Aquaculture 487 (2018) 83–88

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

Determination of optimal fasting time before blood sampling to get baseline data on serum biochemical characteristics in juvenile turbot (*Scophthalmus maximus*)

Yudong Jia[a,*], Yunhong Gao[b], Xiatian Chen[c], Bin Huang[a,*]

[a] Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao Key Laboratory for Marine Fish Breeding and Biotechnology, Qingdao 266071, China
[b] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
[c] Center for Molecular Genetics, Institute for Translational Medicine, College of Medicine, Qingdao University, Qingdao 266021, China

ARTICLE INFO

Keywords:
Fasting
Serum
Biochemical parameters
Turbot *Scophthalmus maximus*

ABSTRACT

Serum biochemical characteristics provide crucial information about the physiological condition of fish and are greatly affected by the sampling conditions. This study examined the effects of short-term fasting periods [in terms of degree-days (°C days)] on the serum biochemical parameters of juvenile turbot (*Scophthalmus maximus*) and revealed the optimal fasting time before blood sampling to obtain the baseline data. Stomach content index (SCI), digestive somatic index (DSI), viscerosomatic index (VSI), hepatosomatic index (HSI), serum thyroid hormones (thyroxine, T_4; triiodothyronine, T_3), cortisol, glucose, lactate, cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, total protein, albumin, globulin and albumin/globulin level of juvenile turbot were determined after different fasting durations (0, 0.25, 0.5, 1, 2 and 3 days). Results showed that the levels of SCI, T_4, T_3, cortisol, glucose, lactate, cholesterol, and triglycerides were significantly affected during fasting, whereas the levels of SCI, glucose, lactate, cholesterol, triglyceride were not significantly different at 1 (16.5 °C days), 2 (31 °C days), and 3 days (48 °C days) of fasting. Meanwhile, no significant difference was found for T_4 and T_3 at 1 day (16.5 °C days) and 2 days (31 °C days) of fasting, and for cortisol at 1 day (16.5 °C days) and 3 days (48 °C days) of fasting. However, DSI, VSI, HSI, high-density lipoprotein, low-density lipoprotein, total protein, albumin, globulin and albumin/globulin levels remained unchanged and showed no significant difference. These results indicate the importance of fasting period on the digestive system and serum biochemical properties of turbot. Therefore, based on these results and considering the fish management, 1 day (16.5 °C days) is the best fasting period prior to blood collection from juvenile turbot for blood biochemistry determination.

1. Introduction

Blood biochemical characteristics provide the basic information on the physiological and nutritional status of fish in wild and captive conditions during their life cycle. However, the blood biochemical parameters used for related physiological research are affected by the sampling conditions, such as water temperature, handling stress, anesthesia, and starvation (Shi et al., 2010; López-Luna et al., 2016). In general, pre-sampling fasting accelerate empties the digestive system of the fish and reduces the oxygen demand and waste production (Robb, 2008). Fasting ensures that the fish is in a post-absorptive state (Wagner et al., 2003). Thus, optimal fasting time is important to obtain the baseline data on blood biochemical parameters. The duration of fasting required to empty the digestive system depends on the species type. In general, 24 h fasting is commonly conducted before blood sampling in Atlantic salmon (Iversen et al., 2003), rainbow trout (Hoseini et al., 2014), common carp (Hoseini and Hosseini, 2010) and Arctic charr (Bystriansky et al., 2010). On the contrary, 48 h, 72 h, or no fasting period can also be selected before blood sampling for different experimental purposes (Cataldi et al., 1998; Wagner et al., 2003; Iversen et al., 2003; Hyvärinen et al., 2004). In addition to the assessment of the time limits (in days or h), acceptable ranges in terms of degree-days are also important (López-Luna et al., 2014). In aquaculture, degree-days are used to estimate the amount of time needed for the different stages of growth, such as during egg incubation, breeding or fattening (From and Rasmussen, 1992). The European Food Safety Authority (EFSA) (2008) indicates that specifying a maximum acceptable duration of fasting is difficult because its effect on welfare is related to the species

* Corresponding authors at: Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, No. 106 Nanjing Road, Qingdao 266071, China.
E-mail addresses: jiayd@ysfri.ac.cn (Y. Jia), huangbin@ysfri.ac.cn (B. Huang).

https://doi.org/10.1016/j.aquaculture.2018.01.009
Received 22 August 2017; Received in revised form 8 December 2017; Accepted 8 January 2018
Available online 09 January 2018
0044-8486/

size, lipid reserves, life stages, and water temperature. Some studies have analyzed the effect of degree-days of fasting on the stress response and flesh quality of rainbow trout (López-Luna et al., 2014, 2016; Bermejo-Poza et al., 2017). EFSA recommends that rainbow trout should not be fasted for over 50 °C days (degree-days). Therefore, the optimal degree-days of fasting time before fish blood sampling must be determined to analyze the biochemical factors.

Turbot (*Scophthalmus maximus*) is a marine fish of high commercial value and is widely cultured in Europe and China. Its annual aquacultured production in China is 50,000 to 60,000 metric tons during the last decade, which is approximately 80% of the world's total output. Thus, turbot makes a principal contribution to the production of land-based tank-cultured marine flatfish in China (Lei et al., 2012). Numerous studies have investigated the nutritional requirements and hormonal induction of spawning, egg quality, metamorphic development, and oocyte development-related gene expressions of turbot during its life cycle (McEvoy, 1989; Suquet et al., 1995; Bromley et al., 2000; Jia et al., 2014, 2015, 2016, 2017; Meng et al., 2016; Hu et al., 2017). However, no detailed studies observed the effect of pre-sampling degree-days of fasting on the serum biochemistry of juvenile turbot. These parameters include thyroxine (T_4), triiodothyronine (T3), cortisol, glucose, lactate, triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein, total protein, albumin, globulin, and albumin/globulin, which serve as indicators of the metabolic and nutritional status in fish. Therefore, this study aims to investigate the effect of fasting period on the blood biochemical parameters in the serum of juvenile turbot and identify the optimal fasting time to obtain baseline values for these parameters.

2. Materials and methods

2.1. Fish and feeding trial

Turbot samples were obtained from Tianyuan Aquatic Co., Ltd. (Yantai, Shandong, China). Prior to the experiment, the fish were acclimatized to the laboratory conditions for two weeks. Basal diets were fed to all fish during the conditioning period. Fish of similar sizes (119.33 ± 9.2 g) were randomly distributed into 18 cylindrical fiberglass tanks (2500 L) with 30 individuals per tank. Each tank was provided with a continuous flow of water (6.5 L min^{-1}) and continuous aeration through air stones to maintain dissolved oxygen at/or near saturation. The fish were fed two times daily at 07:00 and 18:00, until apparent satiation. The commercial diets formation and proximate analysis are shown in Table 1. During the experimental period, photoperiod was 16 h light and 8 h dark. The water temperature ranged from 15.0 °C to 17.0 °C, the salinity ranged from 27‰ to 30‰, and the pH varied from 7.5 to 8.0. Ammonia nitrogen was lower than 0.1 mg L^{-1}, and dissolved oxygen was higher than 6.0 mg L^{-1}. Water temperature was recorded every 2 h during the whole trial using underwater temperature sensors to calculate degree-days.

2.2. Sampling and analyses

After acclimation, the fish were fed at 07:00 and fasted thereafter. Fish were weighted and blood-sampled at 0 day (0 °C day, 0 h), 0.25 day (4 °C days, 6 h), 0.5 day (8 °C days, 12 h), 1 day (15.3 °C days, 24 h), 2 days (31 °C days, 48 h) and 3 days (48 °C days, 72 h) of fasting. Three tanks (replicates) with 30 fish per tank were employed per sample point. Fish handling procedures were conducted according to the guidelines established by the Institutional Animal Care and Use Committee at Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Science. The fish were anesthetized with 100 mg/L of tricaine methane sulfonate (MS-222, Sigma, St. Louis, MO). The blood samples were obtained from the caudal vein of ten fish from each tank using 5 mL syringes and were allowed to clot at room temperature for 4 h and then at 4 °C for 6 h. Blood was subsequently centrifuged at 1000*g* for 10 min. The serum was collected and then stored at −80 °C to analyze the biochemical parameters.

Table 1
Formulation and proximate composition of basal diets (% dry matter).

Ingredients	%	Proximate compositions	
Fish meal[a]	52.00	Moisture (%)	9.28
Soybean meal[a]	16.00	Crude protein (%)	48.85
Wheat meal[a]	15.20	Crude lipid (%)	14.38
Shrimp meal	3.00		
Fish oil	5.00		
Soybean oil	3.00		
Soybean lecithin	2.00		
$Ca(H_2PO_3)_2$	2.00		
Vitamine premix[b]	0.60		
Mineral premix[c]	1.00		
Choline chloride	0.20		
Total	100.00		

[a] Fish meal: crude protein 69.7% dry matter, crude lipid 7.1% dry matter; soybean meal, crude protein 53.3% dry matter, crude lipid 1.9% dry matter; wheat meal, crude protein 14.70 dry matter. These ingredients were supplied by Tech Bang Bio-Tech (Ningbo, China).

[b] Vitamine premix (mg kg^{-1} diet): thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin B12, 0.1; vitamin K3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin, 1.20; retinol acetate, 32; cholecalciferol, 5; choline chloride, 2500; wheat middling, 18.52 g kg^{-1} diet.

[c] Mineral premix (mg kg^{-1} diet): $MgSO_4{\cdot}7H_2O$, 1200; $CuSO_4{\cdot}5H_2O$, 10; $ZnSO_4{\cdot}H_2O$, 50; $FeSO_4{\cdot}H_2O$, 80;$MnSO_4{\cdot}H_2O$, 45; $CoCl_2$ (1%), 50; Na_2SeO_3 (1%), 20; $Ca(IO3)_2$ (1%), 60.

Serum T_3, T_4 and cortisol levels were determined by radioimmunoassay (RIA) using T_3, T_4, and cortisol radioimmunoassay (RIA) kits (Immunotech, Beckman Culture Company, France). Data were expressed as ng mL^{-1}.

The serum glucose, triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein, total protein, albumin, globulin, and albumin/globulin levels were determined using commercially available kits (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen City, China) in an Auto Chemistry Analyzer (BS-200, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen City, China). Serum lactate was measured using the methods described in the detection kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The absorbance was measured at 530 nm using a Multiskan spectrum (Thermo, USA). The data were expressed as mmol L^{-1}.

The fish were sacrificed after blood collection. The viscera and liver were removed from the fish, and were weighted separately to calculate the viscerosomatic index (VSI) and the hepatosomatic index (HSI). In addition, stomach content and digestive tract (from stomach to anus) were weighed to calculate the stomach content index (SCI) and the digestive somatic index (DSI). The VSI, HSI, DSI, and SCI of fish were calculated as follows:

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

$$\text{VSI}\ (\%) = (\text{visceral weight}/\text{body weight}) \times 100$$

$$\text{SCI}\ (\%) = (\text{stomach content weight}/\text{body weight}) \times 100$$

$$\text{DSI}\ (\%) = [(\text{digestive tract weight} - \text{stomach content weight})/\text{body weight}] \times 100$$

2.3. Statistical analysis

Shapiro–Wilk normality test and Bartlett test were used to estimate the data complied or not with normal distribution and homogeneity of variance, respectively (Faraway, 2005). The data were analyzed via one-way analysis of variance (ANOVA) and Tukey's multiple-range tests using SAS 8.0 software. All data are presented as the means ± standard error of the mean. In all statistical tests used, $P < .05$ was considered significantly different.