# Effects of exogenous cortisol on the GH/IGF-I/IGFBP network in channel catfish

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## Abstract

Glucocorticoids are known to hinder somatic growth in a number of vertebrate species. In order to better understand the mechanisms through which they may act in channel catfish, we examined the effects of feeding cortisol on the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein (IGFBP) network. Fish ($30.6 \pm 3.0$ g) were fed once daily for 4 weeks and treatments included: (1) High-cortisol (dietary cortisol provided at 400 mg/kg feed), (2) Low-cortisol (dietary cortisol provided at 200 mg/kg feed), and (3) Control (commercial catfish feed). Fish fed diets with cortisol weighed approximately 50% less than Controls. Feed intake was reduced by approximately 30% in both treatments of cortisol fed fish compared to Controls. A ~20-kDa IGFBP was observed in plasma from High- and Low-treated fish while it was not detected in Control fish plasma. High-cortisol treatment increased pituitary GH mRNA expression approximately 10-fold while liver IGF-I mRNA expression was not different between cortisol-treated fish and Controls. Cortisol treatments decreased plasma levels of IGF-I. These data indicate that feeding cortisol for 4 weeks reduces weight gain, feed intake, and plasma levels of IGF-I and induces a ~20-kDa IGFBP. One mechanism through which cortisol may impede growth of catfish is through an increase in a low molecular weight IGFBP which may lead to inhibitory effects on the action of IGF-I.
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## 1. Introduction

The growth hormone (GH)/insulin-like growth factor-I (IGF-I) network plays an integral role in mammalian growth, and it is becoming clear that this network is highly conserved in fish [1–5]. As in mammals, IGF-I is synthesized predominantly in the liver, while locally produced IGF-I in extrahepatic tissues seems to play autocrine/paracrine roles [3,6]. In mammals, the activity of IGF-I and IGF-II (IGFs) is regulated not only by GH and other endocrine modulators that enhance or suppress local and systemic IGF levels, but also by the presence of IGF-specific binding proteins (IGFBPs). IGFBPs play a central role in prolonging the half-lives of IGFs and coordinating and transporting IGFs in the blood and across the capillary barrier to target tissues [7–10]. In mammals, the IGFBPs are composed of a family of six proteins (IGFBP-1 to -6) that bind to IGFs with high affinity and specificity [9]. Recent evidence suggests that some of the IGFBPs have their own intrinsic biological activities, independent of their ability to interact with IGF-I and IGF-II [10].

In mammals, less than 5% of IGFs circulate in the free form; instead most of the IGFs (90%) are complexed with IGFBP-3 (the predominant IGFBP in serum) or IGFBP-5, and an 85 kDa glycoprotein, the acid-labile subunit (ALS), forming a 150-kDa ternary complex [11–13]. Formation of the ternary complex extends the half-life of IGF-I from approximately 10 min in its free form to 18 h, thereby stabilizing and maintaining plasma IGF-I levels [13].

IGFBPs exist in several teleosts species [2,14–22]. Three to four IGFBPs, ranging in size from ∼20 to ∼50 kDa, are commonly reported in teleost studies. Although it is not known if these IGFBPs function similarly in teleosts as they do in mammals, evidence is growing which suggests IGFBPs may in fact play similar roles. For example, similarly to mammals, the ≤31-kDa fish IGFBPs are increased in catabolic states and inversely correlated with somatic growth [2,14,25,26].

Supraphysiological levels of exogenous glucocorticoids have long been shown to inhibit growth in mammals [27]. The mechanisms through which glucocorticoids retard growth may involve reducing IGF-I gene and protein levels both directly and indirectly by blocking GH-induced IGF-I gene expression through the reduction of GH receptor synthesis [28–30]. In a poorly understood mechanism, glucocorticoids increase IGFBP-1 and -2, and decrease IGFBP-3 in mammals [31–33]. The regulation of IGFBP-1, -2, and -3 may lead to inhibitory effects on IGF-I's action.

Glucocorticoids also decrease growth in teleosts [34]. For example, a reduction in somatic growth after treatment with cortisol has been reported in rainbow trout [35,36], channel catfish [37,38], and goldfish [39]. The mechanisms through which glucocorticoids inhibit growth may also involve the GH/IGF-I/IGFBP network. In a recent study with tilapia, *Oreochromis mossambicus*, cortisol injection increased IGFBPs of four different sizes (24, 28, 30, and 32 kDa) in the plasma by 2 h without affecting plasma IGF-I levels [40]. A reduction of plasma IGF-I and liver IGF-I mRNA was observed 24 and 48 h after cortisol administration [40]. In addition, no change was observed in plasma or pituitary GH at any time point examined [40]. The results of the tilapia study suggest a mechanism whereby cortisol reduces IGF-I sensitivity to GH and increases lower molecular weight IGFBPs. As in mammals, this may be one mechanism through which cortisol inhibits growth.

The upregulation of low molecular weight IGFBPs in catabolic states or when glucocorticoids are administered to teleosts suggests a mechanism similar to the one reported for

mammals. The mechanisms through which glucocorticoids inhibit growth in channel catfish are not known. To better understand the effects of glucocorticoids on the GH/IGF-I/IGFBP network, we examined long term in vivo effects of exogenous cortisol on plasma levels of IGF-I, IGFBPs, and mRNA levels of IGF-I in the liver and GH in the pituitary.

## 2. Materials and methods

Catfish used in this study were from the National Warmwater Aquaculture Center strain (NWAC103). Fish ($30.6 \pm 3.0$ g) were randomly stocked into nine, 76-l aquaria (seven fish/tank) and allowed to acclimate for 2 weeks. Fish were fed two levels of cortisol: (1) High (dietary cortisol provided at 400 mg/kg feed once a day) and (2) Low (dietary cortisol provided at 200 mg/kg feed once a day), and compared to a (3) Control (commercial catfish feed provided once a day) group once a day for 4 weeks. Cortisol-laden feed was prepared by dissolving the appropriate amount of cortisol (Sigma, St. Louis, MO, USA) in ethanol and spraying it on a floating commercial fish feed (Land O Lakes Farmland Feed, Arden Hills, MN, 36% crude protein). Food was weighed back weekly to calculate food intake. At the end of the study, 15 fish per treatment were anesthetized as described below, weighed, bled, and livers and pituitaries were excised after the fish were euthanized. The fish were reared in $26.0\,^{\circ}$C flow-through well water and maintained with a 14 L:10 D h photoperiod. Water quality (pH ~8.5 and dissolved oxygen levels >5.0 mg/l) and flow rates were similar between treatments.

In an effort to obtain cortisol uptake and clearance profiles of catfish fed the cortisol-laden diets over 24 h, a second study was conducted. In the second study, catfish (112.4 g) were randomly stocked into six, 76-l aquaria (9 fish/tank) and allowed to acclimate for 2 weeks. Two fish from each treatment (1/tank) were bled at 0, 0.5, 2, 4, 7, 10, 15, 20, and 24 h after being fed a commercial diet (Control), a cortisol-laden diet fed at 400 mg/kg feed (High), or a cortisol-laden diet fed at 200 mg/kg feed (Low). After the fish were bled, they were removed from their respective tanks. The fish were larger in the second study to ensure that sufficient plasma was collected for the cortisol assay.

### 2.1. Blood collection and plasma preparation

Whole blood was collected from the caudal vasculature into syringes coated with heparin. Fish were initially anesthetized with 0.6 mg/l metomidate hydrochloride prior to blood collection, then euthanized in a 200 mg/l solution of tricaine methanesulphonate (TMS, MS-222) prior to dissection. Metomidate hydrochloride blocks the handling-related release of cortisol into circulation, minimizing endogenous plasma cortisol variability due to sampling [41]. The plasma was analyzed for cortisol, IGF-I, and IGFBPs.

#### 2.1.1. Sample preparation and RNA isolation

The pituitary and a section of liver (~100 mg) were excised for RNA extraction. Samples were immediately placed in 1 ml TRIzol (Life Technologies, Rockville, MD, USA) and flash-frozen in liquid nitrogen and stored at $-80\,^{\circ}$C. Total RNA was isolated according to the manufacturer's recommendations and utilized for analysis of GH and IGF-I mRNA from

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

| Gene | Primer | Sequence |
| --- | --- | --- |
| IGF-I | Sense | CTG TGA GCT GAA ACG ACT CG |
| | Antisense | CCA GAT ATA GGT TTT CTT TGG TG |
| | Probe[a] | CGA GAG CAA CGG CAC ACA GAC ACG C |
| GH | Sense | CAT ATC TCA GAG AAG CTG GC |
| | Antisense | GGT CTG GTA GAA ATC CTC GA |
| | Probe[a] | CCA CAC ATC CCT CGA TAA GTA CGC C |
| 18S | Sense | GAG AAA CGG CTA CCA CAT CC |
| | Antisense | GAT ACG CTC ATT CCG ATT ACA G |
| | Probe[b] | GGT AAT TTG CGC GCC TGC TGC C |

[a] The probes 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, USA).

[b] The probe 18S (GenBank Accession No. AF021880) was dual labeled with a reporter dye Texas red (sulforhodamine 101) at the 5′ end of the probe and a quencher dye (BHQ-1) at the 3′ end (Biosearch Technologies).

pituitary and liver tissues. The integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels. Total RNA was quantified by measuring the absorbance at 260 nm using a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA).

### 2.1.2. Real-time PCR

RNA (1 μg) from liver and pituitary was reverse-transcribed in 10 μl reactions using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA, USA). Real-time PCR was performed using the iCycler iQ (BioRad). The primers, probes, and accession numbers for IGF-I, GH and 18S are listed in Table 1. Primer and probe sequences were designed with Beacon Designer 2.0 (Premier BioSoft International, Palo Alto, CA, USA) software. Each amplification reaction mixture (12.5 μl) contained 300 ng of cDNA; $1 \times$ iQ Supermix (Bio-Rad) which consisted of: 10 mM KCl, 4 mM Tris–HCl, pH 8.4, 0.16 mM dNTPs, 5U/ml iTaq polymerase, 0.6 mM $MgCl_2$ and stabilizers; 10 μM (IGF-I, GH, or 18S) of each primer; and dual-labeled probe (5 μM of IGF-I or GH; 1 μM of 18S). The real-time PCR protocol for IGF-I, GH, and 18S was 3 min at 95 °C; 45 cycles of 95 °C–15 s, 60 °C–1 min. Primer and probe concentrations were optimized using liver and pituitary cDNA as template. Treatment did not affect the 18S values in liver or pituitary samples.

Real-time PCR results were analyzed by subtracting the mean of the 18S (reference sequence) threshold cycle ($C_T$) values from the mean of the IGF-I or GH (target sequences) $C_T$ values for both the treated (cortisol) and the control (no cortisol) samples to obtain $\Delta C_T$ values [42]. The $\Delta C_T$ values of the control samples were then subtracted from the $\Delta C_T$ values of the treated samples to obtain the $\Delta\Delta C_T$ values. The fold induction in levels of IGF-I or GH in cortisol-treated samples as compared to the control samples was obtained by the formula $2^{-\Delta\Delta C_T}$ [42]. This method has been successfully applied to the quantitative

detection of expression levels of IGF-I and IGF-II in common carp (*Cyprinus carpio*) [43] and channel catfish [44].

### 2.1.3. Validation of target and reference amplification

The efficiency of the target amplification and the efficiency of the reference amplification must be similar for the $\Delta\Delta C_T$ calculation to be valid. To assess whether IGF-I, GH, and 18S had similar amplification efficiencies, we examined how changes in relative $C_T$ values ($\Delta C_T$) varied with template dilution [43]. The $\Delta C_T$ values for IGF-I and GH were plotted against the logarithm of the dilution factor of template cDNA, and the slopes were determined.

The relationship between the $C_T$ value and the logarithm of the dilution factor of cDNA template for the target genes IGF-I and GH was 0.98 and 0.97 while 18S was 0.99. The slope of the $\Delta C_T$ versus log dilution factor plot for IGF-I/18S was 0.090 and the slope of the $\Delta C_T$ versus log dilution factor plot for GH/18S was 0.070, less than the recommended value of 0.1 [42]. Therefore, conditions for amplifying IGF-I/18S and GH/18S were reliable in adopting the comparative $C_T$ method.

### 2.2. Plasma IGF-I and cortisol determination

Plasma IGF-I levels were measured using a competitive time-resolved fluoroimmunoassay validated for channel catfish ([45]). Sensitivity of the assay was 0.20 ng/mL and intra- and inter-assay CVs were <7 and <12%. Serial dilutions of plasma was parallel to the standard curve and recovery of IGF-I from spiked plasma samples was >90%. Plasma samples were acid-ethanol extracted prior to assaying and standards were run in triplicate while samples were run in duplicate.

Cortisol was measured using a DELFIA® time-resolved fluroimmunoassay kit (Perkin-Elmer Life Sciences, Boston, MA, USA). This kit has been validated for the quantification of plasma cortisol in channel catfish [46].

### 2.3. Western ligand blotting using digoxigenin-labeled IGF-I

Plasma IGFBP estimates were quantified using Western ligand blotting according to Peterson et al. [23]. GelExpert software version 3.5 (Nucleotech, Westport, CT, USA) was used to calculate peak intensities of IGFBPs. Peak intensity values are reported as arbitrary densitometric units (ADU) and their means. Samples were normalized to a pooled sample that represented all samples. The pooled sample was used in each Western blot.

### 2.4. Statistical analysis

In the first study, significant differences ($P < 0.05$) in tissue gene expression levels, weight, and plasma levels of IGF-I were detected using ANOVA (Statistical Analysis System Version 9.0 software) (SAS Institute, Inc., Cary, NC, USA). Tank served as the experimental unit for each variable measured. The model included the effects of treatment, on tissue gene expression, weight, food intake, feed efficiency, and plasma IGF-I. Least

squares means were generated and separated using the PDIFF option of SAS for main effects. In the second study, the model included the main effect of time (0, 0.5, 2, 4, 7, 10, 15, 20, and 24 h). When the effect of time was significant ($P < 0.05$), least squares means separation was accomplished by the PDIFF option of SAS.

## 3. Results

### 3.1. Final weights, food intake, and food conversion ratio

Table 2 shows that treatment with both levels of cortisol significantly inhibited somatic growth in the long-term study. Final weights of the Control, High, and Low fish were 78.7, 35.3, and 39.9 g, respectively ($P < 0.05$). Cortisol-laden diets decreased food intake approximately 30% compared to fish that received the control diet ($P < 0.05$) (Table 2). Food conversion ratio was similar among the three diets (Table 2).

### 3.2. Plasma levels of IGF-I and IGFBPs

A significant reduction in plasma IGF-I was observed after feeding both levels of cortisol for 4 weeks (Table 2). Circulating levels of IGF-I were approximately twice as high in the Control-treated fish compared to the Low- and High-treated fish ($P < 0.05$).

Fig. 1 shows the effects of cortisol diet on plasma levels of a ~20 kDa IGFBP. Significant increases in a ~20 kDa IGFBP in the High and Low treatments were observed. However, there was no difference between the two cortisol treatments. In all channel catfish plasma, two major bands were consistently detected around 35 and 45 kDa. No differences between treatments were detected in the 35 and 45 kDa (data not shown).

### 3.3. IGF-I and GH gene expression

No difference was observed in liver IGF-I mRNA expression in Low- and High-treated fish compared to Controls ($P > 0.10$) (Fig. 2). Pituitary GH mRNA levels were increased

Table 2
Weight gain, food intake, food conversion ratio and plasma concentrations of IGF-I in Control-, Low-, and High-treated fish

| Treatment[a] | Weight gain[b] (g) | Food intake[c] (g) | FCR[d] | IGF-I (ng/ml) |
|---|---|---|---|---|
| Control | 47.9 ± 4.7[x] | 57.9 ± 5.8[x] | 1.2 ± 0.1 | 3.0 ± 1.6[x] |
| Low | 8.6 ± 2.9[y] | 23.3 ± 0.2[y] | 3.2 ± 0.8 | 1.2 ± 0.2[y] |
| High | 7.5 ± 0.4[y] | 14.9 ± 1.1[y] | 2.0 ± 0.3 | 1.1 ± 0.1[y] |

Within columns, values with different superscript letters (x and y) are different ($P < 0.05$).

[a] Treatments were: (1) High (dietary cortisol provided at 400 mg/kg feed), (2) Low (dietary cortisol provided at 200 mg/kg feed), and (3) Control (commercial catfish feed). Catfish were fed once a day for 4 weeks.

[b] Weight gain represents the average amount of weight gain per fish ± S.E.M.

[c] Food intake represents the average amount of food consumed per fish ± S.E.M.

[d] FCR represents food conversion ratio calculated as ingested food (g)/weight increase (g) ± S.E.M.
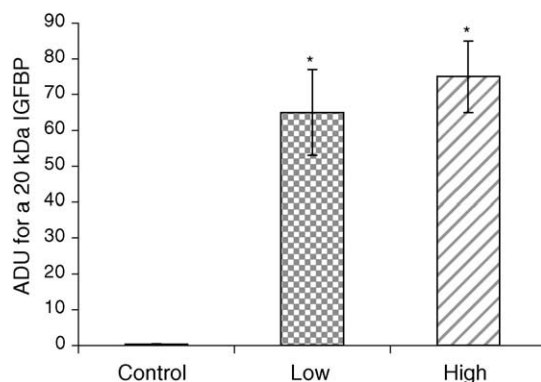
Fig. 1. Levels of a 20-kDa insulin-like growth factor binding protein in Control-, Low-, and High-treated catfish. Fish were fed two levels of cortisol; (1) High (dietary cortisol provided at 400 mg/kg feed once a day) (2) Low (dietary cortisol provided at 200 mg/kg feed once a day) and compared to a (3) Control (commercial catfish feed provided once a day) group for 4 weeks. Levels of the 20 kDa insulin-like growth factor binding protein were not detected in Control-treated fish. Western blots were quantified using GelExpert software and values are expressed as arbitrary densitometric unit (ADU) means. Vertical bars represent means $\pm$ S.E.M. ($n = 9$/treatment). Asterisks indicate significant ($P < 0.001$) differences between Control and Low and High treatments.

approximately 10-fold in High-treated fish compared to Control fish ($P < 0.002$) (Fig. 3). In contrast, there was no significant difference ($P > 0.10$) between pituitary GH mRNA expression in Low and Control fish.

### 3.4. Plasma levels of cortisol over 24 h

Plasma cortisol levels in catfish for 24 h after feeding 200 and 400 mg cortisol/kg feed compared to Control fish are shown in Fig. 4. By 4 h, levels of cortisol peaked to 181 and
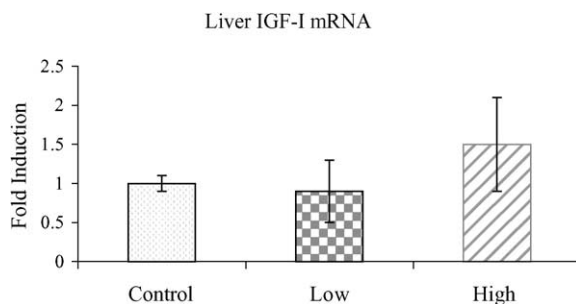


Fig. 2. Liver IGF-I mRNA expression in Control-, Low-, and High-treated catfish. Fish were fed two levels of cortisol: (1) High (dietary cortisol provided at 400 mg/kg feed once a day) (2) Low (dietary cortisol provided at 200 mg/kg feed once a day) and compared to a (3) Control (commercial catfish feed provided once a day) group for 4 weeks. Results are expressed as fold inductions compared to the Control group. Vertical bars represent means $\pm$ S.E.M. ($n = 9$/treatment). There was no difference between Control and Low and High treatments ($P > 0.10$).
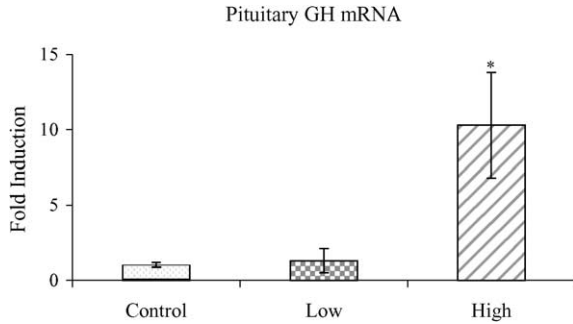
Pituitary GH mRNA



Fig. 3. Pituitary GH mRNA expression in Control-, Low-, and High-treated catfish. Fish were fed two levels of cortisol: (1) High (dietary cortisol provided at 400 mg/kg feed once a day), (2) Low (dietary cortisol provided at 200 mg/kg feed once a day) and compared to a (3) Control (commercial catfish feed provided once a day) group for 4 weeks. Results are expressed as fold inductions compared to the Control group. Vertical bars represent means ± S.E.M ($n = 9$/treatment). Asterisk indicates that GH mRNA expression levels were significantly ($P < 0.002$) higher in the High treatment compared to Control and Low treatments.

520 ng/ml in the Low and High treatments, respectively ($P > 0.002$). Plasma cortisol levels also increased to 42 ng/ml in Controls at 4 h. By 10 h, cortisol levels returned to baseline in Control fish. Interestingly, in both the Low and High treatment, at 15 h, there appeared to be a biphasic response of levels of cortisol. Plasma cortisol levels increased to 395 ng/ml in the High treatment fish, which was significantly higher than Controls. Levels of cortisol
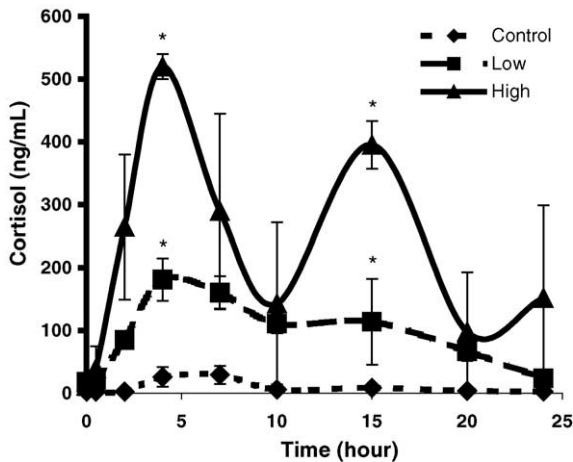


Fig. 4. Plasma cortisol levels in Control-, Low-, and High-treated catfish. Fish were fed two levels of cortisol: (1) High (dietary cortisol provided at 400 mg/kg feed once a day), (2) Low (dietary cortisol provided at 200 mg/kg feed once a day) and compared to a (3) Control (commercial catfish feed provided once a day). Blood was taken at 0, 0.5, 2, 4, 7, 10, 15, 20, and 24 h. Each time point represents two fish per treatment and shows the average level of cortisol in each treatment (mean ± S.E.M.) calculated from two replicate tanks. Asterisks indicate significant ($P < 0.05$) differences between Control and Low and High treatments.

appeared to return to pre-feeding levels at 24 h in the Low treatment but were quite variable in the High treatment.

## 4. Discussion

In the present study we demonstrated that administration of cortisol resulted in a significant inhibition of somatic growth in channel catfish. Final weights were reduced approximately 50% in cortisol-treated fish. This is in agreement with other fish studies, including channel catfish, which have also shown a decrease in growth rate after treatment with cortisol [35–39]. We also demonstrated that exogenous cortisol fed to catfish inhibits feed intake approximately 30% without a significant difference in feed efficiency. Effects of cortisol on feed intake in fish are ambiguous. For example, Davis et al. [37] observed no difference in food acceptability between sham and cortisol fed channel catfish. In a more quantitative study with rainbow trout, Gregory and Wood [36] found that cortisol implanted fish had significantly reduced individual food intakes. Bernier et al. [39] reported no difference in feed intake in gold fish fed a high-cortisol diet while intake was increased in fish fed a low-cortisol diet. In addition, feed conversion efficiency was significantly reduced in both groups of fish fed the cortisol diets [39]. Effects of cortisol on feed efficiency in the current study are not clear. Feed efficiency tended to decrease in catfish fed both cortisol diets, but because of the large variation was not significantly different from controls. More research into mechanisms regulating effects of cortisol on food intake and efficiency in channel catfish is warranted.

We hypothesized that regulation of the GH/IGF-I/IGFBP network maybe one of the mechanisms through which cortisol inhibits growth. Results of this study clearly demonstrated a relationship between cortisol and an increase in a ~20 kDa IGFBP. In a previous study, we showed a relationship between an increase in cortisol in fasted catfish and an increase in plasma levels of a 20-kDa IGFBP [24]. Kelley et al. [15] compared three catabolic states in fish: fasting, untreated insulin-dependent diabetes mellitus, and stress. Under all conditions, cortisol concentrations were increased and an increase in one or both low molecular weight IGFBPs (24 and 30 kDa) (depending on species) was observed. In a study with tilapia, Kajimura et al. [40] demonstrated a relationship between cortisol and four low molecular weight IGFBPs (24, 28, 30, and 32 kDa). The current study as well as other fish studies provides evidence that a relationship exits between cortisol and a 20–32 kDa IGFBP.

Of the three IGFBPs observed in this study, the 35, and 45 kDa IGFBPs were most prominent in the plasma of all fish. We have previously reported the presence of these two IGFBPs in catfish in other studies [23,24]. Cortisol treatment did not affect plasma levels of these two IGFBPs. Based on molecular weight, the 45 kDa IGFBP may be the catfish counterpart of the mammalian IGFBP-3. We have shown that the 45 kDa IGFBP is unique in channel catfish and that it is not affected by GH administration [23]. Others have shown it is actually reduced [22] in catfish administered exogenous GH.

Protein and mRNA levels of IGFBP-3 of mammals are reduced by glucocorticoids [31,32,47–49]. Similarly in the tilapia, a 40 and 42 kDa IGFBP decreased in cortisol-treated fish [38]. In previous studies we observed no differences in plasma levels of the 45 kDa IGFBP in catfish maintained under anabolic (GH treatment) [23] or catabolic con-

ditions (food restriction) [24]. We have only been able to demonstrate an increase in a 20 kDa IGFBP under catabolic conditions (current study and [24]). It is not apparent why we did not detect a decrease in the 45 kDa IGFBP in cortisol-treated catfish fish, similar to that observed in tilapia.

The roles of the lower molecular weight IGFBPs are not clear but they may serve to shut down energy-expensive anabolic processes under catabolic circumstances [49,50]. Based on comparable molecular weight and an assumption of endocrine regulation similar to that in mammals, it has been proposed that the $\leq$31-kDa fish IGFBPs may be counterparts of mammalian IGFBP-1 or -2. In adult mammals, the levels of IGF-I and IGFBPs are appreciably affected in catabolic or stressful conditions such as fasting [50–52]. IGFBP-1 has been shown to inhibit IGF actions in numerous in vitro and in vivo animal studies [50]. In addition, the IGFBP-1 gene possesses glucocorticoid-response elements, and the steroid can potentially increase IGFBP-1 levels [50]. The mechanisms through which IGFBP-1 or -2 inhibit IGF-I action are not clear but may include sequestering IGF-I in the extracellular environment through high IGF binding affinity [53] and curtailing expensive anabolic functions during times of stress or nutritional deficiencies [15].

We observed a reduction in plasma IGF-I after feeding both levels of cortisol to catfish for 4 weeks. Similarly, cortisol injected tilapia were shown to have reduced plasma levels of IGF-I as well as reductions in liver IGF-I mRNA. We were unable to detect differences in liver IGF-I mRNA levels in cortisol-treated fish, which is in contrast to findings in mammals, in which glucocorticoids clearly inhibit IGF-I synthesis and gene expression [31,32]. The reduction in plasma IGF-I levels caused by dietary cortisol may have been due in part to suppression of post-transcriptional events in IGF-I expression. Moreover, increased clearance rates of circulating IGF-I in cortisol-treated fish may also have been involved. Other tissues than the liver could have contributed to the decrease in plasma concentrations of IGF-I.

Higher levels of plasma IGF-I have been reported in faster growing GH-injected catfish [54]. Although the role of IGF-I in catfish is not known, other fish studies have reported positive correlations between somatic growth and circulating IGF-I levels [6,55]. Assuming IGF-I regulates growth in catfish as it does in other species of fish, the reduction in plasma IGF-I observed in the current study may have contributed to the reduction in growth.

Feeding cortisol at 400 mg/kg diet for 4 weeks resulted in an increase in expression of pituitary GH mRNA. However, there was no difference in GH mRNA levels when cortisol was fed at 200 mg/kg diet. Kajimura et al. [40] reported no change in plasma or pituitary levels of GH in cortisol-treated tilapia. In a study with Nile tilapia (*Oreochromis niloticus*), confinement stress increased plasma cortisol and decreased plasma GH [56]. In mammals, it has been shown in vitro that glucocorticoids act at the level of the pituitary to stimulate GH gene expression [57]. Both transcriptional [58,59] and post-translational [59–61] mechanisms have been implicated in glucocorticoid regulation of GH. The interaction between the glucocorticoid receptor and specific sequences in the first intron of the human GH gene in vitro may be responsible for the induction of transcription [62].

In the present study, the High-cortisol dose resulted in a reduction of plasma IGF-I with an increase in gene expression of pituitary GH. The Low-cortisol dose resulted in a reduction of plasma IGF-I without a change in gene expression of pituitary GH. It is not clear from this study or the tilapia studies what effect cortisol has on GH levels in teleost

species. The fact that plasma IGF-I levels declined after Low-cortisol administration, despite unchanged GH mRNA levels, suggests that the sensitivity of IGF-I production to GH is reduced by cortisol. In support of this hypothesis, King and Carter-Su [28] demonstrated that dexamethasone-induced antagonism of GH action by down regulation of GH binding in fibroblasts. In addition, Jux et al. [29] found that dexamethasone impaired GH-stimulated growth by suppression of expression of GH and IGF-I receptor and IGF-I production in rat chondrocytes.

In an effort to obtain cortisol uptake and clearance profiles of catfish fed cortisol-laden diets, we serially bled fish for 24 h. By 4 h, levels of cortisol peaked to 181 and 520 ng/ml in the Low and High treatments, respectively. To put these numbers in perspective, handling and confinement stress typically result in moderate elevation (<100 ng/ml) of circulating cortisol [46,63]; however, higher plasma cortisol levels (>100 ng/ml) have been reported in channel catfish exposed to ammonia [64] and subjected to live-car grading [65]. In general, cortisol levels returned to control levels 24 h after feeding. Davis et al. [63] fed channel catfish cortisol at a dose of 200 mg cortisol/kg diet for 10 days. Feeding cortisol produced a daily peak of plasma cortisol at 6 h, which returned to control levels 24 h after feeding. In the present study, considerable variation was observed within time, which may be explained by the small sample size of two fish or potential differences in feed ingestion rates. Davis et al. [63] also observed large variations in endogenous cortisol over 12 h in catfish fed cortisol at 200 mg/kg feed. The observed biphasic response of cortisol in both the Low and High treatment at 15 h is hard to explain but may be an artifact of the large variation observed between the two fish.

In summary, the present study demonstrated that cortisol administered in the diet for 4 weeks, decreased body weight, decreased feed intake, increased a ~20 kDa IGFBP, and decreased plasma IGF-I levels without observed differences in liver IGF-I mRNA expression. One mechanism through which cortisol may impede growth of catfish is through an increase in a low molecular weight IGFBP which may lead to inhibitory effects on IGF-I's action. These results also provide additional evidence of the conserved nature of the GH/IGF-I/IGFBP-growth network in fish.

## Acknowledgements

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