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Cloning and Analysis of Leptin in *Culter alburnus* in Xingkai Lake and down Regulating its Expression Compared to Cultured Population

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Abstract: Researchers cloned the Leptin of *Culter alburnus* in Xingkai Lake and analyzed the sequence characteristic of Leptin coding protein and detected the relative expression levels of Leptin RNA in wild and cultured *Culter alburnus* in Xingkai Lake of different ages. The result provide reference on studying the regulatory effect and molecular mechanism of Leptin on food intake, digestion and catabolism genes and in *Culter alburnus* in Xingkai Lake. The full length cDNA of *Leptin* gene was got by RT-PCR, 5'-RACE and 3'-RACE. The primer of semi-quantitative RT-PCR and real-time PCR were designed by the acquired cDNA then the gene expression was studied. The full sequence length of Leptin in *Culter alburnus* in Xingkai Lake was 1208 bp, the ORF length is 522 bp which code 173 amino acids. With the increase of age, the expression of mRNA in both wild and cultured *Culter alburnus* in Xingkai Lake show rising trend, from 3 years old, the expression of Leptin in wild population showed down regulation in contrast to the cultured population.

Key words: *Culter alburnus*, Xingkai Lake, Leptin, cloning, expression, wild and cultured population

INTRODUCTION

Carnivorous fishes with high economic value have higher utilization rate in protein and lipid than carbohydrate. Fish is poikilotherm, it storage excess fat in cultured condition which affect its health and water quality. The utilization of feed in fish, including feed take, digestion, absorption, catabolism, synthesis, excretion and a series of complex steps were all proceeding under controlling the expression of specific genes, the obese gene plays key roles in this process. Although, obesity is not the main problem in aquaculture but improvement of meat quality, reproduction and health is the main target in aquaculture. Leptin was found in mice in 1954, the expression product of *obese* gene (ob), secreted by fat tissue in mammals. Zhang *et al*. (1994) first published the *ob* gene homologous sequence between mice and human and foresee ob protein by gene and illuminated the molecular structure and physiological effect of *ob* gene protein-Leptin. Pelleymounter's study showed that Leptin have obviously regulation on animal fat tissue and neuroendocrine, it could promote animals' growth and reproduction by regulating feed intake and energy homeostasis, it is signal factor that reflect internal fat composition and content (Pellymounter *et al*., 1995). In fish, Johnson *et al*. (2000) detect the mice Leptin antibody firstly in *Lepomis macrochirus*, *L. cyanellus*, *Oncorhynchus mykiss*, *Pomonix annularis*, *Ictalurus punctatus*, Leptin was found in their blood, liver, heart, brain. Until 2005, Japanese scientist Kurokawa *et al*. (2005) cloned Leptin sequence of *Takifugu rubripes*. In recent years, study of Leptin in *Cyprinus carpio*, *Arctic charr*, *Oryzias latipes*, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix* (Huising *et al*., 2006; Murashita *et al*., 2008; Froiland *et al*., 2010; Kurokawa *et al*., 2008; Yu *et al*., 2009) were also reported. *Culter alburnus* is cypriniformes, cyprinidae, culterinae, Culter, English name is Topmouth. Culter, widely distributed in main streams, tributaries streams and affiliated lakes of Heilong River, Liao River, Yangtze River, Qiantang River, Min River, Taiwan and Zhu River. The water quality in Xingkai Lake is good, rich of nutrient and its pollution is few because it is far away from cities. So, the large white fish in Xingkai Lake is silvery white and tenderness meat, it is the famous commercially fish have high economic value, it is also one of the four famous freshwater fish (Kun *et al*., 2012). This study cloned *Leptin* gene by RT-PCR, 5'-RACE and 3'-RACE, analyzed the sequence characteristic of Leptin coding protein and detected the relative expression levels of Leptin RNA in wild and cultured *Culter alburnus* in Xingkai Lake of different ages. The result provide reference on studying

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the regulatory effect and molecular mechanism of Leptin on food intake, digestion and catabolism genes and in *Culter alburnus* in Xingkai Lake. It have important affect on solve problems of low feed utilization rate, poor meat quality and serious pollution of water circumstance in aquaculture industry.

MATERIALS AND METHODS

Animals: Wild *Culter alburnus* in Xingkai Lake (2-6 years old) was sampled from Xingkai Lake in Jixi City, Heilongjiang (44°44'~45°24'N, 130°59'~132°51'E, Heilongjiang, China). Cultured *Culter alburnus* in Xingkai Lake (2-6 years old) was sampled randomly from the cultured base of Heilongjiang Nongkenzhenda Xingkaihu big white fish research center. The nutrition facts of feed is: Crude protein ≥42.0%, crude fat ≥8.0%, crude fiber ≤3.0%, crude ash ≤15.0%, Ca ≥1.8%, total phosphorus ≥1.3%, lysine ≥2.0%, moisture ≤10.0%. When the sample was collected in wild, the tissue was separated quickly and store in liquid nitrogen then taken to the laboratory and stored at -80°C.

Synthesis of cDNA first chain and PCR amplification: Total RNA was extracted by one-step procedure. The absorption values of RNA was measured at 260 and 280 nm by UV spectrophotometer and the concentration was culculated. The concentration of RNA (μg/μL) = OD_{260}× dilution multiple×40/1000, OD_{260}/OD_{280} is among 1.8~2.1. Specific first strand cDNA was synthesized were performed with 3', 5'-RACE rapid amplification kit (Takara Biotechnology, Dalian, China). The Leptin code of grass carp was used for design primer LEP1-F and LEP1-R (Table 1). The muscle cDNA was as template of PCR reaction, the reaction system was listed in Table 1, approximately 5 μg RNA was reverse transcribed by Power Script™ reverse transcriptase with Coding Sequences (CDS). Thirty cycles were performed for PCR using an Ex Taq DNA polymerase (TranGen Biotechnology, Beijing, China). Every cycle consisted of denaturation at 94°C for 30 sec, annealing at 55-58°C for 30 sec and extension at 72°C for 1 min. The reaction mixture for PCR was as follows: Each of the forward and reverse primers (20 pmol), 1 μg first strand cDNA as a template, 20 nmol dNTP mixture, 10 μL 10×Ex Taq buffer (Takara) and 1 U Ex Taq DNA polymerase. The final volume was adjusted to 100 μL with sterilized water.

The products were isolated using the Gel Extraction kit (Takara Biotechnology, Dalian, China), cloned into the pMD 18-T vector (Takara Biotechnology, Dalian, China) and transformed into *Escherichia coli* strain DH5α-competent cells, according to manufacturer's instruction.

Table 1: Primers for ob cDNA cloning and analysis in *Culter alburnus* in Xingkai Lake

Primer names	Sequence (5'-3')	Amplicon length (bp)
Lep1-F	GGGGATTCAGAGCTTTACCC	685
Lep1-R	CTTCCCAGCAGAGTCTGACC	
Lep-5'out R	GCAACATTTCTGGCTTTCCGT	426
Lep-5'in R	AATGTAATGTGGTGGGTGGCGTTC	
lep-3'outF	TACCGTCGTTCCACTAGTGATTT	380
Lep-3'inF	CGGATCCTCCACTAGTGATTTCACTATAG	
β-actin F	ACTTCGAGCAGGAGAT	150
β-actin R	ACAGTGTTGGCATACAG	
Lep2-F	CATGCTGTTTGCAGGATGAC	130
Lep2-R	CTTCCCAGCAGAGTCTGACC	

Putative clones were screened by PCR using the above primers under the same cycle conditions and the selected clones were sequenced using the dideoxy chain-termination method on an automatic 3730xl DNA Analyzers (ABI, Foster City, CA, USA). To obtain the full-length cDNA sequence, 5' RACE and 3' RACE were performed using the gene specific primers and adaptor primers (UPM) listed in Table 1. The PCR cycling conditions were 1 cycle at 94°C for 3 min; 20 cycles at 94°C for 30 sec, 55°C for 40 sec and 72°C for 60 sec for outer PCR; 25 cycles at 94°C for 30 sec, 56°C for 40 sec and 72°C for 60 sec for inner PCR; followed by 1 cycle at 72°C for 10 min. The purified fragments were then cloned and sequenced.

Sequence analysis: Sequence assembly of PT-PCR and RACE sequencing result was processed by DANMAN, similarity retrieval of nucleic acid and protein sequence was processed by BLAST on NCBI (http://www.ncbi.nlm.nih.gov), homology analysis were processed by MegAlign in DNAstar. Reading frame analysis and amino acid sequence deduction were processed by NCBI ORF online tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The biochemical property and functional site of Leptin protein were forecasted and analyzed by ExPASy-Tools (http://www.expasy.ch/tools/). Amino acid sequence were compared by Clustal (Thompson *et al*., 1994). Base composition and variation analysis, construction of Neighbor-joining phylogenetic tree were conducted by MEGA4. 0.

Real-time PCR: The primers for real-time PCR were designed according to the cloned *Culter alburnus* in Xingkai Lake Leptin fragment, shown in Table 2. Real-time PCR were proceeded by instruction of Takara PrimeScript RT reagent kit perfect real time, the reaction system is 20 μL, SYBR Premix Ex Taq 10 μL, PCR Forward Primer 0.8 μL, PCR Reverse Primer 0.8 μL, ROX Reference Dye II 0.4 μL, cDNA 2 μL, dH_2O 6 μL. The reaction conditions were pre-denaturation at 95°C for 15 sec; 40 cycles,

denaturation at 95°C 5 sec, annealing at 60°C 34 sec; 95°C for 15 sec, 60°C for 1 min, 95°C for 15 sec. Three replicates of each sample were processed, ddH_2O was as control template. The specificity and reliability of real-time PCR primer were detected by making the standard curve. About 2 μL cDNA of all tissues are taken and mixed as template, target gene primer and reference gene primer were used for PCR, the PCR products were prepared into five gradients 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} by 10-fold dilution which was used to construct standard curve. The transcription levels of target gene mRNA were showed by target gene/muscle actin mRNA (%). The samples repeatability and differences among groups were analyzed by SPSS17.0 Statistical Software.

Table 2: The comparison analysis of sequence similarity

I/S	1	2	3	4	5	6	7	8	9	10
1	-	87.8	90.2	73.7	74.3	19.1	24.0	27.8	22.8	21.6
2	12.7	-	88.4	70.2	71.9	19.7	24.0	26.0	22.2	22.8
3	10.6	12.0	-	73.7	76.6	21.1	25.1	29.6	23.4	23.4
4	32.4	35.4	31.5	-	82.5	19.7	32.2	27.2	23.4	22.2
5	31.5	33.6	28.1	20.0	-	20.4	29.2	27.2	25.1	24.0
6	238.0	214.0	216.0	207.0	207.0	-	23.7	13.2	9.2	8.6
7	191.6	195.6	186.4	165.9	172.2	172.2	-	21.9	17.4	19.2
8	179.4	195.0	170.7	165.4	282.0	282.0	186.2	-	34.1	35.3
9	241.0	233.0	234.0	220.0	289.0	289.0	188.6	133.9	-	82.6
10	241.0	227.0	221.0	227.0	301.0	301.0	196.0	128.3	19.8	-

I = Identity percent; S = Divergence; 1) *Culter alburnus* in Xingkai Lake; 2) *Hypophthalmichthys molitrix*; 3) *Ctenopharyngodon idella*; 4) *Cyprinus carpio I*; 5) *Cyprinus carpio II*; 6) *Takifugu rubripes*; 7) *Oncorhynchus mykiss*; 8) *Xenopus laevis*; 9) *Mus musculus*; 10) *Homo sapiens*

RESULTS

Cloning and analysis of *Culter alburnus* in Xingkai Lake

Leptin sequence: The total RNA of muscle tissue whose OD_{280}/OD_{260} is among 1.8-2.0 were as templates, degenerate primers Lep1 were as primers, to amplify into 685 bp fragment which accord with expection. PCR products were sequenced then analyzed by NCBI BLAST were confirmed to be Leptin sequence. About 426, 380 bp cDNA fragment of the *ob* gene were got by 5'RACE and 3'RACE technology. The results show that, total Leptin length is 1208 bp, 5 'untranslated region is 132 bp, 3' untranslated region is 554 bp, initiation codon is ATG, stop codon is TAA, polyadenylation signal of PolyA is AATAAA. The complete open reading frame is composed of 522 bp which encode 173 amino acids (Fig. 1). The molecular mass is 19565.8Da, isoelectric point is 7.69. The chemical formula is $C_{879}H_{1421}N_{229}O_{255}S_9$, composed of 2793 atoms.

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1       GGGTGGACACATCACTGCGCATGGCTACATGCTGACAGCCTACTGATGATCAGTCGATGG
61      AAAATCATACAATCCTCACATCTCAAAGGTATTGCACTATTGCCAAGATAAAGACCACCA
121     TATACAGGAACAATGTATTCTCCAGTTCTTCTCTACACCTGCTTTTTGAGCATTCTTGGT
                    M  Y  S  P  V  L  L  Y  T  C  F  L  S  I  L  G
181     CTGATCGATGGCCTTTCGATCCCCATTCATCAGGATAGCCTCAAAAACTTGGTCAAACTG
         L  I  D  G  L  S  I  P  I  H  Q  D  S  L  K  N  L  V  K  L
241     CAGGCAGACACCATCATCCACAGAATTAAGGAACACAATGAGAAGCTAAATCTATCTCCA
         Q  A  D  T  I  I  H  R  I  K  E  H  N  E  K  L  N  L  S  P
301     AAGATCCTCATTGGGGATTCAGAGCTTTACCCTGAGGTTCCTGCTGATAAACCTATCCAA
         K  I  L  I  G  D  S  E  L  Y  P  E  V  P  A  D  K  P  I  Q
361     GGGCTTGGGTCCATCATTGACACCCTAACTACCTTCCAGAAGGTCCTTCAAACACTGCCC
         G  L  G  S  I  I  D  T  L  T  T  F  Q  K  V  L  Q  T  L  P
421     AGGGGGTATGCGAGCCAGTTACGCAGTGATGTGTCCACCCTTTTGGACTGCTTTAAAGAT
         R  G  Y  A  S  Q  L  R  S  D  V  S  T  L  L  D  C  F  K  D
481     AGGATGACATTTATGCGTTGTACACCAAAGGAGCCAGCCAATGGGAAGTCACTGGACACT
         R  M  T  F  M  R  C  T  P  K  E  P  A  N  G  K  S  L  D  T
541     TTCATAGAAAAGAACGCCACCCACCACATTACATTTGGGTACATGGCTTTAGACAGACTG
         F  I  E  K  N  A  T  H  H  I  T  F  G  Y  M  A  L  D  R  L
601     ACACAGTTCATGCAAAAGCTGATAGTTAATCTGGACCAGTTGAAGAGCTGCTAATCTGCC
         T  Q  F  M  Q  K  L  I  V  N  L  D  Q  L  K  S  C  *
661     ACTATTATAGCATTATAGTATACTATTTATTATATTTATTTAAAACCCTGTATATTTATA
721     GACCAAAACAGTATTTTTGGCACATTTTAATGTTCAAACAGATTTAAAAAATATTCCCTG
781     CTATTAAACGGAAAGCCAGAAATGTTGCTTGCGACATCATGGAGCGCCAATCAATCTGTC
841     CTTACGATGTAAACAAACACTAGCAATCTGATACCTGCACACATTGCCATGTCATACTTC
901     ATGCTGTTTGCAGGATGACATCAGCACTTGCTGCGCTTGCTCAATGATGTCACTGTGTTT
961     ACCAGAAGAAGCGAGGTCAGACTCTGCTGGGAAGCTGGAATTATGTTCCCATTTTACTTC
1021    TTGCTGCAACACCAGCAATGTAGGACACACACCAATATATATATAAAAATATATATATAT
1081    ATTCCTACACAGTGTGAATCTATGCACTTTGAATATCCTGTGATTGTAAATAGTTTTGTA
1141    TTTTGTGTAATGATGCATTTTTCCATAAAAATAAACCAATAAAATAAATGTATTGTAAAA
1201    AAAAAAAA
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Fig. 1: The full length of Leptin cDNA and predicted amino acid sequences; Initiation codon and termination codon are boxed, the lines under the amino acid sequence indicate the polyadenylation signal

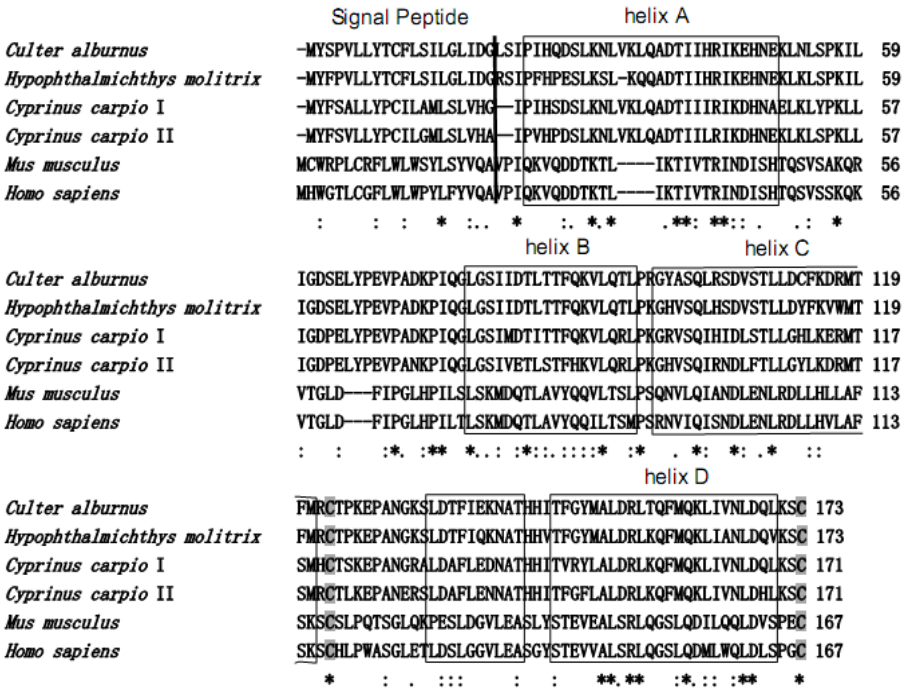

Fig. 2: Leptin amino acid sequence alignment among *Culter alburnus* in Xingkai Lake, *Hypophthalmichthys molitrix*, *Cyprinus carpio*, *Mus musculus* and *Homo sapiens*; The identity amino acid residues are indicated by asterisk; Dashes indicate the amino acid gaps that are necessary to align these sequences; Conservative cysteine residues are marked in shade; Putative signal peptide cleavage site is indicated by a vertical bar, the 4 α-helices domsin inferred from human Leptin are boxed

***Culter alburnus* in Xingkai Lake Leptin amino acid sequence alignment and homology analysis:** Leptin primary structure of different species including *Homo sapiens*, *Mus musculus*, *Cyprinus carpio*, *Hypophthalmichthys molitrix* were analyzed by ClustalX. The result show that Leptin is composed of four α-helical domain, two cysteine residues that form intrachain disulfide bond are relatively conservative (Fig. 2). DNAstar package MegAlign Software were applied to compare homology of Leptin amino acid in *Culter alburnus* in Xingkai Lake, *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Takifugu rubripes*, *Oncorhynchus mykiss*, amphibians, *Mus musculus* and *Homo sapiens*. Results are shown in Table 2, *Culter alburnus* have highest homology with *Ctenopharyngodon idella* at 90.2% have high homology with other carps at 73.7-83.7% have low homology with *Oncorhynchus mykiss* and *Takifugu rubripes* at 24 and 19.1%, respectively have very low homology with mammals and amphibians, <30%. Leptin similarity is high among *cyprinids*, such as the similarity between the *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* is 88.4%, it is 70.2% among *Cyprinus carpio* subtype I similarities.

Phylogenetic analysis of *Culter alburnus* in Xingkai Lake Leptin: Leptin amino acids fragments phylogenetic tree of different species was constructed by Neighbor Joining Method in MEGA4 Software, the result is shown in Fig. 3, in this phylogenetic tree, vertebrate was divided into 2 groups, all teleostean is one group, another one include all mammals. *Culter alburnus* in Xingkai Lake, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* cluster into one branch then cluster into one branch with carp and zebra fish. In teleostean group, all cyprinid fish compose a monophyletic group which show close genetic relationship. The result is consist with the phylogenetic research result in morphology.

Expression levels of Leptin RNA in wild and cultured *Culter alburnus* in Xingkai Lake muscle of different ages: The expression levels of *Leptin* gene in wild and cultured *Culter alburnus* in Xingkai Lake muscle from 2-6 years old were compared, the result show that both wild and cultured population with the increase of age, expression of *Leptin* gene from muscle tissue are increased significantly. The expression of Leptin in wild

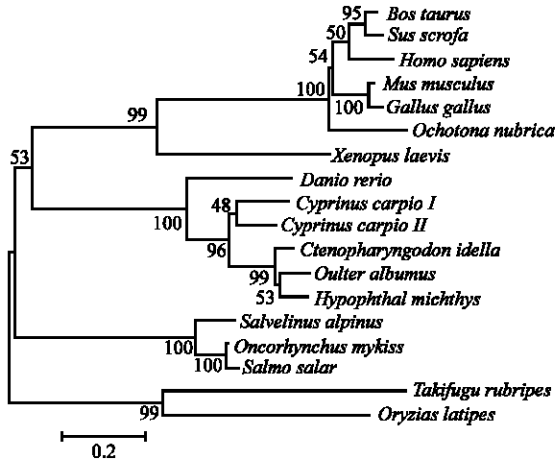

Fig. 3: Phylogenetic tree of and other vertebrates based on Leptin amino acid sequences; *Hypophthalmichthys molitrix* (ACI32423.1); *Ctenopharyngodon idella* (ACI32424.1); *Cyprinus carpio* I (CAI30828.1); *Cyprinus carpio* II (CAI30827.1); *Danio rerio* (CAJ33891.1); *Takifugu rubripes* (BAD94444.1); *Oncorhynchus mykiss* (CAR67819.1); *Oryzias latipes* (BAD94448.2); *Xenopus laevis* (AAI69780.1); *Gallus gallus* (ACL68083.1); *Mus musculus* (ADM72802.1); *Homo sapiens* (AAH69452.1); *Salmo salar* (ACZ02412.1); *Ochotona nubrica* (ABN13965.1); *Bos taurus* (CAD54745.1); *Sus scrofa* (ADK62397.1); *Salvelinus alpinus* (BAH83535.1)

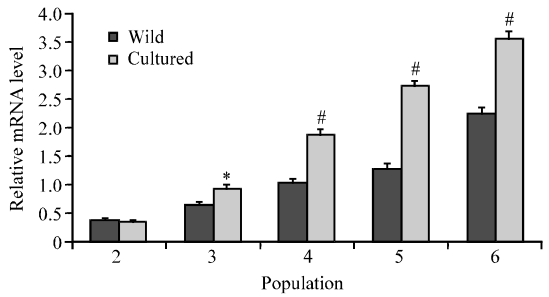

Fig. 4: Comparison of Leptin mRNA level in wild and cultured population of *Culter alburnus* in Xingkai Lake; *Significantly difference ($p<0.05$); #Significantly difference extremely ($p<0.01$)

population showed down regulation in contrast to the cultured population, the difference of 4, 5 and 6 years old is different extremely ($p<0.01$) (Fig. 4).

DISCUSSION

Study of Baker *et al.* (2000) and Londraville and Duvall (2002) showed that mammals Leptin have no effect on feed intake and body weight of silver salmon (*Oncorhynchus kisutch*), catfish (*Ictalurus punctatus*) and green sunfish (*Lepomis cyanellus*) but can inhibit feed intake, control weight on goldfish (Volkoff *et al.*, 2003). This indicates that the function of Leptin is species-specific. Therefore, fish Leptin cloning and functional studies are very important. In this study, it was first reported that Leptin cDNA sequence length *Culter alburnus* in Xingkai Lake is 1208 bp, 5 'untranslated region is 132 bp, 3' untranslated region is 554 bp, ORF length is 522 bp which encode 173 amino acids. The 20~21th enzyme cutting site of Leptin N-terminal form a signal peptide which is consist with mammals (Zhang *et al.*, 1994). The earlier study proposed that there is big difference in Leptin sequence of mammals and non-mammals, gene structure and putative tertiary structure is conservative (Kurokawa *et al.*, 2005; Huising *et al.*, 2006; Murashita *et al.*, 2008; Crespi and Denver, 2006; Boswell *et al.*, 2006). This view is also confirmed in the present study when the homology of amino acid sequence were compared among *Culter alburnus* in Xingkai Lake and other species, it was found that the alburnus have highest homology with *Ctenopharyngodon idella* at 90.2%, have very low homology with mammals and amphibians, <30%. But, the gene structure (which are all composed of four α-helical domain) is conservative relatively, especially the two disulfide bond which is formed by two cysteine residues and follow C and D α-coiled-coil domain, it have key role in maintain Leptin structure and function. This result is the same with *Ctenopharyngodon idella* (Li *et al.*, 2010).

In a recent study, Gorissen *et al.* (2009) cloned and identified two different Leptin paralogs: Leptin-a and Leptin-b from *Danio rerio*, this kind of duplicate gene was also found in another teleost species *Oryzias latipes* which indicates that the fish may have two types of Leptin (Gorissen *et al.*, 2009; Kurokawa and Murashita, 2009). Gorissen *et al.* (2009) found that the expression pattern of Leptin-a and Leptin-b in *Danio rerio* is also different, after a week fasting *Leptin-a* gene expression in the liver did not change significantly while the expression level of Leptin-b showed markedly decreased. These results show that the function of Leptin-a and Leptin-b may be different. In this study, the degenerate primers were designed according to the Leptin-a type of *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, so the *Culter alburnus* in Xingkai Lake Leptin researchers cloned Leptin-a type. Therefore, in the following study, researchers need separate and identify the Leptin-b of *Culter alburnus* in Xingkai Lake and the study its function.

The phylogenetic tree analysis by NJ method show that the *Culter alburnus* in Xingkai Lake,

Hypophthalmichthys molitrix, *Ctenopharyngodon idella* and *Cyprinus carpio* (subtype I and II) and *Danio rerio* is Orthologous. *Oryzias latipes*, *Takifugu rubripes*, *Salmo salar* and *Salvelinus alpinus* were clustered into fish branch; Leptin of mammals, amphibians, birds clustered into one branch independently which is consistent with the species molecular evolutionary relationships. Kumar and Hedges (1998) reported that teleost and tetrapod were evoloved from same ancestor. Taylor *et al*. (2003) reported that the cyprinids was separated from the ancestors of teleost 300 million years ago, these views also support the results of this study.

In Xenopus and mammals, Leptin could regulates energy balance but the role of Leptin in teleost in the energy balance is not clear yet. The main purpose of this study is to get full-length Leptin CDS in *Culter alburnus* in Xingkai Lake and study its phylogenetic, the next step is to study the biological function of the *Culter alburnus* in Xingkai Lake Leptin. The experimental tissue researchers selected is white muscle that related to meat directly. The results showed that Leptin indicated increasing trend with age which is related to the increase of muscle fat content with age which increases with age. Compared with cultured populations, the expression of *Leptin* gene in wild Xingkai Lake *Culter alburnus* in Xingkai Lake showed down regulating patterns, difference is very significant at 6 years old ($p<0.01$). In earlier studies, the cultured space of *Culter alburnus* in Xingkai Lake is small, energy consumption on feeding is few, the fish is provided compound feed which can lead to fat accumulation easily. Therefore, the muscles fat content of cultured *Culter alburnus* in Xingkai Lake population was significantly higher than that of wild population from 3 years old. In this study, these results proved that the expression of Leptin in *Culter alburnus* in Xingkai Lake muscle have a linear relationship with energy state which is the same with mammals. When the energy is in the long-term positive balance, the stable increase of Leptin reflects the increase of body fat deposition (Houseknecht and Portocarrero, 1998), the study result by Ronnestad *et al*. (2010) is also the same. In the study by Friedman and Halaas (1998), regulation of Leptin on body fat and body weight in mammals is a feedback system when the body fat content increase, Leptin levels in blood rise which lead to reduced feeding and increased energy consumption; conversely when the body fat reduced, plasma Leptin levels is also decreased, the Leptin regulate feeding and energy output by centralis (Friedman and Halaas 1998).

In mammals, overcontaining appetite will result in a large increase in Leptin. Exogenous Leptin injection in *Carassius auratus* (De Pedro *et al*., 2006), *Oncorhynchus mykiss* (Murashita *et al*., 2008) and *Xenopus laevis* (Volkoff *et al*., 2003) led to a decrease in food intake which seems to emphasize the conservative role in appetite regulation in vertebrates Leptin. In view of the increase in *Leptin* gene expression in cultured conditions, researchers infer that the the *Leptin* gene may suppress their appetite. However in this study, high levels of endogenous Leptin in *Culter alburnus* cultured populations does not play its role to reduce weight and suppress feeding which may be the same with mammals when fish intake high energy, the body decreased sensitivity to Leptin and produce Leptin resistance, thereby enabling the physiological function of endogenous Leptin to be suppressed and leading to obesity (Burguera *et al*., 2000). However, whether the production or secretion of fish Leptin has a direct relationship with fat content or fat accumulation, it can not be determined.

Therefore, Leptin not only regulate the fat storage of fish and shrimp but also regulate body energy balance and meat quality. This study is about the *Leptin* gene structure of *Culter alburnus* in Xingkai Lake and the differential expression between wild and cultured populations which provide important reference for following studies about regulating function of *Leptin* gene in fish, molecular biology function and mechanism.

CONCLUSION

The expression characteristics of Leptin in wild and cultured *Culter alburnus* in Xingkai Lake of different ages was related to energy catabolism and its special biological function. *Leptin* gene play key roles in energy catabolism, fat storage and meat quality improvement in fish.

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