Insulin-like growth factor I gene polymorphism associated with growth traits in beluga (*Huso huso*) fish

Zeinaddini Meymand Z.[1]; Yeganeh S.[1*]; Rahimi-Mianji Gh.[2]; Farhadi A.[2]

Received: May 2016 Accepted: August 2016

Abstract

The aim of the present study was to detect polymorphism in Insulin like growth factor-I (IGF-I) gene of beluga (*Huso huso*) fish using PCR-SSCP technique and also investigation of its association with growth traits (condition factor, body length and weight). A total of 150 specimens of beluga were randomly selected and DNA was isolated from caudal fin using modified salting out method. Then two fragments of 171 and 362 bp from 5'-UTR and 3'-UTR regions of IGF-I gene were amplified, respectively. Genotyping of individuals by SSCP technique showed five banding patterns of A, B, C, D and E for 5'-UTR region with the frequencies of 29.2, 0.76, 16.92, 51.53 and 10% respectively in one year-old and three banding patterns of A, C and D with the frequency of 45, 10 and 45% for two year-old fish. Also three banding patterns (A, B and C) were seen for 3'-UTR region with the frequency of 62.3, 27.69 and 10.76% in one-year-old and 20, 60 and 20% in two year-old fish. The A banding pattern in 3'-UTR and D banding pattern in 5'-UTR sites were the most frequent pattern in the studied beluga population. The association analysis using SAS statistical software indicated no significant association between observed banding patterns and growth traits (body length, weight, and condition factor) in beluga. Considering the important role of IGF-I as a probable candidate gene affecting growth related traits, these marker sites should be studied more in larger sample sizes and also in other regions of the gene.

Keywords: Polymorphism, Beluga, 3'-UTR, 5'-UTR, PCR-SSCP, Growth traits.

1- Department of Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

2- Laboratory for Molecular Genetics and Animal Biotechnology, Department of Animal Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

*Corresponding author's Email:skyeganeh@gmail.com

Introduction

Among the various Caspian Sea sturgeons, beluga is of great importance in terms of fishing activities because of high caviar quality and superior economic value and also due to the rapid growth and adaptation to cultivation conditions (Peykan Heyrati *et al*., 2010; Bahmani *et al.*, 2016).

Somatogenesis is a polygenic trait that eventuates a series of physiological pathways regulating energy metabolism and muscle growth. Among the possible pathways regulating growth in vertebrates, somatotropic axis genes and a sub-family of transforming growth factors are considered as candidate genes in livestock and fish (De Santis and Jerry, 2007). Although production traits are influenced by several genes, in recent years significant tendency in identifying and implementing major genes associated with production traits have been conducted. From the major genes that have been assessed so far, growth hormone gene, growth hormone receptor and beta growth factor family have been noted. Since the somatotropic axis have a central role in growth regulation, any loci influencing the expression of hormones, growth factors or peptides in this system can be represented as a potential candidate gene with a particular importance in the growth traits (De Santis and Jerry, 2007). In recent decades, significant advances have been made in statistical methods and molecular genetics to identify genes or genomic regions influencing economic traits (Anderson, 2001). IGF-I is a single-chain polypeptide with significant structural similarity to pro-insulin (Peykan Heyrati *et al*., 2010).

IGF impacts mitogenic and metabolic processes such as glucose absorption and it is believed that have an important mediating role between GH and stimulating somatic growth in muscle in vertebrates (De Santis and Jerry, 2007). By affecting muscle, liver, adipose tissue, intestines, brain and many tissues, the IGFs enhance the growth of tissues (Kolangi Miandare *et al*., 2013). IGF-I is produced mainly in the liver. The main promoter of the synthesis and release of the IGF-I from the liver is GH which is released from the anterior pituitary under control of somatotropin and somatostatin hormone, while the IGF-I in particular prevents the secretion of GH through a negative feedback mechanism (Eppler *et al*., 2011). Many studies have shown that the GH acts through the intermediary of the IGF-I. Except the liver, IGF-I is produced and released in many tissues of the fish in auto and -paracrine manners (Salmroudi *et al*., 2013; Yarmohammadi *et al.*, 2013). The GH stimulates the synthesis of the IGF-I from the liver in such a way that some or all of the physiological activities of GH can be done (Mancera *et al*., 1998). IGF-I gene transcription has been observed in many tissues including liver, pancreas, gastrointestinal tract, kidney, anterior kidney, gill, ovary, testis, and the brain

of bony fishes (Bahrami Kamangar *et al*., 2010).

In addition to structural protection, human studies showed that the biological ability of the IGF-I is conserved within a few hundred million years of evolution (Duan, 1998).

There is evidence that suggest that GH regulates the IGF-I gene expression in teleost fish (Duan and Hirano, 1990). It has been shown that the IGF-I, stimulates the synthesis of DNA, sulfation of cartilage and protein synthesis, increasing adaptation to seawater, and stimulates spermatogenesis and the final oocyte maturation in fish. Both IGF-I and II stimulate proteoglycan synthesis in mammals (Duan, 1998).

Due to the important role of IGF-I, it is an important candidate gene for growth traits in aquatic animals. DNA polymorphisms in the IGF-I gene are considered as effective genetic markers for growth traits (Li *et al*., 2009a) and the marker assisted selection (MAS) provides the opportunity to develop a more efficient system by the indirect selection of genetic factors in aquaculture (Hu *et al*., 2012). It has been shown that a polymorphic CA repeats in the first intron of the IGF-I was effective on the plasma IGF concentration in pigs (Estany *et al*., 2007).

The main effort in this research was to identify the IGF-I gene polymorphism with respect to the role of this gene as a candidate gene for growth traits in beluga, an important fish in the production of caviar in Iran and in the world.

Materials and methods

Sampling, DNA extraction and phenotypic measurements

Fish were obtained from the Cooperative Company of Gharahboroun Sturgeon Fish (Mazandaran, Sari), where they were cultured indoor in fiber glass ponds (6 m^3) at a density of 3 fish m^{-3}. Two to three grams of caudal fin tissue was obtained randomly from 150 individuals, including 120 one year olds and 30 two years old beluga. After sampling, the samples were fixed in 96% ethanol and stored in -20 °C until DNA extraction. The DNA was extracted by modified salting out method (Miller *et al*., 1988) and its quality was determined using agarose gel electrophoresis. Also, the weight and length of studied fish were measured to determine condition factor (CF). The CF is one of the factors of obesity that is calculated by the following formula:

$W / L^3 *100$

Where W is the weight of fish in grams and L is the length of fish in centimeters.

PCR amplification of 5'-UTR and 3'-UTR regions of beluga IGF-I gene

To amplify the fragments with 171 and 362 bp from the 5'-UTR and the 3'-UTR regions of the IGF-I gene, respectively, two specific primer pairs were designed by Oligo 7 software from the reference sequence (Accession number:

AB5127701.1). The primer sequences were: 5' TGCTAAATCTCGCTTCCCTC-3' (forward) and 5'-CCCAAAACTACAAAGACCAG-3' (reverse) for 171 bp fragment of the 5'-UTR region and 5'-GGTTCACTTGTATTGTTCATGC-3' (forward) and 5'-CCATTGCTGTATTTGAACCTC-3' (reverse) for 362 bp fragment of the 3'-UTR region.

The PCR thermal condition was as follows: 3 min at 95°C primary denaturation followed by 35 cycles at 95°C for 30s, 55 and 60°C for 30s (annealing temperature for 5'-UTR and 3'-UTR, respectively), 72°C for 45 s and final extension at 72°C for 5 minutes. The PCR reaction mixture contained 150 ng of template DNA, 3 mM $MgCl_2$, 50 pM of each primers, 100 mM dNTP, 1 unit Taq DNA polymerase, 2.5 µL 1x PCR buffer and H2O in a final volume of 25 µL. The accuracy of PCR reaction was confirmed by electrophoresis of PCR products on 1% agarose gel ethidium bromide staining.

The SSCP assay

Single strand conformation polymorphism (SSCP) assay was used to detect the allelic variation in regulatory regions of IGF-I gene. For this purpose, amplified fragments were analyzed on 10% polyacrylamide gel electrophoresis so that, 5 µL of PCR product was mixed with the same volume of SSCP buffer (800 µL formamide, 190 µL glycerol, 2 µL EDTA (0.5 M) and 10 µL bromophenol) and was heated for 5 min at 95°C in a thermo cycler. Then, the mixture was immediately transferred to a cold plate for 10 min before running in polyacrylamide gel. The denatured PCR products were electrophoresed with a voltage of 340 volts at 4°C for 18 hours. After electrophoresis, the gels were stained with Silver nitrate. Genotyping of each sample was done by direct visualization of DNA bands on poly acrylamide gel after silver nitrate staining.

Statistical analysis

The marker-trait association between observed banding patterns and growth traits (body weight and length and also CF) was analyzed by general linear model (GLM) procedure of SAS 9.1 statistical software by the following model:

$$Y_{ij} = \mu + G_i + A_j + e_{ijk}$$

Where Y_{ij} is the value observed for each trait, μ is mean of trait, G_i is effect of the desired banding pattern, A_j is the covariate effect of age and e_{ijk} is the random error. Also, comparisons between the means of each banding patterns were performed using Duncan's multiple range test.

Results

The quality of extracted DNA assessed by agarose gel electrophoresis is shown in Fig. 1.

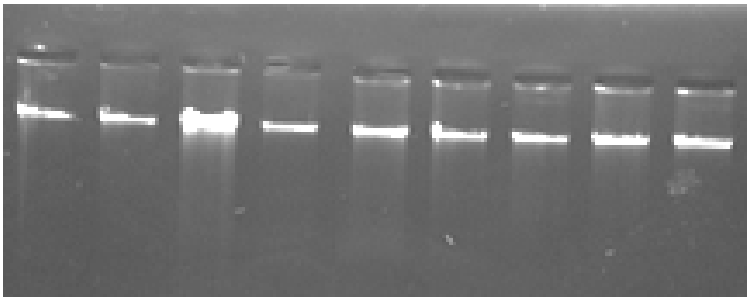

Figure 1: Extracted DNA electrophoresis bands on agarose gel.

Amplification of targets and genotyping

Two fragments of 171 and 362 bp lengths from 5'-UTR and 3'-UTR regions of IGF-I gene were amplified successfully. The accuracy of PCR reaction was determined by electrophoresis of amplified products on 1.5% agarose gel along with DNA size molecular marker (Fig. 2).

The SSCP genotyping showed 5 and 3 banding patterns for 5'-UTR and 3'-UTR of beluga fish IGF-I gene, respectively (Figs. 3 and 4)

Results of statistical analysis

The banding pattern frequencies of studied regulatory regions of IGF-I in beluga were 29.2, 0.76, 16.92, 51.53 and 10% for A, B, C, D and E, respectively (for one-year old fishes) and 45, 10 and 45% for A, C and D, respectively (for two-year old fishes) in 5'-UTR. Also, three banding patterns of A, B and C for 3'-UTR showed frequencies of 62.3, 27.69 and 10.76%, respectively (for one year olds) and 20, 60 and 20%, respectively (for two-year olds). The A genotype at the locus 3'-UTR and D for the locus 5'-UTR were of the highest frequency of genotypes (Tables 1 and 2).

Results of the marker-trait analysis showed no significant association between different banding patterns of beluga IGF-I polymorphisms and body length, weight and CF (Table 3).

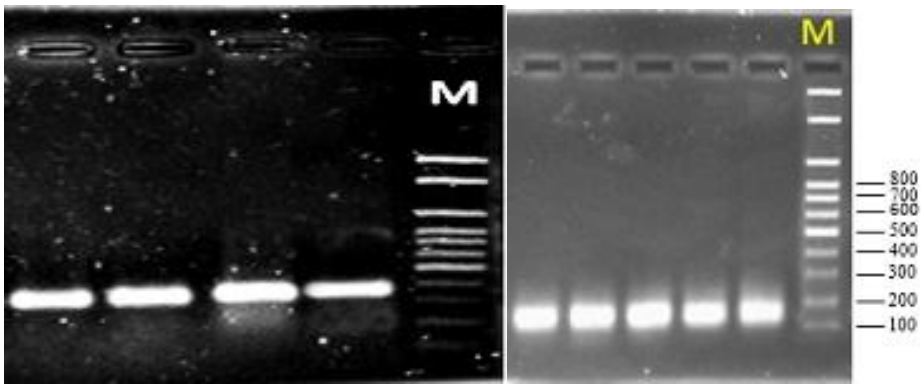

Figure 2: PCR product of the 5'-UTR (right) and 3'-UTR (left) of the IGF-I gene in beluga fish, M: molecular weight marker (mi-E8200s).

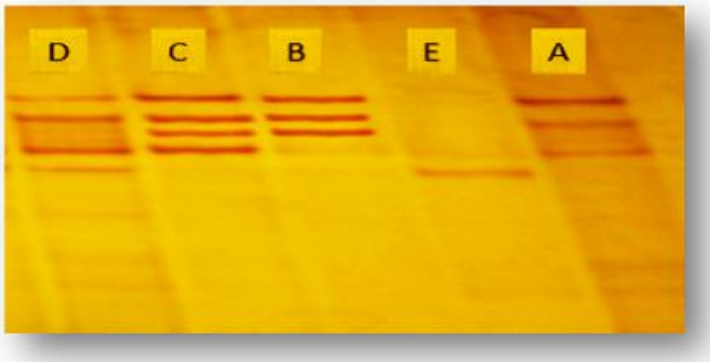

Figure 3: Banding patterns observed for the 5'-UTR region of beluga fish IGF-I.

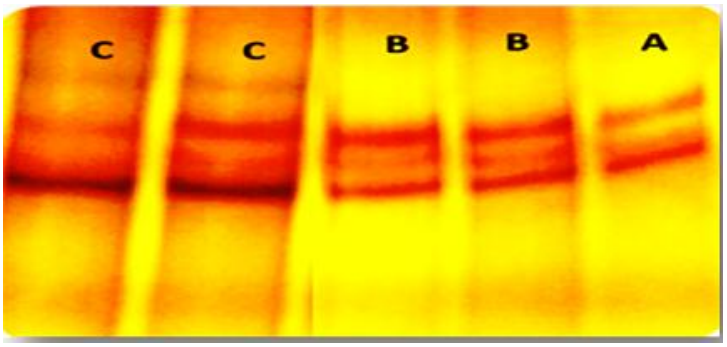

Figure 4: Banding patterns observed for the 3'-UTR region of beluga fish IGF-I.

Table 1: Frequency of observed banding patterns for the 5'-UTR region of beluga fish IGF-I.

Frequency (%) Two year olds (30 pieces)	Frequency (%) One year olds (120 pieces)	Banding patterns
45	29.2	A (38 pieces)
-	0.76	B (1 piece)
10	16.92	C (22 pieces)
45	51.53	D (76 pieces)
-	10	E (13 pieces)

Table 2: Frequency of observed banding patterns for the 3'-UTR region of beluga fish IGF-I.

Frequency (%) Two year olds (30 pieces)	Frequency (%) One year olds (120 pieces)	Banding pattern
20	62.3	A (84 pieces)
60	27.69	B (48 pieces)
20	10.76	C (18 pieces)

Table 3: Means of different banding patterns of IGF-I 3'-UTR and 5'-UTR regions for condition factor (CF) trait of beluga fish.

CF (g cm^{-3})		Banding patterns
3'-UTR	5'-UTR	
0.41	0.46	A (38 pieces)
0.45	0.38	B (1 piece)
0.45	0.4	C (22 pieces)
-	0.4	D (76 pieces)
-	0.4	E (13 pieces)
0.13	0.54	*p*-value
0.0062	0.0095	SEM

Discussion

In several studies it was shown that IGF-I affects weight and body size in pigs, cattle and dogs (Casas Carrillo *et al*., 1997, Machado *et al*., 2003, Sutter *et al*., 2007). Despite the fact that the IGF-I plays an important physiological role in the growth of fish, overall few reviews have been done in the case of the relationship between genetic marker loci in the IGF-I and growth traits in fish (Li *et al*., 2009b).

The regulatory regions of the genes such as 5'- and 3'-UTR sequences regulate gene expression at the transcriptional level, so that polymorphisms in this area may affect the IGF-I plasma concentrations by changing mRNA frequency of the IGF-I gene (Li *et al*., 2009b; Hu *et al*., 2012). In the current research in 3'-UTR of beluga IGF-I gene three banding patterns were observed. As pattern A was the most prevalent in the beluga species there was no significant relationship between the genotype patterns of the 362-bp fragment of the 3'-UTR and growth traits studied in this research. It seems that more work must be done, including a larger sample size and also including other gene regions such as exon and introns to assess more exactly the relationship between polymorphisms and growth traits.

Hu *et al.* (2012) detected an insertion/deletion (in/del) polymorphism of 79 bp of the IGF-I gene by PCR and sequencing in common carp. After genotyping, DD genotypes were mainly associated with maximum body length, body weight and net weight. Polymorphism in the promoter region and also mutations in coding region have a direct association with functional traits affected by candidate gene (Ge *et al*., 2001). Study of allelic polymorphism of the IGF-I in 171 bp fragment from the 5'-UTR showed 5 banding patterns. The genotypes D and A showed the highest frequency.

The relationship between the IGF-I gene promoter polymorphism with different traits have frequently been reported in humans and domestic animals (Curi *et al*., 2005; Islam *et al*., 2009; Li *et al*., 2009b). A single nucleotide polymorphism (SNP) was identified in promoter of the IGF-I in Arctic char (*Salvelinus alpinus* L.) fish but no association were found between this SNP and initial growth of the young fish (Tao and Bolding, 2003). The results of their study were in concordance with the current study. A dinucleotide repeat (CA) polymorphism in the 5 region of the IGF-I gene in cattle and in pigs was detected that was related to annual weight gain in cattle (Moody *et al*., 1994).

The results of the present study showed significant variation in the IGF-I gene of the beluga. No statistical association between the studied sequences and growth traits could be possibly due to the low number of samples or may be due to larger effects of other growth affected genes such as GH, GHR, GHRH and GHIH (Murkaeva, 2008).

Therefore, more studies with a more desirable combination of the number of samples evaluated with exon and intron regions are recommended.

References

Anderson, L., 2001. Genetic dissection of phenotypic diversity in farm animals. *Nature Reviews Genetics*, 2, 130-138.

Bahmani, M., Ghasemi, R. and Yousefi Jourdehi, A., 2016. Effects of dietary isoflavone-genistein on hematological and immunological parameters in pre-brood stock beluga, *Huso huso*. *Iranian Journal of Fisheries Sciences*, 15(**1**), 390-401.

Bahramikamangar, B., MojaziAmiri, B., Rasaee, M.J., Abtahi, B. and Bahmani, M., 2010. Immunolocalization of Insulin-Like Growth factor-I and its immunoreactivity during ovary developmental stages of Persian Sturgeon, *Acipenser Persicus. Yakhteh Medical Journal*, 11(**4**), 441-432.

Casas-Carrillo, E., Prill-Adams, A., Price, S. G., Clutter, A. C. and Kirkpatr- ick, B.W., 1997. Relationship of growth hormone and insulin-like growth factor-1 genotypes with growth and carcass traits in swine. *Animal Genetics*, 28(**2**), 88-93.

Curi, R.A., Oliveira, H.N., Silveira, A.C. and Lopes, C.R., 2005. Effects of polymorphic microsatellites in the regulatory region of IGF-1 and GHR on growth and carcass traits in beef cattle. *Animal Genetics*, 36, 58–62.

De-Santis C. and Jerry D.R., 2007. Candidate growth genes in finfish-where should we be looking? *Aquaculture*, 272, 22–38.

Duan, C. and Hirano, T., 1990. Stimulation of ^{35}S-sulfate uptake by mammalian insulin-like growth factor I and II in cultured cartilages of the Japanese eel, *Anguilla japonica*. *Journal of Experimental Zoology*, 256, 347–350.

Duan, C., 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. *The Journal of Nutrition*, 128(**2**), 306-314.

Eppler, E., 2011. The insulin-like growth factor I (IGF-I) within the bony fish pituitary: New morphofunctional and phylogenetic aspects. *The Open Neuroendocrinology Journal*, 4, 43-50.

Estany, J., Tor, M., Villalba, D., Bosch, L., Gallardo, D., Jiménez, N., Altet, L., Noguera, J.L., Reixach, J., Amills, M. and Sánchez, A., 2007. Association of CA repeat polymorphism at intron 1 of insulin-like growth factor (IGF-I) gene with circulating IGF-concentration, growth, and fatness in swine. *Physiological Genomics*, 31, 236–243

Ge, W., Davis, M.E., Hines, H.C., Irvin, K.M. and Simmen, R.C.M., 2001. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and

growth traits in Angus cattle. *Journal of Animal Science*, 79, 1757–1762.

Hu, X., Li, Ch. and Shi, L., 2012. A novel 79-bp insertion/deletion polymorphism in 3′-flanking region of IGF-I gene is associated with growth-related traits in common carp (*Cyprinus carpio* L.). *Aquaculture Research*, 44(**10**), 1632-1638

Islam, K., Vinsky, K.M., Crews, R.E.E., Okine, S., Moore, S. and Crews, D.H., 2009. Association analyses of a SNP in the promoter of IGF1 with fat deposition and carcass merit traits in hybrid, Angus and Charolais beef cattle. *Animal Genetics*, 40, 766–769.

Kolangi Miandare, H., Farahmand, H., Akbarzade, A., Ramezanpour, S., Kaiyae, H., Miyazatoe, M., Rytkonen, K.T. and Nikinmaa, M., 2013. Developmental transcription of genes putatively associated with growth in two sturgeon species of different growth rate. *General and Comparative Endocrinology*, 182, 41-47.

Li, X.H., Bai, J.J., Ye, X. and Hu, Y.C., 2009a. Polymorphisms of intron 1, 3 and 4 of insulin-like growth factor I gene in largemouth bass. *Journal of Shanghai Ocean University*, 18(**1**), 8-13.

Li, X.H., Bai, J.J., Ye, X., Hu, Y.C., Li, S.J. and Yu, L.Y., 2009b. Polymorphism in the 5´ flanking region of the insulin-like growth factor-I gene are associated with growth traits in largemouth bass *Micropterus salmoides*. *Fisheries Science*, 75(**2**), 351-358.

Machado, M.B.B., Alencar, M.M., Pereira, A.P., Oliveira, H.N., Casas, E., Coutinho, L.L. and Regitano, L.C.A., 2003. QTL affecting body weight in a candidate region of cattle chromosome 5. *Genetics and Molecular Biology*, 26, 259–265.

Mancera, J.M. and McCormick, S.D., 1998. Review Osmoregulatory actions of the GH: IGF axis in non-salmonid teleosts. *Comparative Biochemistry and Physiology Part B*, 121, 43–48.

Miller, S.A., Dykes, D.D. and Polesky H.F., 1988. A simple salting out procedure for extraction DNA from human nucleated cells. *Nucleic Acids Research*, 16(**3**), 1215.

Moody, D.E.D., Pomp, S.N. and MacNeil, M.D., 1994. Characterization of DNA polymorphisms and their associations with growth and maternal traits in line 1 Hereford cattle. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, Canada, 7–12 Aug 1994, 21, 221–224.

Murkaeva, A., 2008. Structure, evolution and expression of the duplication growth hormone genes of common carp (*cyprinus carpio*). Zur erlagung des akademischen grades. Doctor Rerum Naturalium, 1-180.

Peykan Heyrati, F., Mojazi Amiri, B. and Farahmand, H., 2010. Sequencing of IGF-I in Beluga (*Huso huso*), *Modern Genetics Journal*, 4(**3**). 17-25.

Salmroudi, M., Pourkazemi, M., Khoshkholq, M. and Azizzadeh pormehr, L., 2013. Identification the coding sequences of growth hormone gene of Persian Sturgeon (*Acipenser persicus*).12th Iran Genetic Congress.

Sutter, N.B., Bustamante, C.D., Chase, K., Gray, M.M., Zhao, K., Zhu, L., Padhukasahasram, B., Karlins, E., Davis S., Jones, P.G., Quignon, P., Johnson, G.S., Parker, H.G., Fretwell, N., Mosher, D.S., Lawler, D.F., Satyaraj, E., Nordborg, M., Lark, K.G., Wayne, R.K. and Ostrander, E.A., 2007. A single IGF1 allele is a major determinant of small size in dogs. *Science*, 316, 112–115.

Tao, W.J. and Boulding, E.G., 2003. Associations between single nucleotide polymorphisms in candidate genes and growth rate in Arctic char (*Salvelinus alpinus* L.). *Heredity*, 91, 60–69.

Yarmohammadi, M., Shabani, A., Pourkazemi, M., Soltanloo, H., Imanpour, M.R., Ramezanpour, S., Smith, Keune, C. and Jerry, D.R., 2013. Effects of starvation and re-feeding on compensatory growth performance, plasma metabolites and IGF-I gene expression of Persian sturgeon (*Acipenser persicus*, Borodin1897). *Iranian Journal of Fisheries Sciences*, 12(**2**), 465-483.