Effects of Feeding Time on Growth and Insulin-like Growth Factor-I Gene Expression in Juvenile Turbot (Scophthalmus maximus) Postprint

Authors: Wang Yichao, Zheng Keke, Xu Houguo, Liang Mengqing

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Abstract

This study aimed to investigate the effects of feeding time on growth and insulin-like growth factor-I (IGF-I) gene expression in juvenile turbot (Scophthalmus maximus). Three experimental groups were established, with juvenile turbot [initial average body weight (8.95±0.13) g] being fed at three different time points: morning (06:00), noon (12:00), and evening (18:00). Each group comprised three replicates, with 25 fish per replicate. The feeding trial lasted for 45 d. The results indicated that the weight gain rate and specific growth rate of juvenile turbot in the 06:00 feeding group were significantly higher than those in the other two groups ($P<0.05$). No significant differences in feeding rate were observed among the three feeding groups ($P>0.05$); however, the feed efficiency of the 06:00 and 12:00 feeding groups was significantly higher than that of the 18:00 feeding group ($P<0.05$). The relative IGF-I mRNA expression level in the liver of turbot in the 06:00 feeding group was significantly higher than that in the 12:00 and 18:00 feeding groups ($P<0.05$), whereas feeding time had no significant effect on the relative IGF-I mRNA expression level in the brain of juvenile turbot ($P>0.05$). In summary, it is recommended that the feeding time for juvenile turbot with a body weight of approximately 8 g be set at 06:00.

Full Text

Feeding Time Affects Growth and Insulin-like Growth Factor-I Gene Expression of Juvenile Turbot (Scophthalmus maximus)

WANG Yichao, ZHENG Keke, XU Houguo, LIANG Mengqing*
(Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China)

Abstract

This experiment was conducted to investigate the effects of feeding time on growth and insulin-like growth factor-I (IGF-I) gene expression in juvenile turbot (Scophthalmus maximus). Three feeding groups were established, with juvenile turbot [initial average body weight (8.95±0.13) g] fed at three different times: morning (06:00), noon (12:00), and evening (18:00). Each group had three replicates with 25 fish per replicate. The feeding trial lasted for 45 days. The results showed that the weight gain rate and specific growth rate of juvenile turbot in the 06:00 feeding group were significantly higher than those in the other two groups ($P<0.05$). There was no significant difference in feeding rate among the three groups ($P>0.05$), but feed efficiency in the 06:00 and 12:00 feeding groups was significantly higher than in the 18:00 group ($P<0.05$). The relative expression level of IGF-I mRNA in the liver of juvenile turbot in the 06:00 feeding group was significantly higher than that in the 12:00 and 18:00 groups ($P<0.05$), while feeding time had no significant effect on IGF-I mRNA relative expression in the brain ($P>0.05$). In summary, it is recommended that juvenile turbot weighing approximately 8 g be fed at 06:00.

Keywords: juvenile turbot; feeding time; growth; IGF-I gene expression

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Introduction

In modern aquaculture production, feed costs account for approximately 70% of total production costs. Feeding strategy is one of the critical factors affecting growth, feed utilization, and production efficiency in fish and other aquaculture species. Many fish species exhibit natural circadian rhythms, which are major determinants of their feeding behavior and physiological metabolism. Previous studies have shown that selecting feeding times based on the natural feeding rhythms of cultured animals can optimize their growth [1]. Qin et al. [2] investigated the growth and liver transcriptome of *Pelteobagrus vachellii* and found that feeding time significantly affected its growth and liver metabolism. Hossain et al. [3] reported that feeding time significantly influenced the growth and feed utilization of African catfish (*Clarias gariepinus*). Yang et al. [4] demonstrated that feeding time significantly affected feed intake and activity rhythms in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Boujard et al. [5] suggested that fish feeding behavior is closely related to fish ethology and physiology, and their research indicated that blood nutrient composition and hormone levels in fish also have a "biological clock," though the specific physiological mechanisms remain unclear.

However, in current economic fish farming practices, feeding schedules are mostly based on producers' experience, with insufficient attention paid to feeding strategies. The effects of feeding time on growth, feed utilization, and physiological metabolism in aquaculture animals, as well as the mechanisms through which feeding time regulates animal metabolism, require further

in-depth research.

Insulin-like growth factors (IGFs) are peptide hormones with insulin-like metabolic and mitogenic functions that promote cell proliferation, differentiation, and apoptosis, representing an important endocrine system regulating fish growth and development. The IGF system includes two homologous polypeptides: insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II) [6]. Fish IGF-I is highly conserved evolutionarily, with a primary structure composed of 70 amino acid sequences, possessing functions partially similar to insulin, such as reducing blood glucose levels and promoting nutrient digestion and absorption in fish. IGF-I functions through the circulatory system, with approximately 90% of IGF-I synthesized by the liver and entering the bloodstream in an endocrine manner; some tissues such as bone marrow and brain can also synthesize IGF-I, primarily functioning through paracrine and/or autocrine mechanisms. The expression of fish IGF-I is regulated by nutritional status, environmental conditions, and hormone levels.

Turbot (*Scophthalmus maximus*), also known as "Duobao fish," belongs to the order Pleuronectiformes and family Bothidae. Renowned for its delicious taste and rich collagen content, turbot has rapidly developed into one of the most important economic fish species in northern China since its introduction by Academician Lei Jilin of the Yellow Sea Fisheries Research Institute in 1992, due to its rapid growth, easy domestication, strong acceptance of formulated feeds, and suitability for high-density culture. This study investigated the effects of different feeding times on growth, feed utilization, and liver IGF-I gene expression in juvenile turbot, aiming to provide reference for determining feeding strategies in turbot aquaculture.

Materials and Methods

1.1 Experimental Diet The experimental diet was commercial turbot juvenile 专用 No. 2 feed produced by Qingdao Qihao Biotechnology Co., Ltd., with a particle size of 200–300 m. The main nutritional composition was as follows: crude protein 48.83%, crude fat 9.52%, and crude ash 12.53%. The Trizol Reagent kit, Transcriptor First Strand cDNA Synthesis Kit, and FastStart Essential DNA Green Master real-time fluorescent quantitative kit used in the experiment were purchased from Qingdao Saishang Trading Co., Ltd. Agarose, chloroform, isopropanol, ethanol, and other reagents used were domestic analytical pure reagents.

1.2 Experimental Design and Husbandry Juvenile turbot used in the experiment were purchased from the Rizhao Aquaculture Research Institute experimental base. Uniform and healthy juvenile turbot were selected and acclimated for one week before grouping. The fish were fasted for one day before grouping. The experiment was divided into three groups according to feeding time: morning (06:00), noon (12:00), and evening (18:00). Juvenile turbot [initial average body weight (8.95±0.13) g] were fed at the designated times, with three

replicates per group and 25 fish per replicate. The feeding trial was conducted in dark blue 200 L culture tanks at the Rizhao Aquaculture Research Institute using indoor static water culture. Fish were fed to apparent satiation once daily during the acclimation period (at 18:00). During the formal experimental period, feeding occurred at the designated times, with residual feed collected and weighed one hour after feeding to calculate daily feed intake. During the trial, water was exchanged once daily, 1–2 hours before feeding, with 1/2–2/3 of the water volume replaced. Experimental water was sand-filtered natural seawater at 15–18 °C, dissolved oxygen concentration 6.0 mg/L, salinity approximately 30‰, and pH 7.5–8.0. The trial lasted for 45 days. During the experiment, turbot were weighed every week to adjust feed amounts accordingly; feeding was stopped one day before weighing, and culture tanks were regularly cleaned and disinfected.

1.3 Sample Collection At the end of the experiment, juvenile turbot were fasted for 24 hours before sampling. Two fish were randomly selected from each replicate, the abdominal cavity was opened with dissecting scissors, and approximately 1 g of liver tissue was collected from the same location, rapidly frozen in liquid nitrogen, and stored at -80 °C for later analysis. The cranial cavity was opened, the brain was carefully removed, rapidly frozen in liquid nitrogen, and stored at -80 °C.

1.4 Growth Index Calculation Formulas Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0;
Specific growth rate (SGR, %/d) = 100 × (lnWt − lnW0] /t;
Feeding rate (FR, %/d) = 100 × Wf/[t×Wt + W0) /2];
Feed efficiency (FE) = (Wt - W0) / Wf.

Where: Wt is the average final body weight of juvenile turbot (g); W0 is the average initial body weight of juvenile turbot (g); t is the experimental duration (days); Wf is the average feed amount (g).

1.5.1 Total RNA Extraction and cDNA Synthesis Total RNA was extracted from liver and brain tissues of juvenile turbot using the Trizol Reagent kit. RNA concentration and OD values at 260 and 280 nm were measured using a Nanodrop 2000 micro-nucleic acid analyzer, and total RNA quality was assessed using 1% agarose gel electrophoresis. Total RNA samples with OD260 nm/OD280 nm values between 1.8–2.0 and clear gel electrophoresis bands without obvious smearing were selected as templates. cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit in a 20 L reaction system. The synthesized cDNA fragment length was verified by 1% agarose gel electrophoresis to ensure consistency with the target gene, and the cDNA was stored at -80 °C for later use.

1.5.2 Real-Time Fluorescent Quantitative PCR The experiment used the relative fluorescent quantitative method (2-ΔΔCt) to analyze differences in

IGF-I mRNA expression in liver and brain of juvenile turbot. First, reference genes were used to calibrate both control and test samples. Since this experiment involved mutual comparison, one replicate was randomly selected as the control sample to calibrate other test samples. Then, ΔCt values of control and test samples were normalized. Finally, expression differences were calculated as 2-ΔΔCt values. The calculation formulas were as follows:
ΔCt (control sample) = Ct (control sample) - Ct (reference gene);
ΔCt (test sample) = Ct (test sample) - Ct (reference gene);
ΔΔCt = ΔCt (test sample) - ΔCt (control sample).

The reference gene was -actin (accession number: AY008305), and the target gene was juvenile turbot IGF-I (accession number: FJ160587.1). Primers for both genes were designed based on sequences from the GenBank database and synthesized by Shanghai Sangon Biotech Co., Ltd. Primer sequences are shown in Table 1 .

A 20 L reaction system was used according to the FastStart Essential DNA Green Master real-time fluorescent quantitative PCR kit instructions. Amplification and data analysis were performed on an Applied Biosystem 7500 Real-Time PCR System with the following program: 95.0 °C for 10 min; 40 cycles of 95.0 °C for 10 s, 58 °C for 15 s, and 72 °C for 20 s.

1.6 Statistical Analysis Data were analyzed using SPSS 17.0 software package with one-way ANOVA. Duncan' s multiple comparison test was used when significant differences were detected. Data are expressed as mean ± standard deviation, with significance level set at $P<0.05$.

Results

2.1 Effects of Feeding Time on Growth of Juvenile Turbot No mortality occurred during the feeding trial, with 100% survival in all groups. As shown in Table 2 , weight gain rate and specific growth rate of juvenile turbot decreased significantly with later feeding times ($P<0.05$). The 06:00 feeding group achieved the highest weight gain rate (97.23%) and specific growth rate (1.94%/d), which were significantly higher than those in the 12:00 and 18:00 groups ($P<0.05$). The 12:00 group also showed significantly higher values than the 18:00 group ($P<0.05$). Feeding time had no significant effect on feeding rate ($P>0.05$), with values ranging from 1.5%/d to 1.6%/d across the three groups. Feed efficiency in the 18:00 group was only 0.91, significantly lower than in the 06:00 and 12:00 groups ($P<0.05$). The 06:00 group showed slightly higher feed efficiency than the 12:00 group, but the difference was not significant ($P>0.05$).

2.2 Effects of Feeding Time on Relative Expression of IGF-I mRNA in Liver and Brain of Juvenile Turbot Agarose gel electrophoresis of the reverse-transcribed cDNA showed that the synthesized cDNA fragment length was approximately 121 bp, consistent with the theoretical length of the target gene, using a 2,000 bp marker.

As shown in Figure 1 [Figure 1: see original paper], feeding time significantly affected the relative expression level of IGF-I mRNA in the liver of juvenile turbot ($P<0.05$). The 06:00 feeding group showed the highest relative expression level, with a $2\text{-}\Delta\Delta Ct$ value of 2.17, significantly higher than the other two groups ($P<0.05$). The 18:00 group showed the lowest expression level, with a $2\text{-}\Delta\Delta Ct$ value of only 1.13, significantly lower than the other groups ($P<0.05$).

As shown in Figure 2 [Figure 2: see original paper], feeding time had no significant effect on the relative expression level of IGF-I mRNA in the brain of juvenile turbot ($P>0.05$). The highest brain IGF-I mRNA expression was observed in the 06:00 group ($2\text{-}\Delta\Delta Ct$ value of 1.41), followed by the 18:00 group (1.37), with the 12:00 group showing the lowest value (1.24).

Discussion

3.1 Effects of Feeding Time on Growth of Juvenile Turbot Feeding time is often selected based on the natural feeding rhythms of cultured fish. Feeding rhythms are the result of long-term evolutionary adaptation to natural environments. In natural environments, 1–2-year-old turbot primarily feed on small crustaceans such as mysids and polychaetes, gradually shifting to small fish and mollusks as they grow larger, with adult fish consuming fish [7]. Mysid and polychaete larvae exhibit strong phototaxis, with more active daytime than nighttime behavior. Due to prey availability, juvenile turbot are active feeders during daytime. Additionally, turbot is a cold-water fish species, and elevated water temperature can reduce their movement and feeding, increase nitrogen excretion [8], and slow growth. Many scholars have confirmed this phenomenon. Chang et al. [9] reported that juvenile turbot exhibit feeding behavior throughout the day, with strongest feeding activity at dawn. Miao et al. [10] suggested that ecological habit transitions significantly affect feeding rhythms, with flatfish species exhibiting different feeding rhythm characteristics between pelagic and benthic life stages. Furthermore, Sun et al. [11] found that water flow, temperature, and culture systems also significantly influence turbot feeding rhythms. This study found no significant differences in feeding rates among juvenile turbot fed at 06:00, 12:00, and 18:00, but feeding time significantly affected feed efficiency, with the 06:00 group showing significantly higher feed efficiency than the other groups. Correspondingly, the 06:00 group exhibited significantly higher weight gain and specific growth rates, validating previous observations regarding juvenile turbot feeding rhythms.

Arranging feeding times according to natural biological rhythms and feeding cycles can improve feed utilization and production efficiency. Although feeding rates did not differ significantly among groups, daytime feeding groups (06:00, 12:00) showed significantly higher feed efficiency than the evening group (18:00). Similar results have been reported in previous studies. For example, *Pelteobagrus vachellii* fed at night (20:00) and during the day (08:00) showed no significant difference in feeding rate, but the nighttime feeding group exhibited significantly better feed utilization [2]. Marinho et al. [12] studied juvenile Sene-

galese sole (*Solea senegalensis*) and found that compared with nighttime feeding, daytime feeding significantly improved specific growth rate and nitrogen retention while reducing ammonia nitrogen excretion. The authors suggested that daytime-fed Senegalese sole could better utilize dietary protein for body growth.

3.2 Effects of Feeding Time on IGF-I Gene Expression in Liver and Brain of Juvenile Turbot This study demonstrated significant differences in liver IGF-I mRNA relative expression levels among different feeding groups, with the 06:00 group showing the highest expression, corresponding to the growth performance of juvenile turbot. We hypothesize that different feeding times may create varying stimuli for regulating hormone mRNA expression, thereby affecting hormone levels in the circulatory system and subsequently influencing growth, development, and nutritional metabolism in juvenile turbot. Chen et al. [13] also found a positive correlation between nutritional status and liver IGF-I mRNA relative expression levels in largemouth bass.

Feeding time did not significantly affect brain IGF-I mRNA relative expression levels in juvenile turbot. Hua et al. [14] reported that nutritional status did not significantly affect IGF-I mRNA relative expression in tissues other than the liver of juvenile common carp. Studies on coho salmon (*Oncorhynchus kisutch*) suggested that the response of IGF-I mRNA to nutritional status is limited to the liver, with decreased liver IGF-I mRNA expression during starvation but no decrease observed in extrahepatic tissues [15].

Conclusion

Feeding time significantly affected the growth and liver IGF-I mRNA relative expression levels in juvenile turbot. The 06:00 feeding group exhibited the highest weight gain rate, specific growth rate, feed efficiency, and liver IGF-I mRNA relative expression level. Therefore, it is recommended that juvenile turbot weighing approximately 8 g be fed at 06:00.

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Note: Figure translations are in progress. See original paper for figures.