

## Cloning of the Thyroid-Stimulating Hormone Receptor Gene in Yellow Catfish (*Pelteobagrus fulvidraco*) and Its Expression Response to Dietary Potassium Iodide Supplementation - Postprint

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### Abstract

This study aimed to obtain the full-length cDNA sequence of the thyroid-stimulating hormone receptor (TSHR) gene in yellow catfish (*Pelteobagrus fulvidraco*), characterize its tissue expression patterns, and elucidate the effects of dietary potassium iodide supplementation on thyroid TSHR gene expression and growth performance in yellow catfish. The full-length cDNA sequence of the TSHR gene was cloned using rapid amplification of cDNA ends (RACE) technology, and the relative expression level of the TSHR gene was detected by real-time fluorescent quantitative PCR. Four experimental diets with potassium iodide supplementation levels of 0 (control), 10, 50, and 100 mg/kg were prepared for a 27-day yellow catfish feeding trial. The results showed that the full-length cDNA sequence of the TSHR gene with a length of 2 786 bp was successfully cloned in this study, containing an open reading frame of 2 238 bp that encoded 745 amino acids. Blast program analysis indicated that the TSHR amino acid sequence of yellow catfish shared 60-87% similarity with other known fish species. Tissue expression analysis results demonstrated that the TSHR gene exhibited relatively high expression levels in thyroid tissue, followed by liver, muscle, and intestine. The feeding trial results indicated that the relative expression level of thyroid TSHR gene in the 100 mg/kg group was significantly higher than that in other groups ( $P < 0.05$ ), and the weight gain, specific growth rate, and feed conversion ratio in the 50 and 100 mg/kg groups were significantly superior to those of the control group ( $P < 0.05$ ). It was therefore concluded that appropriate dietary supplementation of potassium iodide not only affects TSHR gene expression but also effectively promotes the growth of yellow catfish.

## Full Text

### Thyrotropin Receptor Gene Cloning and Expression Response to Dietary Potassium Iodide in Yellow Catfish (*Pelteobagrus fulvidraco*)

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**Abstract:** This study aimed to obtain the full-length cDNA sequence of the thyrotropin receptor (TSHR) gene in yellow catfish (*Pelteobagrus fulvidraco*), characterize its tissue expression patterns, and investigate the effects of dietary potassium iodide (KI) supplementation on thyroid TSHR gene expression and growth performance. The rapid amplification of cDNA ends (RACE) technique was employed to clone the full-length TSHR cDNA, while quantitative real-time PCR was used to determine relative TSHR expression levels. Four experimental diets were formulated with KI supplementation levels of 0 (control), 10, 50, and 100 mg/kg and fed to yellow catfish for 27 days. The results showed that the full-length TSHR cDNA sequence of 2,786 bp was successfully cloned, containing an open reading frame of 2,238 bp encoding 745 amino acids. Blast analysis revealed that the yellow catfish TSHR amino acid sequence shared 60%-87% similarity with other known fish species. Tissue expression analysis demonstrated relatively high TSHR expression in thyroid tissue, followed by liver, muscle, and intestine. The feeding trial indicated that the 100 mg/kg group exhibited significantly higher thyroid TSHR expression than other groups ( $P < 0.05$ ), while the 50 and 100 mg/kg groups showed significantly improved weight gain, specific growth rate, and feed conversion ratio compared to the control ( $P < 0.05$ ). These findings suggest that appropriate dietary KI supplementation not only affects TSHR gene expression but also effectively promotes growth in yellow catfish.

**Keywords:** yellow catfish (*Pelteobagrus fulvidraco*); potassium iodide; thyrotropin receptor; gene cloning; gene expression

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Thyroid hormones (THs), including thyroxine (T4) and 3,5,3-triiodothyronine (T3), are synthesized and secreted by thyroid tissue. These biologically active substances, though present at extremely low concentrations, act on nearly all parts of aquatic animals. Research has confirmed that THs not only promote growth and development and maintain metabolic balance but also stimulate nervous system development, enhance excitability, and support immune function. The secretion process of THs in fish is similar to that in higher vertebrates, with iodine and the thyrotropin receptor (TSHR) being two crucial factors in the TH synthesis pathway. Iodine is transported into thyroid follicles via the sodium-iodide symporter, where it is incorporated into monoiodotyrosine and diiodotyrosine, which subsequently generate THs. Therefore, iodine is an essential

trace element for growth and metabolic processes, and dietary iodine deficiency has been shown to cause hypothyroidism and goiter in fish. Although NRC standards indicate low iodine requirements for fish, iodine deficiency diseases frequently occur in freshwater areas with low iodine content. Supplementing iodine in feed can effectively increase iodine intake and maintain normal growth and metabolism.

TSHR is a G protein-coupled receptor belonging to the glycoprotein hormone receptor superfamily. It binds with thyrotropin to trigger a series of thyroid responses that promote TH release. The TSHR structure is highly conserved across species, primarily consisting of seven transmembrane helical domains, a short cytoplasmic domain, and a large extracellular domain containing leucine-rich repeats that are responsible for ligand-specific binding. Upon specific binding with thyrotropin, TSHR activates typical G-protein-coupled effectors (adenylate cyclase and phospholipase C), initiating intracellular signal cascades that stimulate thyroid tissue to synthesize and secrete T<sub>4</sub>, which is then converted to T<sub>3</sub> by deiodinases to regulate growth, development, and other vital processes. Consequently, TSHR plays an extremely important role in regulating the pituitary-thyroid axis in all vertebrates. TSHR genes have been studied at the molecular level in several fish species, including amago salmon (*Oncorhynchus rhodurus*), striped bass (*Morone saxatilis*), African catfish (*Clarias gariepinus*), channel catfish (*Ictalurus punctatus*), European sea bass (*Dicentrarchus labrax*), and Senegalese sole (*Solea senegalensis*). However, no studies on the TSHR gene in yellow catfish have been reported.

Given the important roles of both TSHR and iodine, this study first employed reverse transcription PCR (RT-PCR) and rapid amplification of cDNA ends (RACE) to clone the full-length TSHR cDNA from yellow catfish and analyze its differential tissue expression. Based on the importance of iodine for thyroid function and its potential influence on TSHR expression, the second objective was to investigate the effects of different dietary KI levels on TSHR gene expression and growth performance in yellow catfish.

### 1.1 Experimental Materials

Yellow catfish used for gene cloning were purchased from Dongyu Market, Yinzhou District, Ningbo, Zhejiang. One healthy yellow catfish (*Pelteobagrus fulvidraco*) weighing 145.2 g with a body length of 24 cm was selected. After dissection, brain, thyroid, heart, liver, spleen, muscle, kidney, gonad, head kidney, stomach, and intestine tissues were collected, snap-frozen in liquid nitrogen, ground, and partially mixed. The mixed tissues were then added to centrifuge tubes containing Trizol, mixed well, and stored at -80°C for total RNA extraction.

The main reagents used in this study included SuperQuickRT cDNA Kit and 2×Es TaqMasterMix from Kangwei Century Biotechnology Co., Ltd.; SMARTer® RACE cDNA Amplification Kit from Clontech; DL1000 DNA

Marker, DL5000 DNA Marker, DNase I, TaKaRa LA Taq with GC Buffer, pMDTM19-T Vector Cloning Kit, and SYBR® Premix Ex Taq™ II (TliR-NaseH Plus) from TaKaRa; Trans5 $\alpha$  Chemically Competent Cell from Beijing TransGen Biotech Co., Ltd.; GenClean Agarose Gel DNA Recovery Kit (column type) from Shanghai Generay Biotech Co., Ltd.; and potassium iodide from SIGMA (purity >99.5%). Other common chemical reagents were domestic analytical grade.

### 1.2 Feeding Trial

The experimental diets were formulated based on commercial yellow catfish feed (Hangzhou Haihuang Feed Development Co., Ltd., No. 2 feed, crude protein content 41%) supplemented with 0 (control), 10, 50, and 100 mg/kg KI to create four experimental diets. The measured KI contents were 0.31, 8.58, 47.50, and 92.63 mg/kg, respectively, with corresponding iodine contents shown in Table 2 . The supplementation method involved dissolving KI in water, mixing it with crushed commercial feed, extruding it into 3 mm diameter pellets using a meat grinder, low-temperature air-drying, sealing in bags, and storing at -20°C. The main nutritional components of the experimental diets are listed in Table 1 .

Experimental fish were purchased from a yellow catfish farm at Yuyao Fisheries Technology Extension Station, Zhejiang. One hundred twenty healthy yellow catfish with an average weight of 30.3 g were randomly stocked into eight 300-L tanks at 15 fish per tank. The eight tanks were randomly divided into four groups with two tanks per group. Fish were fed twice daily (09:00 and 16:00) to apparent satiation, and uneaten feed was collected and weighed approximately 30 minutes after feeding. Fish activity was monitored daily, and water was regularly disinfected with chlorine dioxide.

The experimental water was aerated tap water under natural light conditions, with continuous aeration using an air blower. Tanks were cleaned and half of the water was exchanged daily at 12:00 to maintain water quality. The trial lasted 27 days. Water temperature, dissolved oxygen, and feed intake were measured during the experiment. Water temperature ranged from 22 to 24°C, and dissolved oxygen concentration exceeded 6.2 mg/L. After 27 days, fish were weighed, and five fish per tank were randomly sampled for individual weight and length measurement. Liver and intestine weights were recorded, and thyroid tissue was collected, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent qPCR analysis.

### 1.3 Total RNA Extraction and First-Strand cDNA Synthesis

Total RNA was extracted from individual tissues (for qPCR) and mixed tissue samples (for gene cloning) using Trizol Reagent. RNA quality was assessed as follows: DNA contamination was removed using DNase I, concentration and purity were measured using a Nanodrop2000 spectrophotometer ( $1.9 < A_{260}/A_{280} < 2.1$ ), and integrity was verified by 1.2% agarose gel electrophoresis. First-strand

cDNA was synthesized using the SuperQuickRT cDNA Kit according to the manufacturer's instructions and stored at -20°C. The 10 L reaction mixture contained 2 L dNTP Mix, 1 L Primer Mix, 1 L RNA template, 2 L RT buffer, 0.5 L reverse transcriptase, and 3.5 L ddH<sub>2</sub>O. Reaction conditions were 37°C for 15 min and 85°C for 5 s. RACE cDNA templates were synthesized from mixed tissue RNA using the SMARTer® RACE cDNA Amplification Kit and stored at -20°C.

#### 1.4 Full-Length TSHR cDNA Cloning

Nucleotide sequences of TSHR genes from channel catfish (NM\_{001200192}.1), African catfish (AY129556.1), striped bass (AF239761.1), tilapia (NM\_{001279621}.1), zebrafish (NM\_{001145763}.2), and Senegalese sole (FN677495.1) were retrieved from NCBI GenBank and aligned to design degenerate primers TSHR-F1 and TSHR-R1 (Table 2) in conserved regions using Bioedit and Oligo 6.0 software. Primers were synthesized by Shanghai Sangon Biotech Co., Ltd. The PCR reaction mixture (10 L) contained 5 L 2×Es TaqMasterMix, 0.2 L mixed tissue cDNA template, 0.4 L each of forward and reverse primers (10 M), and 4 L ddH<sub>2</sub>O. PCR conditions were: 94°C for 2 min; 30 cycles of 94°C for 30 s, 51.2°C for 30 s, 72°C for 45 s; and final extension at 72°C for 10 min. Products were separated on 1.5% agarose gel, purified using the GenClean Agarose Gel DNA Recovery Kit, and sequenced by Shanghai Huada Gene Technology Co., Ltd. The 815 bp product matched the expected size, and BLAST analysis confirmed it as a partial TSHR sequence.

Based on this partial sequence, RACE-specific primers were designed using Oligo 7 software: 3-RACE-F2-1, 3-RACE-F2-2 for the 3' end, and 5-RACE-R2-1, 5-RACE-R2-2, 5-RACE-R3-1, 5-RACE-R3-2, 5-RACE-R3-3, 5-RACE-R4-1, and 5-RACE-R4-2 for the 5' end (Table 2). The 5' end was successfully amplified using three pairs of specific primers. Nested PCR was employed for both 3' and 5' RACE to improve specificity. The 50 L reaction mixture contained 0.5 L TaKaRa LA Taq, 25 L 2×GC Buffer II, 8 L dNTP Mixture, 2 L template cDNA (first-round PCR product), 2 L specific primer, 5 L (or 2 L) UPM (NUP) primer, and ddH<sub>2</sub>O to volume. PCR conditions were: 94°C for 2 min; 30 cycles of 94°C for 30 s, annealing temperature for 30 s, and 72°C for specified time; final extension at 72°C for 10 min. Annealing temperature and extension time were determined based on primer properties and predicted product size. PCR products were purified, cloned into pMDTM19-T Vector, transformed into Trans5α Chemically Competent Cells, and positive clones selected by LB plate (Amp+) culture and colony PCR using M13 and specific primers were sequenced by Shanghai Huada Gene Technology Co., Ltd.

#### 1.5 TSHR Gene Sequence Assembly and Bioinformatics Analysis

The full-length TSHR cDNA sequence was assembled from the partial sequence, 5' RACE sequence, and 3' RACE sequence using Seqman in DNASTar software. The open reading frame and deduced amino acid sequence were predicted

using ExPASy online tool (<http://web.expasy.org/translate/>). Molecular weight and isoelectric point were calculated using EditSeq in DNASTar. Multiple sequence alignment of yellow catfish TSHR amino acid sequence with those of African catfish (AAN01360.1), channel catfish (AY533543), zebrafish (NM001145763.2), Senegalese sole (CBK38913.1), and largemouth bass (AKC32650.1) was performed using MegAlign to analyze similarity and divergence. Signal peptide was predicted using SignalP 4.1 Server (<http://www.cbs.dtu.dk/services/SignalP/>). Transmembrane helices were predicted using TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>). N-glycosylation sites were predicted using NetNGlyc (<http://www.cbs.dtu.dk/services/NetNGlyc/>). Phosphorylation sites were predicted using NetPhos 2.0 Server (<http://www.cbs.dtu.dk/services/NetPhos/>). Secondary structure was analyzed using PSIPRED v3.3 (<http://bioinf.cs.ucl.ac.uk/psipred/>). Conserved domains were analyzed using SMART (<http://smart.embl-heidelberg.de>). A phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 6.0 with 1,000 bootstrap replicates to calculate branch confidence values.

### 1.6 Tissue Expression Analysis of TSHR Gene

To examine TSHR expression in yellow catfish, reference primers GAPDH-F and GAPDH-R for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (JQ068865.1) and TSHR primers TSHR-qF and TSHR-qR (Table 2) were designed based on the cloned TSHR cDNA sequence. The qPCR reaction mixture (12.5  $\mu$ L) contained 6  $\mu$ L 2 $\times$ SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II (TliRNaseH Plus), 0.24  $\mu$ L each of forward and reverse primers, 0.24  $\mu$ L ROX reference Dye II, and ddH<sub>2</sub>O to volume. The qPCR program was: 95°C for 30 s; 40 cycles of 95°C for 5 s and 55.2°C for 30 s; followed by melting curve analysis (95°C for 15 s, 60°C for 60 s, 95°C for 15 s). After reaction completion, melting curve analysis verified PCR specificity, and relative TSHR mRNA expression levels across tissues were determined.

### 1.7 Analytical Methods

Crude protein content was determined by the Kjeldahl method, crude fat by Soxhlet extraction, crude ash according to GB/T 5099.4–2010, moisture by direct drying, and iodine content by GB/T 13882–2010.

### 1.8 Calculations

Specific growth rate (SGR) =  $100 \times (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{feeding days}$ ;

Feed conversion ratio (FCR) =  $\text{feed intake} / \text{weight gain}$ ;

Condition factor (CF) =  $\text{body weight} / \text{body length}^3$ ;

Hepatosomatic index (HSI, %) =  $100 \times \text{liver weight} / \text{body weight}$ ;

Intestine index (%) =  $100 \times \text{intestine weight} / \text{body weight}$ .

## 1.9 Statistical Analysis

qPCR data were calculated using the  $2^{-\Delta\Delta CT}$  method, with TSHR expression in various tissues normalized to muscle expression. Growth trial data were analyzed by one-way ANOVA using SPSS 19.0. All data are presented as “mean  $\pm$  standard deviation.”

## 2 Results and Analysis

### 2.1 Cloning and Sequence Analysis of Yellow Catfish TSHR Gene

Using mixed tissue cDNA as template, a cDNA fragment of the expected size was cloned. Homology analysis of the predicted amino acid sequence in the NCBI database revealed high similarity with TSHR from channel catfish (Siluriformes), confirming it as the yellow catfish TSHR gene. Sequence analysis showed the full-length TSHR cDNA was 2,786 bp, containing a 2,238 bp open reading frame (239–2,476 bp), a 238 bp 5' untranslated region (1–238 bp), and a 310 bp 3' UTR (2,477–2,786 bp), encoding 745 amino acids with ATG start codon and TAA stop codon (Figure 1 [Figure 1: see original paper]). The sequence was submitted to GenBank (accession number KT722732).

The start and stop codons are boxed, the putative signal peptide is underlined, N-linked glycosylation sites are marked in cyan, and transmembrane helix domains are shaded in blue.

DNASar EditSeq analysis revealed the yellow catfish TSHR protein contained 63 strongly basic amino acids (K, R), 65 strongly acidic amino acids (D, E), 293 hydrophobic amino acids (A, I, L, F, W, V), and 221 polar amino acids (N, C, Q, S, T, Y). The predicted molecular weight was approximately 83.8 kDa with a theoretical isoelectric point (pI) of 7.163. SignalP 4.1 Server predicted an N-terminal signal peptide at positions 1–19 (MCLLALVILTFCSTRCTG) with a potential cleavage site between amino acids 19 and 20 (Figure 1).

TSHR is a transmembrane protein spanning the lipid bilayer. The yellow catfish TSHR amino acid sequence contained a hydrophobic seven-transmembrane helical region of 264 amino acids located at positions 421–443, 450–472, 487–509, 540–562, 582–604, 625–647, and 662–684; a 420-amino acid extracellular domain (positions 1–420) including the N-terminal signal peptide; and a 61-amino acid intracellular domain (positions 685–745). Both extracellular and intracellular regions were hydrophilic. The transmembrane helices were connected by three intracellular and three extracellular loops, representing a typical G-protein-coupled receptor structure. Glycosylation site prediction identified three potential N-glycosylation sites (76NISL, 197NGTK, 301NLTE) and 20 conserved cysteine residues (Figure 1). Phosphorylation site prediction revealed 38 protein kinase phosphorylation sites, including 20 serine, 11 threonine, and 7 tyrosine residues. The extracellular domain contained 14 serine, 8 threonine, and 4 tyrosine sites; the second intracellular loop had 1 serine site; the second extracellular loop had 2 tyrosine sites; the third intracellular loop had 2 serine

and 1 tyrosine sites; and the intracellular domain had 3 serine and 3 threonine sites. Secondary structure prediction indicated 21  $\alpha$ -helices, 15  $\beta$ -sheets, and 34 random coils. SMART analysis identified four conserved domains: three leucine-rich repeat domains [LRRNT (56-77), LRR\_8 (78-137), LRR\_5 (149-226)] and one transmembrane helical domain [7tm\_1 (430-677)] (Figure 2 [Figure 2: see original paper]).

Sequence alignment using Clustal X showed that the seven transmembrane helical regions were highly conserved. The yellow catfish TSHR amino acid sequence displayed 57%-87% similarity and 2.0%-11.5% divergence with known TSHR sequences from other fish species (Table 3 ).

The phylogenetic tree based on TSHR amino acid sequences from 16 species including yellow catfish, striped bass, tilapia, Senegalese sole, chum salmon A and B, zebrafish, African catfish, and channel catfish is shown in Figure 4 [Figure 4: see original paper]. The tree divided into two subgroups: fish species clustered in one subgroup, while mammals, amphibians, and birds formed another. Yellow catfish TSHR grouped within the fish branch, showing the closest relationship with channel catfish and African catfish, followed by zebrafish, and a more distant relationship with mammals. The branching pattern was consistent with species evolution, indicating that TSHR proteins have undergone some differentiation during evolution.

## 2.2 Tissue Expression Differences of TSHR Gene in Yellow Catfish

TSHR gene expression across different tissues is shown in Figure 5 [Figure 5: see original paper]. The results demonstrated that TSHR was expressed in all examined tissues with varying expression levels, showing relatively high expression in thyroid, followed by liver, muscle, and intestine, with lower expression in other tissues.

## 2.3 Effects of Dietary Potassium Iodide on Growth Performance of Yellow Catfish

As shown in Table 4 , weight gain and specific growth rate increased initially and then decreased with increasing dietary KI levels. The 50 and 100 mg/kg groups exhibited significantly higher values than the control group ( $P < 0.05$ ). Feed intake did not differ significantly among groups ( $P > 0.05$ ). Feed conversion ratio decreased initially and then increased with KI supplementation, with the 50 and 100 mg/kg groups showing significantly lower FCR than the control ( $P < 0.05$ ). Condition factor, hepatosomatic index, and intestine index did not differ significantly among groups ( $P > 0.05$ ).

## 2.4 Effects of Dietary Potassium Iodide on Thyroid TSHR Gene Expression in Yellow Catfish

qPCR analysis (Figure 6 [Figure 6: see original paper]) showed that thyroid TSHR expression in the 100 mg/kg group was significantly higher than in other

groups ( $P < 0.05$ ), being 2.3-17.5 times higher. No significant differences were observed among the remaining groups ( $P > 0.05$ ). TSHR expression exhibited a decreasing trend followed by an increase with rising dietary KI levels.

### 3 Discussion

#### 3.1 Bioinformatics Analysis of Yellow Catfish TSHR Gene

This study successfully obtained the full-length TSHR cDNA of 2,786 bp from yellow catfish, encoding a 745-amino acid protein. The amino acid sequence showed highest similarity (87%) with channel catfish TSHR and high similarity with other fish TSHR sequences. The sequence exhibited typical characteristics of the glycoprotein receptor family. Segaloff et al. classified TSHR as group A of the G-protein-coupled receptor family, featuring a unique large extracellular domain that regulates thyrotropin binding, rich leucine repeats in the extracellular domain (approximately half of the total sequence length), a 7tm\_1 domain, and three extracellular and three intracellular loops connecting the transmembrane domains—all consistent with our cloned sequence. Rocha et al. reported similar typical glycoprotein family features in TSHR cDNA cloned from gonads. Phylogenetic analysis showed that fish, avian, and mammalian TSHR clustered together, suggesting a common ancestor for TSHR proteins that diverged into different receptor types during evolution.

Comparison of yellow catfish TSHR with other species revealed higher similarity with channel catfish and African catfish, followed by zebrafish and Atlantic salmon, consistent with taxonomic relationships. This indicates that the TSHR gene is evolutionarily conserved and important for physiological functions.

#### 3.2 Tissue Expression Specificity of TSHR Gene in Yellow Catfish

This study detected TSHR expression in both thyroid and non-thyroid tissues, consistent with findings in African catfish, channel catfish, Senegalese sole, striped bass, and European sea bass, where TSHR was expressed not only in thyroid but also in heart, muscle, pituitary, intestine, stomach, kidney, liver, spleen, head kidney, brain, and gonad, albeit with varying expression abundance. Our results showed TSHR expression in heart, liver, spleen, stomach, kidney, head kidney, brain, muscle, intestine, gonad, and thyroid, suggesting broad regulatory roles in normal physiological metabolism. Notably, spleen is an immune organ, implying a potential link between TSHR and immune function. Studies have confirmed that thyroid homeostasis disruption alters immune cell proportions in mammals, and manipulated thyroid hormone status changes leukocyte composition in rainbow trout. Wang et al. demonstrated that thyrotropin connects monocytes, lymphocytes, macrophages, and peripheral T cells during early antigen immune responses, and thyrotropin acts as an immune cell regulator. We hypothesize that TSHR may play an immunomodulatory role in spleen by binding thyrotropin, though the specific mechanism remains unclear.

### 3.3 Effects of Dietary Potassium Iodide on Growth Performance and TSHR Expression

The results demonstrated that dietary KI significantly affected weight gain, specific growth rate, and feed conversion ratio in yellow catfish, with 50 and 100 mg/kg KI promoting growth and improving feed utilization efficiency. These findings align with previous studies showing that environmental iodine content correlates with growth, survival, metamorphosis, and thyroid hormone levels in Pacific threadfin larvae, where appropriate iodine levels enhanced development, growth, and survival. Gensic et al. reported improved growth, higher thyroid hormone levels, and enhanced survival and disease resistance in iodine-supplemented rainbow trout. However, Penglase et al. found that 129 mg/kg iodine was toxic to Atlantic cod larvae, exceeding our maximum KI level, as excess iodine reduces thyroid activity and growth performance. Some studies showed no significant effects of iodine on feed intake, growth, or feed conversion in gilthead seabream, while others reported reduced embryo survival in wall-eye exposed to 100 mg/L iodine solution, possibly due to differences in iodine dosage, chemical form, and developmental stage.

Thyrotropin binds to TSHR on thyroid follicular cells to regulate sodium-iodide symporter expression, which actively transports iodine into follicular tissue to promote TH synthesis and secretion, maintaining normal thyroid activity. TSHR gene plays a critical role in this process; insufficient iodine supply may cause sodium-iodide symporter excess, thereby inhibiting TSHR expression. Therefore, TSHR expression should increase with iodine content within a certain range. Our results showing increased TSHR expression with KI supplementation align with this expectation. Iodine effects on TSHR ultimately influence TH levels and thyroid function. Studies have shown that adequate dietary iodine increases thyroid hormone content and disease resistance while reducing harmful effects on growth. Ribeiro et al. demonstrated that dietary iodine improved survival and reduced thyroid malformations (goiter) in Senegalese sole larvae, enhancing growth rate. Conversely, iodine deficiency reduces thyroid activity during early larval metamorphosis, as confirmed in Atlantic halibut. Our results are consistent with these findings, suggesting that dietary iodine regulates yellow catfish growth performance through the thyroid axis endocrine pathway, providing a feasible approach to improve aquaculture economic benefits.

### Key Findings

1. The full-length TSHR cDNA sequence was successfully cloned from yellow catfish tissues (GenBank accession KT722732).
2. TSHR expression was highest in thyroid tissue, followed by liver, muscle, and intestine, with lower expression in other tissues.
3. Dietary KI supplementation significantly affected weight gain, specific growth rate, feed conversion ratio, and thyroid TSHR gene expression in yellow catfish.

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