Aquaculture 465 (2016) 100–107

Contents lists available at ScienceDirect

Aquaculture

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

Effects of dietary leucine on growth performance, feed utilization, non-specific immune responses and gut morphology of juvenile golden pompano *Trachinotus ovatus*

Xiaohong Tan [a,b], Heizhao Lin [a,c,*], Zhong Huang [a,c], Chuanpeng Zhou [a], Anli Wang [b,*], Changle Qi [a], Shuyan Zhao [a]

[a] Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Ministry of Agriculture, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, PR China
[b] Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, PR China
[c] Shenzhen Base of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shenzhen 518121, PR China

A R T I C L E I N F O

Article history:
Received 28 June 2016
Received in revised form 22 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Trachinotus ovatus
Leucine requirement
Growth performance
Serum biochemical indices
Non-specific immune responses
Gut morphology

A B S T R A C T

The effects of dietary leucine on growth performance, feed utilization, non-specific immune responses and gut morphology were studied in juvenile golden pompano (*Trachinotus ovatus*) using 8-week feeding trials. Six isonitrogenous and isolipidic diets were formulated to contain six graded levels of dietary leucine (16.2, 20.9, 26.0, 30.7, 35.9 and 41.0 g kg^{-1} leucine of dry diet). Each diet was randomly assigned to triplicate groups of 20 juvenile fish (5.76 ± 0.06 g). Maximum weight gain rate (WG), specific growth rate (SGR), feed intake (FI), protein efficiency ratio (PER), microvilli numbers and microvilli length in foregut and midgut were recorded at 30.7 g kg^{-1} dietary leucine. Protein deposition ratio (PDR), hemoglobin (HGB) and hematocrit (HCT) were found to be optimal in fish fed with 35.9 g kg^{-1} leucine of diet. Dietary leucine levels had a significant effect on serum superoxide dismutase (SOD), malondialdehyde (MDA), lysozyme (LZY) and total antioxidant capacity (T-AOC) ($P < 0.05$). Intestinal microstructures were positively affected by dietary leucine at the optimal levels of 26.0 and 30.7 g kg^{-1}, significantly ($P < 0.05$) increasing microvilli numbers and microvilli length in foregut and midgut. Based on the growth performance, feed utilization, proximate composition, serum enzyme activities and gut morphology, the optimum dietary leucine level for optimal growth of juvenile golden pompano was 29.3–32.9 g kg^{-1} of the dry diet, corresponding to 71.5–80.2 g kg^{-1} of dietary protein.

Statement of relevance: The study indicated low or excess amount of dietary leucine decreased growth performance, serum total antioxidant capacity and had a significantly negative effect on gut morphology. The optimum dietary leucine level for juvenile golden pompano was 2.93–3.29% of the dry diet. The results provide a theoretical basis for the designing of feed formulation for golden pompano (*T. ovatus*).

1. Introduction

Proteins and amino acids play a very important role in the structure and metabolism of all living organisms. Fish cannot synthesize essential amino acids (EAAs) and must acquire essential along with several non-essential through the consumption of protein or mixtures of amino acids (National Research Council, NRC, 2011). Ten amino acids have been found to be essential for all fish (Wilson, 1985). EAAs deficiency may cause reduced growth and poor feed efficiency (Wilson and Halver, 1986). Therefore, satisfying the essential amino acids requirements of cultured fish is very important to obtain optimal growth. Leucine, a member of branched-chain amino acids family includes extremely hydrophobic biochemicals found principally in the interior of proteins and enzymes. Leucine is considered between others as essential for the normal growth and reproductive potential of the fish (National Research Council, NRC, 2011). Dietary leucine imbalance leads to a reduction in growth rate and a decrease in the efficiency of feed utilization (Abidi and Khan, 2007). Supplementary leucine in diet can improve whole body protein mass, markers of protein anabolism, insulin release and lipid decomposition (Nair et al., 1992; Pedroso et al., 2014). Leucine plays an important roles in producing hemoglobin, maintaining blood sugar levels, increasing growth hormone production and it also affects stress, energy and muscle metabolism (Abidi and Khan, 2007). When served in the right quantities it also enhances growth performance by improving the feed intake and feed efficiency, and improve the non-specific immune ability of juvenile Jian carp by promoting the phagocytosis ability of leukocytes, body sterilization

* Corresponding authors.
E-mail addresses: linheizhao@163.com (H. Lin), wanganl@scnu.edu.cn (A. Wang).

http://dx.doi.org/10.1016/j.aquaculture.2016.08.034
0044-8486/

ability and increasing resistance to *Aeromonas hydrophila* titers and sim-IgM levels (Wu, 2014). On the one hand dietary supplementation of leucine can also promote the development of intestinal mucosa, enhance gastro-intestinal digestion and absorption capacity of GIFT tilapia (*Oreochromis niloticus*) (Shi et al., 2014). On the other hand leucine deficiency in the diet can cause severe biochemical malfunctions including growth retardation (Abidi and Khan, 2007), animal thymus and spleen atrophy, lymphoid tissue damage, and reduction in animal serum immunoglobulin, complement C3 and iron transport protein levels (Wang and Li, 2011).

Dietary requirements of leucine have been reported for various cultivable fish species such as Japanese sea bass, *Lateolabrax japonicas* (Li, 2010); large yellow croaker, *Pseudosciaena crocea* (Li, 2010); channel catfish, *Ictalurus punctatus*; chinook salmon, *Oncorhynchus tshawytscha*; rainbow trout, *O. mykiss* (National Research Council, NRC, 2011); stinging catfish, *Heteropneustes fossilis* (Farhat and Khan, 2014). The dietary leucine requirements have been estimated for some fish, ranging from 23 to 72.6 g kg^{-1} of dietary protein (Abidi and Khan, 2007; Li, 2010; National Research Council, NRC, 2011; Shi et al., 2014; Farhat and Khan, 2014).

Golden pompano (*Trachinotus ovatus*) belongs to the family Carangidae. It is a carnivorous fish that preys mainly on zooplankton and fish. It is a warm-water species (25–32 °C) and is widely distributed in China, Japan, Australia, and other countries (Cheng and Zheng, 1987; Chen et al., 2007; Tang et al., 2013). It has been widely cultured in the Asia-Pacific region because of its fast growth, high flesh quality and suitability for cage culture. To date, studies on the nutritional requirements of golden pompano are limited. Suitable dietary lysine (Du et al., 2011), methionine (Niu et al., 2013) and arginine (Lin et al., 2015) levels were found to be 29.4 g kg^{-1}, 10.6–12.7 g kg^{-1} and 27.3–27.4 g kg^{-1}of the dry diet for golden pompano, respectively.

Although information on dietary lysine (Du et al., 2011), methionine (Niu et al., 2013) and arginine (Lin et al., 2015) of golden pompano are available, no published data is available on dietary leucine requirement of this fish. Hence, the purpose of this experiment is to determine optimum dietary leucine requirement of golden pompano based on growth performance, serum biochemical indices, immune responses and gut morphology.

2. Materials and methods

2.1. Diet preparation

Six isonitrogenous and isolipidic diets (410 g kg^{-1} crude protein, 120 g kg^{-1} crude lipid), using fish meal, soybean meal, peanut meal as protein sources, and fish oil as lipid source, containing graded levels of L-leucine (16.2, 20.9, 26.0, 30.7, 35.9 and 41.0 g kg^{-1} leucine of diets) were formulated (Table 1). A mixture of crystalline L-amino acids (the purity ≥ 99%, Guangzhou Liyuan Chemical Co., Ltd., Guangzhou, China) was supplemented to simulate the whole body amino acid pattern of golden pompano except for leucine (Table 2). All diets were kept isonitrogenous by decreasing the levels of glutamic acid as the leucine level was increased. All the ingredients were ground into powder, sieved through 60-mesh and thoroughly mixed with oil. To this 350 g kg^{-1} water of diet was added, the dough thus produced was then forced through a pelletizer (F-26, South China University of Technology, Guangzhou, China) and air-dried to about 100 g kg^{-1} moisture of diet. After drying, all diets were sealed in bags and stored at −20 °C until used.

2.2. Fish and experimental conditions

Experimental fish were obtained from a commercial fish hatchery in Shenzhen (Guangdong, China). They were transported to the experimental condition in polythene cages and stocked in an outdoor concrete pond (5.0 m × 4.0 m × 1.5 m). Prior to the feeding trial, the fish were fed with Diet 1 for 2 weeks to acclimate to the experimental diet and conditions. After fasting for 24 h, juvenile gold pompanos of an average weight (5.76 ± 0.06 g) were anesthetized with 100 mg/L Eugenol (Shanghai Medical Instruments Co., Ltd., Shanghai, China) and 20 fishes per cage were randomly stocked into eighteen floating cages (0.8 m × 0.7 m × 1.2 m). Each diet was randomly assigned to cages in triplicate. Fish were fed two times daily at 8:00 and 16:00 until apparent satiation on the basis of visual observation. During the 8 weeks feeding trial, the number and weight of dead fish and feed consumption were recorded every day. The water temperature was maintained at 30.47 ± 1.60 °C. Salinity, pH, ammonia nitrogen and dissolved oxygen ranged between 15 and 17 g/L; 7.6–7.8; 0.05–0.1 mg/L; 6–7.5 mg/L,

Table 1
Composition and nutrient levels of experimental diets (g kg^{-1}).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal	120	120	120	120	120	120
Peanut meal	160	160	160	160	160	160
Soybean meal	60	60	60	60	60	60
Flour	260	260	260	260	260	260
Beer yeast powder	30	30	30	30	30	30
Amino acid mixture[a]	143.3	143.3	143.3	143.3	143.3	143.3
Fish oil	85	85	85	85	85	85
Lecithin	10	10	10	10	10	10
Vitamin premix[b]	10	10	10	10	10	10
Mineral premix[c]	10	10	10	10	10	10
Choline chloride (50%)	5	5	5	5	5	5
Betaine	3	3	3	3	3	3
Vitamin C	6	6	6	6	6	6
Microcrystalline cellulose	72.7	72.7	72.7	72.7	72.7	72.7
Crystalline leucine	0	5	10	15	20	25
Crystalline glutamic acid	25	20	15	10	5	0
Total	1000	1000	1000	1000	1000	1000
Nutrient levels[d]						
Moisture	62.2	62.4	62.1	62.6	62.7	62.2
Crude protein	407.9	412.0	408.4	412.8	412.3	411.1
Crude lipid	117.0	115.9	116.2	115.2	116.8	115.9
Ash	57.7	59.4	58.5	58.6	58.9	59.1

[a] Amino acid mixture provides the following per kg of diet: Thr 5.4 g, Val 12.70 g, Met 9.40 g, Ile 13.20 g, Phe 7.40 g, Arg 12.90 g, His 2.10 g, Lys 16.70 g, Asp 20.50 g, Glu 16.00 g, Pro 1.50 g, Ala 10.80 g, Tyr 1.70 g.
[b] Vitamin premix provides the following per kg of diet: vitamin B_1 25 mg, vitamin B_2 45 mg, vitamin B_6 20 mg, vitamin B_{12} 0.1 mg, vitamin K_3 10 mg, inositol 800 mg, pantothenic acid 60 mg, nicotinic acid 200 mg, folic acid1.2 mg, biotin 32 mg, vitamin D_3 5 mg, vitamin E 120 mg, vitamin C 2.0 g, choline chloride 2.0 g, ethoxyquin 150 mg, manna-croup 4.52 g.
[c] Mineral premix provides the following per kg of diet: NaF 4 mg, KI 1.6 mg, $CoCl_2 \cdot 6H_2O$ (1%) 100 mg, $CuSO4 \cdot 5H_2O$ 20 mg, $FeSO4 \cdot H_2O$ 160 mg, $ZnSO4 \cdot H_2O$ 100 mg, $MnSO4 \cdot H_2O$ 120 mg, $MgSO4 \cdot 7H_2O$ 2.4 g, $ZnSO4 \cdot H_2O$ 100 mg, $Ca(H_2PO_4)_2 \cdot H_2O$ 6.0 g, NaCl 200 mg, zeolite power 0.90 g.
[d] Estimated values.

Table 2
Amino acid composition of experimental diets (g kg^{-1}).

Amino acids	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Threonine	12.7	12.5	12.2	12.4	12.5	12.4
Valine	21.4	21.1	21.7	21.1	21.7	21.6
Methionine	11.4	11.3	11.7	11.5	11.8	11.6
Isoleucine	20.4	20.1	20.0	20.6	20.9	20.9
Leucine	16.2	20.9	26.0	30.7	35.9	41.0
Phenylalanine	16.5	16.3	16.6	16.3	16.4	16.2
Arginine	29.1	29.2	29.0	29.8	29.4	29.5
Histidine	6.5	6.1	6.5	6.3	6.4	6.2
Lysine	28.1	28.7	27.8	28.5	28.0	27.9
Total EAA	162.3	166.2	171.5	177.2	183.0	187.3
Aspartic acid	42.2	41.7	41.4	41.5	41.9	42.1
Serine	11.0	10.1	10.8	10.7	10.8	10.7
Glutamic acid	87.5	82.3	77.2	71.0	66.9	61.6
Proline	13.2	12.9	12.9	13.1	13.3	13.1
Glycine	27.7	27.3	27.0	27.1	27.4	27.5
Alanine	22.9	22.5	22.3	22.5	22.7	22.7
Tyrosine	6.9	6.8	6.4	6.1	6.2	6.5
Total NEAA	211.4	203.6	198.0	192.0	189.2	184.2
Total	373.7	369.8	369.5	369.2	372.2	371.5