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# A moderately high level of dietary lipid inhibited the protein secretion function of liver in juvenile tiger puffer *Takifugu rubripes*

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ABSTRACT

Tiger puffer have a unique lipid storage pattern. They store lipid predominantly in liver. To investigate the hepatic physiology of tiger puffer in response to a moderately high level of dietary lipid, a 74-day feeding trial was conducted, followed by a hepatic transcriptome assay. Two experimental diets which had an optimal (10.44%, Control) or moderately higher (14.64%, high-lipid diet, HL) dietary lipid level was formulated. Each diet was fed to triplicate tanks. No significant difference was observed in growth and feed efficiency between the two groups, but the survival was significantly lower in group HL. The HL diet also resulted in significantly higher lipid accumulation in liver, which was reflected in the hepatic histology. The biochemical parameters in serum confirmed the existence of stress in liver caused by high lipid intake. A total of 63 differentially expressed genes between groups were observed in the hepatic transcriptome assay, which were primarily enriched in Gene Ontology (GO) terms such as Endopeptidase activity, Secretion, and Organic acid transport, as well as in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways such as Protein processing in endoplasmic reticulum and DNA replication. The HL diet tended to inhibit the protein synthesis and secretion in liver, and this was evidenced by activities/concentrations of selected proteins secreted into intestine. Genes related to liver protection and repair were transcriptionally stimulated by diet HL. In conclusion, compared to the optimal lipid requirement, a moderately higher lipid level in the diet for tiger puffer did not compromise the growth performance, but tended to inhibit the protein secretion function of liver and stimulate the processes related to liver protection and repair. The present results will be beneficial to both feed management in tiger puffer farming and elucidation of lipid metabolism characteristics in fish which store lipid in liver.

## 1. Introduction

It is unquestionable that lipid nutrition constitutes an very important component of fish nutrition, especially considering that fish prefer lipid to carbohydrate as their main energy source (Watanabe, 1982), and that fish are the main sources of long chain-polyunsaturated fatty acids for human consumption. Comprehensively understanding the lipid metabolism characteristics of a certain fish species is the base of efficient lipid management in the diet (Rasmussen et al., 2000; Peres and Oliva-Teles, 2001; Williams et al., 2003; Skalli et al., 2004; Ng et al., 2008; Gao et al., 2011; Li et al., 2012a).

Tiger puffer *Takifugu rubripes* is an important aquaculture species in Asia, and also becoming an important model fish in academic research due to the detailed genome information (Fernandes et al., 2005; Kai et al., 2005; Imai et al., 2007; Wongwarangkana et al., 2015). Tiger puffer have a unique lipid storage pattern. They have no adipose tissue and a very low lipid content in muscle, and thus store lipid predominantly in liver (Kaneko et al., 2013). Since liver is a metabolism center of fish and plays vital roles in a wide range of biological processes (Xiong et al., 2014; De Santis et al., 2015; Zhou et al., 2015), high levels of dietary lipid were assumed to significantly affect the physiological status of tiger puffer, especially the hepatic physiology, through increasing hepatic lipid accumulation (Takii et al., 1995; Kikuchi et al., 2009). However, no relevant information has been reported in tiger puffer or other fish species which store lipid in liver. How tiger puffer liver responds to a high dietary lipid level, and to what extent this would affect fish health status remain unclear. Therefore, with a feeding trial followed by hepatic transcriptome assay, the present study was aimed at comprehensively investigating the response of tiger puffer liver to a higher level of dietary lipid (compared to optimal lipid

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**Table 1**
Formulation and proximate composition of the experimental diets (% dry matter basis)[a].

| Ingredients | Control | HL |
|---|---|---|
| Fish meal | 50.00 | 50.00 |
| Soybean meal | 20.00 | 20.00 |
| Wheat meal | 21.28 | 17.28 |
| Vitamin premix[b] | 0.20 | 0.20 |
| Mineral premix[c] | 0.50 | 0.50 |
| L-ascorbyl-2-polyphosphate | 0.50 | 0.50 |
| Choline chloride | 0.50 | 0.50 |
| Monocalcium phosphate | 1.00 | 1.00 |
| Ethoxyquin | 0.02 | 0.02 |
| Betaine | 0.30 | 0.30 |
| Calcium propionate | 0.10 | 0.10 |
| Astaxanthin (10%) | 0.10 | 0.10 |
| Soya lecithin | 1.50 | 1.50 |
| Fish oil | 4.00 | 8.00 |
| Proximate composition | | |
| Crude protein | 50.8 | 51.2 |
| Crude lipid | 10.4 | 14.6 |
| Ash | 10.6 | 13.2 |

[a] All the ingredients were purchased from Qingdao Great Seven Co. Ltd. Peru anchovy meal used in this formulation contained 69.70% crude protein and 7.08% crude lipid (of dry matter); soybean meal, 53.29% crude protein and 1.93% crude lipid (of dry matter); wheat meal, 12.99% crude protein and 1.70% crude lipid (of dry matter).

[b] Vitamin premix (mg/kg diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin $B_{12}$, 0.1 mg; vitamin $K_3$, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin, 1.2 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; wheat middling, 661.7 mg.

[c] Mineral premix (mg or g/kg diet): $MgSO_4{\cdot}7H_2O$, 1200 mg; $CuSO_4{\cdot}5H_2O$, 10 mg; $ZnSO_4{\cdot}H_2O$, 50 mg; $FeSO_4{\cdot}H_2O$, 80 mg; $MnSO_4{\cdot}H_2O$, 45 mg; $CoCl_2{\cdot}6H_2O$ (1%), 50 mg; $NaSeSO_3{\cdot}5H_2O$ (1%), 20 mg; $Ca(IO_3)_2{\cdot}6H_2O$ (1%), 60 mg; zoelite, 3.485 g.

**Table 2**
Fatty acids composition of experimental diets (% total fatty acids).

| Fatty acids | Control | HL |
|---|---|---|
| C14:0 | 6.31 | 5.79 |
| C16:0 | 22.65 | 20.73 |
| C18:0 | 4.25 | 4.33 |
| C20:0 | 0.41 | 0.52 |
| ΣSFA | 33.61 | 31.37 |
| C16:1n-7 | 5.90 | 5.45 |
| C18:1n-9 | 9.33 | 9.40 |
| C18:1n-7 | 2.66 | 2.68 |
| ΣMUFA | 17.89 | 17.52 |
| C18:2n-6 | 10.18 | 7.93 |
| C20:4n-6 | 0.72 | 0.81 |
| Σn-6 PUFA | 10.89 | 8.75 |
| C18:3n-3 | 1.89 | 1.78 |
| C18:4n-3 | 1.95 | 2.02 |
| C20:5n-3 | 8.22 | 8.12 |
| C22:5n-3 | 0.93 | 0.99 |
| C22:6n-3 | 9.67 | 11.27 |
| Σn-3 PUFA | 22.66 | 24.17 |
| Σn-3LC-PUFA | 18.82 | 20.37 |
| Σn-3/Σn-6 | 2.08 | 2.76 |

SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; n-6 PUFA: n-6 poly-unsaturated fatty acids; n-3 PUFA: n-3 poly-unsaturated fatty acid.

requirement). The results will be beneficial to feed management in tiger puffer farming and may provide new insight into understanding the lipid metabolism characteristics of fish which store lipid in liver.

## 2. Methods

### *2.1. Experimental diets*

Two isonitrogenous experimental diets with different lipid contents were formulated (Table 1). The control diet had a lipid content (10.44%) which has been demonstrated to be suitable for juvenile tiger puffer (Takii et al., 1995; Kikuchi et al., 2009). The treatment diet had a higher lipid content (14.64%, diet HL), with more fish oil added. The diets were made, packed and stored following the standard procedures in our laboratory (Xu et al., 2016b). The dietary fatty acid compositions were presented in Table 2.

### *2.2. Experimental fish and feeding procedure*

Juvenile tiger puffer with an average initial body weight of 11.93 g, which were purchased from Huanghai Aquaculture Co. Ltd. (Haiyang, China), were used in the present study. The feeding trail was conducted in an indoor flow-through seawater system. Prior to the start of the feeding trial, experimental fish were reared in polyethylene tanks and fed the control diet for 7 days to acclimate to the experimental conditions. At the onset of the feeding trial, experimental fish were distributed into 6 polyethylene tanks (200 L) in the same flow-through seawater system, and each diet was randomly assigned to triplicate tanks. Each tank was stocked with 30 fish. Fish were hand-fed to apparent satiation four times each day (8:00, 12:00, 16:00, and 20:00). The feeding trial lasted for 74 days. Fish were reared under the natural photoperiod and ambient temperature of Haiyang, Shandong, China (N36°41′, E121°07′). During the experiment, the water temperature ranged from 20 to 26 °C; salinity, 29–32; pH, 7.4–8.6; and dissolved oxygen, > 5 mg $L^{-1}$. The tanks were cleaned daily by siphoning out residual feed and feces.

At the end of the feeding trial, after anesthesia with eugenol (1:10000, Shanghai Reagent, Shanghai, China), number and weight of fish in all tanks were recorded to calculate the weight gain and survival of fish. Body length of 5 fish from each tank was also recorded to calculate the condition factor (CF). After that, 5 randomly selected whole fish were collected from each tank for the analysis of proximate composition. Ten more randomly selected fish per tank were dissected to collect the samples of serum, liver, muscle, and intestine for the other assays. After dissection, before collection of tissue samples weight of liver and viscera from 5 fish, as well as that of the corresponding whole fish were recorded first to calculate the hepatosomatic index (HSI) and viscerasomatic index (VSI). From each fish, four small pieces of liver tissue, two pieces of muscle tissue (dorsal muscle, about 3 cm × 1.5 cm), two pieces of middle intestine tissue (about 0.5 cm in length), and all blood were collected for subsequent potential use. Blood samples, which were collected from the caudal vein, were allowed to clot firstly at room temperature for 2 h and then at 4 °C for 6 h. After that, centrifugation (836 ×*g*, 10 min, 4 °C) was conducted and the straw-colored supernatants were collected as serum samples. All the sampling procedures were handled on ice plate, and all tissue samples collected were frozen with liquid nitrogen immediately, and then stored at −86 °C prior to analysis. All sampling protocols, as well as fish rearing practices, were reviewed and approved by the Animal Care and Use Committee of Yellow Sea Fisheries Research Institute.

### *2.3. Analysis of proximate composition, fatty acids, biochemical parameters of serum, and intestinal enzymes*

The analysis of proximate composition of experimental diets (triplicate assays for each diet) and whole fish (five individual fish per tank) were performed in accordance with the standard methods of AOAC (1995). Samples of diets and fish were oven-dried at 105 °C to a constant weight for moisture assay. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method; lipid by ether extraction using Soxhlet method; and ash by combustion at 550 °C. The extraction and analysis of lipid in liver and muscle were done with

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