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Response of the somatotropic axis to alterations in feed intake of channel catfish (*Ictalurus punctatus*)

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ABSTRACT

To better understand the effects of reduced feeding frequency on the GH–IGF-I axis, channel catfish (*Ictalurus punctatus*), were either fed (Fed control, commercial diet fed daily), fed every other day (FEOD, commercial diet fed every other day), or not fed (Unfed, no feed). Pituitary GH mRNA increased whereas hepatic growth hormone receptor (GHR), IGF-I mRNA, and plasma IGF-I decreased in the FEOD and Unfed fish ($P<0.05$). In another study, fish were either continually fed (Fed) or fasted and then re-fed (Restricted) to examine the physiological regulation of somatostatin-14 (SS-14) and SS-22 mRNA. Fasting increased ($P<0.05$) levels of SS-14 mRNA in the hypothalamus and pancreatic islets (Brockmann bodies) at d 30 while re-feeding decreased SS-14 mRNA to control values in all tissues examined by d 45. Fasting had no effect on levels of SS-22 mRNA in the pancreatic islets whereas SS-22 mRNA was not detected in the stomach or hypothalamus. The results demonstrate that feeding every other day has similar negative impacts on components of the GH–IGF-I axis as fasting. The observed increase in SS-14 mRNA in the hypothalamus and pancreatic islets suggests a role for SS-14 in modulating the GH–IGF-I axis in channel catfish.

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1. Introduction

The growth hormone–insulin-like growth factor (GH–IGF) axis has been implicated in the regulation of somatic growth and metabolism in teleost fishes. The GH–IGF axis begins in the anterior pituitary gland under the control of multiple hypothalamic hormones, including growth hormone releasing hormone (GHRH), pituitary adenylate cyclase-activating peptide (PACAP), and somatostatins (SSs). Growth hormone acts on target tissues and stimulates a range of mitogenic processes that are mediated by IGF-I and probably IGF-II as administration of exogenous GH increases levels of IGF-II mRNA in teleosts (Shamblott et al., 1995; Greene and Chen, 1999; Vong et al., 2003; Peterson et al., 2005). Insulin-like growth factor binding proteins (IGFBPs) play a central role in prolonging the half-lives of IGFs and coordinating and transporting IGFs in circulation (Moriyama et al., 2000). The IGFBP family has recently expanded to include the IGFBP-related proteins (IGFBP-rPs) that may also play a role in regulating IGF activity (Rodgers et al., 2008). The IGFs evoke their biological responses through their respective receptors on target tissues resulting in growth promotion.

Much of the research on the regulation of vertebrate growth has focused on the production of GH. Major inhibitors of GH release are the SSs, a functionally and structurally diverse family of peptides (Very and Sheridan, 2004). Somatostatin was originally isolated from ovine hypothalamus as a 14-amino acid peptide and found to inhibit release of GH (Brazeau et al., 1973). Depending on species, SS peptides ranging in size from 14- to 37-amino acids have been isolated from the central and peripheral nervous systems, pancreatic islets, gastrointestinal tract, and thyroid tissue (Conlon et al., 1997). The structural heterogeneity of SS results from processing of the precursor preprosomatostatin (PPSS) as well as from the presence of multiple PPSSs (Very et al., 2008). Fish examples include, PPSS-I that contains the highly conserved 14-amino acid sequence at its C-terminus (SS-14-I), two nonallelic genes that encode separate forms of PPSS-II that each contain (Tyr^7, Gly^{10})-SS-14 at its C-terminus (SS-14-II), and PPSS-III that contains (Pro^2)-SS-14 at its C-terminus (Lin et al., 2000; Sheridan et al., 2000; Ehrman et al., 2002; Nelson and Sheridan, 2005).

Somatostatins have been shown to modulate GH and thus growth of teleosts species. For example, exogenous treatment with SS-14-I decreases plasma GH concentrations in rainbow trout, coho salmon, and goldfish (Cook and Peter, 1984; Sweeting and McKeown, 1986; Diez et al., 1992; Very and Sheridan, 2001; Peterson et al., 2003). The N-terminally extended mammalian SS, SS-25-I and SS-28-I have also been shown to decrease plasma levels of GH in coho salmon (Diez et al., 1992). *In vitro* studies have shown that mammalian SS-28-I, SS-14-I, goldfish brain SS-28-I, and SS-14-III inhibit GH release whereas salmonid SS-25-II and catfish SS-22 do not (Oyama et al., 1980; Marchant et al., 1987; Marchant and Peter, 1989; Yunker et al., 2003; Cameron et al., 2005). No *in vivo* studies have examined the physiological functions of SS-14 and SS-22 in channel catfish.

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Our previous research focused on understanding the regulation of GH, GHR, IGF-I, and IGFBPs in fed or fasted catfish (Small and Peterson, 2005; Small et al., 2006, Peterson et al., 2008). The role of SS-14 and SS-22 in modulating the GH–IGF-I axis in channel catfish is not known. The objectives of these studies were to investigate the regulation of GH, GHR, IGF-I, and IGF-I receptor a (IGF-IRa) mRNA in channel catfish that were either fed, fasted, or fed every other day, as well as to better understand the physiological role(s) of SS-14 and SS-22 mRNA during periods of fasting-associated growth restriction.

2. Materials and methods

2.1. Treatments

The channel catfish (*Ictalurus punctatus*) used in this study were from the USDA103 strain that originated from broodstock maintained at the USDA–ARS Catfish Genetics Research Unit, Stoneville, MS aquaculture facility. In the first study, 120 fish (60.0 ± 0.2 g) were randomly assigned to one of three treatments: 1) Fed control (36% crude protein floating commercial catfish diet, Farmland Industries, Inc., Kansas City, MO (USA) fed daily); 2) Fed every other day (FEOD; commercial diet fed every other day); and 3) Fasted (no feed). The fish were maintained in 76-L tanks (10 fish/tank), and allowed to acclimate for 10 d. All fish were fed during the acclimation period. The aquaria were supplied with flow-through well water (1.0 L/min) and continuous aeration. Water temperature averaged 26.7 ± 0.2 °C and a diel light:dark cycle was set at 14 h:10 h. Four replicate groups of Fed fish were fed to visual satiety per day while four replicate groups of FEOD fish were fed to visual satiety every other day. Visual satiation was obtained by feeding each tank as much as they would eat during a 20 min. period. Feed was withheld 24 h prior to handling fish. All fish were anesthetized with 0.1 g/L tricainemethane sulfonate (MS-222; Western Chemical Inc., Ferndale, WA, USA), and weighed and bled from the caudal vasculature on d 0, 21, and 42. On d 42, catfish were euthanized with an overdose (0.3 g/L) of MS-222 and muscle, liver, and pituitary were excised from 8 fish per treatment (2 fish/tank).

In the second study, 100 fish (60.9 ± 1.5 g) were randomly assigned to one of two treatments with five replicates each. The treatments were: 1) Fed control (36% crude protein floating commercial catfish diet, Farmland Industries, fed daily) and 2) Restricted (not fed for 30 d followed by feeding a commercial diet for 15 d). The fish were acclimated and maintained as described above. Ten fish per treatment (2 fish/tank) were sampled on d 0, 30, and 45. Brockmann bodies (pancreatic islets), gut (stomach), and hypothalamus were excised on each of the three sampling days. Both studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, USDA/ARS Catfish Genetics Research Unit.

2.2. Sample preparation and RNA isolation

Approximately 100 mg of liver, pituitary, stomach, Brockmann bodies, and hypothalamus was taken for RNA extraction. Samples were immediately placed in 1 ml TRI-Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) and then flash-frozen in liquid nitrogen. Total RNA was isolated according to manufacturer's instructions (Molecular Research Center) and quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). The integrity of the RNA was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels.

2.3. Real-time PCR

One microgram of total RNA from each tissue was reverse-transcribed using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA, USA) according to the protocol provided by the manufacturer. Quantitative real-time PCR was performed using the iCycler iQ real-time PCR detection system (BioRad) to quantify IGF-I, IGF-I Receptor a, 18S, and GH, and growth hormone receptor (GHR) mRNA as previously described (Peterson et al., 2004; Small and Peterson 2005; Small et al., 2006; Peterson et al., 2008). Primer and probe sequences for SS-14 and SS-22 are listed in Table 1. PCR fragments for these two genes were cloned into the pCR®4-TOPO vector (Invitrogen, Carlsbad, CA, USA) and introduced into One Shot® TOP10 Chemically Competent *E. coli* (Invitrogen) cells and the identity of the cloned inserts was confirmed by using an automated capillary DNA sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). The DNA concentration of each resulting recombinant plasmid was measured using the NanoDrop ND-1000 spectrophotometer. Each amplification reaction mixture (12.5 μL) contained 400 ng of cDNA; 1X iQ™ Supermix (BioRad), 2.5 μM dual-labeled probe, and 10 μM (SS-14, SS-22) of each primer. The real-time PCR protocol for SS-14 and SS-22 was 3 min at 95 °C; 45 cycles of 95 °C–15 s, 60 °C–1 min. All amplifications were performed in triplicate. The standard curve showed a linear relationship between cycle threshold values and the logarithm of input gene copy number. All specific quantities were normalized against the amount of 18S amplified after it was determined 18S levels did not change with respect to treatment.

2.4. IGF-I fluoroimmunoassay

Plasma IGF-I levels were measured using a competitive time-resolved fluoroimmunoassay validated for channel catfish (Small and Peterson, 2005). Plasma samples were acid-ethanol extracted prior to assaying and standards were run in triplicate while samples were run in duplicate.

2.5. Statistical analysis

For study 1, fish weight, plasma IGF-I, and tissue gene expression levels were analyzed by using the GLM procedures of SAS (Version 9.1 software) for a repeated measures design. The model included the main effect of time (0, 30, and 42 d) and when the main effect was significant ($P<0.05$), least squares means separation was accomplished by the PDIFF option. For study 2, tissue gene expression levels for both days (30 and 45 d) were subjected to nested analyses of variance (ANOVA) with treatment as a fixed effect and tank within treatment as a random effect, followed by Tukey's post hoc test. Normalized gene expression data passed Levene's test for homogeneity of variance. Tank served as the experimental unit for each variable measured.

3. Results

3.1. Study 1

Fed control fish gained more weight than both the FEOD and Unfed treatments throughout the 42-d study (Table 2; $P<0.001$). Differences

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

Gene	Primer	Sequence	PCR product length (base pair)
SS-14			99
	Sense	GGT CGA TTC TTG CAC CGT CT	
	Antisense	AGT CCA GCA CCT CGT TTT GC	
	Probe[1]	AGC TCA CCA GGT ACA CGC TCG CAG	
SS-22			86
	Sense	TCA CCA GCT ACC AAG AAG ATG TC	
	Antisense	GAT ATG ACA CCG ACG GCT GAG	
	Probe[1]	TCA CCA CTC CGT CTC GCT CTT GCC	

[1]The probes somatostatin 14 (SS-14) (GenBank accession no. M25903) and somatostatin 22 (SS-22) (GenBank accession no. M25904) 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).

Table 2
Mean mass (g) and plasma IGF-I levels in Fed (commercial catfish diet fed daily), FEOD (commercial diet fed every other day), and Unfed (no feed) catfish sampled on d 0, 21, and 42.

	Treatments[a]		
	Fed	FEOD	Unfed
Mass (g)[b]			
Day 0	60.0 ± 0.38	59.9 ± 0.29	60.0 ± 0.26
Day 21	84.2[d] ± 2.40	69.1[e] ± 1.14	44.5[f] ± 0.71
Day 42	110.4[d] ± 1.99	78.8[e] 1.26	40.6[f] 1.62
IGF-I (ng/mL)[c]			
Day 0	5.3 ± 0.62	4.7 ± 0.48	4.6 ± 0.56
Day 21	7.3[d] ± 0.66	5.5[d] ± 0.55	1.7[e] ± 0.58
Day 42	4.8[d] ± 0.12	1.8[e] ± 0.15	1.0[e] ± 0.10

[d,e,f]Within rows, values with different letters are different ($P<0.001$).
[a] Treatments include: Fed (commercial catfish diet fed daily), FEOD (commercial diet fed every other day), and Unfed (no feed).
[b] Mass represents the average mass (g) of each fish at d 0, 21, and 42.
[c] Mean plasma IGF-I values at d 0, 21, and 42. All fish (10 fish/tank) were sampled at each time point.

in weight were apparent by d 21 in the FEOD treatment and continued through d 42. Plasma IGF-I decreased ($P<0.05$) by d 21 in the Unfed fish and was lower in both the FEOD and Unfed treatments by d 42. On d 42, liver IGF-I mRNA was lower in both the Unfed and FEOD treatments compared to Fed fish (Fig. 1A; $P<0.05$). Muscle IGF-IRa mRNA was similar among treatments (Fig. 1B). Liver GHR mRNA was lower in the catfish FEOD and Unfed group compared to Fed controls (Fig. 2A; $P<0.001$). Pituitary GH mRNA levels were higher in both the Unfed and FEOD treatments compared to the Fed group (Fig. 2A; $P<0.05$).

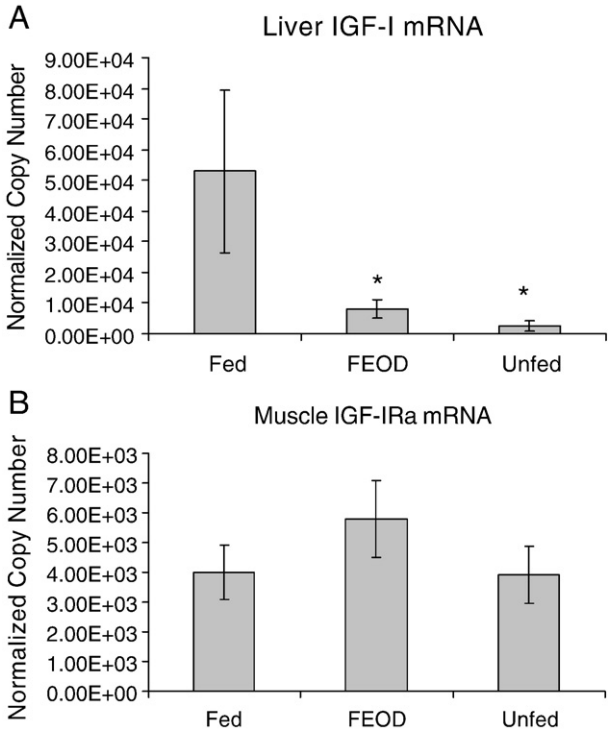

Fig. 1. A. Liver IGF-I mRNA levels in Fed control (commercial catfish diet fed daily); Fed every other day (FEOD, commercial diet fed every other day); Unfed (no feed). $N=8$ per treatment. IGF-I copy number was normalized as a ratio to the amount of 18S. Standard error bars represent standard error of the mean and significant differences ($P<0.01$) are denoted by asterisks. B. Muscle IGF-I receptor a (IGF-IRa) mRNA levels in Fed control (commercial catfish diet fed daily); Fed every other day (FEOD, commercial diet fed every other day); Unfed (no feed). $N=8$ per treatment. IGF-IR copy number was normalized as a ratio to the amount of 18S. Standard error bars represent standard error of the mean.

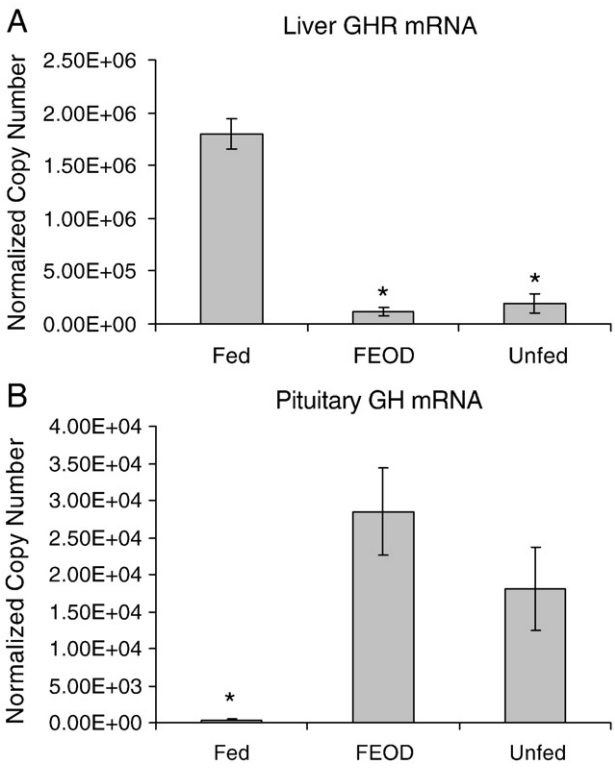

Fig. 2. A. Liver GH receptor (GHR) mRNA levels in Fed control (commercial catfish diet fed daily); Fed every other day (FEOD, commercial diet fed every other day); Unfed (no feed). $N=8$ per treatment. GH receptor copy number was normalized as a ratio to the amount of 18S. Standard error bars represent standard error of the mean and significant differences ($P<0.001$) are denoted by asterisks. B. Pituitary GH mRNA levels in Fed control (commercial catfish diet fed daily); Fed every other day (FEOD, commercial diet fed every other day); Unfed (no feed). $N=8$ per treatment. GH copy number was normalized as a ratio to the amount of 18 S. Standard error bars represent standard error of the mean and significant differences ($P<0.05$) are denoted by asterisks.

3.2. Study 2

Fed fish gained 91.6 g/fish whereas Restricted fish gained 14.2 g/fish over the 45-d study (Fig. 3). Abundance of SS-14 mRNA increased ($P<0.05$) in the hypothalamus and Brockmann bodies of Restricted fish compared to Fed fish at d 30 while abundance of SS-14 mRNA was similar in the stomach (Fig. 4A, B, C). After 15 d of re-feeding, abundance of SS-14 mRNA was similar in all tissues between the Fed and

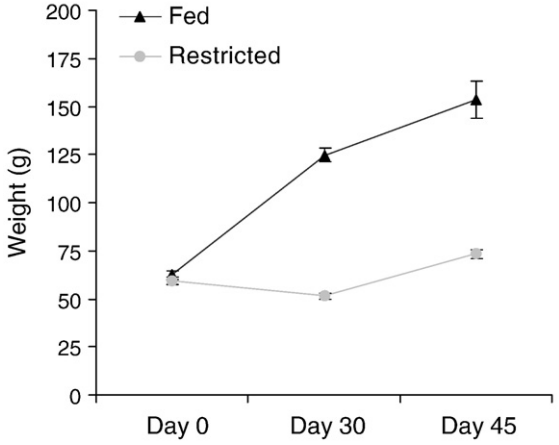

Fig. 3. Weight (g) of Fed control (commercial catfish diet fed daily) and Restricted (not fed for 30 d followed by feeding a commercial diet for 15 d. Fish were sampled on d 0, 30, and 45. Each time point shows the average (10 fish/tank) weight in each treatment (mean ± SEM) calculated from 5 replicate tanks.

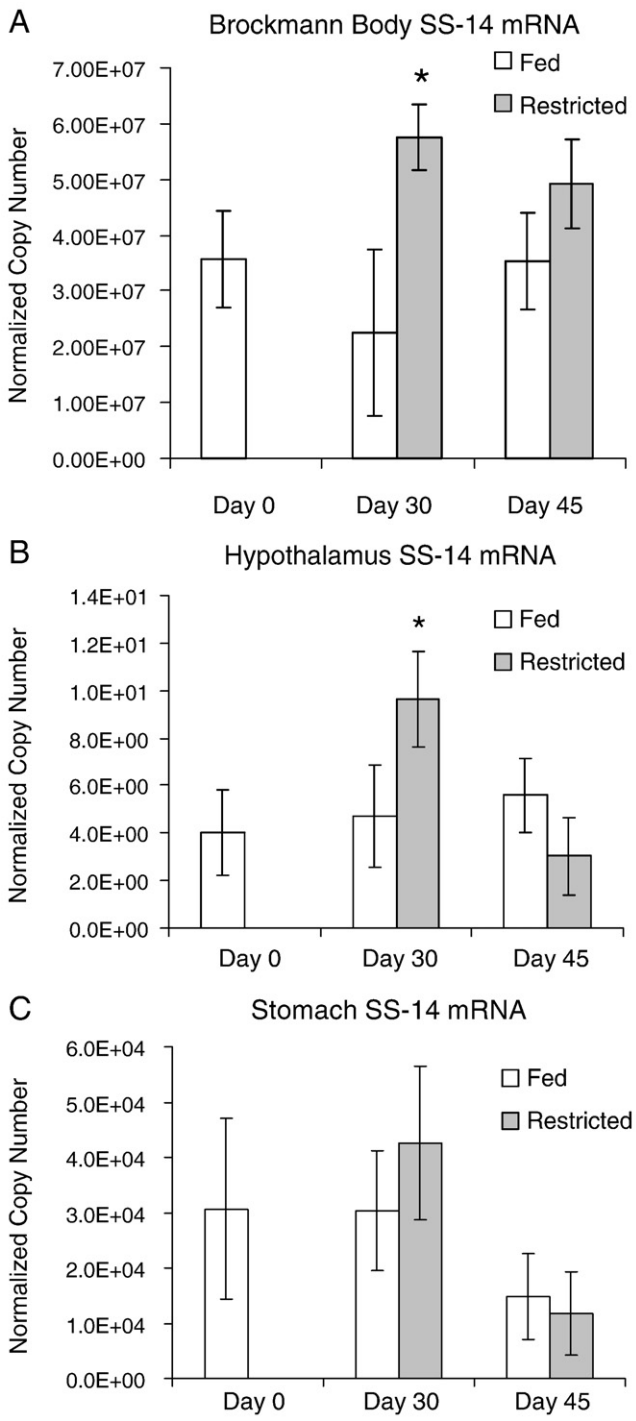

Fig. 4. (A) Brockmann body somatostatin 14 (SS-14) mRNA, (B) hypothalamus SS-14 mRNA, and (C) stomach SS-14 mRNA levels of Fed control (commercial catfish diet fed daily) and Restricted (not fed for 30 d followed by feeding a commercial diet for 15 d) catfish. Fish were sampled on d 0, 30, and 45. $N=8$ per treatment per time point. Standard error bars represent standard error of the mean and significant differences ($P<0.05$) are denoted by asterisks.

Restricted fish. Abundance of SS-22 mRNA was similar in the pancreas of Restricted and Fed fish at d 30 and 45 while SS-22 mRNA was not detected in the stomach or hypothalamus (Fig. 5).

4. Discussion

In the first study, catfish were fed daily, fed every other day, or fasted for 6 weeks. Feeding catfish daily, every other day, or every third day are common management strategies for feeding catfish under certain economic conditions (e.g., high feed costs) (Li et al., 2004). It is known that feeding every other day or every third day improves feed efficiency but also reduces growth (Li et al., 2004, 2005). What is not known is what effect decreasing feeding frequency has on the GH–IGF-I axis of channel catfish.

As expected, fed fish gained more weight than unfed fish or fish fed every other day. Differences in weight were apparent by 3 weeks and were magnified by 6 weeks. Unfed fish lost 47% and 63%, respectively after 3 and 6 weeks of restricted feeding while fish fed every other day gained 18% and 28%, respectively less weight compared to the fed fish. Interestingly, plasma IGF-I was significantly lower by 3 weeks only in the Unfed treatment. By 6 weeks, circulating levels of IGF-I were lower in both the Unfed and FEOD groups. Previous studies in salmon have shown that plasma IGF-I levels adjust to the amount of ration in 2–4 weeks (Larsen et al., 2001; Pierce et al., 2001). In another salmon study, fasting decreased plasma IGF-I at day 4 and completed decreasing by 2 weeks (Pierce et al., 2005). The plasma IGF-I response in catfish and salmon is slower than in mammals and chickens, where plasma IGF-I responds to fasting or re-feeding within 1–2 days (Frystyk et al., 1999; Isley et al., 1983; Morishita et al., 1993; Thissen et al., 1994). Pierce et al. (2005) suggested that the difference may be due to the slower metabolic rate of fish compared to mammals.

Liver IGF-I mRNA was also reduced after 6 weeks of feeding every other day or fasting. Fasting has been shown to reduce plasma IGF-I and liver IGF-I mRNA levels in several species of fish such as seabream (Perez-Sanchez et al., 1995), barramundi (Matthews et al., 1997), coho salmon (Moriyama et al., 1994; Duan, 1997), tilapia (Uchida et al., 2003; Fox et al., 2006); channel catfish (Small and Peterson, 2005), chinook salmon (Pierce et al., 2005), and grouper (*Epinephelus coioides*) (Pedroso et al., 2006). The correlation between liver IGF-I mRNA and plasma IGF-I levels after 6 weeks of feeding every other day or fasting supports a role for liver IGF-I production in the regulation of plasma IGF-I level. Furthermore, we have shown that liver IGF-I mRNA and plasma IGF-I are significantly reduced after 2 and 4 weeks of fasting in channel catfish (Small and Peterson, 2005). Liver samples were not taken at 3 weeks so it is not known if liver IGF-I mRNA levels in the fish fed every other day would have been similar to fed fish. In fasted salmon, liver IGF-I gene expression levels explained only 25 to 50% of the variation in circulating IGF-I levels (Pierce et al., 2005). A detailed time course examination into other components of the GH–IGF-I axis such as the insulin-like growth factor binding proteins may provide further insight into the regulation of IGF-I in channel catfish.

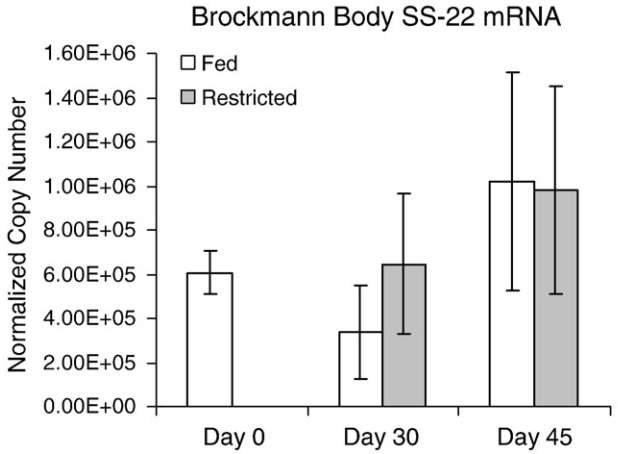

Fig. 5. Stomach somatostatin 22 (SS-22) mRNA levels of Fed control (commercial catfish diet fed daily) and Restricted (not fed for 30 d followed by feeding a commercial diet for 15 d) catfish. Fish were sampled on d 0, 30, and 45. $N=8$ per treatment per time point. Standard error bars represent standard error of the mean.

Less is known about the regulation of IGF-IRs in teleosts. We found that muscle IGF-IRa mRNA was similar among fed, unfed, and fish fed every other day. Two IGF-IR mRNAs (IGF-IRa and IGF-IRb) have been identified in species such as zebra fish (Maures et al., 2002) and rainbow trout (Norbeck et al., 2007) whereas only one receptor has yet been identified in channel catfish (Peterson et al., 2008). In two studies, fasting did not alter IGF-IRa or IGF-IRb mRNA levels in the muscle of rainbow trout (Gabillard et al., 2003; Norbeck et al., 2007). However, Chauvigne et al. (2003) showed that the IGF-IRa mRNA decreased while IGF-IRb mRNA remained relatively constant after 10 weeks of fasting in rainbow trout. Peterson et al. (2008) found no difference in abundance of muscle IGF-IRa mRNA in fast and slow-growing families of channel catfish The significance of the differential response in IGF-I sensitivity to fasting among different tissues is not clear. Unchanged levels of IGF-IRa in the muscle of growth restricted channel catfish may reflect protein sparing of the muscle during food restriction (Norbeck et al., 2007).

Similar to mammals, GH is regulated in part by negative feedback from IGF-I (Fruchtman et al., 2000; Perez-Sanchez et al., 1992). Many fish studies have reported that food restriction causes elevation of plasma GH levels in rainbow trout (Storebakken et al., 1991; Sumpter et al., 1991; Farbridge and Leatherland, 1992), gilthead sea bream (Perez-Sanchez et al., 1995; Meton et al., 2000), chinook salmon (Beckman et al., 2004); tilapia (Fox et al., 2006), hybrid stripped bass (*Morone chrysops* x *Morone saxatilis*) (Picha et al., 2008; Small et al., 2002), and channel catfish (Small and Peterson, 2005). Similarly, we found that pituitary GH mRNA levels were higher in both the fasted and fish fed every other day compared to the fed fish.

It is generally agreed that fasting contributes to a general state of GH resistance. Prior to week 6 in the current study, it is not known when pituitary GH mRNA was first elevated, but we have previously shown that pituitary GH mRNA does not change between 2 and 4 weeks of fasting, despite low levels of IGF-I at both 2 and 4 weeks (Small and Peterson, 2005). These results suggest that GH resistance developed between 2 and 4 weeks in fasted fish whereas GH resistance developed between 3 and 6 weeks in fish fed every other day. These results suggest that low plasma IGF-I levels alone do not account for the observed state of GH resistance. Other factors such as insulin-like binding proteins also may play a role in regulating levels of GH.

Fasting also induces a down regulation of growth hormone receptor (GHR) in most teleosts investigated to date. This has been demonstrated in black sea bream (*Acanthopagrus schlegeli*) (Deng et al., 2004), coho salmon (*Oncorhynchus kisutch*) (Fukada et al., 2004), and channel catfish (Small et al., 2006). In contrast, no effect of fasting was observed on hepatic GHR mRNA levels in tilapia (*Oreochromis mossambicus*) (Fox et al., 2006). In the current study, we found that liver GHR mRNA was lower in fasted catfish as well as the fish fed every other day. It should be pointed out that the catfish GHR has low sequence homology to both the type 1 and type 2 GHR reported for other species of fish. It is too early to speculate whether catfish have only one or two GHRs. The findings of this study suggests that fasting or even feeding every other day for 6 weeks alters the GH–IGF-I axis by increasing pituitary GH mRNA, down regulating GHR mRNA, decreasing plasma IGF-I, as well as hepatic IGF-I mRNA. Possible explanations as to why the GH–IGF-I axis was altered in fish that were fed every other day may include that mRNA does not always correspond to protein levels or that local (muscle) IGF-I may be more important than circulating levels or may play a compensating role when plasma IGF-I is reduced (Sjogren et al., 1999). Changes in locally-produced IGF-I may be more important than receptor levels, especially if the receptor is not regulated by nutritional state.

The objective of the second study was to understand the physiological functions of SS-14 and SS-22 in fed, fasted, and re-fed catfish. *In vivo* and *in vitro* inhibition of GH release by SS has been demonstrated in both mammals and teleost fish (Nelson and Sheridan, 2005). It has been shown that fasting modifies the SS signaling system by increasing plasma levels of SS and the mRNAs encoding them, altering the patterns of SS receptor mRNA expression, and increasing the number of high affinity hepatic SS-14 binding sites in rainbow trout (Ehrman et al., 2002; Slagter et al., 2005; Pesek and Sheridan, 1996). There is no clear explanation of why SS increases during fasting, despite high levels of GH that are also known to increase during fasting. It has been proposed that SSs may have extra-pituitary influences on the growth axis (Ehrman et al., 2002). For example, SS-14 and salmon SS-25 have been shown to reduce GH receptor number in rainbow trout hepatocytes *in vitro* (Very et al., 2008). Very et al. (2008) speculated that the extra-pituitary level of regulation functions to add a fine control to growth regulation, such that the growth-promoting action of GH would be uncoupled (e.g., IGF-I synthesis). Also, is it possible that high hypothalamic SS-14 levels during fasting may reflect more stored protein instead of levels actually released onto pituitary somatotrophs.

In the present study, fed fish gained 91.6 g/fish whereas Restricted fish gained 14.2 g/fish over the 45-d study. Fasting increased abundance of SS-14 mRNA in the hypothalamus and Brockmann bodies while abundance of SS-14 mRNA was unchanged in the stomach. After 15 d of re-feeding, abundance of SS-14 mRNA was similar in the Fed and Restricted fish in all tissues examined. The increase in levels of SS-14 mRNA in the hypothalamus and Brockmann bodies suggests that nutritional state regulates SS-14 in channel catfish, similarly as has been reported in rainbow trout. The function of SS-14 in the hypothalamus, Brockmann bodies, and stomach in catfish is not clear but SSs have been shown to modulate GH and thus growth of teleosts species. For example, exogenous treatment with SS-14-I decreases plasma GH concentrations in rainbow trout, coho salmon, and goldfish (Cook and Peter, 1984; Sweeting and McKeown, 1986; Diez et al., 1992; Very and Sheridan, 2001; Peterson et al., 2003). Further studies on the regulation of somatostatin receptor expression will also be needed to better understand the roles of SS-14 in the regulation of growth and metabolism.

Fasting did not change the abundance of SS-22 mRNA in the Brockmann bodies and SS-22 mRNA was not detected in the stomach or hypothalamus. Little is know about the function of SS-22 in channel catfish. It is known that the catfish SS-22 amino acid peptide differs in 7 out of 14 residues from SS-14 (Andrews et al., 1984; Oyama et al., 1980) and the cDNA for SS-22 have been identified in catfish (Magazin et al., 1982). The precursor encoding catfish SS-22 shows less than 30% similarity to goldfish PPSS-I, -II, and -III (Nelson and Sheridan, 2005). It has been demonstrated in goldfish, mammalian SS-14 and SS-28 were equipotent in inhibiting GH release from the pituitary; whereas catfish SS-22 had no effect on GH release (Marchant et al., 1987; Marchant and Peter, 1989). It is unclear from the current study the physiological function of SS-22 in food restricted catfish.

In summary, these studies provide evidence that nutritional state differentially regulates components of the GH–IGF-I axis in channel catfish. While verifying the effects of fasting, the first study also showed that feeding every other day for 6 weeks alters the GH–IGF-I axis by increasing pituitary GH mRNA, down regulating GHR mRNA, decreasing plasma IGF-I as well as hepatic IGF-I mRNA, with no change in muscle IGF-IRa mRNA. The second study showed that fasting increases levels of SS-14 mRNA in the hypothalamus and pancreatic islets and re-feeding restores their levels similar to fed control levels. SS-22 mRNA was not affected by fasting and its function is not known. Future work will need to examine SS receptors in specific tissues and determine if SS and their respective receptors are regulated in a coordinate fashion.

Acknowledgments

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