generations from the base strain of Nile tilapia (Li et al. 2010). They are widely recommended to fish farmers in southern China because of their better growth performance, tolerance to crowding, high marketability, high disease resistance, high fecundity, and broad diet (Ng and Romano 2013).

Over the past few years, we have acquired some data on dietary nutrient requirements of tilapia with different body weight or size such as protein(Liu et al. 2017), lipid(Tian et al. 2015), phosphatidylcholine(Tian et al. 2018), carbohydrate (Wu et al. 2011, Wu et al. 2012), niacin (Jiang et al. 2014, Huang et al. 2013), vitamin C (Wu et al. 2015), zinc (Huang et al. 2015), cholin (Shao et al. 2013) and phosphorus (Jiang et al. 2013, Yao et al. 2013). Our results have demonstrated that nutrient requirements are dependent of fish size (Ng and Romano 2013). However, there are few studies showing the iron requirement in adult fish. In this study, six semi-purified diets with different levels of dietary iron were formulated and artificially fed to GIFT (initial body weight 175 g) to detect the impact of dietary iron levels respectively on growth, feeding efficiency, whole body composition, iron contents in different tissues and some hematological parameters of adult GIFT strain of Nile tilapia. Simultaneously, the dietary iron demand of grown-up tilapia was estimated on account of hematological parameters.

Materials and Methods

Experimental diets.

Six semi-purified diets were prepared with graded levels of available iron at 45.55 (the control group), 94.91, 193.62, 292.34, 391.05 and 489.77 mg iron/kg diet. The six diets had a similar composition of protein, lipid, and energy. Also, 33% dietary protein from casein, gelatin, and soybean meal was included in the diets. Corn oil to soybean oil = 1:1, which were the main lipid sources, provided 7.3% dietary lipid. $FeSO_4 \cdot 7H_2O$ was used as the iron source. Cellulose was used to adjust the desired iroj level in the 6 experinantalk diets. Dry ingredients were weighed accurately, blended thoroughly, and pelleted with a medium-size meat grinder with a 3 mm diameter template. The pellets were dried at room temperature for approximately 48 h, and the selected pellets were sealed and frozen at -20°C. Diet ingredients and proximate composition as well as available iron content were respectively shown in Tables 1 and 2.

Table 1. Formulation and chemical proximate composition of experimental diets

Ingredients (g/kg diet)	*Groups*					
	D1	*D2*	*D3*	*D4*	*D5*	*D6*
Basal diet[1]	860	860	860	860	860	860
Cellulose	104	103.75	103.25	102.75	102.25	101.75
$FeSO_4 \cdot 7H_2O$	0	0.25	0.75	1.25	1.75	2.25
Vitamin	10	10	10	10	10	10
Mineral	20	20	20	20	20	20
Choline	5	5	5	5	5	5
Yi_2O_3	1	1	1	1	1	1
Proximate composition (g/kgdiet)						
Dry matter	886.8	890.2	889.3	884.6	887.7	888.6
Crude protein	328.1	328.0	327.6	327.7	327.8	329.4
Crude lipid	69.4	68.4	69.4	68.7	68.9	70.1
Crude ash	68.2	68.0	67.9	67.8	68.1	68.3
Gross energy	18.02	18.14	17.94	18.03	18.18	18.15

[1] Basal diet (g/kg diet): casein, 200; gelatin, 50; soybean meal, 150 ; corn starch, 380; soybean oil, 30;corn oil, 30; Monocalcium phosphate, 20.
[2] Vitamin premix contained (mg/g mixer) thiamin hydrochloride, 5 mg; riboflavin, 5 mg; calcium pantothenate,10 mg; nicotic acid, 6.05 mg; L-ascorbyl-2-monophosphate-Mg, 3.95 mg; pyridoxine hydrochloride, 4 mg; folic acid, 1.5 mg; inositol, 200 mg; menadione, 4 mg; alpha-tocopherol acetate, 50 mg; retinyl acetate, 60 mg; biotin, 0.6 mg. All ingredients were diluted with alpha-cellulose to 1 g.
[3] Mineral premix contained (g kg^{-1} diet) calcium biphosphate, 13.58 g; calcium lactate, 32.7 g; magnesium sulfate, 13.7 g; potassium phosphate dibasic, 23.98 g; sodium biphosphate, 8.72 g; sodium chloride, 4.35 g; $AlCl_3 \cdot 6H_2O$, 0.015 g; KI, 0.015 g; $CuCl_2$, 0.01 g; $MnSO_4 \cdot H_2O$, 0.08 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.3 g.
[4] Energy was determined by direct combustion in an adiabatic bomb calorimeter (SDC311, Hunan Sundy Science and Technology Development Co., Ltd, Changsha, Hunan province, China).

Table2. The available iron levels of the experimental diets

Index	*Dietary available iron level (mg kg-1diet)*					
	D1	*D2*	*D3*	*D4*	*D5*	*D6*
Added iron level (mg/kg)	0	50.36	151.08	251.80	352.52	453.24
Total iron level (mg/kg)	64.59	114.95	215.67	316.39	417.11	517.83
ADCiron (%)	70.52	82.57	89.78	92.24	93.75	94.58
Available iron level(mg/kg)	45.55	94.91	193.62	292.34	391.05	489.77

Experimental procedure.

The trial was carried out in the experimental base of Yangtze River Fisheries Research Institute (Jingzhou, China). The GIFT strain of Nile tilapia was acquired from a commercial farm in Nanning (Guangxi Zhuang Autonomous Region, China). After transportation, only male GIFT tilapia were selected for the experiment. These males were sterilized by soaking in 2% physiological saline for 10 min, then transferred to freshwater tanks (with a diameter of 2.0 m, and a depth of 0.8 m). Fish were acclimated for a fortnight during which they were fed the control diet to reduce the iron level stored in their body.

Before the beginning of the experiment, the fish were fasted overnight and anesthetized, and weighed individually. Adult fish (initial weight: 174.58±3.49 g) were randomly distributed into 18 tanks (water volume 400 L) with 15 fish per tank. Each diet was randomly assigned to triplicate tanks. Experimental tanks were supplied with a continuous flow of filtered water (8 L/h) to reduce the iron content in the water, and provided with continuous aeration, keeping the dissolved oxygen level above saturation. Fish were hand fed at 2.0-3.0% of their respective average body weight, at 8:20, 12:20, and 16:20 every day. The fish were slowly hand-fed until apparent satiation. All fish were weighed every two weeks following 24-h starvation and their feed ration regulated

accordingly to the fish weight. The fish were maintained under natural photoperiod conditions and ambient temperature for 11 weeks. Water quality parameters were monitored every Monday morning. During the experimental period, water temperature was 28.0±3.0°C, dissolved oxygen was above 6.0 mg/L, pH was 7.30±0.10; NH_4^+-N and NO_2-N was 0.45±0.10 mg/L and 0.026±0.013 mg/L respectively, and total iron in the water was below 0.10 mg/L.

Sampling.

At the end of feeding trial, fish were weighed and counted after 24 h fasting and were anesthetized and sacrificed with 200 mg MS-222/L water for sampling body parts and tissues for this experiment. Three fish from each tank were randomly sampled, cut into small pieces, and then minced with a meat grinder and mixed thoroughly, 100g samples were taken from each fish and stored at -80°C until analysis of whole body composition. An additional three fish were removed from each tank, measured for body weight and length. The blood samples were then collected from the caudal vein using a 5-ml vacuum blood collection tube, placed in anticoagulant tube, and hematological parameters were determined immediately. These three fish were sacrificed, their liver and visceral weight determined to calculate the hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor K (CF). The livers were frozen at -80°C for anayziung their iron content. Subsequently, the remains of these three fish were placed in a microwave oven and cooked for 10 minutes. Whole vertebrae collected from each fish were dried at 105°C for 12 hrs and transferred to a Soxlhet apparatus to get rid of fat remnants by ether extraction for 12 hrs, dried again, ground, and combusted in a muffle furnace at 550°C for 12 hrs. The iron content from the ash was subsequently determined.

From the eighth week of the growth trial, feces from the bottom of each tank was siphoned at 20:00 every night and the samples quickly frozen and stored at -20°C for iron and yttrium analysis. The apparent iron digestibility was calculated using this formula: $ADC=[1-(C_1/C_2)(N_2/N_1]\times100\%$

Where C_1 indicates diet Yi_2O_3 concentration, C_2 the Yi_2O_3 concentration in feces, N_1 is the concentration of iron in diet, N_2 is the concentration of iron in feces.

Biochemical analyses.

Micro-Kjeldahl, Soxhlet, and ignition methods (AOAC 2000) was used respectively to measure the contents of crude protein, crude lipid, and ash. Samples were placed in a vacuum freeze dryer for 48 h according to the freeze-drying method to measure the Moisture content (Christ Beta 2-4 LD plus LT, Marin Christ Corporation, Osterode, Germany). Gross energy content was measured in an adiabatic bomb calorimeter through direct combustion (SDC311, Hunan Sundy Science and Technology Development Co., Ltd, Changsha, Hunan province, China).

In accordance with the method described by Agrawal and Mahajan (Agrawal and Mahajan, 1983), the main blood parameters measured were number of red blood cell (RBC), hemoglobin content (Hb) and packed cell volume (PCV).

To determine the iron and yttrium contents, the samples were disolved in 5 ml concentrated nitric acid at a temperature of 60°C for 4 h and then diluted to 20 ml. Finally plasma emission spectrophotometry (ICP-AES, IRIS, TJA) was utilized to determine the Iron and yttrium content. All tests were run in triplicate.

Statistical analysis.

Results were expressed as mean ± S.E.M. (standard error of mean) in tables and figures. All data were analyzed with one-way analysis of variance by SPSS 17.0 (SPSS Company, New York, USA). $P < 0.05$ indicates a significant difference. Duncan's test was utilized to make comparisons among individual treatments when overall differences were significant. The requirement of dietary available iron was estimated through the method of Broken-line model (Robbins et al. 1979).

Results

Growth performance and biometric parameters

For 11 weeks, no mortality occurred. The apparent digestibility of dietary iron increased with in relation to added iron levels. From these results, iron content (mg/kg diet) in the experimental diets was 45.55 (control diet, D1), 94.91 (D 2), 193.62 (D3), 292.34 (D4), 391.05 (D5) and 489.77 (D6), respectively.

As shown in Table 3, weight gain ratio (WGR), feed efficiency rate (FER), and specific growth rate (SGR) were reduced with increasing dietary iron level. After 11 weeks, WGR, SGR, and FER in D6 group were significantly lower than in the control group ($P < 0.05$). Following the increasing dietary irons levels (i.e., from 45.55 to 292.34 mg/kg), HSI increased from 2.34 to 2.82% ($P < 0.05$) and then dropped to 2.39% when irons content in diets reached to 489.77 mg/kg, but these groups showed no significant difference in VSI and CK ($P > 0.05$).

Table 3. Growth performance and biometric parameters for Nile tilapia fed diets containing different iron levels for 11 weeks.

Index	*Dietary available iron level (mg/kg diet)*					
	45.55	94.91	193.62	292.34	391.05	489.77
Initial weight	175.65±1.00	176.00±1.33	175.48±0.89	176.50±1.08	176.07±1.08	174.80±0.44
Final weight (g)	558.14±12.33	551.67±20.45	538.70±29.61	530.76±21.57	524.51±19.91	511.37±23.80
Weight gain	218.29±9.28a	213.49±5.42ab	206.96±8.37ab	201.47±12.76ab	197.15±10.28b	192.53±8.45b
Specific growth	1.50±0.04a	1.48±0.02ab	1.46±0.04ab	1.43±0.05ab	1.41±0.05ab	1.39±0.04b
Feed efficiency	68.48±1.32a	71.42±3.47a	69.04±2.35a	64.06±2.80b	64.29±2.12b	62.98±2.05b
Hepatosomatic	2.34±0.15b	2.55±0.21ab	2.75±0.17a	2.82±0.24a	2.60±0.11ab	2.39±0.15b
Viscerosomatic	8.90±0.33	8.73±0.70	9.35±0.48	9.14±0.77	8.80±0.68	8.81±0.72
Condition	4.39±0.33	4.44±0.21	4.23±0.39	4.35±0.19	4.52±0.24	4.20±0.18

a. Weight gain ratio (WGR, %) = 100 × (final body weight − initial body weight) / initial body weight.
b. Specific growth ratio (SGR, %/d) = 100 × ln(final weight / initial weight) / days.
c. Feed efficiency rate (FER, %) = 100 × wet weight gain / dry feed consumed.
d. Hepatosomatic index (HSI, %) =100 × (hepatosomatic weight / whole body weight).
e. Viscerosomatic index (VSI, %) =100 × (viscera weight / whole body weight).
f. Condition factor (CF, %/cm^3) = 100× (body weight/ body length^3).

Whole body composition

Table 4 displays the impacts of dietary irons on whole body composition. Whole body lipid levels increased in relation to the supplemented dietary iron, and were significantly higher in comparison to the control group when dietary iron level was over 292.34 mg/kg ($P < 0.05$). While the content of moisture, crude protein, ash in whole body among groups exhibited no significant differences ($P > 0.05$).

Table 4. The proximate compositions of whole body of Nile tilapia fed diets containing different iron levels for 11 weeks (%) (wet mass).

Index	*Dietary available iron level (mg/kg diet)*					
	45.55	*94.91*	*193.62*	*292.34*	*391.05*	*489.77*
Moisture	68.94±1.27	69.65±0.57	69.26±0.95	67.83±1.46	68.46±1.52	68.64±1.07
Crude protein	17.07±0.48	16.94±0.65	16.64±0.41	16.03±0.20	16.80±0.25	16.65±0.25
Crude fat	9.16±0.29^b	9.46±0.37^{ab}	9.52±0.39^{ab}	10.11±0.59^a	10.12±0.20^a	9.96±0.77^a
Ash	3.78±0.23	3.72±0.20	3.68±0.19	3.64±0.20	3.81±0.17	3.89±0.17

Data shown as means ± SD (n = 3). Figures in the same row with different letters indicate a significant difference ($P < 0.05$).

Tissue iron content

Figures 1A, B, and C show the effect of dietary iron levels on the tissue iron content. The dietary iron levels were positively correlated to iron concentrations in whole body, vertebrae, and liver. Compared to the control group, the D6 group had higher iron levels in whole body, vertebrae, and liver ($P < 0.05$).

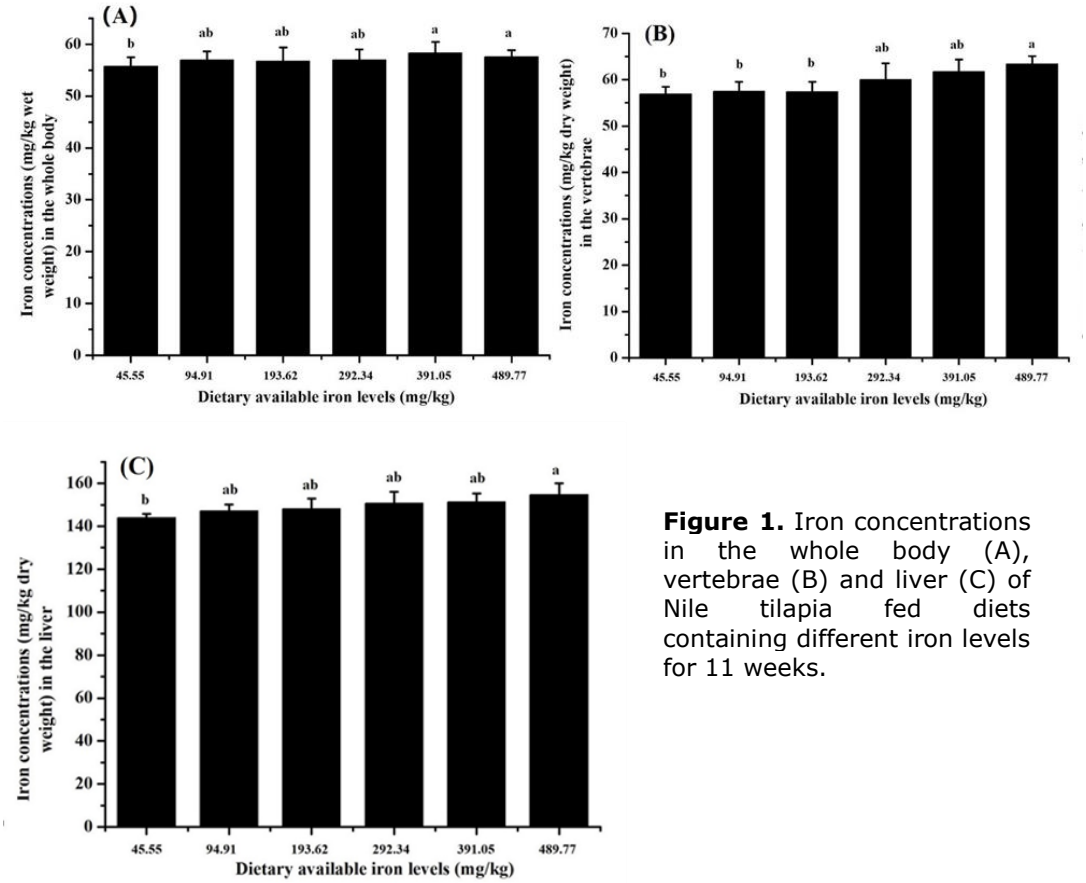

Figure 1. Iron concentrations in the whole body (A), vertebrae (B) and liver (C) of Nile tilapia fed diets containing different iron levels for 11 weeks.

Hematological parameters

There were significant differences in RBC, Hb, and PCV among the treatment groups (Table 5). RBC and Hb tended to increase when dietary iron increased to 193.62 mg/kg ($P < 0.05$), while when dietary iron level was more than 193.62 mg/kg, RBC and Hb levels decreased and were similar to the control group. Broken-line regression analysis was used to determine the optimum iron requirement for maximum Hb level (Figure 2). The results indicate that the optimum iron requirement of adult GIFT is 120.94 mg/kg for Hb.

Table 5. The hematological parameters of Nile tilapia fed diets containing different iron levels for 11 weeks.

Index	*Dietary available iron level (mg/kgdiet)*					
	45.55	*94.91*	*193.62*	*292.34*	*391.05*	*489.77*
RBC (10^9/ml)	2.19±0.14^b	2.53±0.07^a	2.36±0.07^{ab}	2.33±0.09^a	2.27±0.14^b	2.22±0.18^b
Hb (mg/ml)	122.75±3.90^b	137.33±2.89^a	133.50±3.87^{ab}	130.75±3.97^{ab}	126.75±4.90^b	123.50±5.79^b
PCV (%)	38.03±2.17^b	41.53±0.83^a	39.73±1.09^{ab}	40.65±0.98^a	39.47±2.57^{ab}	38.30±1.73^b

Data shown as means ± SD (n = 3). Figures in the same row with different letters indicate a significant difference ($P < 0.05$).

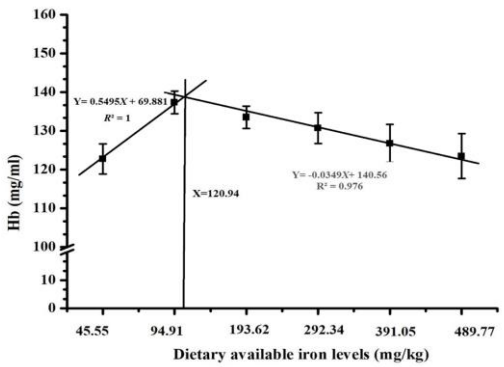

Figure 2. Broken-line analysis of hemoglobin of Nile tilapia fed diets containing different iron levels for 11 weeks.

Discussion

In this study, dietary iron had no obvious beneficial effect on growth performance, therefore according to our findings no additional iron needs to be added to the adult Nile tilapia (174.6–558.1 g) feed based on the growth parameters. Similarly, reports on Atlantic salmon (Andersen et al. 1996), channel catfish (Lim et al. 1996), and juvenile gibel carp (Pan et al. 2009) showed that dietary iron did not enhance growth and feed efficiency. This phenomenon was not observed in studies on hybrid tilapia (Shiau and Su 2003), juvenile grouper (Ye et al. 2010), and Jian carp (Ling et al. 2010), which found that reduced growth were associated with dietary iron deficiency. These results suggest that dietary iron deficiency is affected by fish species, body size, and basal diets containing iron levels. As observed in the results, feed efficiency rates (FER) also were similar to growth performance in the present study. FER reduction may explain the relatively low weight gain although there was adequate iron in the diets. The results showed that WGR and FER decreased following increased supplementation of dietary iron, which suggests that it is not necessary to supplement iron for normal growth and feed efficiency conversion of adult Nile tilapia.

In the present study, dietary iron did not significantly affect the level of moisture, crude protein, and ash in whole body, but crude fat content apparently increased in relation to the level of iron up to 292.34 mg iron /kg diet, then stabilized. In juvenile Jian carp, the feed containing an appropriate level of iron significantly improved crude protein content, however there were no differences in the contents of moisture, lipid, and ash (Ling et al. 2010). No other reports have analyzed the effects of dietary iron levels on whole body composition in fish, so whether the dietary iron level affects the composition of fish needs further study.

Some studies have suggested that insufficient dietary iron intake could contribute to the depression of hepatic iron concentration (Shiau and Su 2003, Lim et al. 1996) Dietary iron levels were positively correlated to iron concentrations in whole body, vertebrae, and liver in our study, which was in consistent with the results for Jian carp in serum (Ling et al. 2010), Atlantic salmon (Andersen et al. 1996), and juvenile grouper in whole body and liver (Ye et al. 2010).

A perfect indicator of mineral status ought react straightforwardly to dietary treatments and provide for extensive contrasts in concentrations between saturated and insufficient states (Wekell et al. 1986). Many nutrient deficiencies commonly result in growth retardation, growth retardation still may be not viewed as an appropriate indicator for investigating the requirement of mineral (Baker 1987). Furthermore, hematological parameters are preferred indicators than weight gain for iron requirement (Chu et al, 2007; Shiau and Su, 2003; Lim et al. 1996; Ling et al., 2010). Hemoglobin is an accepted parameter when detecting iron status and it plays an important role as an indicator for iron requirement, which can be exemplified by the fact in Atlantic salmon (Andersen et al. 1996), hybrid tilapia (Shiau and Su 2003), and gibel carp (Pan et al. 2009). In this study, hemoglobin was used as indicator of iron status in adult tilapia. The hemoglobin content had significant impact on the dietary iron content, and showed rising tendency with increasing dietary iron up to 193.62 mg/kg. With dietary lipid levels >193.62 mg/kg, hemoglobin contents declined and were similar to the result in the control group. Therefore, as presented in Fig. 2, the iron status remains at an optimum value when dietary iron content reaches 120.94 mg iron /kg diet.

In conclusion, our study clearly stated that dietary iron did not improve growth performance, but improved hematopoietic function of adult Nile tilapia. No additional iron needs to be added to the adult Nile tilapia (174.6–558.1 g) feed based on the growth, whereas the dietary iron requirement for hemoglobin was 120.94 mg iron/ kg diet with ferrous sulfate as an iron source.

Acknowledgements

This work was supported by the China Agriculture Research System [grant number CARS-46], and Hubei University Student Innovation and Entrepreneurship Project (No.2017085).

Conflict of interest

Weihua Gao, Juan Tian and Hua wen designed the research; Yingfei Yao, Fan Wu, Wei Liu, Li-Juan YU, Xing LU, and Ming Jiang conducted the experiments and analyzed the data; Xiaoli Cheng and Weihua Gao wrote the paper; all the authors have read and approved the final manuscript. The authors declare no conflict of interest.

Ethics statement

This study was carried out in strict accordance with the Standard Operation Procedures (SOPs) of the Guide for the Use of Experimental Animals of Yangtze River Fisheries Research Institute. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Yangtze River Fisheries Research Institute (according to YFI 2018-40 of July 20, 2018). Fish were anesthetized with 80 mg MS-222/L water to minimum suffering before being assigned to cages and sampling.

References

Agrawal NK, Mahajan CL (1983) Haematological and haematopoietic studies in pyridoxine deficient fish, *Channa punctatus Bloch*. *J Fish Biol* 22:91-103. doi:doi:10.1111/j.1095-8649.1983.tb04729.x

Andersen F, Maage A, Julshamn K (1996) An estimation of dietary iron requirement of Atlantic salmon, *Salmo salar* L., parr. *Aquacult Nutr* 2: 41-47. doi:doi:10.1111/j.1365-2095.1996.tb00006.x

Association of Official Analytical Chemists (AOAC) (2000) Official methods of analysis of official analytical chemists international. 17th ed. *AOAC International, Gaithersburg, MD*.

Baker HD (1987) Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. *J Nutr* 116:2339-2349. doi:10.1097/00017285-198501000-00002

Chu JH, Chen SM, Huang CH (2007) Effect of dietary iron concentrations on growth, hematological parameters, and lipid peroxidation of soft-shelled turtles, *Pelodiscus sinensis.* Aquaculture 269: 532-537. doi: https://doi.org/10.1016/j.aquaculture.2007.03.004

FAO (Food and Agriculture Organization of the United Nations) (2018) Aquaculture Department. *The state of world fisheries and aquaculture*, 56-58.

Huang F, Jiang M, Wen H, Wu F, Liu W, Tian J Yang C (2015) Dietary zinc requirement of adult Nile tilapia (*Oreochromis niloticus*) fed semi-purified diets, and effects on tissue mineral composition and antioxidant responses. *Aquaculture* 439: 53-59. doi:https://doi.org/10.1016/j.aquaculture.2015.01.018

Huang F, Wen H, Wu F, Jiang M, Liu W, Tian J, Shao H (2013) The dietary niacin requirement of large GIFT tilapia. *Journal of South China Agricultural University* 34:235-240.

Jiang M, Huang F, Wen H, Yang C, Wu F, Liu W, Tian J (2014) Dietary Niacin Requirement of GIFT Tilapia, *Oreochromis niloticus*, Reared in Freshwater. *J World Aquacult Soc* 45:333-341. doi:doi:10.1111/jwas.12119

Jiang M, Yao Y, Wen H, Wu F, Liu W, Tian J, Yang C. (2013) Dietary available phosphorus requirement of adult GIFT strain of *Oreochromis niloticus* reared in freshwater. *Journal of Fisheries of China* 37: 1725-1732.

Li SF, Tang SJ, Cai WQ (2010) RAPD-SCAR Markers for Genetically Improved NEW GIFT Nile Tilapia (*Oreochromis niloticus* L.) and Their Application in Strain Identification. *Zoological Research* 31: 147-153.

Lim C, Sealey WM, Klesius PH (1996) Iron methionine and iron sulfate as sources of dietary iron for channel catfish *Ictalurus punctatus*. *J World Aquacult Soc* 27:290-296. doi:doi:10.1111/j.1749-7345.1996.tb00610.x

Ling J, Feng L, Liu Y, Jiang J, Jiang WD, Hu K, Li SH, Zhou XQ (2010) Effect of dietary iron levels on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquacult Nutr* 16: 616-624. doi:doi:10.1111/j.1365-2095.2009.00699.x

Liu W, Jiang M, Wu JP, Wu F, Tian J, Yang CG, Wen H (2017) Dietary protein level affects the growth performance of large male genetically improved farmed tilapia, *Oreochromis niloticus*, reared in fertilized freshwater cages. *J World Aquacult Soc* 48: 718-728. doi:doi:10.1111/jwas.12384

Ng WK, Romano N (2013) A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. Rev Aquacult 5: 220-254. oi:doi:10.1111/raq.12014

Pan L, Xie S, Zhu X, Lei W, Han D, Yang Y (2009) The effect of different dietary iron levels on growth and hepatic iron concentration in juvenile gibel carp (*Carassius auratus gibelio*). *J Appl Ichthyol* 25: 428-431. doi:10.1111/j.1439-0426.2009.01210.x

Robbins E, Pederson T (1970) Iron: its intracellular localization and possible role in cell division. *P NATL ACAD SCI USA* 66: 1244-1251.

Shao, H., Wen, H., Liu, W., Jiang, M., Wu, F., Tian, J. and Huang, F. (2013) Dietary choline requirements of adult GIFT tilapia (*Oreochromis niloticus*). *Journal of Fishery Sciences of China* 20: 1007-1014.

Shiau SY, Su LW (2003) Ferric citrate is half as effective as ferrous sulfate in meeting the iron requirement of juvenile tilapia, *Oreochromis niloticus* x *O-aureus*. *J Nutri* 133: 483-488. doi:10.1093/jn/133.2.483

Tian J, Wen H, Lu X, Liu W, Wu F, Yang CG, Jiang M, Yu LJ (2018) Dietary phosphatidylcholine impacts on growth performance and lipid metabolism in adult Genetically Improved Farmed Tilapia (GIFT) strain of Nile tilapia *Oreochromis niloticus*. *Brit J Nutr* 119: 12-21. doi:10.1017/s0007114517003063

Tian J, Wu F, Yang CG, Jiang M, Liu W, Wen H (2015) Dietary lipid levels impact lipoprotein lipase, hormone-sensitive lipase, and fatty acid synthetase gene expression in three tissues of adult GIFT strain of Nile tilapia, *Oreochromis niloticus*. *Fish Physiol Biochem* 41:1-18. doi:10.1007/s10695-014-0001-1

Wekell CJ, Shearer KJ, Gauglitz Jr E (1986) Zinc supplementation of trout diets: tissue indicators of body zinc status. *The Progressive Fish-Culturist* 48:205-212. doi:10.1577/1548-8640(1986)482.0.CO;2

Wu F, Huang F, Wen H, Jiang M, Liu W, Tian J, Yang C G (2015) Vitamin C requirement of adult genetically improved farmed tilapia, *Oreochromis niloticus*. *Aquacult Int* 23:1203-1215. doi:10.1007/s10499-014-9877-0

Wu F, Wen H, Jiang M, Liu w, Liu B, Tian J (2011) Effects of different dietary carbohydrate levels on growth performance, body composition and serum biochemical indices of juvenile hybrid tilapia (*Oreochromis niloticus*♀× *O. aureus*♂). *Journal of South China Agricultural University* 32:91-95.

Wu F, Wen H, Jiang M, Liu W, Liu B, Tian J, Yang C (2012) Effects of different dietary carbohydrate levels on growth performance and blood biochemical parameters of juvenile GIFT tilapia (O*reochromis niloticus*). *Journal of Northwest Agriculture and Forestry University* 40: 8-14.

Yao YF, Jiang M, Wen H, Wu F, Liu W, Tian J, Yang CG (2013) Dietary phosphorus requirement of GIFT strain of Nile tilapia *Oreochromis niloticus* reared in freshwater. *Aquacult Nutr* 20: 273-280. doi:10.1111/anu.12075

Ye CX, Liu YJ, Mai KS, Tian LX, Yang HJ, Niu J, Huang JW (2010) Effect of iron supplement on growth, haemotology and microelements of juvenile grouper, *Epinephelus coioides*. *Aquacult Nutr* 13:471-477.