# マダイ飼料用鉄給源としての各種鉄剤の利用性

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# Availabilities of Three Iron Compounds as Dietary Iron Sources for Red Sea Bream*1

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Two experiments were conducted to compare the availability of ferrous chloride, ferric chloride, and ferric citrate as the iron sources in the diet for red sea bream, *Chrysophrys major*. Iron contents of the test diets were adjusted to approximately 15 mg per 100 g of diet in experiment I, and 20 mg per 100 g in experiment II. Fish were reared on the test diets at 25°C over a 73-day period (Exp. I) and over a 90-day period (Exp. II). At the end of the feeding trial, hematological examinations and chemical analyses of the blood serum were conducted. At the 15 mg Fe per 100 g diet level, fish fed the diet supplemented with ferric citrate showed a slight iron deficiency anemia, but fish fed the diets with supplemental ferrous chloride and ferric chloride did not. On the other hand, at the 20 mg Fe per 100 g diet level, no anemia was found in the ferric citrate group. Therefore, it is concluded that the amount of ferrous chloride and ferric chloride required for the prevention of iron deficiency anemia corresponds to 15 mg Fe per 100 g diet, and that of ferric citrate corresponds to 20 mg Fe per 100 g diet.

In the previous paper[1], it was proven that an iron deficiency anemia of red sea bream could be prevented by the supplementation of ferrous chloride to the diet at a 15 mg Fe per 100 g diet level. However, in the cases of terrestrial animals[2–4], it is well known that the physicochemical form of iron affects iron absorption. Therefore, in this study, two experiments were conducted to compare the availabilities of three iron compounds in the diet for red sea bream.

## Materials and Methods

Ferrous chloride, ferric chloride, and ferric citrate were used as the dietary iron sources. These iron compounds were supplemented to test diets at a 15 mg Fe per 100 g diet level in experiment I, and a 20 mg Fe per 100 g diet level in experiment II. The composition of test diets is the same as that of the diet YR-2[5] except for mineral mixture (Table 1). The composition of mineral mixtures used in test diets is listed in Table 2. The fish were reared on the test diets over a 73-day period (Exp. I) and over a 90-day period (Exp. II) at 25°C. The methods of fish selection, grouping, care, preparation of diets, and feeding were the same as those reported previously[5]. At the end of feeding trial, 10 fish from each group were selected. A blood sample was taken from each fish by a cardiac puncture for the hematological examinations. Hemoglobin content (Hb) and hematocrit value (Ht) were determined by the cyanmethemoglobin method[6] and microhematocrit method[7], respectively. Erythrocytes were counted on a hemocytometer (improved Neubauer type) after dilution in a Thoma pipette with the physiological salt solution for marine teleostei[7]. From these values, mean corpuscular constants (MCH, MCV, and MCHC) were calculated. A mean corpuscular diameter (MCD) of 1000 cells from each group

**Table 1.** Composition of test diet

| | | | |
|---|---|---|---|
| Casein | 52.0 | L-Lys·HCl | 0.6 |
| Gelatin | 11.0 | L-Val | 0.7 |
| L-Phe | 0.6 | Gly | 0.4 |
| L-Arg·HCl | 1.3 | P. L. Oil*1 | 9.0 |
| L-Cys | 0.7 | Dextrin | 8.0 |
| L-Try | 0.2 | Vitamins*2 | |
| L-His·HCl·$H_2O$ | 0.2 | +$\alpha$-Cellulose | 3.0 |
| DL-Ala | 1.3 | Mineral mix.*3 | 8.0 |
| L-Asp·Na | 1.0 | $\alpha$-Cellulose | 2.0 |

*1 Alaska pollack liver oil purified by molecular distillation.
*2 HALVER's vitamin mixture (1957).
*3 Shown in Table 2.

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**Table 2.** Composition of mineral mixtures

| Experiment | I | | | |
|---|---|---|---|---|
| Iron source | None | Fe-cit. | $FeCl_2$ | $FeCl_3$ |
| $Fe^{3+}$-citrate | — | 1.120 | — | — |
| $FeCl_2 \cdot 4H_2O$ | — | — | 0.667 | — |
| $FeCl_3 \cdot 6H_2O$ | — | — | — | 0.907 |
| KCl | 5.18 | 5.18 | 5.18 | 5.18 |
| $NaH_2PO_4 \cdot 2H_2O$ | 30.805 | 30.805 | 30.805 | 30.805 |
| Trace metals* | 0.294 | 0.294 | 0.294 | 0.294 |
| $\alpha$-Cellulose | 64.309 | 63.189 | 63.642 | 63.402 |
| Fe (mg/100 g diet) | 0.1 | 14.9 | 14.9 | 14.9 |

| Experiment | II | | | |
|---|---|---|---|---|
| Iron source | None | Fe-cit. | $FeCl_2$ | $FeCl_3$ |
| $Fe^{3+}$-citrate | — | 1.485 | — | — |
| $FeCl_2 \cdot 4H_2O$ | — | — | 0.889 | — |
| $FeCl_3 \cdot 6H_2O$ | — | — | — | 1.203 |
| KCl | 5.18 | 5.18 | 5.18 | 5.18 |
| $NaH_2PO_4 \cdot 2H_2O$ | 30.805 | 30.805 | 30.805 | 30.805 |
| Trace metals* | 0.294 | 0.294 | 0.294 | 0.294 |
| $\alpha$-Cellulose | 64.309 | 62.824 | 63.420 | 63.106 |
| Fe (mg/100 g diet) | 0.1 | 19.9 | 19.9 | 19.9 |

* $AlCl_3 \cdot 6H_2O$, 9.0 mg; $ZnSO_4 \cdot 7H_2O$, 178.5 mg; $MnSO_4 \cdot 7H_2O$, 40.0 mg; CuCl, 5.5 mg; KI, 8.5 mg; $CoCl_2 \cdot 6H_2O$, 52.5 mg.

was obtained by measuring the longest diameter of each cell with a micrometer on a smeared preparation stained with May-Giemsa[8]. Specific gravity was determined by the copper sulfate method[9]. Serum from the remaining blood was used for the chemical analyses. Iron content and total iron binding capacity (TIBC) of the blood serum were determined by the bathophenanthroline[10] and magnesium carbonate method[11], respectively. The unsaturated iron binding capacity (UIBC) and iron saturation index were calculated by subtracting serum iron content from TIBC and by dividing serum iron content by TIBC, respectively. Serum triglyceride and phospholipid content were determined by the acetylacetone method[12] and the modified HOEFLMARY-FRIED method[13]. The levels of cholesterol, bilirubin, protein, glucose, and urea-N (BUN), and the activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH), leucine aminopeptidase (LAP), and alkaline phosphatase (ALP) were determined with Rapid Blood Analyzer 3010 and Unikit (Chugai Pharmaceutical Co.). After the blood sampling, the fork length and the weight of entire body, liver, spleen, and heart were measured. From these values, the condition factor and the percentage of each organ weight to body weight were calculated.

## Results and Discussion

As shown in Table 3, no significant differences were recognized between the ferrous chloride group and the other groups in the growth rate, feed efficiency, condition factor, hepatosomatic index, and the percentages of spleen and heart weight to body weight, in both experiments. Also, at a 15 mg per 100 g dietary iron level (Exp. I), little differences were found between the ferrous chloride group and the ferric chloride group in the following: the Hb and Ht of the whole blood; the blood serum levels of Fe, phospholipid, triglyceride, and cholesterol; and the Fe saturation index, and the activities of LDH and LAP in the blood serum. However, these values of the group with ferric citrate were higher than those of the group without iron supplementation, and lower than those of other two groups with ferrous or ferric chloride (Table 4). Thereby, it is presumed that the ferric citrate group exhibited a slight iron deficiency anemia at this dietary iron level. On the other hand, at a 20 mg per 100 g dietary iron level, no significant differences were recognized among these three groups in the following determinations; Hb, Ht, red blood cell count (RBC), MCH, MCV, MCHC, MCD, and specific gravity of the whole blood (Table 5). From these results, it is evident

**Table 3.** Effect of supplemental iron compounds on the growth rate, feed efficiency, condition factor, and percentages of organ weight to body weight at a 15 and 20 mg per 100 g dietary iron level

| Experiment | I | | | |
|---|---|---|---|---|
| Iron source | None | Fe-cit. | $FeCl_2$ | $FeCl_3$ |
| No. of fish | | | | |
| at start | 20 | 20 | 20 | 20 |
| after 73 days | 18 | 20 | 20 | 20 |
| Average body weight (g) | | | | |
| at start | 17.4±0.9*1 | 17.4±0.9 | 17.4±0.9 | 17.4±0.9 |
| after 73 days | 34.6±4.8 | 35.0±5.3 | 34.0±5.9 | 34.3±4.5 |
| "t" test (5%) | NS*2 | NS | — | NS |
| Feed efficiency (%) | 66.3 | 71.2 | 65.9 | 66.8 |
| Condition factor*3 | 2.29±0.11 | 2.31±0.14 | 2.29±0.09 | 2.31±0.10 |
| "t" test (5%) | NS | NS | — | NS |
| Hepatosomatic index (%)*4 | 2.53±0.33 | 2.59±0.38 | 2.48±0.47 | 2.42±0.81 |
| "t" test (5%) | NS | NS | — | NS |
| Spleen Wt×$10^2$/BWt (%) | 0.06±0.02 | 0.08±0.02 | 0.07±0.02 | 0.08±0.03 |
| "t" test (5%) | NS | NS | — | NS |
| Heart Wt×$10^2$/BWt (%) | 0.16±0.01 | 0.15±0.02 | 0.15±0.02 | 0.16±0.03 |
| "t" test (5%) | NS | NS | — | NS |

| Experiment | II | | | |
|---|---|---|---|---|
| Iron source | None | Fe-cit. | $FeCl_2$ | $FeCl_3$ |
| No. of fish | | | | |
| at start | 25 | 25 | 25 | 25 |
| after 90 days | 24 | 24 | 25 | 25 |
| Average body weight (g) | | | | |
| at start | 14.7±0.7 | 14.7±0.8 | 14.7±0.7 | 14.7±0.7 |
| after 90 days | 40.6±7.3 | 39.3±5.8 | 40.2±8.5 | 39.0±7.7 |
| "t" test (5%) | NS | NS | — | NS |
| Feed efficiency (%) | 67.5 | 63.5 | 64.3 | 60.5 |
| Condition factor | 2.12±0.08 | 2.14±0.12 | 2.15±0.10 | 2.17±0.11 |
| "t" test (5%) | NS | NS | — | NS |
| Hepatosomatic index (%) | 2.50±0.41 | 2.20±0.43 | 2.48±0.45 | 2.21±0.43 |
| "t" test (5%) | NS | NS | — | NS |
| Spleen Wt×$10^2$/BWt (%) | 0.07±0.02 | 0.06±0.02 | 0.06±0.01 | 0.07±0.03 |
| "t" test (5%) | NS | NS | — | NS |
| Heart Wt×$10^2$/BWt (%) | 0.18±0.02 | 0.18±0.06 | 0.18±0.02 | 0.18±0.03 |
| "t" test (5%) | NS | NS | — | NS |

*1 Standard deviation. *2 Non-significant.
*3 Body weight (g)×$10^2$/Fork length (cm)$^3$.
*4 Liver weight (g)×$10^2$/Body weight (g).

that ferric chloride can prevent the development of iron deficiency anemia at a 15 mg per 100 g dietary iron level as well as ferrous chloride, and that the amount of dietary ferric citrate required for the prevention corresponds to approximately 20 mg Fe per 100 g diet. Therefore, ferric citrate is less available than the other two iron compounds. In the terrestrial animals[3], it was proven that the soluble iron is more effective than the insoluble iron for the prevention and therapy of iron deficiency, because the soluble iron is absorbed more easily. Therefore, it is supposed that the low anti-anemia effect of ferric citrate for red sea bream is due to its lower solubility than the other two iron compounds. Also, the terrestrial animals absorbed ferrous iron in dose at a larger amount than ferric iron[3]. The sword-tail, *Xiphophorus helleri,* and platy-fish, *Xiphophorus maculatus,* utilized ferrous iron dissolved in water more effectively than ferric iron[14]. On the other hand, in the

**Table 4.** Effect of supplemental iron compounds on the hematological characteristics, levels of chemical components, and activities of enzymes in blood serum at a 15 mg per 100 g dietary iron level

| Iron source | None | Fe-cit. | $FeCl_2$ | $FeCl_3$ |
|---|---|---|---|---|
| Whole blood | | | | |
| Hb (g/d*l*) | 4.4±0.7 | 5.9±1.1 | 7.0±0.5 | 6.6±0.6 |
| "t" test (5%) | S*1 | S | — | NS |
| Ht (%) | 19.8±2.9 | 26.1±5.0 | 30.9±3.2 | 28.8±4.6 |
| "t" test (5%) | S | S | — | NS |
| Blood serum | | | | |
| Fe (μg/d*l*) | 25 | 42 | 52 | 52 |
| TIBC (μg/d*l*) | 308 | 332 | 320 | 300 |
| UIBC (μg/d*l*) | 283 | 290 | 268 | 248 |
| Fe saturation index (%) | 8.1 | 12.7 | 16.3 | 17.3 |
| Phospholipid (mg/d*l*) | 359 | 449 | 496 | 512 |
| Triglyceride (mg/d*l*) | 79 | 120 | 187 | 193 |
| Total cholesterol (mg/d*l*) | 151 | 181 | 202 | 210 |
| Total bilirubin (mg/d*l*) | 0.40 | 0.57 | 0.56 | 0.60 |
| Total protein (g/d*l*) | 3.38 | 3.29 | 3.51 | 3.32 |
| Glucose (mg/d*l*) | 73 | 77 | 76 | 73 |
| BUN (mg/d*l*) | 4.8 | 5.0 | 4.0 | 4.7 |
| GOT (K. U.)*2 | 111 | 133 | 117 | 145 |
| GTP (K. U.)*2 | 25 | 20 | 25 | 26 |
| LDH (W. U.)*3 | 862 | 981 | 1111 | 1246 |
| LAP (G.-R. U.)*4 | 455 | 480 | 522 | 519 |
| ALP (K.-A. U.)*5 | 2.3 | 2.5 | 2.9 | 2.1 |

*1 Significant. *2 Karmen Unit. *3 Wroblewski Unit.
*4 Goldberg-Ruteinburg Unit. *5 King-Armstrong Unit.

**Table 5.** Effect of supplemental iron compounds on the hematological characteristics at a 20 mg per 100 g dietary iron level

| Iron source | None | Fe-cit. | $FeCl_2$ | $FeCl_3$ |
|---|---|---|---|---|
| Hb (g/d*l*) | 4.1±0.4 | 7.2±0.4 | 7.1±0.7 | 7.2±0.7 |
| "t" test (5%) | S | NS | — | NS |
| Ht (%) | 25.1±2.9 | 33.9±1.9 | 33.8±2.7 | 33.4±2.9 |
| "t" test (5%) | S | NS | — | NS |
| RBC ($\times 10^6$/mm$^3$) | 3.07±0.43 | 3.48±0.23 | 3.39±0.45 | 3.28±0.30 |
| "t" test (5%) | NS | NS | — | NS |
| MCH (γγ)*1 | 13.52±2.06 | 20.80±1.50 | 21.21±2.15 | 22.11±2.44 |
| "t" test (5%) | S | NS | — | NS |
| MCV (μm$^3$)*2 | 83.1±14.0 | 97.9±6.6 | 100.7±10.4 | 102.1±8.5 |
| "t" test (5%) | S | NS | — | NS |
| MCHC (%)*3 | 16.34±1.17 | 21.26±0.62 | 21.12±1.30 | 21.64±0.83 |
| "t" test (5%) | S | NS | — | NS |
| Specific gravity | | | | |
| ($x$–1.0)×1000 | 35±2 | 43±2 | 43±2 | 42±1 |
| "t" test (5%) | S | NS | — | NS |
| MCD (μm) | 8.44 | 9.73 | 9.77 | 9.75 |

*1 Mean corpuscular hemoglobin: Hb (g/d*l*)×10/RBC ($\times 10^6$/mm$^3$).
*2 Mean corpuscular volume: Ht (%)×10/RBC ($\times 10^6$/mm$^3$).
*3 Mean corpuscular hemoglobin concentration: Hb (g/d*l*)×100/Ht (%).

present study the dietary ferrous and ferric iron were equally effective to prevent the iron deficiency anemia in red sea bream. The reason for this difference may be understood on the basis of the following review by CONRAD[3]: "Iron is absorbed poorly from test doses of ferric salts unless it is

reduced or chelated. Ferric iron is insoluble in aqueous solutions more alkaline than pH 5, whereas some ferrous iron remains soluble at pH 8. However, commonplace constituents of the diet and intestinal secretions bind iron to maintain it in a soluble monomeric form at the alkaline pH of the small intestine. Sugars, amino acids, and amines are examples of dietary ingredients which decrease both the precipitation and polymerization of iron in aqueous solutions. These compounds interfere with the formation of water bridges between iron molecules to form relatively unstable metal coordinating complexes. Certain chelators, such as ascorbic acid, even make ferric iron more soluble than ferrous iron over a wider range of pH." Test diets used in this study contained sugar, amino acids and ascorbic acid. Furthermore, hydrochloric acid, which makes ferric iron more available for chelation, is secreted in the stomack of red sea bream. Accordingly, it is presumed that ferric chloride was changed to an available form by these factors in red sea bream.

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