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# Requirement of Tiger Puffer *Takifugu rubripes* for Dietary Iron

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Two experiments were conducted to determine the dietary iron (Fe) requirement of tiger puffer at a water temperarure of 19–29.5 °C. In experiment I, the fish with average body weight of 2.5 g were fed the purified diets with and without Fe supplement for 12 weeks. Growth, Ht value and Hb content were significantly low in fish fed the diet without Fe supplement. Reduced values of Fe contents of plasma and vertebrae were also observed in fish fed the Fe–unsupplemented diet. On the other hand, unsaturated iron binding capacity (UIBC) of plasma was higher in fish fed the Fe–unsupplemented diet. In experiment II, the fish averaging 10.9 g were fed the five diets with different levels of Fe (55, 92, 140, 197 and 263 mg/kg diet) for 13 weeks. No substantial effect on growth performance was observed in all the groups fed the diets with different Fe levels. However, hematological charateristics and Fe contents of vertebrae and liver were significantly different between the fish fed the diet containing 55 mg Fe/kg and other groups. These results suggest that Fe deficiency developes hypochromemia in tiger puffer and the dietary Fe requirement is 90–140 mg/kg diet.

## INTRODUCTION

Iron (Fe) is one of the essential minerals that fishes require in diets. Studies on mineral requirements of fishes have indicated that dietary iron deficidency causes anaemic symptoms such as microcytic hypochromemia and the anisocytosis of erythrocyte, and taht its requirements are in the range of 30–170 mg per kg diet in most of fishes (Arai *et al.*, 1975; Sakamoto and Yone, 1978a; Gatlin and Wilson, 1986; Iida *et al.*, 1991; Anderson *et al.*, 1996). However, there are only a few reports with marine fishes. The present study was conducted to determine the dietary Fe requirement of tiger puffer, which is one of the important marine culture species in Japan.

## MATERIALS AND METHODS

### Experimental Diets

Compositions of the experimental diets are shown in Table 1. Two experiments were conducted. Protein source was 55% vitamin–free milk casein in experiment I and 45% vitamin–free milk casein and 10% squid meal in experiment II. In experiment I, two diets were prepared by adding mineral mixtures with and without iron (Fe) supplement from

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**Table 1.** Compositions of the experimental diets for tiger puffer

| Experiment | I | | II | | | | |
|---|---|---|---|---|---|---|---|
| Diet no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Supplemental Fe (mg/kg diet) | 200 | 0 | 0 | 75 | 125 | 150 | 200 |
| Dietary Fe (mg/kg) | 220 | 19 | 55 | 92 | 140 | 197 | 263 |
| Ingredient(%) | | | | | | | |
| Casein*1 | 55 | 55 | 45 | 45 | 45 | 45 | 45 |
| Squid meal | 0 | 0 | 10 | 10 | 10 | 10 | 10 |
| Dextrin | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| $\alpha$-Starch | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Pollack liver oil | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Vitamin mix.*2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Mineral mix.*3 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Fe-citrate | 0.12 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ferrous chloride | 0 | 0 | 0 | 0.027 | 0.044 | 0.053 | 0.071 |
| Guar gum | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Feeding stimulant*4 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| $\alpha$-Cellulose | 3.88 | 4 | 5 | 4.973 | 4.956 | 4.947 | 4.929 |

*1 Vitamin-free milk casein.

*2 Vitamin mixture (Halver, 1957).

*3 Fe-free mineral mixture (in 100 g): KCl, 6.54; $MgSO_4 \cdot 7H_2O$, 6.82; $NaH_2PO_4 \cdot 2H_2O$, 25.22; Ca-lactate, 24.53; cellulose, 36.60 (g); $AlCl_3 \cdot 6H_2O$, 8; $ZnSO_4 \cdot 7H_2O$, 177; $MnSO_4 \cdot 4\text{-}6H_2O$, 40; CuCl, 5; KI, 8; $CoCl_2 \cdot 6H_2O$, 52 (mg).

*4 Alanine 0.3, sodium aspartate 0.3, sodium glutamate 0.032, 5'-ribonucleotide·Na 0.368 g/g.

Fe-citrate. In experiment II, five diets were formulated to contain different levels of dietary Fe from ferrous chloride. The diets were pelleted according to the method as previously reported (El-Zibdeh *et al.*, 1995). The Fe contents of diets (1 and 2) with and without Fe supplement in experiment I were 220 and 19 mg/kg, and those of diets 3 to 7 in experiment II were 55, 92, 140, 197 and 263 mg/kg, respectively.

**Fish and Rearing**

Juvenile tiger puffer (*Takifugu rubripes*), which were produced from eggs at the Fishery Research Raboratory of Kyushu University, were acclimatized to indoor laboratory conditions for 3 weeks and were sorted into two replicate groups of 30 fish each in experiments I and II. Initial average body weights of fish were 2.5 g in experiment I and approximately 11 g in experiment II. The rearing was conducted in 150 $\ell$ rectangular flow-through aquaria with a flow rate of approximately 1.5 $\ell$/min. Water temperarue ranged from 19 to 29.5 °C. The fish were fed each diet three times a day to satiation for 12 weeks in experiment I and 13 weeks in experiment II. Body weight of individual fish was measured at biweekly intervals. Other rearing methods were the same as those described previouly (El-Zibdeh *et al.*, 1995).

**Analytical Methods**

At the end of the feeding trials, weight gain, feed efficiency, condition factor and

hepatosomatic index were measured. Blood samples were taken by cardiac puncture from 10 fish randomly selected from each group. Hemoglobin content, Hematocrit value and plasma protein content were determined by the cyanmethemoglobin method, a Kubota hematocrit reader and an ATAGO serum protein refractometer, respectively. Red blood cells were counted using a hemocytometer (improved Neubauer type). Plasma triglyceride and mineral contents (P, Ca, K and Mg) of pooled samples from each group were determined using a Rapid Blood Analyzer (RaBA–Super) and Unikits (Chugai Pharmaceutical Co.). Plasma Fe and unsaturated iron binding capacity (UIBC) were determined by the Nitroso–PSAP method (Fe C–test Wako and UIBC–test Wako, Wako Pure Chemicals Co.). A composite sample of liver and vertebrae from each group was subjected to proximate composition and mineral analyses. Mineral contents of the samples were determined with a Perkin–Elmer 3000 atomic absorption spectrophotometer (Perkin–Elmer Co., USA) except that P was measured by the molybdenate blue method.

**Statistical Analysis**

Student's T–test was employed to determine the significance in difference between the treatment means in growth, hepatosomatic index and condition factor data. Hematological data were subjected to analysis of variance, and significance of difference ($P<0.05$) was determined by the Fisher's PLSD test.

## RESULTS

**Experiment I**

Tiger puffer fed the diet without Fe supplement manifested significantly reduced growth compared to the control group while feed efficiency was comparable to each other (Table 2). Distinguishable pale yellow color of the liver and significantly low hepatosomatic index were also observed in the Fe–unsupplemented group. However, no effect was observed on the condition factor. Significantly reduced values of Ht, Hb, and plasma total protein were observed in fish fed the Fe–unsupplemented diet (Table 3). Plasma Fe

**Table 2.** Effect of dietary Fe supplement on growth performance of tiger puffer (Expt. I)

| Diet no. | 1 (Cont.) | 2 (–Fe) |
|---|---|---|
| Average body weight (g) | | |
| Initial | 2.5±0.2 | 2.5±0.2 |
| Final*[1] | 33.9±4.5[a] | 27.4±4.7[b] |
| Average weight gain (%) | 1124 | 994 |
| Feed efficiency (%)*[2] | 76 | 74 |
| Condition factor*[3] | 3.23±0.19 | 3.01±0.11 |
| Hepatosomatic index*[4] | 8.24±0.36[a] | 6.29±0.58[b] |

*[1] Values in the same row with different superscript letters are significantly different ($P<0.05$).
*[2] Wet weight gain (g) × 100/dry feed intake (g).
*[3] Body weight (g) × 100/(body length in cm)$^3$.
*[4] Liver weight (g) × 100/ body weight (g).

**Table 3.** Effect of dietary Fe supplement on blood profile of tiger puffer (Expt. I)

| Diet no. | | 1 (Cont.) | 2 (-Fe) |
|---|---|---|---|
| Ht | (%)*1 | 12.4±1.7a | 3.3±0.4b |
| Hb | (g/100 ml) | 3.6±0.4a | 1.2±0.4b |
| Total protein | (g/100 ml) | 3.3±0.3a | 2.6±0.1b |
| Triglyceride | (mg/100 ml) | 137 | 135 |
| P | (mg/100 ml) | 11.4 | 8.0 |
| Ca | (mg/100 ml) | 11.3 | 9.7 |
| Mg | (mg/100 ml) | 2.6 | 1.8 |
| Fe | (μg/100 ml) | 72 | 28 |
| UIBC | (μg/100 ml)*2 | 229 | 412 |

*1 Values in the same row with different superscript letters are significantly different ($P<0.05$).
*2 Unsaturated iron binding capacity.

**Table 4.** Effect of dietary Fe supplement on mineral contents of the vertebrae of tiger puffer (Expt. I)*

| Diet no. | | 1 (Cont.) | 2 (-Fe) |
|---|---|---|---|
| Ca | (%) | 28.1 | 28.2 |
| P | (%) | 10 | 9.9 |
| Mg | (%) | 0.36 | 0.38 |
| K | (%) | 0.04 | 0.05 |
| Fe | (μg/g) | 22.2 | 4.6 |
| Zn | (μg/g) | 77.2 | 75.3 |
| Mn | (μg/g) | 24.0 | 36.3 |
| Cu | (μg/g) | 2.4 | 3.0 |

* Data are on dry weight basis.

**Table 5.** Effect of dietary Fe supplement on proximate compositions of the liver of tiger puffer (Expt. I)*

| Diet no. | | 1 (Cont.) | 2 (-Fe) |
|---|---|---|---|
| Moisture | (%) | 42.7 | 40.7 |
| Crude protein | (%) | 10.8 | 12.3 |
| Crude lipid | (%) | 84.1 | 82.7 |
| Crude ash | (%) | 3.0 | 2.8 |

* Data are on dry weight basis.

content also markedly decreased and UIBC increased in the Fe–unsupplemented group. Furthermore, Fe content of vertabrae was markedly low in the Fe–unsupplemented group (Table 4). On the other hand, no influence of Fe supplementation was observed on proximate compositions of the liver (Table 5). These results indicate that serious anaemic symptoms are developed due to Fe deficiency in tiger puffer.

## Experiment II

No substantial effects on growth, feed efficiency, and condition factor were observed

**Table 6.** Effect of dietary Fe levels on growth performance of tiger puffer (Expt. II)

| Diet no. | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|
| (Dietary Fe, mg/kg) | (55) | (92) | (140) | (197) | (263) |
| Average body wt. (g) | | | | | |
| Initial | 10.9±1.6 | 10.9±1.7 | 11.0±1.7 | 11.1±1.6 | 11.1±1.6 |
| Final | 64.9±13.8 | 65.6±14.3 | 71.1±18.5 | 69.1±13.9 | 70.2±16.4 |
| Average wt. gain (%) | 492 | 497 | 545 | 522 | 536 |
| Feed efficiency (%)* | 87 | 79 | 79 | 75 | 81 |
| Condition factor* | 3.2±0.2 | 3.2±0.2 | 3.3±0.3 | 3.4±0.3 | 3.2±0.2 |
| Hepatosomatic index* | 8.1±1.4 | 8.3±0.8 | 8.9±1.4 | 8.8±1.7 | 9.0±1.3 |
| Mortality (%) | 21.7 | 11.7 | 12.8 | 14.2 | 8.5 |

* See Table 2.

**Table 7.** Effect of dietary Fe levels on hematological characteristics and mineral contents of the plasma of tiger puffer (Expt. II)

| Diet no. | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|
| (Dietary Fe, mg/kg) | (55) | (92) | (140) | (197) | (263) |
| Ht (%)*1 | 19.7±3.8$^{b}$ | 26.1±2.6$^{a}$ | 27.3±2.1$^{a}$ | 25.1±2.7$^{a}$ | 26.5±1.9$^{a}$ |
| Hb (g/100ml) | 1.8±0.9$^{b}$ | 3.8±1.0$^{a}$ | 3.5±0.6$^{a}$ | 3.7±0.8$^{a}$ | 4.3±0.8$^{a}$ |
| RBC ($\times 10^{6}$/mm$^{3}$) | 1.25±0.17$^{c}$ | 2.62±0.57$^{a}$ | 2.07±0.42$^{b}$ | 2.16±0.18$^{b}$ | 2.73±0.58$^{a}$ |
| MCH($\gamma\ \gamma$)*2 | 14.1±3.9 | 15.5±5.4 | 17.0±3.3 | 17.0±4.7 | 16.3±4.7 |
| MCV($\mu^{3}$)*3 | 167±14$^{a}$ | 105±22$^{b}$ | 137±37$^{ab}$ | 107±18$^{b}$ | 103±14$^{b}$ |
| MCHC (%)*4 | 7.5±3.1$^{c}$ | 14.6±4.3$^{ab}$ | 12.8±2.1$^{b}$ | 15.3±3.3$^{ab}$ | 16.0±2.7$^{a}$ |
| Ca (mg/100ml) | 10.3 | 11.6 | 12.4 | 10.8 | 10.6 |
| P (mg/100ml) | 6.1 | 5.4 | 6.5 | 5.4 | 6.1 |
| Mg (mg/100ml) | 3.0 | 2.3 | 1.9 | 1.8 | 1.9 |
| K (mEq/l) | 2.6 | 1.9 | 1.1 | 1.4 | 1.0 |
| Fe ($\mu$g/100ml) | 50 | 115 | 109 | 107 | 112 |
| UIBC ($\mu$g/100ml)*5 | 369 | 274 | 267 | 260 | 256 |
| TIBC ($\mu$g/100ml)*6 | 419 | 389 | 376 | 367 | 268 |
| ISI (%)*7 | 11.9 | 29.6 | 28.9 | 29.2 | 30.4 |

*1 Values in the same row with different superscript letters are significantly different ($P<0.05$).
*2 Mean corpuscular hemoglobin.
*3 Mean corpuscular volume.
*4 Mean corpuscular hemoglobin concentration.
*5 Unsaturated iron binding capacity.
*6 Total iron binding capacity.
*7 Iron saturation index.

in all the groups fed different levels of Fe supplement (Table 6). Hepatosomatic index increased with increasing levels of dietary Fe, but the significant difference was not recognized among these values. Ht value, Hb content, and RBC of fish fed 55 mg Fe/kg diet were significantly lower than those of the other groups (Table 7). MCH was also lowest in fish fed 55 mg Fe/kg diet, but there were no significant differences among the treatment groups. However, MCV was significantly high and MCHC was significantly low in fish fed the diet containing 55 mg Fe/kg. The minimum values of plasma Fe and ISI and

**Table 8.** Effect of dietary Fe levels on mineral contents of the vertebrae of tiger puffer (Expt. II)*

| Diet no. (Dietary Fe, mg/kg) | 3 (55) | 4 (92) | 5 (140) | 6 (197) | 7 (263) |
|---|---|---|---|---|---|
| Crude ash (%) | 56.9 | 56.1 | 56.9 | 58.6 | 57.4 |
| Ca (%) | 27.3 | 26.3 | 26.3 | 26.4 | 26.4 |
| P (%) | 5.9 | 5.8 | 5.9 | 6.3 | 6.3 |
| Mg (%) | 1.3 | 1.3 | 1.1 | 1.4 | 1.3 |
| Fe (μg/g) | 22.2 | 22.9 | 26.6 | 26.5 | 27.1 |
| Zn (μg/g) | 51.8 | 51.3 | 61.7 | 60.9 | 60.6 |
| Mn (μg/g) | 17.1 | 18.5 | 23.9 | 24.6 | 24.7 |

* Data are on dry weight basis.

**Table 9.** Effect of dietary Fe levels on proximate and mineral compositions of the liver of tiger puffer (Expt. II)*

| Diet no. (Dietary Fe, mg/kg) | 3 (55) | 4 (92) | 5 (140) | 6 (197) | 7 (263) |
|---|---|---|---|---|---|
| Moiture (%) | 34.4 | 31.2 | 32.2 | 31.9 | 28.7 |
| Crude protein (%) | 4.2 | 6.2 | 6.8 | 6.4 | 6.0 |
| Crude lipid (%) | 85.4 | 88.6 | 86.4 | 87.4 | 90.5 |
| Crude ash (%) | 8.5 | 7.3 | 7.6 | 7.0 | 8.2 |
| Ca (%) | 24.8 | 29.3 | 31.6 | 34.2 | 32.0 |
| P (%) | 61.0 | 62.3 | 60.1 | 60.4 | 60.2 |
| Mg (%) | 29.9 | 39.8 | 43.7 | 45.6 | 42.3 |
| Fe (μg/g) | 5.0 | 10.3 | 13.1 | 12.6 | 11.8 |
| Zn (μg/g) | 8.0 | 8.9 | 11.0 | 9.4 | 8.7 |

* Data are on dry weight basis.

the maximum values of UIBC and TIBC were detected in fish fed the 55 mg Fe diet, while similar values of these parameters were found in other treatment groups except that TIBC tended to decrease with the increase of dietary Fe. Plasma K and Mg contents were slightly high in fish fed the diets containing 55 mg and 92 mg Fe/kg, but plasma Ca and P contents were similar in all treatment groups. The minneral contents of vertebrae are shown in Table 8. There were no differences in Ca, P, and Mg contents of vertebrae among the treatment groups, however, vertebral Fe, Zn, and Mn contents were slightly lower in fish fed the diets containing less than 140 mg Fe/kg diet. Reduced liver Fe and Mg contents were also observed in fish fed the 55 mg Fe diet (Table 9).

## DISCUSSION

The present study demonstrated that dietary Fe deficiency induced hypochromemia in tiger puffer. Fe deficiency in tiger puffer was characterized by reduced Hb, Ht, RBC, plasma and vertebral Fe, and ISI as reported for brook trout (Kawatsu, 1972), yellowtail (Ikeda *at al.*, 1973), Japanese eel (Arai *et al.*, 1975), red sea bream (Sakamoto and Yone, 1976, 1978a), carp (Sakamoto and Yone, 1978b), channel catfish (Gatlin and Wilson,

1986), and Atlantic salmon (Anderson *et al.*, 1996). In most fish species, growth and feed efficiency were not affected by Fe deficiency. However, Gatlin and Wilson (1986) and Lim and Klesius (1997) observed decreased growth and feed efficiency in channel catfish fed Fe–deficient diets. In the present study, growth performances of Fe–unsupplemented groups were different between the two experiments. The Fe level of the Fe–unsupplemented diet in experiment I was 19 mg/kg diet, but that in experiment II was 55 mg/kg diet due to the difference in the composition of the basal diets in experiments I and II. Therefore, Fe deficiency might be more severe in fish fed the diet containing the least amount of Fe as observed in experiment I. Furtheremore, it is possible that the difference in fish size affects the growth of fish in the present study, because, initial body weight of fish used in experiment I was smaller than that in experiment II. Poor growth was also observed in smaller size (17.5 g) of yellowtail but not in larger size (42 g) of yellowtail (Makino *et al.*, 1989; Iida *et al.*, 1991). Based on the present study, the dietary Fe requirement of tiger puffer is considrered to be 90–140 mg/kg diet.

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