MOLECULAR MECHANISM OF GROWTH DIVERSITY FOR THE FIRST HYBRID GENERATION INDIVIDUALS OF GRASS CARP (*CTENOPHARYNGODON IDELLUS*) (♀) × BARBEL CHUB (*SQUALIOBARBUS CURRICULUS*) (♂)

LV, L. G.[1,2#] – XU, Y.[1,2#] – XU, B. H.[1,2*] – LIU, Q. L.[1,2*] – XIAO, T. Y.[1,2] – LIU, Y.[1,2] – SU, H.[1,2]

[1]*Hunan Engineering Technology Research Center of Featured Aquatic Resources Utilization, Hunan Agricultural University, Changsha 410128, China*

[2]*Collaborative Innovation Center for Efficient and Health Production of Fisheries in Hunan Province, Changde, Hunan 415000, China*

[#]*The authors contributed equally to this work*

[*]*Corresponding authors*
e-mail: xbht568@126.com; phone: +86 15802616494 (Xu, B. H.)
e-mail: qiaolinliu2017@hunau.edu.cn; phone: +86 18374893286 (Liu, Q. L.)

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Abstract. The first hybrid generation (F1) individuals of grass carp (*Ctenopharyngodon idellus*, ♀) × barbel chub (*Squaliobarbus cunrriculus*, ♂) commonly show obvious growth diversity, even though they are from the same parents and cultured in the same environment. To characterize the molecular mechanism of the growth diversity, the present study compared the expressions of growth-related genes of the fast-growth population (FGP) and slow-growth population (SGP). The expression of growth-inhibiting *SRIF* gene was significantly higher in the SGP hypophysis than in the FGP. The expressions of growth-promoting *GHR* and *IGF-I* genes in blood, *GHR*, *IGF-I* and *IGF-II* genes in liver, and *IGF-I* and *IGF-II* genes in muscle of the FGP were significantly higher than those of the SGP in liver. The results implied that hypernomic-expression of *SRIF* gene in hypophysis caused the expressions of *GHR* and *IGF-I* genes in blood, *GHR*, *IGF-I* and *IGF-II* genes in liver, and *IGF-I* and *IGF-II* genes in muscle of the SGP to be significantly lower than those in the FGP, which caused the lower growth of the SGP. These results provided valuable reference for studying the relationship between growth-related genes and fish growth, and the molecular mechanism of fish growth.
Keywords: *fish, growth axis, quantitative reverse transcriptional PCR, growth-inhibiting gene, GHR gene, expression analysis*

Introduction

Grass carp (*Ctenopharyngodon idellus*) is already the largest freshwater aquaculture product worldwide (Ni and Yu, 2013; Ni et al., 2014) and it is an important aquaculture species in Asia. Approximately 5.90 million tons of grass carp was produced ever year only in China (Department of Fisheries of Ministry of Agriculture of China, 2017). However, frequent occurrence of diseases in grass carp culture has caused serious loss and severely restricts sustained development of grass carp culture (Nie and Pan, 1985; Chen et al., 2012). Hybridization between different fish species is extensively used as their offspring exhibit growth and disease-resistant superiority compared to their parents. For instance, the first hybrid generation (F1) of Kaluga sturgeon (*Huso dauricus*, ♀) × sterlet (*Acipenser ruthenus*, ♂) exhibits fast-growth and disease-resistant superiority (Yin

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et al., 2004). The hybrid offspring of rainbow trout (*Oncorhynchus mykiss*, ♀) × speckled trout (*Salvelinus fontinalis*, ♂) exhibit disease-resistant superiority (Wu et al., 2014). The F1 of grass carp (♀) × topmouth culter (*Erythroculter ilishaeFormis*, ♂) also exhibits disease-resistant superiority (Aquaculture Research Group of Jianhu County of Jiangsu Province, 1974).

Barbel chub (*Squaliobarbus curriculus*) as well as grass carp belongs to the Leuciscinae subfamily of fish. The shape of barbel chub is similar to grass carp, and barbel chub exhibits strong adaptability and disease-resistant superiority (Liu et al., 2012). In addition, a previous study has showed that disease resistance of the F1 of grass carp (♀) × barbel chub (♂) was significantly higher than that of grass carp (He et al., 2015). However, the F1 individuals of grass carp (♀) × barbel chub (♂) commonly emerge obvious individual difference in size during culture process, even though they are from the same parents and cultured in the same environment (Zhou et al., 2017).

Fish growth is influenced by various internal (such as incretion) and external factors (such as environment and nutrition) (Su et al., 2012; Valente et al., 2013). The incretion factors regulate fish growth through *GH/IGF-I* axis, which involves the hypothalamus, the hypophysis, and the liver (Lin, 1996; Peng and Peter, 1997). Hypothalamus secretes stimulators and inhibitors. The stimulators, such as growth hormone releasing factor (*GRF*), gonadotropin-releasing hormone (*GnRH*), pituitary adenylate cyclase activating polypeptide (*PACAP*), dopamine (*DA*) and neuropeptide Y (*NPY*), stimulate the release of growth hormone (*GH*) in the hypophysis (Parker et al., 1997; Montero et al., 1998), while the inhibitors, such as somatostatin (*SRIF* or *SS*), 5-serotonin (*5-HT*), norepinephrine (*NE*) and glutamate (*Glu*), inhibit the release of *GH* that induces the secretion of *GRF*, *GnRH*, *PACAP*, and *NPY* (Peter and Marchant, 1995; Peng and Peter, 1997). Secretion of stimulators and inhibitors regulates the secretion of *GH*, and *GH* is transmitted to the surface of liver cell membrane through the circulation system and then it binds to growth hormone receptor (*GHR*) to trigger the transduction of insulin-like growth factors (*IGFs*). Then *IGFs* are transmitted to each tissue through the circulation system to promote fish growth.

Considering the F1 individuals of grass carp (♀) × barbel chub (♂) commonly emerge obvious individual difference in size during culture process, to characterize the molecular mechanism of the growth diversity of the F1 populations, the present study compared the expressions of growth-related genes of the fast-growth population (FGP) and slow-growth population (SGP) using fluorescence quantitative reverse transcriptional PCR (qRT-PCR). The results provided valuable reference for studying the relationship between growth-related genes and fish growth, and the molecular mechanism of fish growth.

Materials and Methods

Sample collection

The F1 samples were collected from Wulong Fishing Ground located at Beisheng Town, Liuyang City of China (28.295 N, 113.436 E). The F1 fish were cultured 150 days from 1^+ years of F1 offspring with the same parents. They were fed with commercial puffed compound feed-8110 (Dabeinong, China), which contains equal more than 36.0% of crude protein, equal more than 4.0% of crude fat, and equal more than 15.0% of crude ash. The F1 samples were distinguished to two populations, i.e. the FGP (their body weights were more than 500 g) and the SGP (their body weights were equal or less than

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500 g), according to their body weight. Each population was collected 7 healthy samples, and the samples were anaesthetized using an overdose of neutralized MS222 (ethyl 3-aminobenzoate methane-sulfonic acid). Then the samples were dissected as approximately 80 mg of their hypothalamus, hypophysis, liver and muscle, and 2 ml of blood was collected and stored in liquid nitrogen.

RNA extraction and synthesis of cDNA

The tissues stored in liquid nitrogen were put in homogenizing pipe with 1 ml TRK lysis buffer and homogenized three times (15 s per time with 6000 rpm/min, 5 s of interval between homogenizing). Subsequently, RNAs were extracted from the homogenates using an E.Z.N.A. total RNA kit I (OMEGA, USA) according to the manufacturer instructions. The RNAs were used to synthesize the first strand of cDNA by a RevertAid first strand cDNA synthesis kit (Thermo, USA), with Oligo$(dT)_{18}$ and random primers.

qRT-PCR

To design the primers for qRT-PCR, fragments of *GH*, *GHR*, *IGF-I*, *IGF-II*, *PACAP*, *SRIF*, *MSTN-1* and *MYOG* genes of the F1 were cloned and sequenced. To clone these gene fragments, the primers (*Appendix 1*) were designed using primer 6.0 referenced cDNA sequences of these genes from Cyprinidae in GenBank. The fragments were amplified using the first strand of cDNA of the F1 as templet. Each 50 μl of the PCR reaction mixture contained 1×Ex Taq Buffer (TaKaRa, China), 1.25 U of Ex Taq polymerase (TaKaRa, China), 10 nmol of each primer, 40 nmol of each NTP, and 2 μl of cDNA. The PCR amplified procedure was carried out at 94°C for 5 min; at 94°C for 30 s, at 52~56°C (β-actin: 54°C; EF-1α: 54°C; GH: 56°C; GHR: 54°C; *IGF-I*: 55°C; *IGF-II*: 55°C; *PACAP*: 52°C; *SRIF*: 52°C) for 30 s, at 72°C for 90 s, in 30 cycles; and finalized at 72°C for 5 min. Then the fragments were sequenced using AB3730 sequencer at Beijing Aokedingsheng Bio-Science Ltd., China (*Appendix 2*). Finally, the primers that used to qRT-PCR were designed based on these fragments using primer 6.0 (*Table 1*).

Table 1. *Primers for gene expression of growth-related genes*

Gene name	Primer name	Primer sequence (5'-3')	Length of target sequence (bp)	Amplification efficiency
β-actin	β-actin-RT-F β-actin -RT-R	GCTATGTGGCTCTTGACTTCG GGGCACCTGAACCTCTCATT	124	95~97
EF-1α	EF-1α-RT-F EF-1α-RT-R	GCTATGTGGCTCTTGACTTCG GGGCACCTGAACCTCTCATT	124	96~99
GH	GH-RT-F GH-RT-R	ACAGTTTGACCGTCGGGAACCC CAGCGGCAGGGAGTCGTTATCA	131	95~99.8
GHR	GHR-RT-F GHR-RT-R	TGTGTGGAAACGGACTGGTGTCTG CAGCAACGGAAGGTCTCCTGTTCT	115	101~105
IGF-I	IGF-I-RT-F IGF-I-RT-R	ACATTGCCCGCATCTCATCCTCT CCCTGGAAGAAATGACCGCTAGAC	114	95~97.7
IGF-II	IGF-II-RT-F IGF-II-RT-R	GTTTCAGCCACATCCCTACAGGTCA CCGTTGCCACACGTCATATTTGGA	108	97~99.6
PACAP	PACAP-RT-F PACAP-RT-R	AGCCTTGAGGGACATCCTGGTTCA CCGATTCGTCTTCCTCGCTGCTT	101	96~98.6
SRIF	SRIF-RT-F SRIF-RT-R	TGCTTGGACGAGGTCTGTGAGC ACGCCAAACTCCGCCAACTTCT	108	95~97.1
MSTN-1	MSTN-1-RT-F MSTN-1-RT-R	AGGACTTCGGCTGGGACTGGATTA GCGGATTGGCCTTGTTCACCAGAT	126	95~96.5
MYOG	MYOG-RT-F MYOG-RT-R	AAGCCGCCACATTGAGGGAGAAG GGCAGCCTCTGGTTGGGATTCAT	100	98~101.5

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The expressions of *PACAP* and *SRIF* genes in the hypothalamus, *PACAP*, *SRIF* and *GH* genes in the hypophysis, *GH*, *GHR*, *IGF-I* and *IGF-II* genes in the blood, *GHR*, *IGF-I* and *IGF-II* in the liver, and *IGF-I*, *IGF-II*, *MYOG* and *MSTN-1* genes in the muscle were tested through qRT-PCR using a SYBR Premix Ex Taq™ II kit (TaKaRa, China). The first strand cDNA from the tissues was used as templet. The *β*-actin and EF-1α genes were used as internal controls. The qRT-PCR was conducted using a CFX96Touch TM real-time PCR detection system (Bio-Red, USA). Each 10 μl of the qRT-PCR reaction mixture contained 5 μl of SYBR Green PCR master mix (CWBIO, China), 0.4 μl of each primer (10 μM), 1 μl of cDNA, and 3.2 μl of ddH_2O. The PCR amplified procedure was carried out at 94°C for 10 min, followed by 35 cycles of 95°C for 10 s, 60°C for 10 s, and 72°C for 15 s. The solubility curve was obtained by raising 0.5°C per 5 s from 65°C to 95°C. Seven samples in each group and triplicate of each sample were analyzed. Data were collected and analyzed using CFX manager software 3.1 (Bio-Rad, USA).

Data analysis

The results are presented as the mean ± standard error (S.E.) for each group. One-way ANOVA and t-test was conducted using R 3.5.1 (R Core Term, 2014). Differences for which *P* values were < 0.05 were considered statistically significant.

Results and Discussion

The body lengths of the FGP and the SGP were 38.043 ± 0.44 and 26.243 ± 0.48 cm, respectively (*Fig. 1A*). The body weights of the FGP and the SGP were 932.86 ± 50.41 and 312.43 ± 22.70 g, respectively (*Fig. 1B*). The average body length and body weight of the FGP were significantly higher than those of the SGP, respectively (independent t-test, $p < 0.001$).

Expressions of *PACAP* (Welch two sample t-test, $t = 1.393$, $P = 0.230$) and *SRIF* (Welch two sample t-test, $t = 0.448$, $P = 0.670$) genes in the hypothalamus of the FGP were not detected significantly different to the SGP (*Fig. 2A*). However, the relative expression of the *SRIF* gene in the hypophysis was significantly increased in the SGP than those in the FGP (Welch two sample t-test, $t = -13.818$, $P < 0.001$; *Fig. 2B*). The results implied that the high expression of *SRIF* gene in the SGP hypophysis was probably the major reason that caused the slow growth of the SGP, as *SRIF* is considered as an inhibitor that inhibits secretion of *GH* in the hypophysis (Peter and Marchant, 1995; Peng and Peter, 1997; Lin and Peter, 2001). Meanwhile, *SRIF* also reduces the combination of *GHR* to *GH* and transcriptional level of *IGF-I* gene in the liver (Tanaka et al., 1995; Masini et al., 1999). The *GH* gene was rarely expressed in the hypophysis and in the blood, and its expressions were not detected significantly different between the hypophysis of FGP and SGP (Welch two sample t-test, $t = 1.266$, $P = 0.250$; *Fig. 2B*) and in the blood (Welch two sample t-test, $t = -0.576$, $P = 0.605$; *Fig. 2C*). However, the expressions of *GHR* (Welch two sample t-test, $t = 3.162$, $P = 0.014$) and *IFG-I* genes (Welch two sample t-test, $t = 5.663$, $P = 0.002$) in the FGP blood were significantly higher than those in the SGP (*Fig. 2C*).

The expressions of *GHR* (Welch two sample t-test, $t = 3.334$, $P = 0.039$), *IGF-I* (Welch two sample t-test, $t = 5.157$, $P < 0.001$) and *IGF-II* (Welch two sample t-test, $t = 5.995$, $P = 0.004$) genes in the FGP liver were significantly higher than those in the SGP liver (*Fig. 2D*). In addition, the expressions of *IGF-I* (Welch two sample t-test,

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t = 7.744, P < 0.001) and *IGF-II* (Welch two sample t-test, t = 3.594, P = 0.012) genes in the FGP muscle were significantly higher than those in the SGP muscle. *IGF-I* and *IGF-II* genes are mainly expressed in the liver. Therefore, these genes were higher expressed in the liver than in the muscle (*Fig. 2E*). Fish growth mainly shows as fast growth of muscle. The growth of muscle was regulated by *GH-IGF* axis. *GH* regulates expression of genes that promote muscle growth, such as myostatin and muscular atrophy genes. *IGFs* mainly regulate expression of genes that regulate muscle form (Fuentes et al., 2013). In addition, the external factors, such as environment and nutrition, also influence fish growth through the *GH/IGF-I* axis (Reinecke, 2010; Su et al., 2012). However, the expressions of *MYOG* and *MSTN-1* genes in the muscle were not significantly different between the FGP and the SGP (Welch two sample t-test, t = 0.569, P = 0.589 for *MYOG* gene, and t = -2.006, P = 0.090 for MSTN-1 gene).

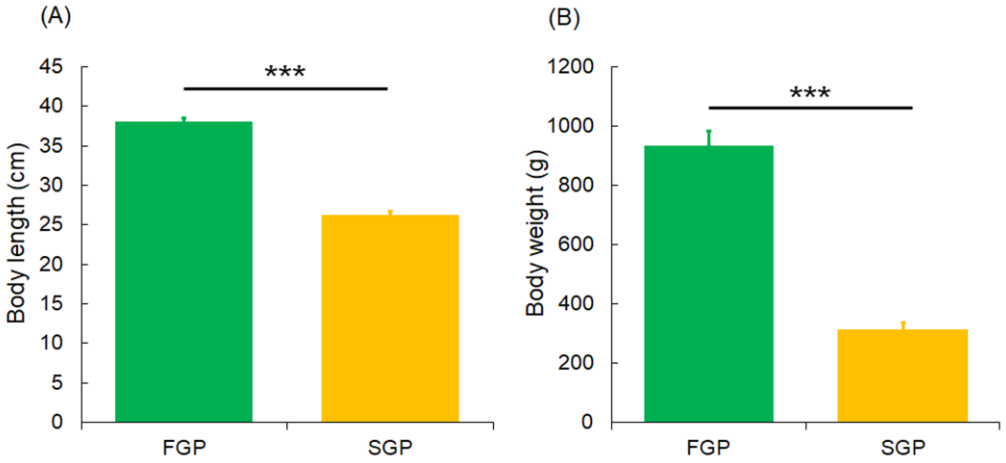

Figure 1. *Body length (A) and body weight (B) of the samples. ***, P < 0.001*

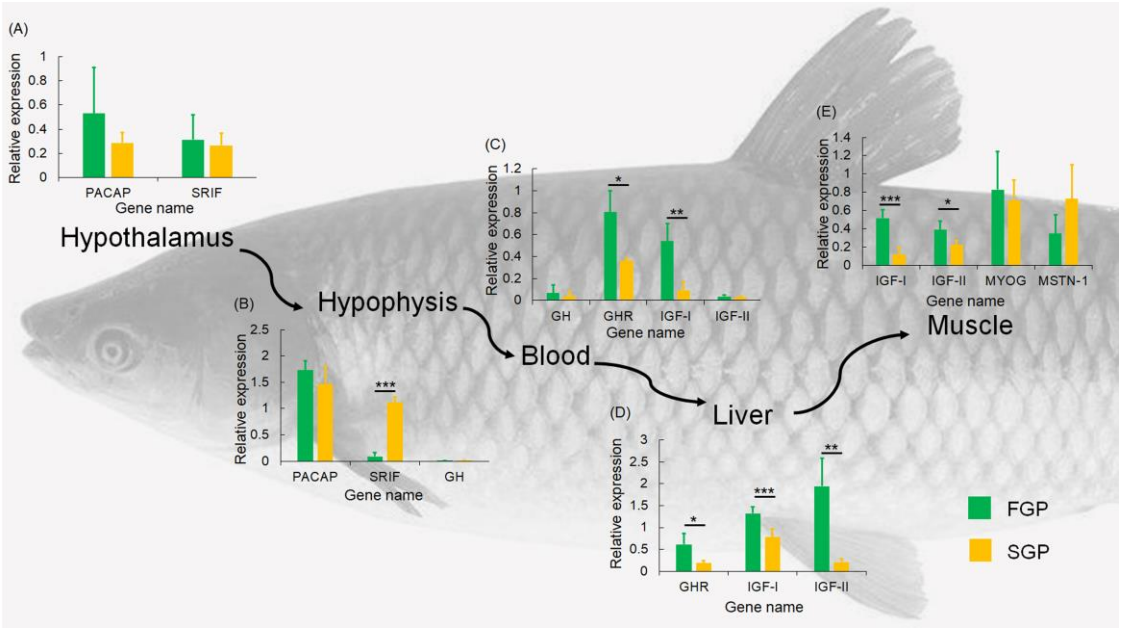

Figure 2. *Expression analysis of genes in growth axis of direct cross F1. *: P < 0.05; **: P < 0.01; ***: P < 0.001*

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GH is synthetized in the hypophysis and transported to target tissues through the circulatory system. The growth-promoting performance of *GH* is vitally needed to mediate *GHR* and *IGFs*. Therefore, fish growth could not be expressed only by the expression of *GH* gene, but the expressions of *GHR* and *IGF* genes are also vital. In the present study, the expressions of *GHR*, *IGF-I* and *IGF-II* genes in the FGP liver were significantly higher than those in the SGP, consistent with previous studies in goldfish (*Carassius auratus*) (Zhong et al., 2012), Japanese pufferfish (*Takifugu rubripes*) (Kaneko et al., 2011), mud carp (*Cirrhinus molitorella*) (Zhang et al., 2006), Nile tilapia (*Oreochromis niloticus*) (Cruz et al., 2006), European eel (*Anguilla anguilla*) and half-smooth tongue sole (*Cynoglossus semilaevis*) (Degani et al., 2003; Ma et al., 2012). In addition, *GH* also regulates muscle growth through regulating the expressions of myogenic factors. For instance, expressions of *MYOG*, *MYOD2*, *MYF5V* and *MEF2A* genes in the muscle of the fast-growth transgenic fish were significantly higher than those of wildtype fish (Devlin et al., 2013). However, we did not detect significant difference between the expression of *MYOG* and MSTN-1 genes in the muscle of the FGP and the SGP.

Conclusion

The significantly higher expression of *SRIF* gene in the SGP hypophysis than in the FGP hypophysis caused the expressions of *GHR* and *IGF-I* genes in the blood, *GHR*, *IGF-I* and *IGF-II* genes in the liver, and *IGF-I* and *IGF-II* genes in the muscle of the SGP to be significantly lower than those in the FGP, which caused the lower growth of the SGP than the FGP. However, whether increasing the expression of *SRIF* gene in the hypophysis could increase growth in other fishes still needs further study.

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REFERENCES

[1] Aquaculture Research Group of Jianhu County of Jiangsu Province. (1974): Hybrid test report of Ctenopharyngodon idellus (♀) × Erythroculter ilishae Formis (♂). – Freshwater Fisheries 4(3): 22-23.

[2] Chen, J., Li, C., Huang, R., Du, F., Liao, L., Zhu, Z., Wang, Y. (2012): Transcriptome analysis of head kidney in grass carp and discovery of immune-related genes. – BMC Veterinary Research 8: 108.

[3] Cruz, E. M. V., Brown, C. L., Luckenbach, J. A., Picha, M. E., Bolivar, R. B., Borski, R. J. (2006): Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, Oreochromis niloticus. – Aquaculture 251(2-4): 585-595.

[4] Degani, G., Tzchori, I., Yom-Din, S., Goldberg, D., Jackson, K. (2003): Growth differences and growth hormone expression in male and female European eels (Anguilla anguilla). – General and Comparative Endocrinology 134(1): 88-93.

[5] Department of Fisheries of Ministry of Agriculture of China. (2017): China Fisheries Yearbook 2017. – Beijing, China Agricultural Press.

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DOI: http://dx.doi.org/10.15666/aeer/1801_11511159

[6] Devlin, R. H., Sakhrani, D., White, S., Overturf, K. (2013): Effects of domestication and growth hormone transgenesis on mRNA profiles in rainbow trout (Oncorhynchus mykiss). – Journal of Animal Science 91(11): 5247-5258.
[7] Fuentes, E. N., Valdes, J. A., Molina, A., Björnsson, B. T. (2013): Regulation of skeletal muscle growth in fish by the growth hormone-insulin-like growth factor system. – General and Comparative Endocrinology 192(9): 136-148.
[8] He, M., Xiao, T., Liu, Q., Li, D., Li, W., Deng, Y. (2015): Morphological characteristics analysis of Ctenopharyngodon idellus, Squaliobarbus curriculus and their reciprocal hybrids F1. – Journal of Hunan University of Arts and Sciences (Science and Technology) 27(4): 36-42.
[9] Kaneko, G., Furukawa, S., Kurosu, Y., Yamada, T., Takeshima, H., Nishida, M., Mitsuboshi, T., Otaka, T., Shirasu, K., Koda, T., Takemasa, Y., Aki, S., Mochizuki, T., Fukushima, H., Fukuda, Y., Kinoshita, S., Asakawa, S., Watabe, S. (2011): Correlation with larval body size of mRNA levels of growth hormone, growth hormone receptor I and insulin-like growth factor I in larval torafugu Takifugu rubripes. – Journal of Fish Biology 79(4): 854-874.
[10] Lin, H. (1996): The regulation of growth and growth hormone secretion in fish. – Acta Zoologica Sinica 42(1): 69-79.
[11] Lin, X., Peter, R. E. (2001): Somatostatins and their receptors in fish. – Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 129(2-3): 543-545.
[12] Liu, Q., Xiao, T., Liu, M., Zhou, W. (2012): Research progress of biology in Squaliobarbus curriculus. – Fisheries Science 31(11): 687-691.
[13] Ma, Q., Liu, S., Zhuang, Z., Lin, L., Sun, Z., Liu, C., Ma, H., Su, Y., Tang, Q. (2012): Genomic structure, polymorphism and expression analysis of the growth hormone (GH) gene in female and male Half-smooth tongue sole (Cynoglossus semilaevis). – Gene 493(1): 92-104.
[14] Masini, M. A., Sturla, M., Uva, B. (1999): Somatostatin in the ovary of an African lungfish (Protopterus annectens): an in situ hybridisation, immunohistochemical, and autoradiographical study. – General and Comparative Endocrinology 114(2): 287-292.
[15] Montero, M., Yon, L., Rousseau, K., Arimura, A., Fournier, A., Dufour, S., Vaudry, H. (1998): Distribution, characterization, and growth hormone-releasing activity of pituitary adenylate cyclase-activating polypeptide in the European eel, Anguilla anguilla. – Endocrinology 139(10): 4300-4310.
[16] Ni, J., Yu, Y. (2013): Intestinal microbiota changes of grass carp (Ctenopharyngodon idellus) in different months. – Journal of Fisheries of China 37(10): 1558-1563.
[17] Ni, J., Yan, Q., Yu, Y., Zhang, T. (2014): Factors influencing the grass carp gut microbiome and its effect on metabolism. – FEMS Microbiology Ecology 87(3): 704-714.
[18] Nie, D., Pan, J. (1985): Diseases of grass carp (Ctenopharyngodon idellus Valenciennes, 1844) in China, a review from 1953-1983. – Fish Pathology 20(2/3): 323-330.
[19] Parker, D. B., Power, M. E., Swanson, P., Rivier, J., Sherwood, N. M. (1997): Exon skipping in the gene encoding pituitary adenylate cyclase-activating polypeptide in salmon alters the expression of two hormones that stimulate growth hormone release. – Endocrinology 138(1): 414-423.
[20] Peng, C., Peter, R. E. (1997): Neuroendocrine regulation of growth hormone secretion and growth in fish. – Zoological Studies 36(2): 79-89.
[21] Peter, R. E., Marchant, T. A. (1995): The endocrinology of growth in carp and related species. – Aquaculture 129(1): 299-321.
[22] R Core Term. (2014): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/.

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DOI: http://dx.doi.org/10.15666/aeer/1801_11511159

[23] Reinecke, M. (2010): Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. – Journal of Fish Biology 76(6): 1233-1254.
[24] Su, J., Zhang, Y., Lou, Z., Jiao, W., Yang, J., Wei, Y. (2012): Progress on fish growth traits improvement and its regulation. – Sichuan Journal of Zoology 31(1): 165-169.
[25] Tanaka, M., Hayashida, Y., Wakita, M., Hoshino, S., Nakashima, K. (1995): Expression of aberrantly spliced growth hormone receptor mRNA in the sex-linked dwarf chicken, Gifu 20. – Growth Regulation 5(4): 218-223.
[26] Valente, L. M. P., Moutou, K. A., Conceição, L. E. C., Engrola, S., Fernandes, J. M. O., Johnston, I. A. (2013): What determines growth potential and juvenile quality of farmed fish species. – Reviews in Aquaculture 5(s1): S168-S193.
[27] Wu, S., Li, J., Ou, Y., Lv, G., Liu, J. (2014): Allometric growth of hybrid grouper (Epinephelus coioides ♀ × E. lanceolatus ♂) larvae and juveniles. – Journal of Fishery Sciences of China 21(3): 503-510.
[28] Yin, H., Sun, Z., Sun, D., Qiu, L. (2004): Comparison of nutritive composition in muscles among six farmed sturgeon species. – Journal of Dalian Fisheries University 19(2): 92-96.
[29] Zhang, D. C., Huang, Y. Q., Shao, Y. Q., Jiang, S. G. (2006): Molecular cloning, recombinant expression, and growth-promoting effect of mud carp (Cirrhinus molitorella) insulin-like growth factor-I. – General and Comparative Endocrinology 148(2): 203-212.
[30] Zhong, H., Zhou, Y., Liu, S., Tao, M., Long, Y., Liu, Z., Zhang, C., Duan, W., Hu, J., Song, C., Liu, Y. (2012): Elevated expressions of GH/IGF axis genes in triploid crucian carp. – General and Comparative Endocrinology 178(2): 291-300.
[31] Zhou, Z., Li, X., Wang, R., Jin, S., Xiao, T. (2017): Growth test report of 1+ year hybrid F_1 of grass carp (Ctenopharyngodon idellus ♀) × barbel chub (Squaliobarbus curriculus ♂). – Scientific Fish Farming 33(6): 79-80.

APPENDIX

Appendix 1. *Primers used for partial fragment cloning of growth-related genes*

Primers	Sequences (5'-3')
GH-F	GGATGGGAGTTGGAGGAGAAA
GH-R	GGCTGACCGTCTGACACAA
GHR-F	GCCATTCAGGACGAGGAGATA
GHR-R	TGGTTGGGATTACAGGGAGATG
IGF-I-F	TCTCACTTCTCCACAACGA
IGF-I-R	CTTCTGATGAACCTCCTTACA
IGF-II-F	GTCGAACAGTCGGCGTCCTCAA
IGF-II-R	CTGTGGTGGTGCAGTTGCTCCT
PACAP-F	AGAATGGCTRYRCAAACCYTGG
SRIF-F	GTCCGAGCAAAGAGAACT
SRIF-R	GGTTAGGATGGAGAATGTGA
MSTN-1-F	GTGTTGCTTTTTCTCCTTCAGTC
MSTN-1-R	CACAGCGGTCTACTACCATCG
MYOG-F	AGGCGGCGATAACTTCTTCCA
MYOG-R	CTTGCTCATGTTCCTGCTGGTT

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Appendix 2. *Partial target fragment used for cloning*

Gene name	Sequences (5'-3')
IGF-I	CCACAACGAGCCTGCGCAATGGAACAAAGTCGGAATATTGAGATGTGACATTGCCCGCATCTCATCCTC TTTCTCGCTTTTTAATGACTTCAAACAAGTTCATTTTTGCTGGGCTTTTGCTGGAGACCCAAGGGGATGT CTAGCGGTCATTTCTTCCAGGGGCAYTGGTGTGATGTCTTTAAGTGTACCATGCGCTGTCTCTCGTGCAC CCACACCCTCTCACTGGTGCTGTGCGTCCTCGCGTTGACTCCCGCGACACTGGAGGCRGGGCCGGAGA CGCTGTGCGGGGCGGAGCTTGTAGACACGCTGCAGTTTGTGTGTGGAGACAGGGGCTTTTATTTCAGCA AACCAACAGGATATGGGCCTAGTTCGAGRCGGTCGCACAACCGCGGCATTGTGGACGAATGCTGCTTTC AGAGCTGCGAACTGCGGCGCCTCGAGATGTACTGTGCACCCGTGAAAACCGGCAAAWCTCCACGATCC CTACGAGCGCAACGGCACACAGATATCACCAGGACAGCAAAGAA
IGF-II	GACGCGCTACAGTTTGTGTGCGAAGACAGAGGCTTCTATTTCAGTCGACCAACTAGTAGGTCGAACAGT CGGCGTCCTCAAAATCGTGGGATTGTGGAAGAGTGTTGTTTTAGCAGTTGTAACCTAGCTCTACTAGAAC AATACTGCGCTAAACCTGCCAAGTCAGAGAGGGACGTTTCAGCCACATCCCTACAGGTCATCCCGGTGA TGCCCGCATTAAAACAGGAGGTCCCAAGAAAACATGTGACCGTGAAATATTCCAAATATGACGTGTGGC AACGGAAGGCCGCACAGAGGCTACGAAGGGGCGTCCCTGCCATCCTGCGGGCCAAGAAGTTTAGGCG GCAGGCGGAGAGAATCAAGGCCCAGGAGCAACTGCACCACCACAGGCCTCTCATCACGCTTCCCAGCA A
PACAP	GCAGGGCAAGGTCTAGTAGAGCGACTTTAGCGTTGCTCATCTACGGAATCATGATGCATTACAGCGCCTA CTGCACGCCTATTGGGATGGCTTTTCCTAAGATGAGACTAGACAACGATGTATTTGACGAAGACGGAAA CTCGTTAAGCGACCTGGCTTTTGGCACGGATCAAATTGCTATACGAAGTCCTCCTTCTCTTACGGATGAC CTATACACGCTATACTATCCTCCAGAGAAAAGAACGGAAAGGCATGCAGATGGATTATTAGATAGAGCCT TGAGGGACATCCTGGTTCAGTTATCAGCACGAAAATATCTGCATTCTCTGATGGCAGTTCGCGTAGGCGG AGGAAGCAGCGAGGAAGACGAATCGGAACCATTATCAAAAAGGCATTCGGATGGGATCTTCACCGACA TTTACAGTCGCTACCGAAAACAGATGGCCGTCAAGAAGTATTTAGCAGCCGTCCTGGGAAGAAGGTACA GACAGAGAATTAAAAACAAAGGACG
SRIF	GACGTAACGGTAAGTTTCAGAGAGTTCTTGCATCCGGCTTTGCGCTCGCGAGGTGCCAGCATGGGACCG GCGGCGCGCTCGAGCTCCAAACGAACGTCATCTTTCTCCACAGCGCGAGACAGATCCTCGGGCTCCAG CACCTCGTTTTCTGCTTGGACGAGGTCTGTGAGCAAATCTGCAAGTGTGTATCTTGCGAGTTCCTGTTTT CCAGCCGGGTTGAGGAGAGATCTCTGCAGAAGTTGGCGGAGTTTGGCGTA
MSTN-1	GTGTTGCTTTTTCTCCTTCAGTCCGAAAATCCAAGCGAACCGGATCGTAAGAGCGCAGCTCTGGGTTCA TCTGAGACCGGCGGAAGAAGCGACCACCGTCTTCTTACAGATATCACGGCTGATGCCCGTTACGGACGG AGGAAGACACATACGAATACGATCCCTGAAGATCGATGTGAACGCAGGAGTCACGTCTTGGCAGAGTAT AGACGTAAAGCAGGTGCTCTCGGTGTGGTTAAGACAACCGGAGACCAACTGGGGCATCGAGATAAACG CGTATGACGCGAAGGGAAACGACTTGGCCGTCACCTCAGCTGAGGCTGGAGAGGATGGACTGCTCCCC TTTATGGAGGTGAAAATCTCAGAGGGCCCAAAGCGAATCCGGAGGGACTCCGGACTGGACTGCGACGA GAATTCCTCAGAGTCTCGATGCTGCAGATACCCTCTCACTGTGGACTTCGAGGACTTCGGC
MYOG	AGGATATCAGGACAGAAGCTCCATGATGGGCTTGTGTGGAGACGGACGGCTGCTGTCTAATGGAGTGGG GTTGGAGGACAAACCGTCTCCATCATCTAGCCTCGGTCTGTCCATGTCTCCTCACCAGGAGCAGCAGCA CTGTCCGGGTCAGTGTCTGCCTTGGGCCTGCAAGGTGTGTAAGCGCAAGTCGGTGACCATGGACCGAC GGAAAGCCGCCACATTGAGGGAGAAGAGGAGGTTGAAGAAGGTCAACGAGGCCTTTGAGGCTCTTAA GAGGAG

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