Effects of starvation and re-feeding on compensatory growth performance, plasma metabolites and IGF-I gene expression of Persian sturgeon (*Acipenser persicus*, Borodin 1897)

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Abstract

The effects of starvation and subsequent re-feeding on compensatory growth performance, blood serum metabolites and IGF-ImRNA expression in liver and muscle were investigated in juvenile Persian sturgeon. Growth indices including body weight, SGR, CF, and HSI significantly decreased after starvation. However, after re-feeding sturgeons that were starved for 1 week reached the same weight as the control, indicating that complete compensatory growth had occurred. Conversely, sturgeon in longer periods of starvation showed only partial growth compensation. HSI values decreased significantly during starvation, although they returned to the control fish levels after re-feeding. Plasma levels of glucose and insulin during starvation and re-feeding did not significantly change. This suggests that sturgeon is able to maintain glycaemia during starvation, probably due to their non-carbohydrates source dietary. Plasma total lipid level in un-fed treatments, however, was found to increase, possibly as a mechanism to utilise lipids as a fuel during starvation. IGF-I mRNA expression in liver and muscle increased during starvation and decreased after re-feeding. However, changes in the IGF-I mRNA expression were not significantly different among treatments. These results indicate that a periodic short-term starvation in Persian sturgeon does not adversely sacrifice overall fish weight gain and sturgeon can realise compensatory growth.

Keywords: Persian sturgeon, Feeding regime, Compensatory growth, Blood metabolite, IGF-I mRNA expression

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Introduction

Fish are able to cope with long periods of feed restriction due to limitation in forage base, weather events and during certain phases of their reproductive cycle (Davis and Gaylord, 2011). During starvation or fasting, fish employ various behavioural, physiological, and structural responses to mobilise energy reserves to cover their metabolic requirements. These may include allowing overall condition factor to decrease, changes in lipid content and blood metabolites, and/or muscle and organ mass (Navarro and Gutierrez, 1995). Overall decreases in growth are observed. However, when re-feeding resumes, fasted fish exhibit an exceptionally fast growth rates, known as compensatory (catch up) growth (Ali et al., 2003). Although not fully understood, this phenomenon may be through hyperphagia (increase in appetite) (Saether and Jobling, 1999; Chatakondi and Yant, 2001; Ali et al., 2003; Nikki et al., 2004), rapid protein synthesis (Quinton and Blake, 1990), and replenishment of energy reserves (Alvarez and Nicieza, 2005). From a practical perspective, exploitation of the compensatory growth response has been suggested as a way to increase productivity of finfish aquaculture (Hayward et al., 1997), as feeding programmes can be designed to limit rations of food while still overall achieving acceptable long-term growth trajectories of fish, thereby minimizing feeding costs (Hayward et al., 1997).

Growth in vertebrates, including fishes, involves a complex array of neuroendocrine processes [growth hormone (GH) – insulin- like growth factor I (IGF-I) axis] in relation to nutritional status. Growth hormone acts directly on target tissues by stimulating mitosis and other aspects of energy metabolism, and indirectly by regulating the production and release of IGF-I in the liver and other tissues (Fox et al., 2010). IGF-I has been shown to promote growth in teleosts (Chen et al., 2000; Degger et al., 2000), with studies indicating a significant and positive correlation between circulating IGF-I and growth rates (Jones and Clemmons, 1995; Larsen et al., 2001; Fox et al., 2006; De Santis and Jerry, 2007). The positive correlation between IGF-I levels and growth rate is more consistent than that between GH and growth, with GH levels being shown to become dissociated under certain conditions such as malnutrition, while the correlation between IGF-I and growth persisted (Duan et al., 2010). Food deprivation studies in fish show that the liver is one of the first organs to be affected (Power et al., 2000) and in several fish species prolonged fasting causes a reduction in plasma IGF-I and liver expression of IGF-I mRNA (Pierce et al., 2005; Small and Peterson, 2005), as well as subsequent activation of hepatic gluconeogenesis and reduction in the rate of glucose utilization (Navarro and Gutierrez, 1995). For these reasons it can be expected that nutritional status can regulate IGF-I expression in fish (Wood et al., 2005) and this gene may be a possible indicator of growth rate in fishes (Shimizu et al., 2000; Larsen et al., 2001).

While the relationship between IGF-I and nutritional condition has been well-

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characterized in teleost fish (Fox et al., 2010), there is no information about the role of IGF-I in regulating the nutrition status of non-teleost fishes such as sturgeons. In fact it is currently unknown whether IGF-I participates in maintaining metabolic homeostasis in sturgeon. Consequently, in the present study we partially cloned Persian sturgeon (*Acipenser persicus*) IGF-I cDNA and examined its level of expression using real time quantitative real-time PCR (qRT-PCR when sturgeon were subjected to various food deprivation treatments. We also investigated if the level of IGF-I mRNA and other biochemical parameters such as blood glucose could be used as a possible indicator of compensatory growth in Persian sturgeon.

Materials and methods

Animals and experimental conditions

375 juvenile Persian sturgeon (7 months old, mean initial weight 108 ± 0.63 g and length 31.73 ± 0.28 cm) were adapted to rearing conditions at the Shahid Beheshti Sturgeon Propagation Center, located in north of Iran. To study the effects of re-feeding on fasting, we used the experimental design previously described by Montserrat et al. (2007 a) with some modifications. Fish were randomly distributed to ensure no starting weight differences in 15 circular 1000-l outdoor tanks supplied with a mix of aerated well water and water originating from the Sefidrood River. Water quality parameters including DO, pH and temperature (using a Multi 34Oi meter, USA) were monitored in the experimental tanks daily at 8 am.

After initial acclimatization for 2 weeks, 25 fish per tank were randomly allocated to one of four fasting treatments (1 week (W1), 2 weeks (W2), 3 weeks (W3) and 4 weeks (W4)), or to a control (C) treatment. Three replicate tanks were created for each feeding treatment. Control treatment fish were fed until satiety with commercial pellets (BIOMAR, 1.9 mm) throughout the experiment. In the second phase of the trial, fish in the fasted treatments were subsequently fed to satiation 3 times a day for the last 4 weeks of the experiment. Fasted treatments were deprived of food in a manner so that all groups finished their fasting period at the same time and were re-fed simultaneously during the last 4 weeks of the experiment.

Sample collection

All fish were weighted individually to the nearest 0.1 g at the start and end of each experimental period (fasting and re-feeding). The fish were not fed 12 h prior to weight and were anaesthetized during sampling (Clove powder, 0.5 g/l). Fifteen fish per treatment were rapidly anaesthetized (Clove powder, 0.5 g/l) and killed by a blow to the head. Blood samples were taken from caudal vessels with heparinised syringes. Plasma samples were obtained after blood centrifugation (3000 rpm, 10 min, 4°C) and then stored at -20 °C until further analyses. Liver and white muscle samples were sampled, snap frozen and stored at -80 °C until analysis. Standard length (cm) and body weighting (g) were measured. Specific growth rates (SGR) for fish in the different treatments

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were calculated according to Houde and Schekter (1981) formula as follows:

SGR (SGR, %g day $^{-1}$) =100 × l n (w_2-w_1)/ t,

Where:

w_2 = weight after fasting or re-feeding and w_1 = weight before feeding (g), t is the interval (days) between weightings.

Condition factor (CF) - an indicator of body shape and percentage of weight gain was calculated as

CF=100 × [body weight / (body length$^{)3}$]

Finally hepato-somatic Index (HSI) was calculated as

HSI= 100 × [Liver weight (g)/body weight (g)]

RNA isolation and cDNA synthesis

RNA extraction was performed using BIOZOL reagent (Bioflux-Bioer, China) according to the manufacturer's instructions. Briefly total RNA was extracted by homogenizing frozen muscle tissues directly in BIOZOL reagent (Bioflux-Bioer, China) and precipitating into a solution containing 0.5 vol of RNA precipitation solution (1.2 M sodium chloride, 0.8 M disodium citrate, (Sambrook and Russell, 2001) and 0.5 vol of isopropyl alcohol. RNA quality and quantity was verified using a Nanodrop spectrophotometer (Nanodrop technology, Wilmington, DE, USA) via examination of absorbance ratios at OD 260/280 (range 1.98-2.06) and OD 260/230 (range 1.96-2.07) and by visual inspection of the integrity of both the 18S and 28S ribosomal RNA bands on a 1.5 % agarose gel. Samples were adjusted to a final concentration of 200 ng/µL and treated with *DNa*se I, RNase-free followed by an ammonium acetate precipitation. First strand cDNA synthesis was performed on 5 µg of DNase treated total RNA using a *M-MuLV* Reverse Transcriptase kit with 2.5 µM oligo(dT)20 (Resuehr , 2003). Complete removal of contaminating DNA was verified by PCR amplification of RNA samples using gene specific primers as a no-amplification control (NAC)

($C_{q\ (NAC\ control)} - C_{q\ (cDNA\ synthesis)} > 10$). After cDNA synthesis, RNA template was digested with 1 µL of RNase H within 20 µL of cDNA incubated at 37 °C for 30 min followed by enzyme deactivation at 70 °C for 10 min. To remove any potential PCR inhibitors and facilitate quantification, single strand cDNA was purified using Nucway spin columns (Ambion). The cDNA was then quantified using the Nanodrop (ND 1000, USA) and diluted to a final standardized concentration of 2 ng/µL prior to real time PCR analyses.

Real-Time PCR: Primer Design

To enable species specific qPCR primers, we first designed primer pairs for the IGF-I gene from cross-species comparative alignments of other sturgeon sequences available from Gene bank (Table 1). For the IGF-I, full or partial length sturgeon specific sequences were available for *Acipenser guldenstaedtii* (GenBank no.DQ201138), *A. ruthenus* (GenBank no.DQ329352), *Huso huso* (GenBank no.AB512770) and *A. barii* (GenBank

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no.FJ428828). Universal primers were designed for IGF-I within conserved regions in related species using (Primer3: http://frodo.wi.mit.edu/primer3/). Persian sturgeon specific sequence was PCR amplified in a 25 µL reaction containing 1 x NH_4-based reaction buffer , 2.0 mM $MgCl_2$, 200 µM dNTP mix, 0.2 µM of both forward and reverse primers, 1 u/µL of Taq DNA polymerase (Fermentas) and approximately 4 ng of Persian sturgeon liver cDNA. Amplification was performed in an Eppendorf thermal cycler (Mastercycler ep gradient, 96 plus, eppendorf, Germany) using standard conditions [3 min at 95 °C, 35 x (30 s at 95 °C, 30 s at primer specific annealing temperature, 45 s at 72 °C), 10 min at 72 °C]. Each PCR reaction was visualized for the amplification of a single product on a 1.5 % agarose gel before being cloned into Clone-Jet vector and sequenced (Bioneer, Daejeon, South Korea) in both directions. Sequence specificity was confirmed via a comparison of the homology to other sturgeon IGF-I sequences in the BLASTN database (WWW. http://blast.ncbi.nlm.nih.gov) (Altschul et al., 1990). To verify the efficiency of the reverse transcription (RT) and to exclude possibility of genomic DNA contamination, a β-2-microglubulin (β *2m*) fragment of 1900 bp primer was designed to span an intron (Table 1) (Patruno et al., 2008).

RT-qPCR validation and optimization

RT-qPCR reactions were performed in a final reaction volume of 12 µL using 1 x SYBR GreenER qPCR Supermix Universal (Invitrogen), 2.5 µM of ROX reference dye, 0.2 µM of each primer with 2 ng of cDNA template. All reactions were performed on an MJ research DNA engine with a Chromo 4 detector and utilizing Opticon Monitor 3.0 software (Bio-Rad). Each reaction was amplified in triplicate and consisted of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension for 30 s at primer specific temperatures (Table 1).

To validate real-time PCR primers, each gene's specific primer pair was run in duplicate, along a temperature gradient (58-68˚C) in the same plate. The primer specificity assay revealed that at 65 ˚C the IGF-I and two other housekeeping genes (*Eef*1 and β *2m* – see below) had low affinity for non-specific product (Table 2.a). A melting curve analysis was performed after every amplification program to verify specificity of target and absence of primer dimers and a no-template control (NTC) was included with each assay to verify that PCR master mixes were free of contamination (NTC > 39). To ensure PCR conditions were optimal, a log $_{10}$ dilution series was produced starting from undiluted cDNA pooled together from five randomly selected treatments for both tissues and two experimental steps. The dilution series was used to generate a standard curve by plotting the quantification cycle (C_q) for each dilution point against the starting quantity of cDNA. Standard curves were used to estimate efficiency (*E*) and reproducibility of the assay and were run in triplicate on each PCR run (Rasmussen,

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2001, Bustin et al., 2009) (Table 2.b). Reproducibility was represented by the R^2 value of the standard curve and was always greater than 0.99. Repeatability (intra-assay variability) was also measured as the standard deviation (SD) for the C_q variance (mean Cq= 25.90, SD= 0.40 and CV= 1.56) as indicated in MIQE guidelines (Bustin et al., 2009).The expression of each replicate was analysed on a single plate that contained all samples from all time points as well as a standard curve for determining amplification efficiency thereby ensuring that technical variation (inter-assay variability) was not confused with biological variation.

Elongation factor 1 (*Eef*1) and β-2-microglubulin (β *2m*) were initially investigated as possible reference genes for RT-PCR (Table1) through examination of mRNA expression stability in three liver and muscle samples for each time point (W1, W2, W3, W4 and C) chosen randomly from across the three replicate tanks. However these genes were observed to vary in their expression level due to treatment effects and were not used as normalisation genes in the current study.

Table 1: Name, sequence, and annealing temperature (T) of primers used in the present study to isolate the partial sequence of the *Acipenser persicus IGF-I* gene and to quantify this gene's transcript abundance through Real-time PCR.

Primer name	Primer sequence (5'-3')	Annealing T (°C)	Amplicon size (bp)	Application
APIGF1-F	GGC ATT CCG TCT TCC ATC AG	58	466	Gene discovery
APIGF1-R	CGG TAG TTC CTG TTG CCT GT	58		
B2m with intron-F	GTA CTT TCG TGG CGG CTC T	55	1900	Determining of DNA contamination
B2m with intron-R	TGC TCC ACT TTG CAG GAG TA	55		
APIGF-I-qPCR-F	CAG TTT GTG TGT GGG GAG AG	65	183	Real-Time PCR
APIGF-I-qPCR-R	GGC ACG TAC AGA GCG TGA G	65		
*Eef*1-qPCR-F	AGG AGG CCG CTG AGA TGG GGA AAG	65	155	Real-Time PCR
*Eef*1-qPCR-R	GTG GCC GGG AGC ATC AAT GAT GGT	65		
β *2m* - qPCR-F	AGC TGG GCA AGC CCA ACA CC	65	127	Real-Time PCR
β *2m* - qPCR-R	TGG TGG AAG GCC AGG TCG CT	65		

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Table 2.a: Primer specificity assays (raw C_q values) for the primer pairs IGF-I, *Eef*1 and β *2m*. The primer specificity assay revealed that at 65˚C, all 3 primer pairs had low affinity for the non-specific product respect to melting curve.

	Primer	IGF-I	*Eef*1	β *2m*
Temperature	58.00 ˚C	32.39	18.37	24.46
	58.9 ˚C	31.61	18.32	24.55
	60.08 ˚C	32.81	18.38	24.16
	62.3 ˚C	31.89	18.11	24.12
	64 ˚C	32.47	18.05	24.41
	65.4 ˚C	33.20	18.13	21.38
	66.5 ˚C	32.47	17.85	23.89
	67.2 ˚C	32.52	17.72	24.80
	68 ˚C	32.91	17.63	24.53

Table 2.b: Standardization of pool of muscle and liver tissues in different fasting and re-feeding treatments of IGF-I gene expression

	Serial dilution	Mean Cq	SD	CV (%)	E-1(%)	r^2 (%)
1	10 ng	24.38	0.03	0.13	101.4	99.2
2	5 ng	25.29	0.15	0.60		
3	2.5 ng	26.09	0.03	0.11		
4	1.25 ng	27.10	0.08	0.33		
5	0.625ng	28.09	0.16	0.59		

Mean quantification (Cq), standard deviation (SD), coefficient of variation (CV), amplification efficiency (E-1) and correlation coefficients (r^2 values).

Normalization Method

Since the two reference genes investigated did not have constant expression the raw C_q data was analysed using the ΔC_q method where the relative expression value was calculated according to the equation [Ratio $_{(test/calibrator)}$ = E $^{Cq(calibrator) - Cq(test)}$] where individual fish represent the test value and the average of all nine control fishes represent the calibrator value. This normalisation method was chosen as the preferred method as it has been previously shown to be a robust alternative when there is doubt about the stability of reference genes (De Santis et al., 2010).

Biochemical analyses of plasma parameters

As well as RT-PCR expression of IGF-I we also examined how several blood parameters responded to our fasting treatments. All blood assays were carried out using commercial diagnostic kits. Plasma glucose concentration was determined by a kit based on colorimetric glucose oxidase – peroxidase reaction (Pars azmoon, Iran) and Auto Analyzer (Technicon, USA) methods while plasma insulin levels were measured by immunoradiometery (IRMA) (Biosource, France) using a Gama counter (LKB, Finland).. Total lipid was determined by the enzymatic method (Pars Azmoon, Iran)

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and using an Auto analyser (Technicon, USA) (Thomas, 1998).

Statistical analysis

All data are reported as mean values ± standard error (S.E.). IGF-I mRNA expression data were expressed as the ratio. A Kolmogorov-Smirnov test was used to assess for normality of distributions. Normalized gene expression data passed Levenes test for homogeneity of variance before one-way ANOVA. In cases where variances were not uniform, a Kruskall-Wallis test was used. Post hoc multiple comparisons of means were performed using Dunnett's T3 test. Whenever appropriate, comparison of two means was also performed using a *T*-test or Mann-Whitney *U* test depending on homogeneity of variances between treatments. All statistical analyses were undertaken using IBM SPSS Statistics version 19 software. Differences between treatments were considered significantly different at $p<0.05$.

Results

Growth performance

The means of fish Treatment did not significantly differ in their initial weights at commencement of the experiment ($p<0.05$). Throughout the experiment, fish survival was 100%.

Mean final body weight of juveniles was significantly impacted by fasting period (Table 3). Fasting caused weight loss, with the mean weight of fish subjected to the three different fasting regimes, W2 (108.94 ±1.18 g), W3 (100.36 ±1.8 g) and W4 (99.68 ±2.3 g), being significantly lower than that of the control group (139.08±2.27 g) ($p<0.05$). After re-feeding, only the W1 treatment (125.02 ±5.06 g) was not significantly lighter than the final weight of control fish.

In addition, SGR was significantly higher in control (0.88) than in W2 (0.025), W3 (-2.28) and W4 (-0.26) treatments during fasting ($p<0.05$), while W1 (0.47) did not differ from control fish ($p>0.05$). Following 4 weeks re-feeding, SGR in all four groups, W1 (2.01%), W2 (2.01%), W3 (2.07%) and W4 (2.32%) when compared to the control group (C) (1.61%) significantly increased ($p<0.05$).

The condition factor (CF) during fasting periods were significantly higher in C and W1 groups (0.31%) than W2 (0.28%), W3 (0.28%) and W4 (0.27%). After the re-feeding period, the fish in 3 treatments (W1, W2 and W3, 0.35, 0.34 and 0.34, respectively) did not statistically differ from control (0.35) in their condition factor ($p>0.05$), while the W4 (0.33) treatment was statistically different from control group ($p<0.05$).

HSI values significantly decreased from that in Control fish during the fasting period in all non-feeding treatments (W1, W2, W3 and W4 ($p<0.05$). However, after the 4 week re-feeding period HSI values of fasted fish had rebounded to approach similar levels to that of controls, although slight significant differences still were evident between control and W3, W1 fasted treatments (Table 3).

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Table3: Mean weight, Specific Growth Rate (SGR), Condition Factor (CF) in Persian sturgeon reared under different fasting treatments: C (control treatment), W1 (1 week fasting), W2 (2 weeks fasting), W3 (3 weeks fasting) and W4 (4 weeks fasting), and after 4 weeks re-feeding period.

Treatment	Weight (g)		SGR (%day $^{-1}$)		CF (%)		HSI (%)	
	Fasting	Re-feeding	Fasting	Re-feeding	Fasting	Re-feeding	Fasting	Re-feeding
C	139.08±2.27^{b}	218.56±8.11^{b}	0.880±0.09^{b}	1.61±0.12^{a}	0.31^{b}	0.35^{c}	4.81±0.50^{b}	3.88±0.14^{a,b}
W1	125.02±5.06^{b}	219.33±2.76^{b}	0.470±0.15^{b}	2.01±0.10^{b}	0.31^{b}	0.35^{b,c}	2.51±0.30^{a}	4.24±0.11^{b}
W2	108.15±1.18^{a}	190.23±3.01^{a}	-0.025±0.05^{a}	2.01±0.02^{b}	0.28^{a}	0.34^{a}	1.99±0.12^{a}	3.64±0.10^{a}
W3	100.36±1.80^{a}	179.60±5.2^{a}	-2.280±0.05^{a}	2.07±0.04^{b}	0.28^{a}	0.33^{a,b}	1.93±0.05^{a}	3.92±0.11^{a,b}
W4	99.68±2.30^{a}	191.01±2.01^{a}	-0.260±0.02^{a}	-0.26±0.02^{a}	0.27^{a}	0.33^{a}	2.18±0.15^{a}	3.84±0.09^{a}

Note: Values are means (± SE) correspond to measurements of at least 15 fish at end of fasting and the end of 4 weeks re-feeding. The *p* value corresponds to the results of one way analysis of variance tests. Each different superscript(a,b,c) in the same columns indicate significant ($p < 0.05$) difference between groups.

Effects of fasting and re-feeding on plasma metabolites and insulin

Different fasting treatments did not significantly affect plasma glucose levels ($p>0.05$) (Fig.1a). Moreover, after the re-feeding period, the level of plasma glucose significantly increased from that of the fasting period ($p<0.05$), but its level between groups was similar.

Plasma total lipid level in the W3 treatment was significantly higher than other treatments. Following 4 weeks re-feeding, levels were significantly decreased compared to fasting treatments, but showed similar level between experimental treatments ($p>0.05$) (Fig. 1 b).

Different fasting periods likewise did not have a significant effect on plasma insulin (Fig.1c), after re-feeding periods treatment (W) groups showed similar insulin level to the C group and also fasting period ($p>0.05$).

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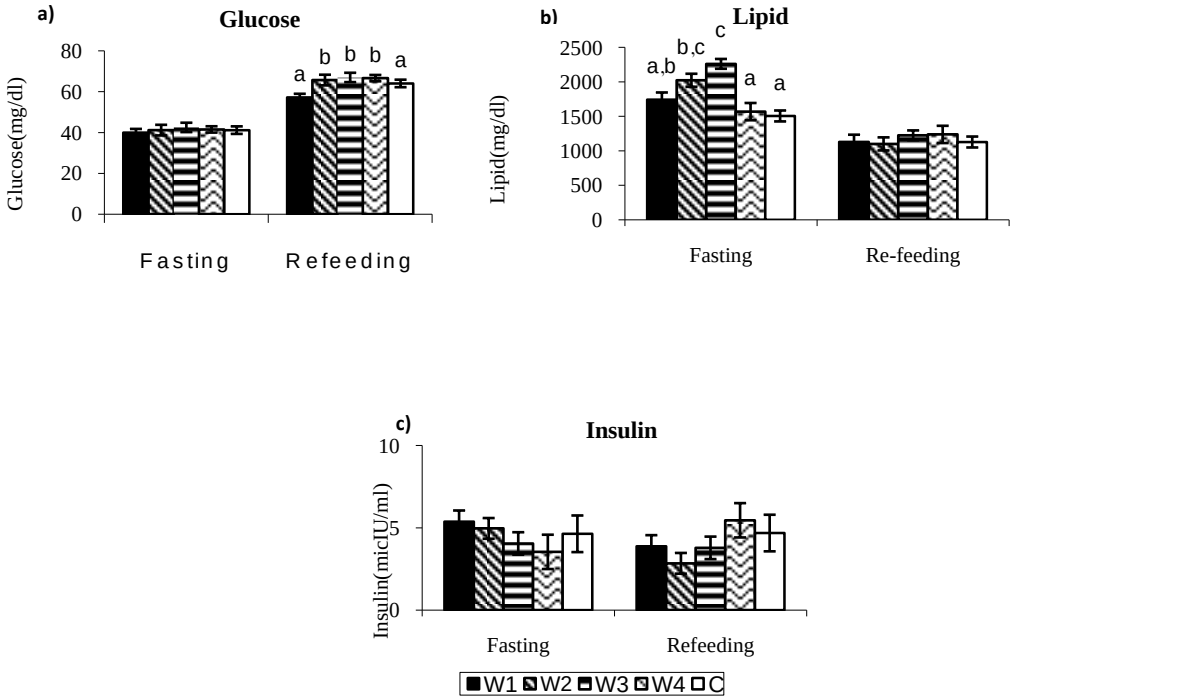


Figure 1: Changes in circulating level of (a) blood glucose, (b) lipid and (c) insulin of Persian sturgeon from controls (C), and from fasted fish for 1 (W1), 2 (W2), 3 (W3) and 4 (W4) weeks and after 4 weeks re-feeding period. Data is presented as mean ± SE. Differences between subscripts indicate significantly different means at p<0.05.

Gene expression

During fasting IGF-I mRNA levels in liver (Fig. 2a) increased in all 4 fasting treatments compared to controls. Likewise, after 4 weeks re-feeding IGF-I mRNA levels were still found to be elevated with the highest fold difference being 6.9 in W1. However, due to wide variation in expression level the transcript levels of IGF-I in liver were not significantly different between treatments in fasting and re-feeding and also fed versus fasted fish after 4 weeks of fasting (p>0.05). White muscle expression of IGF-I mRNA increased during fasting in W1, W2, W3 and W4 treatments and was at their highest level in W2 (4.7 fold change). After one month re-feeding, IGF-I mRNA levels in W1, W3 and W4 decreased (0.6, 0.6 and 0.4 fold, respectively) compared to control fish (1.5 fold change), and in W2 (1.9 fold) compared to controls (1.5 fold) (Fig.2b). Interestingly, IGF-I mRNA levels were not significantly different between different treatments in fasting and re-feeding and also fed versus fasted fish after 4 weeks of fasting in muscle (p>0.05).

Although there are IGF-I mRNA level fold changes between different treatments in fasting and re-feeding for both tissues (liver and white muscle), the results showed, these differences in expression to not be statistically significant (p>0.05).

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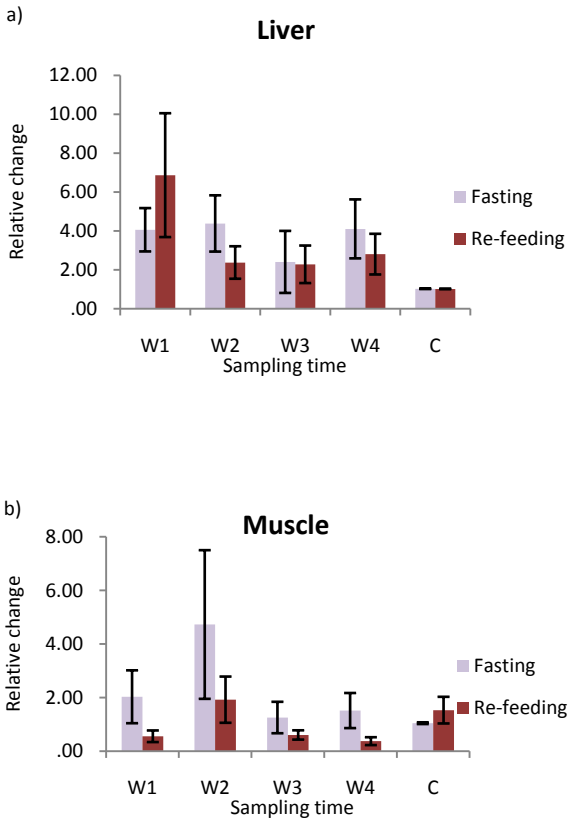


Figure 2: Changes in mean expression level of IGF-I mRNA in (a) liver and (b) muscle of Persian sturgeon juveniles from control (C), 1 (W1), 2 (W2), 3 (W3) and 4 (4W) weeks fasting or re-feeding.

Discussion

In fish, as in higher vertebrates, the endocrine control of growth is controlled in a large part by the growth hormone(GH)/ insulin-like growth factor-I (IGF-I) axis in relation to available nutrients (Fox et al., 2010). In this study, we investigated how nutritional restriction and subsequent re-feeding to induce compensatory growth affects the regulation of one of these genes, IGF-I, in liver and muscle tissues of the Persian sturgeon, the most important species endemic to Iranian coastlines. We also investigated how metabolites change with different starvation periods and subsequent re-feeding. In the present study, fasting for 2, 3 and 4 weeks resulted in significant reduction in body weight, specific growth rate (SGR) and condition factor in Persian sturgeon juveniles. After re-feeding for one month *ad libitum*, the W1 group achieved the same weight as controls and also showed higher SGR, indicating full growth compensation. However, W2, W3 and W4 showed higher SGR after re-feeding; their final weight was less than that of controls, suggesting only a partial compensation in growth over the period evaluated. However, fish in these treatments were exhibiting a higher SGR than controls and it is possible that if the experiment involved a longer period of re-feeding fish may have reached similar weights. The increase in SGR in fasting groups after re-feeding suggests that fixed periods of food deprivation and re-feeding may be useful strategies to induce

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compensatory growth in this species. The findings of the current experiment were similar to that shown in trout (*Onchorhynchus mykiss*) whereby full compensation after 1 week and partial compensation in 2 and 3 weeks were observed after fasting (Montserrat et al., 2007a). Similar findings have also been observed in barramundi (*Lates calcalifer*) (Tian and Qin, 2003) and hybrid tilapia, (*Oreochromis mossambicus* × *O. niloticus*) (Wang et al., 2000). However, in Atlantic cod (*Gadus morhua*) after 3 weeks fasting, complete compensation was observed (Jobling et al., 1994). We also observed decrease in HSI values during fasting periods, although HSI restored to those of the control treatment after 4 weeks re-feeding. This reduction in HSI during fasting suggests that the hepatic glycogen store of energy is mobilised and becomes reduced (Meton et al., 2003; Perez-Jimenez et al., 2007). Consequently, as also seen in teleosts, liver glycogen mobilised by food deprivation acts as a promptly available energy reserve in Persian sturgeon (Navarro and Gutierrez, 1995; Power et al., 2000; Meton et al., 2003; Perez-Jimenez et al., 2007).

Regulation in plasma glucose level in response to fasting is variable in fish. We found that glucose plasma levels for Persian sturgeon juveniles remained relatively constant over the 4 weeks of feed deprivation. The maintenance of blood glucose concentration during food deprivation indicates an active gluconeogenic process in the liver. Since glucose is an essential fuel for a number of tissues, it is particularly important that glucose levels be maintained throughout starvation (Gillis and Ballantyne, 1996). Consistent with the results of Hung (1991), the maintenance of the plasma glucose of sturgeon during four weeks of starvation suggests that sturgeon prefer to use dietary non-carbohydrates source instead of mobilizing their muscle protein for gluconeogenesis. The ability of Persian sturgeon to maintain plasma glucose levels in the fasting state is consistent with results for Lake sturgeon (*A. fulvescens*), in which a maintenance in glycaemia over 60 days of starvation was evident (Gillis and Ballantyne, 1996). In contrast to the present study, white sturgeon (*A. transmontanus*) showed a significant reduction in plasma glucose levels during eight weeks of starvation (Hung et al., 1997). The maintenance of glycaemia during starvation has been observed in many species of teleost (Hochachka, 1962; Zamit et al., 1979; Davis and Gaylod, 2010; Wood et al., 2010; Chatzifotis et al., 2011). However, many other teleosts species are unable to maintain plasma glucose level at extended starvation periods (Peterson and Small, 2004; Montserrat et al., 2007 a, b).

In most animals, fat stores are known to be mobilized in response to feed deprivation. As a result of fat mobilization, glycerol and free fatty acids (FFAs) are produced. Feed deprivation causes FFAs changes and their level is variable between different species. In the current research, total plasma lipid (FFAs) increased during the fasting period so that its level in W3 was significantly greater than in other groups.

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Increases in plasma lipid levels suggested that the lipids were the preferred nutrients for mobilization in juvenile Persian sturgeon during starvation periods. These observations are inconsistent with findings in sub yearling white sturgeon, in which lipid was the preferred nutrient for mobilization during starvation (Hung et al., 1997). However, in Lake sturgeon (*A. fulvescens*), starved fish had an ability to metabolise amino acids to produce energy (Gillis and Ballantyne, 1996). Other studies in finfish, reported increases in FFAs in fasting groups (Albalot et al., 2005; Montserrat et al., 2007a ; Preze-Jimnez et al., 2007), and decreases after re-feeding. Furthermore, in Persian sturgeon re-feeding after fasting restores total lipids (FFAs) to the levels observed in control fish, indicating that lipid mobilization had finished (Pottinger et al., 2003).

Insulin is an anabolic hormone which induces glucose uptake in liver and muscle (Machado et al., 1988). Insulin plasma levels throughout our experiment showed a decrease in W groups. The insulin plasma levels of the W4 group were the least; however, no significant differences between the W groups and control (p>0.05) were evident. After re-feeding, insulin plasma levels did not appear to change. Stability of insulin levels during fasting is consistent with that of plasma glucose. In contrast to our study, reduced plasma insulin levels following starvation and complete recovery after re-feeding are commonly observed in other species of teleosts, including cod and rainbow trout (Navarro and Gutireeez, 1995; Larsen et al., 2001).

The IGF-I peptide plays an important role in muscle somatic growth and development in vertebrates (Yakar et al., 2005), including fish (Castillo et al., 2004). Several studies have shown that the response of fish to fasting is an increase in GH mRNA, and decrease in hepatic IGF-I mRNA (Ayson et al., 2007). The response, however, varies depending on the species and duration of the fasting periods. As shown in Fig. 2, the IGF-I mRNA expression pattern in Persian sturgeon is influenced by both fasting periods and subsequent re-feeding. Although the levels were not statistically significant between different fasting and control treatment in both tissues (liver and muscle), hepatic IGF-I mRNA reached highest levels after 2 weeks of fasting and it remained higher during fasting. After re-feeding IGF-I expression decreased, but still remained higher compared to control fish (Fig. 2a). Studies in fish have indicated a different response to fasting and subsequent re-feeding in the case of IGF-I mRNA expression in liver and white muscle. Ayson et al. (2007) found an increase of hepatic IGF-I mRNA levels after 3 and 6 days of starvation before a reduction in IGF-I mRNA level at day 9 in rabbitfish (*Siganus guttatus*). Increases in hepatic IGF-I mRNA expression have also been reported in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) after fasting (Pitcha et al., 2008), whereas no significant changes were observed in IGF-I mRNA hepatic expression after fasting in carp (Tse et al., 2006). Gabillard et al. (2006) found significant differences after starvation in IGF-I mRNaA levels in hepatic and white muscle in rainbow trout. However, its expression had

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similarly recovered after re-feeding in both tissues.

In the present study, white muscle expression of IGF-I mRNA increased during fasting in W1, W2, W3 and W4 treatments and was highest in W2 compared to other groups. After one month re-feeding, IGF-I mRNA levels in W1, W3 and W4 decreased compared to controls, but was higher in W2 (Fig. 2b). Interestingly, IGF-I mRNA levels were not significantly different between different treatments in fasting and re-feeding and also fed versus fasted fish after 4 weeks of fasting in muscle (p>0.05). It can be concluded that longer periods of starvation are required to observe significant changes in IGF-I mRNA expression in non-teleosts species such as sturgeon. In mammals, e.g. rats, 3 days of starvation caused significant decreases (Thissen et al., 1994); however, in salmon, 3 to 4 weeks were required to observe a significant change in hepatic IGF-I mRNA (Duan and Plisetskaya, 1993).

Regarding to increased IGF-I mRNA levels during fasting in Persian sturgeon in the present study, this suggests a particular need for this hormone for the chemical break down of food (energy reserves) by including the secretion of the key digestive enzymes and facilitates the absorption of nutrients (Ayson et al., 2007). There is evidence in humans that exogenous IGF-I can induce lipase activity (Tremblay et al., 2001).

Our findings regarding liver and muscle IGF-I mRNA expression in Persian sturgeon are not consistent with what has been observed in teleosts whereby IGF-I mRNA decreased with starvation (Fox et al., 2006; Terova et al., 2007; Fox et al., 2009).

However, this is the first observation of its kind in a Acipenseriformes and further studies are needed in this species and other closely related species to confirm if the pattern of regulation of IGF-I that was observed is unique to sturgeon. In conclusion, re-feeding of previously fasted Persian sturgeons juveniles induced compensatory growth and the degree of response was dependent on the length of food deprivation. However, one week fasting and 4 weeks re-feeding appears to be the best feeding deprivation strategy to recover weight. Our data indicates that fat stores and liver glycogen, are clearly mobilized during fasting in order to assume metabolism. The differential regulation in liver and muscle expression of IGF-I mRNA did not change significantly during starvation and re-feeding. It can also be concluded that a periodic short-term starvation in Persian sturgeon juveniles may be a useful strategy to lower feeding costs, or to deal with short-term water quality problems that may benefit from reductions in feeding, as sturgeon rapidly recover weight losses through compensatory growth once re-feeding commences.

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