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Establishment of a time-resolved fluoroimmunoassay for measuring plasma insulin-like growth factor I (IGF-I) in fish: effect of fasting on plasma concentrations and tissue mRNA expression of IGF-I and growth hormone (GH) in channel catfish (*Ictalurus punctatus*)

Brian C. Small*, Brian C. Peterson

USDA/ARS Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, P.O. Box 38, Stoneville, MS 38776, USA

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Abstract

A time-resolved fluoroimmunoassay (TR-FIA) was established and validated that allows for the determination of plasma concentrations of insulin-like growth factor I (IGF-I) in three domestically cultured fishes: channel catfish (*Ictalurus punctatus*), hybrid striped bass (*Morone chrysops* × *M. saxatilis*), and rainbow trout (*Oncorhynchus mykiss*). Sensitivity of the assay was 0.20 ng/ml. Intra- and inter-assay coefficients of variation (CV) were <7 and <12%, respectively. Serial dilutions of plasma from each species were parallel to the standard curve. Recovery of IGF-I from spiked plasma samples was >90% for all three species of fishes. The IGF-I TR-FIA was biologically validated via its use to determine the effect of fasting on circulating IGF-I levels in channel catfish. Fasting-induced changes in plasma growth hormone (GH), hepatic IGF-I mRNA expression, and pituitary GH mRNA expression were also determined. Fasted channel catfish lost 5.6 and 15.6% body mass after 2 and 4 weeks of fasting, respectively. Plasma IGF-I concentrations were depressed ($P < 0.05$) relative to fed controls following 2 and 4 weeks of fasting. Plasma GH concentrations were not different ($P > 0.05$) in fasted fish after 2 weeks, but significantly increased ($P < 0.05$) by 4 weeks of fasting. Hepatic IGF-I mRNA expression after 2 and 4 weeks of fasting was reduced ($P < 0.05$) relative to fed controls.

* Corresponding author. Tel.: +1 662 686 3586; fax: +1 662 686 3567.
E-mail address: bsmall@ars.usda.gov (B.C. Small).

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Pituitary GH mRNA expression was similar ($P > 0.05$) between 2-week-fasted catfish and fed controls, but was increased ($P < 0.05$) in 4-week-fasted catfish. The IGF-I TR-FIA was sensitive, accurate, and precise for all three species of fishes, and provided a low-cost, and non-radioisotopic method for quantifying plasma IGF-I levels in fed and fasted channel catfish.
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Keywords: IGF-I; GH; Fluoroimmunoassay; Fasting; Channel catfish

1. Introduction

Insulin-like growth factor-I (IGF-I) is a single-chain polypeptide of approximately 7.5 kDa with sequence and structural similarity to proinsulin [1]. IGF-I is primarily produced in the liver and released into circulation for transport to target tissues. One of the primary functions of IGF-I is to mediate many of the growth-promoting actions of growth hormone (GH) [2,3]. Because of this, circulating levels of IGF-I vary as a result of growth and metabolic changes.

The development of a radioimmunoassay (RIA) to measure circulating concentrations of IGF-I in salmonid fishes [4] was a crucial step towards defining the role of IGF-I in fish. Since then the role of IGF-I in regulating salmonid growth and development has been well documented [5,6]. Shimizu et al. [7] validated the use of commercially available components (GroPep, Adelaide, Australia) for the measurement of salmon IGF-I. The complete validation of a commercially available fish IGF-I RIA kit (GroPep) was later published by Dyer et al. [8], in which they compared cross-reactivity of the assay with several fish species including barramundi (*Lates calcarifer*), coho salmon (*Oncorhynchus kisutch*), Southern bluefin tuna (*Thunnus maccoyii*), tilapia (*Oreochromis mossambicus*), and seabream (*Pagrus auratus*). The level of cross-reactivity reported between species suggests that the assay might prove useful in IGF-I research involving less commonly studied species.

Channel catfish, *Ictalurus punctatus*, are highly susceptible to infection by the bacterium *Edwardsiella ictaluri* [9], which is responsible for up to 50% of total annual losses to catfish farmers [10]. A prominent management tool for controlling losses to *E. ictaluri* is the withdrawal of feed during disease outbreaks [11]. Little is known, however, concerning the physiological ramifications of short-term fasting. In many vertebrate species, catabolic states such as fasting result in low circulating IGF-I levels [1]. In contrast, fasting can have the opposite effect on circulating GH concentrations. Several studies with fish have demonstrated the increased concentrations of plasma GH during food deprivation [12–18]. This apparent paradox between increased concentrations of circulating GH and decreased concentrations of circulating IGF-I might be explained by tissue resistance to GH [19,20] and by a reduction in hepatic GH-receptors (GHR) during starvation [21–23].

Since fasting is a common disease management technique in commercial catfish aquaculture, and studies with several fish species have demonstrated profound effects of fasting on the somatotropic axis, we sought to validate a time-resolved fluoroimmunoassay (TR-FIA) for quantifying circulating IGF-I in channel catfish during a short-term fast. The TR-FIA

was further validated for two other domestically cultured species of fishes, hybrid striped bass (*Morone chrysops* × *M. saxatilis*) and rainbow trout (*Oncorhynchus mykiss*). The effect of fasting on the somatotropic axis of channel catfish was further examined by measuring changes in plasma GH concentrations, hepatic IGF-I mRNA expression, and pituitary GH mRNA expression.

2. Materials and methods

2.1. Peptides, antibodies, and plasma

Recombinant barramundi IGF-I and polyclonal rabbit anti-barramundi (fish) IGF-I were purchased from GroPep, Australia. Europium (Eu)-labeled IGF-I tracer was prepared by Perkin-Elmer Life Sciences (Norton, OH) via N-terminal labeling of recombinant barramundi IGF-I with DELFIA® Eu-N1 ITC lanthanide chelate (Ref 1244-302, Perkin-Elmer). The number of Eu-chelates per protein was one, and initial concentration of Eu-labeled IGF-I tracer was 32 μg/ml. Channel catfish insulin and rainbow trout plasma were provided by Dr. Jeffrey Silverstein, USDA/ARS, National Center for Cool and Cold Water Aquaculture, Kearneysville, West Virginia. Hybrid striped bass plasma was provided by Dr. Kenneth Davis, USDA/ARS, Stuttgart National Aquaculture Center, Stuttgart, Arkansas.

2.2. IGF-I fluoroimmunoassay

Quantitative determination of IGF-I in standards and fish plasma was conducted using a competitive time-resolved fluoroimmunoassay (TR-FIA) according to dissociation enhanced lanthanide fluorescence immunoassay (DELFIA®) methodology (Ref 1244-1126-06; Perkin-Elmer) under non-equilibrium conditions in a 96-well format. The entire assay was conducted in Reacti-Bind™ goat anti-rabbit coated clear 96-well plates (15135; Pierce, Rockford, IL). Tracer (16 ng Eu-IGF-I/ml), recombinant barramundi IGF-I standards (in 125 μl), and anti-barramundi IGF-I polyclonal antiserum (20 μl diluted 1:2072) were diluted in DELFIA® assay buffer (1244-111; Perkin-Elmer). Plasma samples were acid–ethanol-extracted prior to assaying [24]. Briefly, 50 μl of plasma was treated with 200 μl acid–ethanol (12.5% HCl, 87.5% ethanol), vortexed, and incubated at room temperature for 30 min. The solution was then neutralized with the addition of 100 μl of 0.855 M Tris-base, centrifuged (10 000 × *g*) for 10 min at 4 °C, and the supernatant (plasma extract) was assayed for IGF-I. The standard curve was generated in triplicate by adding 125 μl of known IGF-I standard, 125 μl of A/E blank solution (14.3% assay buffer, 28.6% Tris-base, and 57.1% acid–ethanol), 20 μl of tracer, and 20 μl of anti-barramundi antiserum per well. Unknown plasma concentrations were determined in triplicate by adding 125 μl of plasma extract, 125 μl of assay buffer, 20 μl of tracer, and 20 μl of anti-barramundi antiserum per well. Non-specific binding was determined by replacing the binder (anti-barramundi IGF-I antiserum) with assay buffer in a zero-standard (B_0) sample. Loaded assay plates were incubated overnight at 4 °C with slow shaking. Bound and free tracer were separated the next day by washing the reaction wells three times with DELFIA® wash solution (1244-

114; Perkin-Elmer) on an EL_X50 Auto Strip Washer (Bio-Tek Instruments, Winooski, VT). Following washing, 200 μl of DELFIA® enhancement solution (1244-105; Perkin-Elmer) was added to each well. The plates were then shaken slowly for 5 min at room temperature and read in a Victor^2 1420 Multilabel Counter (Perkin-Elmer).

2.2.1. Data acquisition and reduction

Fluorescence data acquisition, data reduction, and preliminary statistical analysis were performed using StatLIA® Immunoassay Workflow and Analysis Software version 3.1 (Brendan Scientific, Gross Point Farms, MI). The standard curve was generated by plotting percent bound ($(B/B_0) \times 100$) against known IGF-I concentrations using a five-parameter-logistic equation [25]. Calculation of IGF-I concentrations in the unknown samples, means, and coefficients of variation (CV) for replicate samples were calculated.

2.2.2. Sensitivity, precision, reproducibility, and specificity

The minimum detectable limit of the assay was determined by calculating the mean concentration at B_0 minus 2 standard deviations (S.D.), when interpolated from the standard curve. Precision and reproducibility of the assay were determined by calculating the intra- and inter-assay CV. The intra-assay precision was obtained by calculating the CV of six measurements of pooled plasma each from channel catfish, hybrid striped bass, and rainbow trout analyzed within the same assay. The inter-assay reproducibility was obtained from triplicate samples of pooled plasma each from channel catfish, hybrid striped bass, and rainbow trout analyzed in three consecutive assays. Accuracy of the assay and potential non-specific effects of fish plasma on the IGF-I TR-FIA were tested by recovery and parallelism tests, respectively. To determine recovery of IGF-I from acid–ethanol-extracted plasma samples, recombinant barramundi IGF-I was spiked into plasma extract of each species at 1.2, 10.4, and 90.0 ng/ml. In the parallelism test, plasma samples were serially diluted with assay buffer. The antiserum displays less than 0.5% cross-reactivity with recombinant human IGF-I, less than 1% cross-reactivity with recombinant human IGF-II, and non-detectable cross-reactivity with recombinant salmon insulin (Ref 4001, GroPep).

2.3. Plasma GH determination

Growth hormone concentrations were determined using a homologous ELISA validated for quantifying GH in channel catfish plasma [26].

2.4. RNA isolation and cDNA synthesis

Total RNA was isolated from hepatic and pituitary tissues using TRI-reagent® (Molecular Research Center, Cincinatti, OH). Total RNA was quantified based on absorbance at 260 nm, and purity was assessed based on absorbance ratios at 260 and 280 nm. All samples had 260/280 ratios above 1.8. Integrity of the RNA was verified by visualization of the 18S and 28S ribosomal bands after electrophoresis on a 1.0% agarose gel stained with ethidium bromide. Total RNA was reverse-transcribed in 20 μl reactions using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA).

Table 1
Nucleotide sequences of PCR primers and probes used to assay gene expression by real-time quantitative PCR

Gene	Primer	Sequence
IGF-I	Sense	CTGTGAGCTGAAACGACTCG
	Anti-sense	CCAGATATAGGTTTTCTTTGGTG
	Probe[a]	CGAGAGCAACGGCACACAGACACGC
GH	Sense	CATATCTCAGAGAAGCTGGC
	Anti-sense	GGTCTGGTAGAAATCCTCGA
	Probe[a]	CCACACATCCCTCGATAAGTACGCC
α-Tubulin	Sense	AGCCATACAATTCCATCCTGACC
	Anti-sense	GCGGCAGATGTCGTAGATGG
	Probe[b]	CCACACCACACTTGAGCACTCCGAC

[a] The probes for IGF-I (GenBank accession no.: AY353852) and GH (GenBank accession no.: S69215) were dual labeled with a reporter dye (FAM, 6-carboxyfluorescein) at the 5′ end and a quencher dye (BHQ-1, Black Hole quencher-1) at the 3′ end (Biosearch Technologies, Novato, CA).

[b] The probe for α-tubulin (TIGR Gene Index: TC8342) was dual labeled with a reporter dye (HEX, hexachlorofluorescein) at the 5′ end and a quencher dye (BHQ-1) at the 3′ end (Integrated DNA Technologies, Coralville, IA).

2.5. IGF-I and GH mRNA expression

Gene expression was determined by real-time quantitative PCR using the iCycler iQ™ (Bio-Rad). Standards were developed by generating PCR fragments using the primers listed in Table 1, cloning them into the pCR®4-TOPO vector (Invitrogen, Carlsbad, CA), and introducing them into One Shot® TOP10 chemically competent *Esherichia coli* (Invitrogen) cells. Cloned inserts were sequenced to confirm sequence identity. Concentration of each resulting plasmid was measured spectrophotometrically, and serial dilutions of each plasmid were used to make the standard curves for quantification. Primer and probe sequences for the target genes are listed in Table 1. The standard curve showed a linear relationship between cycle threshold values and the logarithm of input gene copy number.

Each 12.5 μl PCR contained 1X Supermix (Bio-Rad), 300 ng cDNA template, sense and antisense primers (10 μM each), and probe (5 μM each). The PCR protocol was 3 min at 95 °C; 45 cycles of 95 °C-15 s, and 60 °C-1 min. PCR efficiencies of all reactions were between 90 and 100%. All measurements were performed in triplicate. All specific quantities were normalized against the amount of α-tubulin amplified.

2.6. Fasting experiment

The effect of fasting was investigated in NWAC103-line channel catfish maintained at the USDA-ARS Catfish Genetics Research Unit, Stoneville, MS, aquaculture facility following accepted standards of animal care, approved by the Institutional Animal Care and Use Committee (IACUC) according to USDA-ARS policies and procedures. One month prior to starting the experiment, 60 catfish (mean weight = 115 g) were randomly stocked into ten 76-l aquaria, and allowed to acclimate. During the 1-month acclimation period, all fish were fed a commercial floating catfish feed (36% crude protein; land O'Lakes

Farmland Feed, Fort Dodge, IA) 3 days per week. Throughout the acclimation and experimental periods, the fish were reared in 26 °C well-water under a 14:10 h light–dark photoperiod.

At the start of the experiment, all the fish were weighed, and five aquaria were randomly assigned to each of two treatments. Fish in five aquaria were fed once daily to satiety (fed control) and the fish in the remaining five aquaria were fasted for 4 weeks. After 2 weeks, three fish per aquaria were euthanized in a solution of tricainemethane sulfonate (0.2 g/l; Finquil; Argent Chemical Laboratories, Richmond, WA), weighed, bled from the caudal vasculature into syringes coated with heparin, and dissected. Plasma was separated, stored at −80 °C, and later analyzed for IGF-I and GH. Hepatic and pituitary tissue specimens for total RNA extraction were rapidly excised from each fish, flash frozen in liquid nitrogen, and stored at 80 °C until RNA isolation. After 4 weeks of experimentation, the remaining three fish per aquaria were euthanized, weighed, bled and dissected as previously described. Plasma, hepatic, and pituitary tissues were treated and stored for later analysis as described above.

2.7. Statistical analyses

The experimental data were analyzed using SAS software system version 8.00 (SAS Institute, Cary, NC). Plasma hormone concentrations and tissue RNA expression data were subjected to two-way analysis of variance (ANOVA) mixed-model procedures with treatment (fed and fasted), time (2 and 4 weeks), and treatment × time as the fixed effects and aquarium within treatment as the random effect. When significant differences were found using ANOVA, pairwise contrasts were made using an LSD test to identify significant differences at the 5% level. Results are presented as mean ± standard error (S.E.). Inter- and intra-assay CV were calculated as the standard deviation (S.D.) divided by the mean. Mean recoveries at each concentration were calculated as a percentage of the expected value. Statistical full curve parallelism analyses of the displacement curves for recombinant barramundi IGF-I standards and serially diluted fish plasma were conducted using StatLIA® Immunoassay Workflow and Analysis Software version 3.1 (Brendan Scientific).

3. Results

3.1. IGF-I TR-FIA

The minimum detectable limit of the assay was calculated to be 0.20 ng/ml. The displacement curve for the barramundi standard was parallel to the displacement curves of serially diluted plasma from channel catfish ($P = 0.956$), hybrid striped bass ($P = 0.870$), and rainbow trout ($P = 0.904$) (Fig. 1). The anti-barramundi IGF-I antiserum showed no detectable cross-reactivity with native channel catfish insulin (Fig. 1). The intra-assay CV was less than 7% and the inter-assay CV was less than 12% for repeated measurements of pooled plasma from channel catfish, hybrid striped bass, and rainbow trout (Table 2). Recovery of IGF-I from acid–ethanol extracted plasma samples spiked with recombinant barramundi IGF-I was greater than 90% (Table 3).

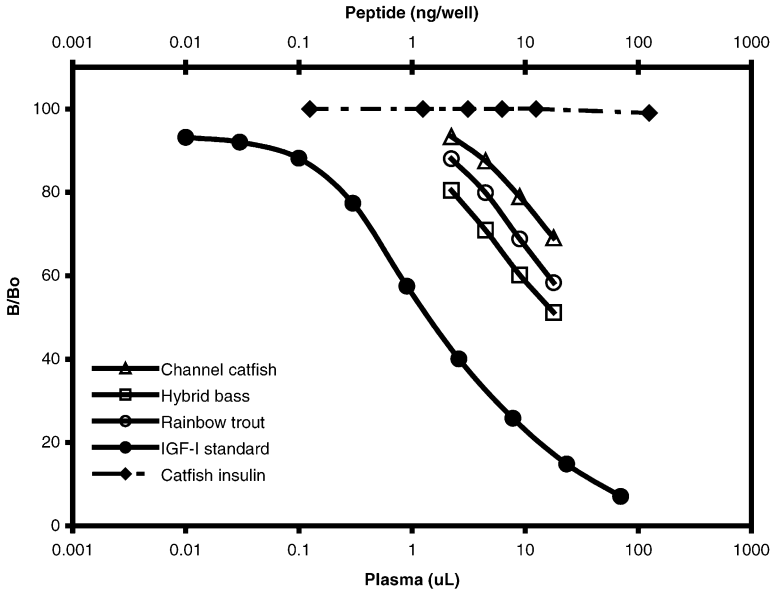

Fig. 1. Displacement curves for recombinant barramundi IGF-I assay standards, serially diluted channel catfish, hybrid striped bass, rainbow trout plasma, and channel catfish insulin. Values are means of triplicate determinations.

Table 2
Intra- and inter-assay coefficients of variation (CV) in the IGF-I TR-FIA for mean pooled plasma concentrations from channel catfish, hybrid striped bass, and rainbow trout

Species	Mean (ng/ml)	Intra-assay CV[a] (%)	Inter-assay CV[b] (%)
Channel catfish	19.5	5.1	9.8
Hybrid bass	53.9	5.9	7.6
Rainbow trout	45.7	6.5	11.1

[a] $n = 6$.
[b] $n = 3$.

Table 3
Recovery of recombinant barramundi IGF-I added to acid–ethanol-extracted plasma

Added IGF-I[a] (ng/ml)	Recovered IGF-I (ng/ml)			Recovery[b] (%)		
	Channel catfish	Hybrid bass	Rainbow trout	Channel catfish	Hybrid bass	Rainbow trout
1.2	1.1	1.2	1.1	95.1	96.6	100.9
10.4	11.1	11.2	10.8	107.0	107.3	103.8
90.0	87.5	90.7	81.4	97.2	100.8	90.4

[a] Fish plasma was spiked with known quantities of recombinant barramundi IGF-I.
[b] Mean recoveries ($n = 6$) at each concentration were calculated as a percentage of the expected value for each species.

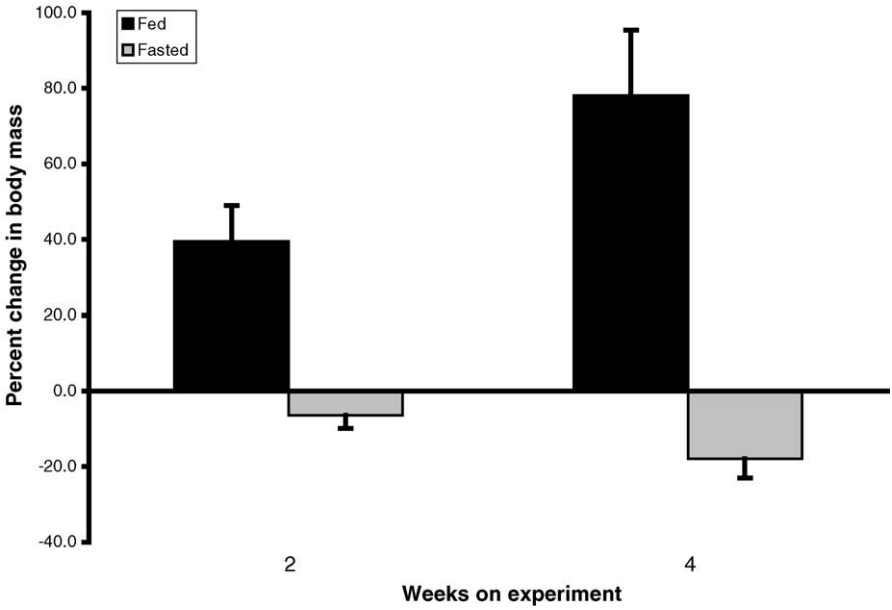

Fig. 2. Percent change in body mass of channel catfish fed or fasted for 2 and 4 weeks (mean ± S.E.; $n = 5$). Fasting had a significant ($P < 0.05$) effect on body mass. The effects of time and fasting × time interaction were non-significant ($P > 0.05$).

3.2. Fasting experiment

Channel catfish fasted for 2 and 4 weeks lost an average of 5.6 and 15.6% body mass, respectively, compared to catfish fed daily to satiety, which gained 34.3 and 67.9% body mass after 2 and 4 weeks, respectively (Fig. 2). After 2 and 4 weeks of fasting, circulating concentrations of IGF-I were reduced ($P < 0.05$) relative to fed controls (Fig. 3a). Circulating concentrations of GH were similar ($P > 0.05$) between 2-week fasted catfish and fed controls, but were increased ($P < 0.05$) in 4-week fasted catfish relative to fed controls (Fig. 3b). Hepatic IGF-I mRNA expression after 2 and 4 weeks of fasting was reduced ($P < 0.05$) relative to fed controls (Fig. 4a). Pituitary GH mRNA expression was similar ($P > 0.05$) between 2-week fasted catfish and fed controls, but was increased ($P < 0.05$) in 4-week fasted catfish relative to fed controls (Fig. 4b).

4. Discussion

4.1. IGF-I TR-FIA

This study reports the establishment and validation of a heterologous time-resolved fluoroimmunoassay (TR-FIA) for quantifying circulating IGF-I levels in three commercially important, domestically cultured fish species using commercially available components.

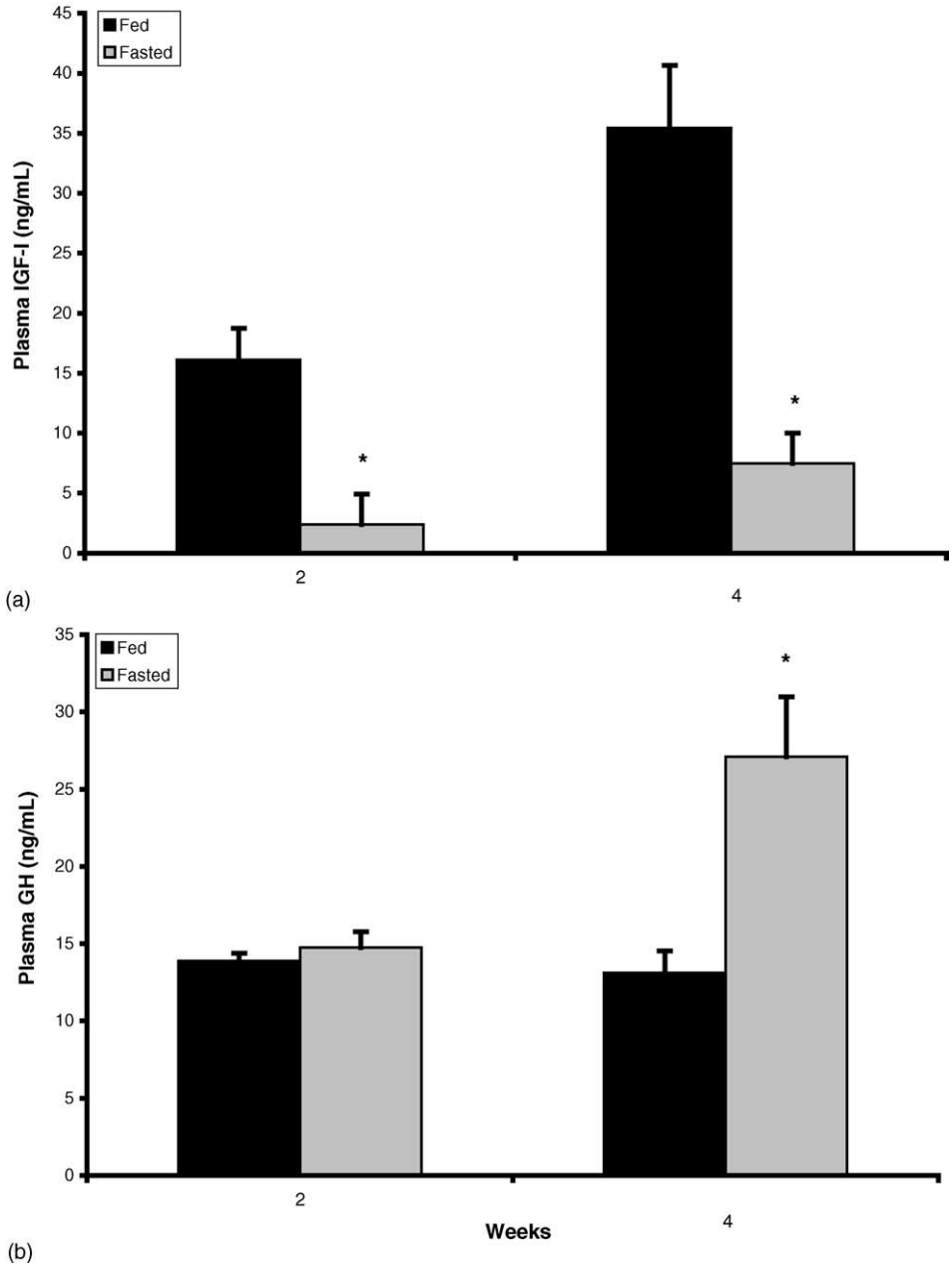

Fig. 3. Effect of fasting channel catfish for 2 and 4 weeks on plasma IGF-I (a) and GH (b). The effects of fasting, time, and fasting × time were significant ($P < 0.05$) for plasma IGF-I and GH concentrations. Asterisks indicate differences ($P < 0.05$) between treatments within time.

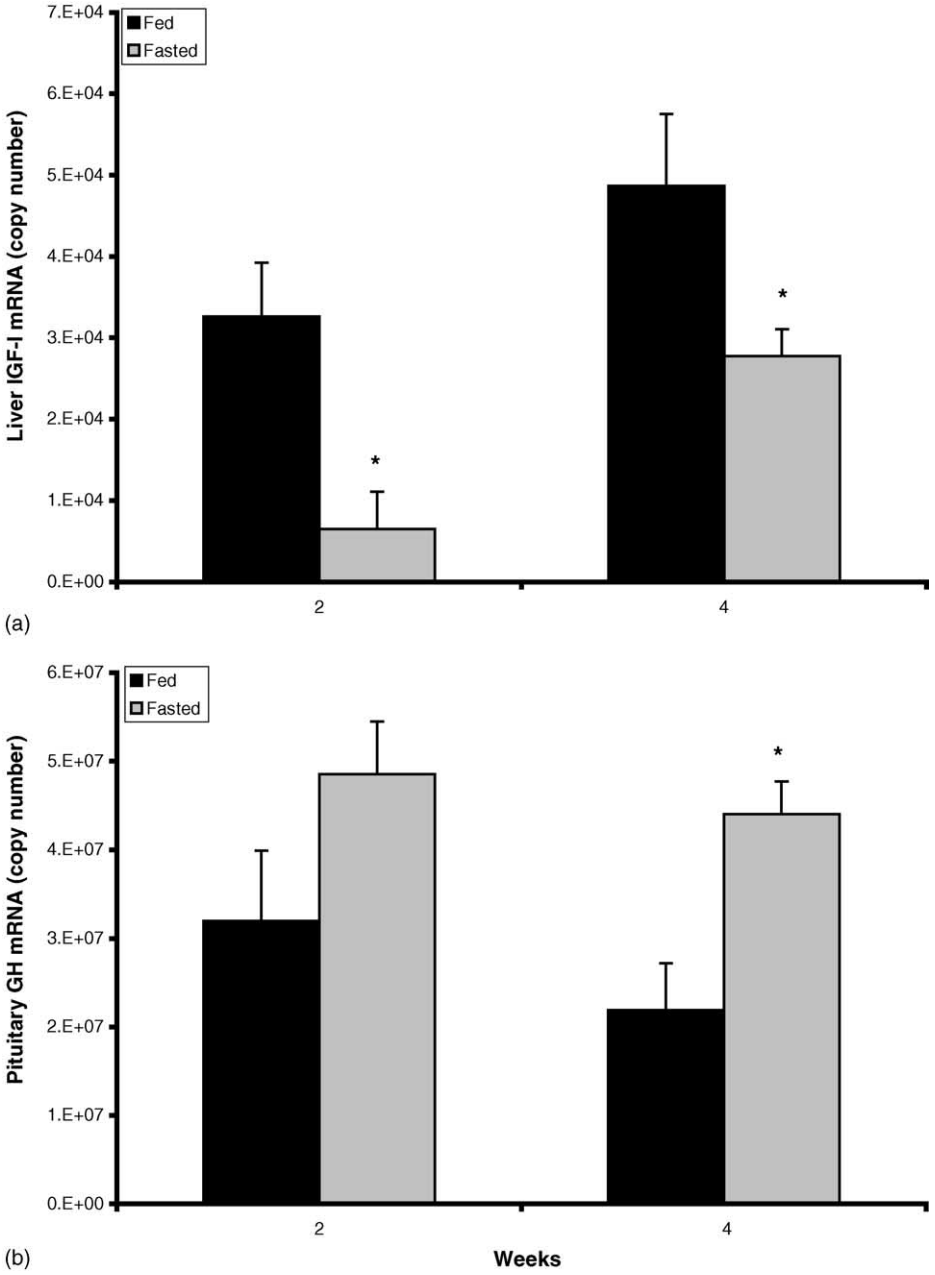

Fig. 4. Effect of fasting channel catfish for 2 and 4 weeks on mRNA expression of hepatic IGF-I (a) and pituitary GH (b). Copy number for both genes was normalized against α-tubulin. The effects of fasting and time were significant ($P < 0.05$) for hepatic IGF-I expression. The effect of fasting was significant ($P < 0.05$) and the effect of time was non-significant ($P > 0.05$) for pituitary expression. The interaction between fasting and time was non-significant ($P > 0.05$) for both genes. Asterisks indicate differences ($P < 0.05$) between treatments within time.

To our knowledge, this is the only TR-FIA that has been established to measure plasma IGF-I concentrations in teleost fishes. Sensitivity, precision, reproducibility, and specificity of this TR-FIA are comparable with the commercially available Fish IGF-I RIA kit (GroPep) which also utilizes recombinant barramundi IGF-I antigen and anti-barramundi IGF-I antiserum.

One major disadvantage of the commercial RIA is the use of radioisotopes and the associated potential health hazards and waste disposal issues. While both the RIA and TR-FIA depend on competition between labeled and unlabeled IGF-I for a limited number of antibody binding sites, the TR-FIA utilizes the unique fluorescence property of Europium as an alternative to radioisotopes for labeling and detection. Our experience also indicates that the Eu-label is stable for a year or more.

Increasingly, cumbersome and expensive regulatory controls over the use of radioactive materials make the TR-FIA particularly attractive. Additionally, the TR-FIA is an economically viable alternative to the existing commercial RIA. Cost of the commercial Fish IGF-I kit (GroPep) currently is $750 per 100 samples run in triplicate. Total cost of the TR-FIA established and validated in this study was approximately $210 per 100 samples run in triplicate.

4.2. Effect of fasting on channel catfish IGF-I and GH

The effect of fasting on the GH/IGF-I axis in channel catfish appears to be very similar to that reported for other teleost species. Fasting results in reductions of plasma IGF-I and hepatic IGF-I mRNA levels in coho salmon [4,19], gilthead seabream (*Sparus aurata*) [23,27], and tilapia [28]. In this respect, teleost fish, including channel catfish, appear to respond in a manner similar to fasted mammals [29,30].

GH is the primary positive regulator of hepatic IGF-I synthesis. However, this study and others demonstrate increased circulating GH concentrations during food deprivation in teleost fishes [17–19,31]. This apparent paradox has also been observed in other species, including humans, and supports the conclusion that decreased circulating IGF-I concentrations during fasting are not the result of impaired GH secretion [32]. In salmon and gilthead seabream, reduced hepatic binding capacity for GH appears to be one of the mechanisms responsible for the decline in circulating IGF-I during fasting [22,23,33]. No information is currently available regarding channel catfish GH-receptors (GHR) or hepatic binding capacity. However, the increased circulating GH concentrations and pituitary GH mRNA expression coupled with decreased circulating IGF-I concentrations and hepatic IGF-I mRNA expression are suggestive of diminished hepatic binding capacity for GH in fasted catfish as well.

It could also be hypothesized that the observed increase in channel catfish plasma GH after 4 weeks of fasting is due to low circulating IGF-I, which could result in reduced negative feedback on GH synthesis and release. Uchida et al. [28] suggested that increased plasma GH during fasting observed in several teleost species may partially be due to decreased IGF-I concentrations. In mammals, IGF-I inhibits GH synthesis and release at the somatotrope and the central nervous system via hypothalamic somatostatin release[34,35], and in rainbow trout, IGF-I has been shown to negatively regulate GH release [36].

Withdrawal of feed is a prominent tool for managing *E. ictaluri* outbreaks on commercial catfish farms [11]. Our research shows that 2 weeks of fasting can result in moderate

weight loss compared to potentially high weight gain in fish fed daily to satiety. In channel catfish, GH production (mRNA expression and plasma concentration) was not significantly affected after 2 weeks of fasting, but was increased by 4 weeks of fasting. Changes in IGF-I production were much faster, with significant reductions in both hepatic mRNA expression and plasma concentrations occurring by 2 weeks of fasting. These changes in GH and IGF-I production have the potential to affect not only growth and metabolic functions but other physiological processes as well. Involvement of the GH/IGF-I axis in fish has been implicated in numerous physiological processes, including growth [37], osmoregulation [26,38–40], and reproduction [41–44].

4.3. Summary

We have described the establishment and validation of a time-resolved fluoroimmunoassay (TR-FIA) for quantifying IGF-I concentrations in channel catfish, hybrid striped bass, and rainbow trout. These three fishes are commercially important and make up a significant portion of United States' aquaculture production. The IGF-I TR-FIA provides a low-cost, and non-radioactive alternative to the RIA. Our results demonstrated that the TR-FIA was specific, sensitive, precise, and accurate. Biological validation was accomplished by using the TR-FIA to assess the effect of fasting on channel catfish circulating IGF-I concentrations. Furthermore, in addition to the observed reduction in plasma IGF-I concentration, fasting was shown to have a negative effect on IGF-I hepatic mRNA expression. Fasting-induced changes in GH production were found to occur more slowly than changes in IGF-I production. The IGF-I TR-FIA will be a valuable tool for future IGF-I research in fish.

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