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Iron status of channel catfish *Ictalurus punctatus* affected by channel catfish anemia and response to parenteral iron

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ABSTRACT: Originally reported in 1983, channel catfish anemia (CCA), also 'white lip' or 'no blood,' is a major idiopathic disease affecting commercial production in the Mississippi Delta region of the USA. Affected individuals are characterized by lethargy, anorexia, extreme pallor, and packed cell volumes often below 5%, but a definitive cause for CCA remains elusive. Records from the National Warmwater Aquaculture Center (NWAC) reveal that, on average, CCA accounted for 4.7% of case submissions from 1994 to 2012. Known infectious agents, parasites, and perturbations in commonly measured water quality variables have been largely excluded, and research has focused on potential feed-related etiologies, particularly folic acid deficiency. No natural or anthropogenic contaminants have been found in feeds, and no associations have been made to any particular feed brand or formulation, or to the age or condition of the feed itself. Contrary to reports indicating a short clinical course, NWAC records indicate an insidious condition where certain ponds have contained fish diagnosed with CCA for up to 4 consecutive years and individual outbreaks have persisted for at least 5 mo. Investigation into the iron status of CCA-affected fish revealed values consistent with iron deficiency anemia, including low-packed cell volume (mean ± SE, 5.6 ± 1.0 vs. 24.8 ± 2.4%), serum iron (35.2 ± 3.5 vs. 104.4 ± 18.5 µg dl^{-1}), liver iron (12.2 ± 2.6 vs. 23.3 ± 4.6 µg g^{-1}), and percent transferrin saturation (14.5 ± 2.7 vs. 26.9 ± 3.1%) in anemic and healthy controls, respectively. Administration of parenteral iron produced complete recovery and returned iron indices to within the ranges of normal controls. Despite these findings, factors predisposing a state of hypoferremia remain unknown.

KEY WORDS: Channel catfish · Anemia · Iron · Hypoferremia · Therapy

INTRODUCTION

Originally reported as 'no blood' by Lovell (1983), channel catfish anemia (CCA) is also known as 'white lip,' or simply 'anemia.' Extreme pallor of the gills and viscera, with packed cell volumes often below 5%, characterize the disease. Affected individuals are lethargic, often swimming listlessly at the water's surface or resting on the bottom along pond banks. In affected ponds, feeding responses are typically poor, and fish may congregate behind aerators. Infections with *Flavobacterium columnare* and *Saprolegnia* sp. are common complicating factors.

Estimates of disease incidence and overall losses vary. Lovell (1983) reported numbers of anemic fish in affected ponds as 1% or less, with only a few daily mortalities. Weekly and overall losses of 5 and 10% were observed by Butterworth et al. (1986) and Klar

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et al. (1986), respectively. A higher incidence of disease is reported in late spring and early summer as water temperatures approach 21°C and fish are feeding actively (Lovell 1983, Klar et al. 1986). Mortalities occur for 1 to 2 wk and then cease (Lovell 1983).

Known infectious agents, parasites, and routine water quality anomalies have been largely ruled out as possible etiologies. No natural or anthropogenic contaminants have been found in feeds during epizootics, including heavy metals, pesticides, and mycotoxins (Lovell 1983, Klar et al. 1986, Plumb et al. 1986). *Bacillus thuringiensis* toxin (Burtle et al. 1998), elevated environmental nitrite (Tucker et al. 1989), and aflatoxin B-1 (Jantrarotai & Lovell 1990) have been shown to cause mild declines in hematocrit but not of the magnitude seen in CCA.

Research has focused on feed-related causes, both nutritional deficiencies and dietary antagonisms. Lovell (1983) considered that the disease could be linked to diet but concluded that the factor responsible would have to be common to all feeds. Plumb et al. (1986) and Klar et al. (1986) could make no associations with feed brand, formulation, or condition and encountered outbreaks associated with fresh and year-old feeds. However, both groups found that mortalities ceased when feed was replaced, brands were changed, or feeding was stopped. Yearling fish recovered from CCA, then placed back on the offending feed, had hematocrits of 1 to 9% in as little as 2 wk, while cohorts fed a fresh diet had hematocrits above 26% (Plumb et al. 1986). Klar et al. (1986) produced severe anemia in fry but not in subadults fed an anemia-associated diet.

A role for folic acid has been suggested in the pathogenesis of CCA. Butterworth et al. (1986) described CCA as a megaloblastic anemia and postulated that the condition resulted from the microbial degradation of folic acid to the folate antagonist pteroic acid. Fingerling catfish fed high pteroic acid diets failed to gain weight but developed only mild anemia. In contrast, Noyes et al. (1991) described necrosis of hematopoietic tissues. Both studies found erythrocytes with bilobed nuclei in peripheral blood, similar to folic acid deficiency in other species.

In this report, records of the Aquatic Diagnostic Laboratory, National Warmwater Aquaculture Center (NWAC) in Stoneville, Mississippi (USA), were compared to published information concerning CCA. In addition, evidence is presented that links CCA with iron deficiency. During outbreaks on commercial operations, the iron status of anemic fish was examined and compared to that of healthy individuals using indicators of normal iron homeostasis (Centers for Disease Control and Prevention 1998). The role of iron deficiency in the pathogenesis of CCA was further investigated by evaluating the response of anemic fish to the parenteral administration of iron in controlled laboratory trials.

MATERIALS AND METHODS

Fish and pond survey

Anemic and control channel catfish *Ictalurus punctatus* used in all studies were grown under typical industry practices on commercial farms and collected from adjacent ponds receiving the same diet. Fish were collected by seine net and snag line for survival and terminal studies, respectively. In the absence of published criteria, packed cell volumes of 10% or less were interpreted as diagnostic of CCA. Fish were anesthetized in a 100 mg l^{-1} solution of tricaine-methanesulfonate (MS-222, Western Chemical), and blood was collected via the caudal vein using a 20 g needle into vacutainers containing either no additive or K_3 EDTA (Becton Dickinson). Terminal fish were then euthanized in 1000 mg l^{-1} of MS-222 and necropsied immediately.

In April 2005, 20 anemic and 10 non-anemic fish were randomly collected as described above. Variables determined for each included packed cell volume (PCV), serum iron (SFe), total iron binding capacity (TIBC), liver iron (LFe), and percent transferrin saturation (%Sat). PCV was measured from unclotted blood using a Crit Spin microhematocrit centrifuge (StatSpin). Clotted blood was centrifuged and 200 µl of serum removed for SFe and TIBC determination. A 0.5 g portion of liver was frozen at −20°C for LFe analysis.

Trial 1

An initial study in June 2005 investigated the response of anemic catfish to iron therapy. Twenty-four anemic fish, average weight 0.62 kg, were collected and transported to the NWAC wet lab facility. Fish were maintained individually, in aerated, 114 l glass aquaria, receiving a 1 l min^{-1} constant flow of 27 ± 0.5°C well water and offered a 32% protein feed once daily (Arkat Feeds). Twelve fish were injected with 2.5 mg Fe kg^{-1} body weight of iron dextran (Dexferrum, American Regent Labs), and 12 with a similar volume of physiologic saline. Blood for PCV determination was collected every 14 d for 70 d.

Trial 2

In October 2005, 20 anemic and 20 healthy catfish (mean weight ± SD = 1.25 ± 0.07 kg) were collected and maintained as described above. Fish were randomized to 1 of 4 treatment groups in a factorial arrangement and injected with iron, erythropoietin (EPO), iron + EPO, or saline. Five anemic and 5 control fish were initially randomized to each treatment. However, with the exception of the iron and EPO group, 1 anemic and 1 control fish died in each of the other groups. Only fish that survived the entire study period were included in the analysis.

Assigned groups received 2 doses of iron dextran (2.5 mg Fe kg^{-1} body weight) by deep intramuscular injection 3 d apart and 3 doses of human recombinant EPO (65 units EPO kg^{-1} body weight, Procrit®, Amgen) by intraperitoneal injection 3 d apart. Control fish received identical volumes of saline by the same injection routes. Blood was collected at 9 to 14 d intervals for PCV determination. At the completion of the study, surviving fish were euthanized and tissues collected for iron analysis as above. A subset of head kidney tissues was fixed in 10% buffered formalin, processed routinely for histopathology, and stained by the Perls Prussian blue method for iron (Prophet et al. 1994).

Analytical and statistical methods

SFe and TIBC analyses were performed using a Cobas Mira analyzer and reagents as per the manufacturer's instructions (Roche Diagnostics). %Sat was calculated by dividing SFe by the TIBC. LFe levels were determined using the colorimetric method of Torrance & Bothwell (1980) and Stanbio Laboratory-Iron procedure 0370 reagents on a Spectramax-340PC microplate reader (Molecular Devices).

For the pond survey, body weight, PCV, and iron status measurements were compared between groups using a 2-sample *t*-test. Equal variances were not assumed. In the treatment study (Trial 2), factorial univariate repeated-measures ANOVA was used to compare PCV over time for groups defined by their anemia and treatment status. A factorial general linear model was used to compare iron status measures at the end of the 6 wk study period. Main effects and all possible interactions were evaluated for iron treatment, EPO treatment, and anemia status. Analyses were implemented using commercially available software (SPSS version 15.0.1), and all testing was performed assuming a 2-sided alternative hypothesis. p-values ≤0.05 were considered statistically significant.

RESULTS

Review of NWAC records revealed that on average, CCA was diagnosed in 4.7% (range 2.1–10.7%) of annual case submissions, or 46/973 cases yr^{-1}, from 1994 to 2012. Producers reported most outbreaks in food and brood size fish, often in association with poor feeding responses. Disease occurred year round, with spring and fall peaks at water temperatures of 17 to 27°C. The greatest numbers of cases was consistently diagnosed in October at an average water temperature of 20.4°C (Fig. 1). Overall, CCA occurred sporadically and at random in individual farm ponds. Anemia was diagnosed in certain ponds for up to 4 consecutive years and some outbreaks persisted for at least 5 mo. The disease was also seen during winter when no feed was being offered.

Pond survey

Nine female and 11 male catfish were collected from a pond diagnosed with anemia. Five female and 5 male fish from an unaffected pond were used as normal controls. Body weight, PCV, and iron-related data are presented in Table 1. The mean weight of anemic fish was significantly greater than that of control fish, but controls had significantly higher values than anemic fish for all other measures except LFe, which was marginally nonsignificant.

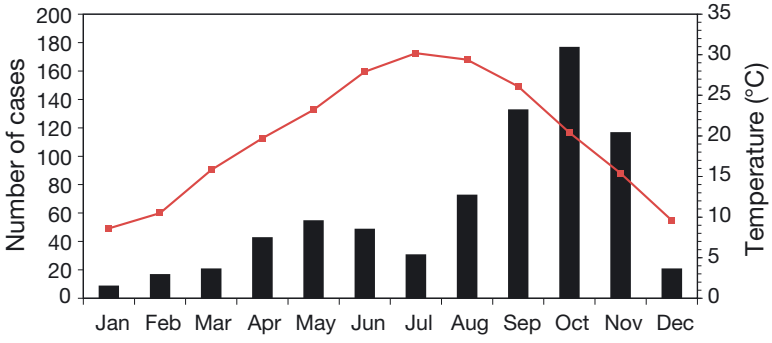

Fig. 1. *Ictalurus punctatus*. Monthly incidence of channel catfish anemia (CCA) cases (bars) from 1994 to 2012 versus water temperature (red line) seen at the Aquatic Diagnostic Laboratory, National Warmwater Aquaculture Center, Stoneville, Mississippi, USA

Table 1. *Ictalurus punctatus*. Mean (SE) weights, packed cell volumes, and iron indices for 20 pond fish diagnosed with anemia and 10 fish from an adjacent unaffected pond. p-values are based on a comparison of group means using a 2-sample *t*-test (equal variances not assumed). PCV: packed cell volume; TIBC: total iron binding capacity; %Sat: percent transferrin saturation

Variable	Anemic	Control	p
Weight (kg)	1.26 (0.18)	0.83 (0.04)	0.030
PCV (%)	5.6 (1.0)	24.8 (2.4)	<0.001
Serum iron (µg dl^{-1})	35.2 (3.5)	104.4 (18.5)	0.005
Liver iron (µg g^{-1})	12.2 (2.6)	23.2 (4.6)	0.053
TIBC (µg dl^{-1})	271 (17)	373 (22)	0.002
%Sat	14.5 (2.7)	26.9 (3.1)	0.007

Table 2. *Ictalurus punctatus*. Summary of mean (SE) packed cell volume percentages for anemic and control fish receiving 1 of 4 randomly allocated treatments over a 6 wk period. EPO: erythropoietin

Treatment	n	Day 1	Day 14	Day 23	Day 32	Day 44
Control						
Saline	4	36.6 (1.3)	24.0 (1.4)	22.3 (2.3)	20.4 (2.5)	31.5 (1.1)
Iron	4	40.0 (1.8)	30.8 (0.4)	27.9 (1.8)	27.5 (0.3)	30.1 (2.3)
EPO	4	34.4 (1.1)	29.5 (1.7)	26.0 (1.4)	24.1 (0.6)	27.1 (0.7)
Iron + EPO	5	36.0 (2.3)	27.4 (1.0)	24.8 (1.0)	25.4 (1.8)	27.8 (1.3)
Anemic						
Saline	4	9.3 (1.2)	7.6 (1.6)	7.6 (1.7)	11.3 (2.5)	17.9 (4.7)
Iron	4	5.5 (1.2)	9.8 (1.2)	12.5 (0.3)	16.0 (2.0)	25.5 (0.9)
EPO	4	6.1 (1.6)	6.9 (1.9)	7.5 (1.3)	13.1 (2.3)	17.8 (3.0)
Iron + EPO	5	6.5 (0.9)	9.8 (1.2)	16.0 (1.7)	17.8 (2.7)	27.6 (3.2)

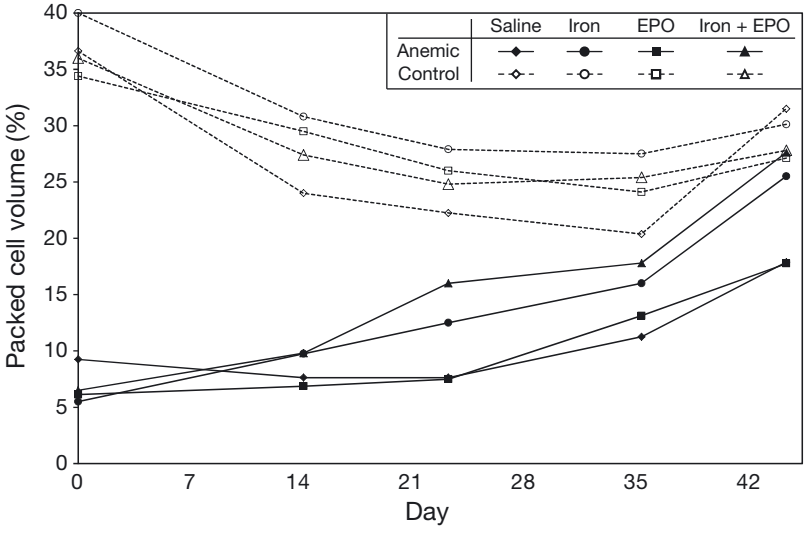

Fig. 2. *Ictalurus punctatus*. Changes in the mean packed cell volumes of anemic (solid lines) and normal control catfish (dashed lines) over a 6 wk period. Fish were randomly allocated to 1 of 4 treatment groups and injected with either saline, iron, erythropoietin (EPO), or iron + EPO

Trial 1

Initial PCV values averaged (±SD) 5.0 ± 0.46% and 5.6 ± 0.37% for the iron treatment and saline control groups, respectively. Only 2/12 untreated controls survived, while 6/12 iron-treated fish survived the 70 d study. In both groups, most deaths occurred during the first 14 d, consistent with intolerance of the fish to handling at high (28.5°C) temperatures. Due to the poor survival, detailed statistical analysis was not performed. However, iron indices at the end of the trial (data not shown) were in agreement with those seen for the pond study above. Average PCV values in the iron treatment group rose to 14 ± 1.78% (n = 7) by Day 14 and 27 ± 1.52% (n = 6) on Day 70. In the untreated controls, PCV increased slowly to 10 ± 1.15% by Day 42 (n = 3) and 15.5 ± 0.49% (n = 2) on Day 70. Fish typically remained anorectic until PCV exceeded 10%.

Trial 2

The second trial was conducted at cooler (18°C) water temperatures. Twenty anemic and 20 healthy catfish were divided into 4 treatment groups. Mean PCV values and standard errors are shown in Table 2. At baseline, the marginal mean PCV of the control group was significantly higher than that of the anemic group (36.7 versus 6.8%; $p < 0.001$). Within the anemic and control groups, there were no significant differences among treatment groups at the time of treatment allocation ($p > 0.20$).

Responses in PCV differed markedly for the anemic and control groups (Fig. 2). For anemic fish, there was a significant interaction between iron treatment and time ($p = 0.005$). In anemic fish, PCV increased significantly regardless of their iron treatment status ($p < 0.01$), but those that received iron had a greater increase than those that did not (20.6 vs. 10.1%). There were no significant interactions between EPO treatment and time ($p = 0.588$), or between the iron and EPO treatments ($p = 0.492$). There was also

no significant main effect of EPO treatment in anemic fish that either did (p = 0.442) or did not receive iron (p = 0.845).

For control fish, we found no significant interactions between iron treatment and time (p = 0.317) or between EPO treatment and time (p = 0.081), but there was a significant interaction between the iron and EPO treatments (p = 0.022). In controls that received EPO, there was no difference in the mean PCV of fish that received iron versus those that did not (28.3 vs. 28.2%), whereas in controls that did not receive EPO, iron treatment was associated with a slightly higher mean PCV (31.3 vs. 27.0%). Over the 6 wk study, the PCV of controls decreased on average 7.6% compared to baseline levels (p < 0.001).

Comparing the PCV of groups at the end of the 6 wk study, there was a significant interaction between anemia status and iron treatment (p = 0.018). Anemic fish treated with iron had significantly higher PCV than anemic fish not treated with iron (26.6 vs. 17.8%; p = 0.020), whereas iron treatment was not significantly associated with PCV in control fish ($29.0\%_{(Treated)}$ vs. $29.3\%_{(Untreated)}$; p = 0.812). EPO treatment was not significantly associated with the PCV of anemic fish ($22.7\%_{(Treated)}$ vs. $21.7\%_{(Untreated)}$; p = 0.768), but control fish that received EPO had significantly lower PCV than control fish that did not ($27.4\%_{(Treated)}$ vs. $30.8\%_{(Untreated)}$; p = 0.037).

Measurements of iron status are summarized in Table 3. For SFe, the marginal mean of the control group was significantly higher than that of the anemia group (102 vs. 66 µg dl^{-1}; p = 0.011), and the marginal mean of fish that received iron was higher than the mean of fish that did not (97 vs. 70 µg dl^{-1}; p = 0.050). There was no significant effect of EPO on SFe levels (p = 0.814). Fig. 3 demonstrates the presence and absence of iron in head kidney macrophages of anemic catfish that had and had not received iron, respectively.

For LFe, there was a significant interaction between anemia group status and iron treatment (p = 0.001). Controls had higher marginal mean LFe than anemic fish regardless of whether or not iron treatment was used, but the magnitude of the difference was much larger in fish that received iron (583 vs. 155 µg g^{-1}; p < 0.001) compared to those that had not (41 vs. 18 µg g^{-1}; p = 0.034). EPO had no significant effect on LFe levels (p = 0.744).

Table 3. *Ictalurus punctatus*. Mean (SE) iron status measurements obtained for anemic and control fish receiving 1 of 4 randomly allocated treatments at the end of a 6 wk period. EPO: erythropoietin, TIBC: total iron binding capacity, %Sat: percent transferrin saturation

Treatment	n	Serum iron (µg dl^{-1})	Liver iron (µg g^{-1})	TIBC (µg dl^{-1})	%Sat
Control					
Saline	4	107 (14)	38 (16)	406 (43)	26.3 (1.9)
Iron	4	114 (26)	675 (194)	300 (53)	38.0 (3.8)
EPO	4	92 (14)	44 (8)	300 (39)	31.3 (4.6)
Iron + EPO	5	95 (8)	491 (60)	257 (12)	36.8 (2.7)
Anemic					
Saline	4	48 (5)	21 (5)	297 (34)	16.8 (2.4)
Iron	4	73 (20)	99 (24)	296 (40)	25.5 (7.1)
EPO	4	35 (11)	16 (5)	289 (27)	12.0 (3.2)
Iron + EPO	5	108 (29)	211 (69)	414 (72)	24.6 (4.2)

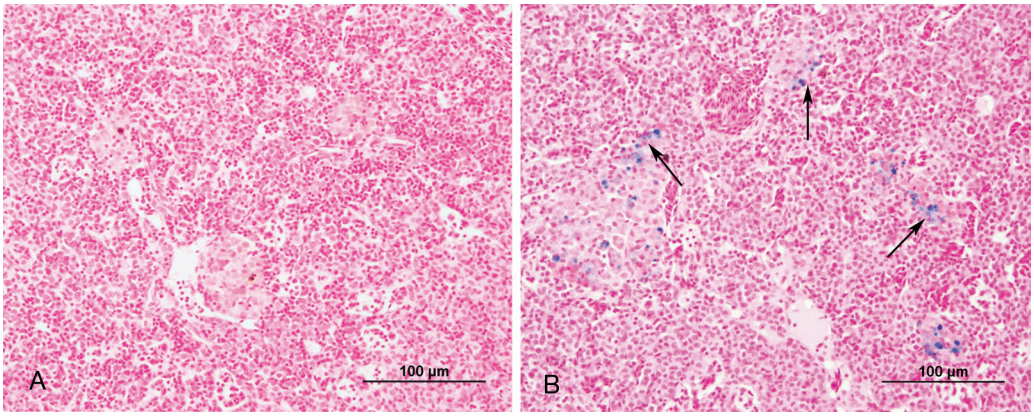

Fig. 3. *Ictalurus punctatus*. Histological sections of channel catfish head kidney prepared with Perl's iron stain (Prophet et al. 1994). (A) Anemic fish showing no iron (negative Prussian blue reaction) within macrophages. (B) Recovered iron-treated fish showing positive Prussian blue staining of ferric iron within macrophages (arrows)

Analysis of TIBC suggested significant interactions between anemia group status and iron treatment status ($p = 0.033$), and also between anemia group status and EPO treatment status ($p = 0.043$). However, separate follow-up analyses of the anemia and control groups failed to find any significant effects for either the iron or EPO treatments. Consequently, the significant interactions may have been caused by spuriously large TIBC values in the saline-treated control group and the iron+EPO-treated anemia group.

The %Sat paralleled results of the SFe comparisons. The marginal mean of controls was significantly higher than that of the anemia group (33.1 vs. 19.7%; $p < 0.001$), and the marginal mean of fish that received iron was higher than the mean of fish that did not (31.2 vs. 21.6%; $p = 0.002$). EPO had no significant effect ($p = 0.872$).

DISCUSSION

Channel catfish anemia is an enigmatic condition affecting *Ictalurus punctatus* pond aquaculture in the southeastern USA. Disease occurs sporadically throughout the industry and at random on individual farms (Lovell 1983, Butterworth et al. 1986, Klar et al. 1986, Plumb et al. 1986, Noyes et al. 1991). Representing 4.7% of reviewed case submissions, CCA is the sixth most frequently diagnosed disease at the NWAC. Seen most commonly in food and brood size fish, CCA can have a significant impact on profitability. However, the true incidence and severity of losses is difficult to assess. Ponds with low mortalities may receive little attention (Lovell 1983), and production and accurate mortality data are seldom available. Diagnoses are often made subjectively and signs of anemia may be overlooked due to the presence of concomitant disease.

In contrast to brief late spring to early summer disease episodes, when fish are actively feeding (Lovell 1983, Klar et al. 1986), NWAC records indicate that CCA can persist in ponds for months to years, including winter months when feed is not being offered. There is a small spring peak, but most cases occur in October at an average water temperature of 20.4°C. All 20 fish randomly collected in the pond survey had PCV below the 19.4% average for April reported by Leard et al. (1998), and 17 had PCV ≤10%. Findings suggest that numbers of anemic fish in a pond can approach 100%, an assumption supported by poor feeding responses that often first alert producers to the problem.

Common denominators associated with outbreaks are also difficult to elucidate, and anemias of the severity seen with CCA have not been reproduced experimentally. Notably, adjacent identically managed ponds may be diseased and unaffected, a phenomenon first observed by Lovell (1983). This is the first study to investigate a potential role for iron in the pathogenesis of the disease.

Iron deficiency is the most common cause of anemia in humans (Centers for Disease Control and Prevention 1998, Thomas & Thomas 2002). When availability and uptake are sufficient, approximately 70% of the body's iron is in use, mostly in hemoglobin (Hb). The remainder is stored, primarily as ferritin, or carried in blood by transferrin. In deficient states, iron is not partitioned to erythroid precursors, compromising red cell production (Krause 1988).

Regulation of iron balance occurs in the gastrointestinal tract. Normally, absorption and loss are tightly coupled, demands are met, and stores established (Centers for Disease Control and Prevention 1998, Higgins & Rockey 2003, Handelman & Levin 2008). The key regulator of absorption is the peptide hormone hepcidin (Hu et al. 2007, Handelman & Levin 2008). Uptake is also influenced by the rate of erythrocyte production, amount and kind of iron in the diet, and the presence of absorption enhancers and inhibitors. Heme iron from meat is more absorbable than the non-heme iron found in plants. Additionally, non-heme iron availability is influenced by other diet components. Heme-iron and vitamin C enhance absorption, while polyphenols, tannins, phytates, and calcium inhibit it (Centers for Disease Control and Prevention 1998).

In mammals, the gold standard for diagnosis of iron deficiency is direct Prussian blue staining of bone marrow for the absence of iron (Krause 1988), although indirect measurements are more commonly used and have been applied to catfish (Gatlin & Wilson 1986, Lim et al. 1996, Lim & Klesius 1997). However, no single test is diagnostic for iron deficiency (Centers for Disease Control and Prevention 1998). PCV and SFe are late indicators, falling only after iron stores and Hb levels are depleted. TIBC typically increases, while the %Sat declines as deficiency progresses and more binding sites become available (Thomas & Thomas 2002, Handelman & Levin 2008). Serum ferritin, the most specific early indicator of iron depletion, could not be determined, due to the lack of a channel catfish specific antibody.

The iron status of catfish in these studies was evaluated using the above indicators. However, reference intervals do not exist for these indices in *Ictalu-*

rus punctatus, and it is anticipated that like PCV, values would vary with time of year (Leard et al. 1998), under different husbandry conditions (Fig. 2), and levels of dietary iron. As an alternative to serum ferritin, hepatic iron levels were determined, as the liver is a major site of iron storage, and hematopoietic tissues of the head kidney were evaluated histologically for the presence of iron.

In human iron deficiency, SFe, normally 55–165 µg dl^{-1}, declines to <33 µg dl^{-1} and, although more variable, TIBC typically increases above 400 µg dl^{-1} (Higgins & Rockey 2003). %Sat, normally around 30%, declines below 16% (Centers for Disease Control and Prevention, 1998). For comparison, fingerling catfish fed the 30 mg Fe kg^{-1} of feed minimum dietary iron requirement had average values of 60.7 ± 4.5 µg dl^{-1} for plasma iron, 224.4 ± 23.5 µg dl^{-1} TIBC, 27.8 ± 3.8 %Sat, and PCV of 31.8 ± 0.2%. Fish fed a basal diet with 9.6 mg Fe kg^{-1} of feed for 10 wk had significantly lower plasma iron and %Sat values, but TIBC did not change significantly. The minimum PCV was 25.7 ± 0.8% (Gatlin & Wilson 1986). The fish had decreased growth and feed efficiency, but there was no effect on mortalities. In a similar study by Lim et al. (1996), survival, feed conversion, SFe, TIBC, and %Sat were not significantly affected by dietary iron levels, although a significantly lower PCV (16.3 ± 0.8%) was reported.

Considering species variations (Mahaffey 2003), with the exception of TIBC, parameters measured in the pond survey approximated the above values and trends in healthy and iron-deficient states (Table 1). Furthermore, PCV and iron indices in treated anemic fish returned to ranges of healthy untreated controls after receiving iron injections (Table 3). Human EPO, reported to induce erythropoiesis in goldfish *Carassius auratus* (Taglialatela & Corte 1997), had no significant effects in these catfish, regardless of iron status. EPO, the principal stimulator of erythrocyte production, has been identified putatively in the channel catfish (GenBank accession number BM-438685), but it is not commercially available.

Iron deficiency anemia results from 3 general mechanisms: inadequate dietary levels, inadequate uptake from the diet, or through blood loss (Thomas & Thomas 2002). While findings indicate that CCA is associated with an iron deficient state, they provide only limited insight into potential predisposing mechanisms. However, the responsiveness of anemic fish to intramuscularly administered iron and the restoration of hepatic stores imply that the pathways involved in iron transport, storage, and Hb synthesis are functioning adequately.

Practical diets formulated with >5% animal protein should contain adequate iron, and supplementation is generally not necessary (Gatlin & Wilson 1986, Robinson et al. 2004). Previous work has shown that hepcidin levels are decreased in anemic fish (Hu et al. 2007). Responsive to hypoxia, hepcidin levels decrease with anemia to increase intestinal absorption. Decreased hepcidin levels also rule out CCA as an anemia of chronic disease (Handelman & Levin 2008). If dietary iron levels are sufficient, this may suggest a failure of iron uptake, either as a result of poor dietary bioavailability or from an unknown malabsorptive process.

Findings do not exclude concurrent deficiencies of other nutrients required for effective erythropoiesis, such as folic acid, long suspected of playing a role in CCA (Butterworth et al. 1986, Noyes et al. 1991, Duncan et al. 1993). In humans with inadequate vitamin B_{12} and folic acid levels, iron deficiency is also common (Koury & Ponka 2004). Signs of iron deficiency tend to predominate, confounding test results for folate and B_{12} deficiency (Herbert 1987).

Findings also do not resolve questions regarding the 'feed related anemia' reported by Plumb et al. (1986) and Klar et al. (1986), where the precipitous declines in PCV are more indicative of a hemolytic process (Aster 2005). In contrast to the acute onset and rapid recoveries described with feed change, the anemia investigated here is characterized by depletion of iron pools, a process requiring months in mammals and more compatible with a deficiency disease (Handelman & Levin 2008). As mentioned previously, CCA shows no relation to particular feeds or feeding practices in general.

Channel catfish anemia has been a part of commercial catfish aquaculture since the early 1980s. Although a number of potential etiologies have been investigated, a definitive cause has remained elusive. A nutritional anemia must meet 2 criteria: deficiency of a specific nutrient must produce the condition, and providing the nutrient must correct it (Krause 1988). Our studies evaluated the iron status of channel catfish suffering from CCA and revealed close similarities to iron deficiency anemia in humans and in experimental catfish. Conclusions were further supported by the recovery of affected fish that received iron by intramuscular injection. Results suggest that 'white lip' or 'no blood' anemia as seen on catfish production operations in the Mississippi Delta is a chronic deficiency disease that may represent a separate entity from the 'feed related anemia' described by previous authors (e.g. Plumb et al. 1986).

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