Iron Methionine and Iron Sulfate as Sources of Dietary Iron for Channel Catfish *Ictalurus punctatus*

CHHORN LIM, W. M. SEALEY AND P. H. KLESIUS

USDA-ARS, Fish Diseases and Parasites Research Lab, P.O. Box 952, Auburn, Alabama 36831-0952 USA

Abstract

The dietary iron requirement for normal growth and optimum hematological values and bioavailability was determined for channel catfish *Ictalurus punctatus* fingerlings using egg-white based diets supplemented with 0, 5, 10, 20, 60, and 180-mg iron/kg from iron methionine or 20, 60, and 180-mg iron/kg from iron sulfate. The basal diet which contained 9.2-mg iron/kg, 34% crude protein and 3.1 kcal of digestible energy/g was fed to channel catfish fingerlings (8.5 g) in triplicate flow-through aquariums to satiation twice daily for 8 wk. Fish fed the basal diet without iron supplementation exhibited poor growth throughout the 8-wk period. Fish fed iron-supplemented diets did not differ with regard to final weight gain. Survival, feed conversion, total blood cell count, mean corpuscular hemoglobin concentration, serum iron, total iron binding capacity, and transferrin saturation were not significantly affected by dietary iron level. Hemoglobin, hematocrit, mean corpuscular hemoglobin, and mean corpuscular volume were significantly lower in fish fed the basal diet. These values were also consistently lower for fish fed diets with 5 and 10-mg iron/kg from iron methionine. However, differences were not always significant. Results of this study indicate that supplementation of 5-mg iron from iron methionine was sufficient for growth. However, a supplemental iron level of 20-mg/kg diet or a total iron level of 30-mg/kg of diet appeared to be needed for optimum hematological values. Iron methionine and iron sulfate were equally effective in preventing anemia in channel catfish.

Iron is an essential element in all higher animals including fish because of its importance in cellular respiration and mitosis (Robbins et al. 1972). Although fish can absorb soluble iron across the gill membrane and intestinal mucosa (Roedar and Roedar 1968), feed is considered as the major source of iron for fish due to low concentrations of soluble iron in natural waters (NRC 1993). Dietary iron requirements have been determined for several species of fish including red sea bream *Chrysophrys major* (Sakamoto and Yone 1976, 1978a), yellowtail *Seriola quinqueradiata* (Ikeda et al. 1973), common carp *Cyprinus carpio* (Sakamoto and Yone 1978b), eel *Anguilla japonica* (Nose and Arai 1979), Atlantic salmon *Salmo salar* (Lall and Hines 1987), and channel catfish *Ictalurus punctatus* (Gatlin and Wilson 1986).

The total dietary iron requirement for channel catfish fingerlings was determined by Gatlin and Wilson (1986) to be not more than 30 mg/kg diet. Fingerlings fed iron-deficient diets had decreased weight gain, feed efficiency, hematocrit, hemoglobin, transferrin saturation and erythrocyte values. This requirement was determined with purified diets utilizing iron sulfate as the dietary iron source.

Studies with mammals have shown that chelation of minerals to amino acids may increase their absorption rate in the intestine (Ashmead 1992). More recently, Paripatananont and Lovell (1995) reported that using zinc methionine as the dietary zinc source reduced the dietary zinc requirement of channel catfish in both purified and practical diets as compared to diets containing zinc sulfate. No studies have been conducted on the dietary iron requirement of channel catfish using a chelated source of iron. The objective of this study was to deter-

1 Corresponding author.

© Copyright by the World Aquaculture Society 1996

290
TABLE 1. Composition of diet containing different levels of iron.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet (dry matter basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg white</td>
<td>399.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>475.8</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>32.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>32.5</td>
</tr>
<tr>
<td>Iron-free mineral mix*</td>
<td>40.0</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>15.0</td>
</tr>
<tr>
<td>Ethoxyquin</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Contains (as g/kg of premix): calcium carbonate, 300.0; potassium phosphate, dibasic, anhydrous, 319.0; sodium phosphate, monobasic, 200.34; magnesium sulfate heptahydrate, 132.0; zinc sulfate heptahydrate, 3.00; sodium chloride, 43.50; cobalt chloride, 1.00; manganese sulfate monohydrate, 0.80; cuprous chloride, 0.20; potassium iodide, 0.15; sodium selenite, 0.011.

*Contains (as g/kg of premix): Retinyl acetate, 1.20; cholecalciferol, 0.17; menadione, 3.33; dl-alpha tocopheryl acetate, 4.00; L-ascorbyl-2-polyphosphate (15% vitamin C activity), 600; inositol, 10.00; choline chloride, 150.0; niacin, 9.00; riboflavin, 2.00; pyridoxine hydrochloride, 2.00; thiamin hydrochloride, 2.00; d-calcium pantothenate, 6.00; biotin, 0.31; folic acid, 0.18; cyanocobalamin, 0.0027; celufil, 295.62.

mine the dietary iron requirement of channel catfish fingerlings utilizing iron methionine (FeM) as the dietary iron source and to compare the bioavailability of FeM and iron sulfate (FeS) using a purified diet.

Materials and Methods

Experimental Diets

The egg-white-based diet used in this study was modified from Gatlin and Wilson (1986) and is given in Table 1. The basal diet was supplemented with 0, 5, 10, 20, 60, or 180 mg of iron/kg of diet from FeM (Zinpro, Chaska, Michigan, USA) or 20, 60, or 180 mg of iron/kg of diet from FeS (Sigma Chemical Co., St. Louis, Missouri, USA) while removing corresponding amounts of cellulose. Because it had been found that 20 mg of supplemental iron from FeS was optimum for growth and hematological values, this value was used as the lowest level of supplementation for the FeS diet. The diet was formulated to contain approximately 34% crude protein and 3.1 kcal of digestible energy/g based on feedstuff values reported in the NRC (1993). Diets were prepared by first mixing the dry ingredients then adding oil and deionized water, and blending in a Hobart mixer. The resulting mixture was extruded through a 2.38-mm (3/32-inch)-diameter die in a Hobart meat grinder and cooled to room temperature. Pellets were broken into small pieces and stored at -18 C until needed. Iron content of the basal diet without iron supplementation was determined to be 9.2-mg iron/kg of diet by an inductively-coupled argon plasma (ICAP) spectrometer according to the method of Campbell and Plank (1992).

Experimental Fish and Feeding

Channel catfish fingerlings from a single Auburn x Kansas spawn which had been maintained at the U.S.D.A., Fish Diseases and Parasites Research Laboratory and fed on a commercial diet to an average weight of 8.5 g were randomly stocked into 27, 132-L aquaria at a density of 60 fish per aquarium. Aquaria were supplied with flow-through well-water at a rate of 0.6–0.8 L/min. Water temperature was maintained by individual 300-watt aquarium heaters at 25–27 C. Water was continuously aerated and a photoperiod was maintained at 12:12-h light:dark schedule. Water quality parameters determined for well water were: pH 7.6; nitrite, 0.4 mg/L; sodium, 21.9 mg/L; silicon, 13.4 mg/L; calcium, 18.6 mg/L; potassium, 1.9 mg/L; magnesium, 1.3 mg/L; and less than 0.5 mg/L for iron, manganese, zinc, boron, molybdenum, aluminum, barium, cobalt, chromium, copper, and phosphorus.

Fish in triplicate aquaria were randomly assigned to each of the nine experimental diets and fed their respective diets twice daily (between 0730–0800 and 1430–1500) to satiation for an acclimation period of 2 wk and then for an additional 8-wk period.
During each feeding, feed was offered by hand two to three times until satiation was reached. The quantity of feed consumed was recorded daily by calculating the differences in weights of feeds prior to the first and after the last feeding. Water flow rates were checked and adjusted daily to insure proper water exchange rate. All aquaria were cleaned once weekly by scrubbing and siphoning accumulated wastes. On cleaning days fish were fed only once in the afternoon. Fish in each aquarium were counted and weighed biweekly. When fish were removed for weighing, the aquaria were cleaned thoroughly and drained. No feeding was done on sampling days.

**Blood Analysis**

Blood samples were obtained from fish prior to the 2-wk acclimation period and following the 8-wk feeding period. Ten fish from each aquarium were randomly chosen and anesthetized with tricane methanesulfonate (MS-222, Argent Chemical Company, Redmond, Washington, USA) at 125 mg/L. Blood samples were collected from the caudal vein with a 27-gauge needle and tuberculin syringe. Serum samples for determination of iron and total iron binding capacity were collected following centrifugation of whole blood from five fish at 1000 × g. Sera from each of the five fish from the same aquarium were pooled to obtain one composite sample. Total iron was determined by the colorimetric assay using the Stanbio Total Iron Kit (Stanbio Laboratory, Inc., San Antonio, Texas, USA). In this procedure, iron is released from its combination with transferrin in acid medium, reduced to its ferrous form by hydroxylamine and reacted with ferrozine to form a violet-colored complex that is measured at 560 nm. Transferrin saturation was determined as described by Sherman and Moran (1984).

The remaining five fish from each replicate tank were bled for determination of hematocrit, total cell count, and hemoglobin with heparinized needles and syringes. Hematocrit was determined by the microhematocrit method described by Brown (1988). Total cell counts were determined by diluting whole blood and enumeration in a hemacytometer. Hemoglobin was determined by the total hemoglobin kit (Sigma Diagnostics, Sigma Chemical Co., St. Louis, Missouri, USA) which is a standardized procedure of the cyanomethemoglobin method. Hemoglobin values were adjusted by the cyanomethemoglobin correction factor for channel catfish described by Larsen (1964). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by standard formulas described by Brown (1988).

**Statistical Analyses**

Data were analyzed by one-way analysis of variance and Duncan's multiple-range test was used to determine differences between treatment means (SAS Institute, Inc. 1993). Differences were considered significant at the 0.05 probability level.

**Results**

The average final weight gain, survival and feed conversion are given in Table 2. Decreased feed consumption was observed during the second week of feeding for fish fed the basal diet without iron supplementation. This group of fish had consistently lower weight gain than fish fed iron-supplemented diets over the 8-wk period. By the end of week 8, the weight gain of this group of fish was significantly (P < 0.05) lower than those of the other treatments. There were no significant differences among the weight gain of fish receiving iron-supplemented diets over the 8-wk feeding period. Feed conversion ratios were not affected by the dietary treatments. Fish fed the diet without iron supplementation and the 180-FeS diet had the highest survival rates which were significantly higher than that of fish fed the 20-FeM diet. However, these values were not significantly different from those of fish in other treatments.
Mean hematological values of channel catfish fed diets containing different levels of FeM or FeS following the 8-wk feeding period are shown in Table 3. There seems to be a trend of increased hemoglobin value with increasing levels of iron supplementation from either FeM or FeS. Fish fed the diets with 0 or 5-mg iron/kg supplemental iron had significantly (P < 0.05) lower hemoglobin value than those receiving other dietary treatments. The hemoglobin value for fish fed the 10-FeM diet also was low, although not significantly different from those of the 20-FeM, 60-FeM, or 20-FeS treatments. Hematocrit value was lowest for fish fed the basal diet and was followed by fish fed the 5-FeM and 10-FeM diets. These values were significantly lower than those of fish fed other iron-supplemented diets. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were lowest for fish fed the basal diet but were not significantly lower than those of fish fed the 5 and 10-FeM diets. No significant differences were observed among these values for fish fed diets with 10 FeM or higher and 20 FeS or higher.

The average value of serum iron, total iron binding capacity and transferrin saturation are presented in Table 4. Except for the 60-FeM diet, the serum iron and transferrin saturation tended to be lowest for fish.
fed the basal diet without iron supplementation. The relatively low values for fish fed the 60 FeM diet could not be explained since the corresponding hematological values (Table 3) were not low. No discernable trend was observed for the total iron binding capacity.

**Discussion**

Results of this study showed that channel catfish fed a purified diet without supplemental iron had reduced appetite, feeding activity and reduced weight gain as has been reported by Gatlin and Wilson (1986). Robbins et al. (1972) reported decreased protein synthesis in mammals suffering from severe iron deficiency. A significant decrease in feed efficiency was observed by Gatlin and Wilson (1986) in catfish fed iron-deficient diets. In our study, however, since feed conversion was not affected, the reduced growth was more likely due to reduced feed consumption. Decreased feeding activity also was observed by Gatlin and Wilson (1986). Studies with red sea bream (Sakamoto and Yone 1976), yellowtail (Ikeda et al. 1973) and common carp (Sakamoto and Yone 1978b) showed that growth and feed efficiency were not affected by iron-deficient diets. In contrast, Suzuki et al. (1982) reported a significant improvement in growth and feed efficiency of eel fed a standard eel feed supplemented with 250-mg iron/kg from iron amino acid chelate.

The survival rate in the present study was generally high and was not affected by dietary iron level. The lowest survival observed in fish fed the 20-FeM diet could not be attributed to the dietary iron level or source since there was no discernable trend among the values obtained from various treatments. Likewise, Gatlin and Wilson (1986) indicated that dietary iron level had no effect on the mortality of channel catfish.

Supplementation of 5-mg iron/kg with FeM was sufficient to prevent significant growth depression. No additional increase in weight gain was observed when dietary iron level was increased, suggesting that growth depression only occurs in severe iron deficiency. Gatlin and Wilson (1986) reported that 10 mg of supplemental iron/kg of diet from FeS was necessary to maintain optimum growth. In their study, levels of iron supplementation below 10 mg/kg of diet was not examined. In the present study, levels of supplemental iron from FeS below 20 mg/kg of diet were not used. Thus, due to the unavailability of data on the use of lower levels of supplemental FeS, the bioavailability of iron from FeM and FeS for growth of catfish could not be determined, but it can be speculated that the requirement of iron from FeS could possibly be lower

**Table 4.** Mean serum iron, total iron binding capacity (TIBC), and transferrin saturation in channel catfish fed diets containing different levels of iron from iron methionine (FeM) or iron sulfate (FeS). Means with different superscripts in each column are significantly different at \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Dietary iron added (mg/kg)</th>
<th>Serum iron (µg/dL)</th>
<th>TIBC (µg/dL)</th>
<th>Transferrin saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.87 ± 6.35ᵇ</td>
<td>361.6 ± 19.8</td>
<td>8.48 ± 2.20</td>
</tr>
<tr>
<td>5 FeM</td>
<td>33.29 ± 3.18ᵇ</td>
<td>272.7 ± 45.3</td>
<td>13.01 ± 2.52</td>
</tr>
<tr>
<td>10 FeM</td>
<td>35.07 ± 0.52ᵇ</td>
<td>286.6 ± 31.5</td>
<td>12.41 ± 1.54</td>
</tr>
<tr>
<td>20 FeM</td>
<td>61.44 ± 25.64ᵇ</td>
<td>286.0 ± 63.6</td>
<td>17.84 ± 3.49</td>
</tr>
<tr>
<td>60 FeM</td>
<td>23.42 ± 8.20ᵇ</td>
<td>381.5 ± 146.6</td>
<td>8.18 ± 3.32</td>
</tr>
<tr>
<td>180 FeM</td>
<td>89.96 ± 50.49ᵃ</td>
<td>367.4 ± 105.4</td>
<td>22.39 ± 7.33</td>
</tr>
<tr>
<td>20 FeS</td>
<td>30.68 ± 13.41ᵇ</td>
<td>225.8 ± 138.1</td>
<td>23.17 ± 9.68</td>
</tr>
<tr>
<td>60 FeS</td>
<td>73.32 ± 32.09ᵇ</td>
<td>653.5 ± 388.4</td>
<td>14.66 ± 3.29</td>
</tr>
<tr>
<td>180 FeS</td>
<td>65.26 ± 25.49ᵇ</td>
<td>329.3 ± 12.3</td>
<td>19.84 ± 2.74</td>
</tr>
</tbody>
</table>
than that reported by Gatlin and Wilson (1986).

Fish fed the diet without supplemental iron developed hypochromic microcytic anemia characterized by decreased hemoglobin, hematocrit, MCV and MCH. The groups of fish fed the 5-FeM and 10-FeM diets also showed reduced hematological values, but the differences were not always significant, indicating less severe iron deficiency. Iron-deficiency anemia has been observed in brook trout (Kawatsu 1972), yellowtail (Ikeda et al. 1973), red sea bream (Sakamoto and Yone 1978a), and channel catfish (Gatlin and Wilson 1986) fed iron-deficient diets.

Hematological values did not differ among the groups of fish fed diets supplemented with 20 mg or higher iron/kg from either FeM or FeS. This indicates that both sources of iron are equally available for fingerling channel catfish and supplementation of 20-mg iron/kg diet or a total dietary iron level of approximately 30-mg iron/kg was sufficient to maintain normal hematological values. This is in agreement with Gatlin and Wilson (1986) who determined that the dietary iron requirement of catfish was 30 mg/kg diet. This requirement is considerably lower than values reported for Atlantic salmon (Lall and Hines 1987), eel (Nose and Arai 1979) and red sea bream (Sakamoto and Yone 1978a) which were 60, 170, and 150-mg iron/kg of diet, respectively.

It has been reported that trace minerals chelated with organic compounds, such as amino acids, have higher bioavailability for various animals than inorganic forms. Paripatananont and Lovell (1995) found that the bioavailability of zinc from zinc methionine for growth and zinc deposition in channel catfish was 305-352% and 482-586% of that of zinc sulfate in egg-white and soybean diets, respectively. In our study, however, we found that FeM (ferric methionine complex) was equally effective as FeS (ferrous sulfate heptahydrate) for prevention of iron-deficiency anemia. A reason for the difference between the efficacy of chelated zinc and chelated iron as mineral sources for channel catfish is that calcium binds dietary zinc and decreases its absorption through the intestinal mucosa (Lewis et al. 1994). Hardy and Shearer (1992) reported that feeding rainbow trout a zinc amino acid chelate resulted in higher body zinc content than zinc sulfate or zinc sulfate EDTA in low calcium-phosphorus diets but not in high calcium-phosphorus diets. Iron is not similarly inhibited; therefore, chelation may not be as effective for iron as for zinc. The low dietary iron requirement for normal growth observed in this study and the ability of channel catfish to absorb iron from the water to meet part of their requirement (NRC 1993) also may have contributed to the lack of differences between the availability of iron from FeM and FeS. Recently, Li and Robinson (in press) showed that zinc methionine and zinc sulfate were equally bioavailable for channel catfish when fish were fed a typical practical diet.

Results of the present study indicate that a supplemental level of 5 mg of iron/kg from FeM was sufficient for growth while a supplemental level of 20 mg of iron/kg from FeM or FeS appeared to be required for adequate hematological parameters. Both iron methionine complex and iron sulfate heptahydrate were equally effective in preventing iron-deficiency anemia.

**Literature Cited**


Gatlin, D. M. III and R. P. Wilson. 1986. Characterization of iron deficiency and the dietary iron...


