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Short communication

Effects of food availability on growth performance and immune-related gene expression of juvenile olive flounder (*Paralichthys olivaceus*)

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ABSTRACT

Unfavorable environmental conditions and inappropriate culture practices have increased the vulnerability of cultured fish to disease infection. Up to date many studies have aimed to determine a feeding regimen to maximize productivity; however, very little information on immune responses of cultured fish in response to underfeeding or overfeeding is available. Therefore, a preliminary study was conducted to evaluate effects of graded feeding levels (i.e., food availability) on growth performance and immune-related gene expression of juvenile olive flounder (*Paralichthys olivaceus*). Six different feeding rates including 1, 4, 7, 10, 13, and 16% body weight per day (BW/d) were randomly assigned to three replicate tanks stocking 150 fish (average initial body weight: 0.27 ± 0.02 g; mean ± SD) per tank. A feeding trial lasted for two weeks. Based on the results of the weight gain, nutrient gain, and whole-body compositions and energy content, the feeding rate of 10%, 13%, and 16% BW/d resulted in high nutritional status, whereas the feeding rate of 1% and 4% BW/d resulted in low nutritional status. Intermediate nutritional status was observed at the feeding rate of 7% BW/d. In the given rearing conditions the optimum feeding rate resulting in the maximum growth was estimated to be 11.9% BW/d based on the quadratic broken-line regression model, chosen as the best-fit model among the tested models. Expression of immune-related genes including IL-8 and IgM was significantly down-regulated in the flounder fed at 1% BW/d in comparison to those fed at 7% BW/d. Interestingly, expression of these genes in the flounder fed at 10%, 13%, and 16% BW/d was relatively down-regulated in comparison to that of the flounder fed at 7% BW/d. Although no statistical difference was detected, overall response patterns of other immune-related genes, including TLR3, polymeric Ig receptor, lysozyme C-type, GPx, SOD, and Trx followed what IL-8 and IgM exhibited in response to the various feeding rates. Given the current challenges in aquaculture of the flounder our findings suggest to prohibit underfeeding or overfeeding (i.e., ad-libitum feeding) when culturing the young flounder.

1. Introduction

Olive flounder (*Paralichthys olivaceus*) are an economically important fish species in Asian countries including Korea, China, and Japan [1]. The aquaculture production of this species in 2015 was 45,759, 30,000 and 2,500 metric tons by Korea, China, and Japan, respectively [2,3]. In Korea, culture of this species has significantly contributed to the marine fish aquaculture production (85,449 metric tons in 2015) since the late 1990s [4]. Despite of its importance in Korea aquaculture industry, the increase in production of this species has been stagnant in recent decades for several reasons. First, deteriorated water quality caused by the increase in population and demand for agricultural products has rendered cultured fish susceptible disease infection such as scuticociliatosis, streptococcosis, VHS (viral hemorrhagic septicemia), vibriosis, and gliding bacterial disease [5]. Second, feeding farm-made feeds containing low-quality raw fish and overfeeding of fish held at high stocking density that have been widely practiced by fish farmers are considered to increase risk of exposing fish to disease infection and chronic stress [6,7]. Last, frequent occurrence of extreme climatic events such as localized heavy rain and record-breaking temperature rise, driven by global and local climate change have increased vulnerability to disease infection as well as may likely reduce physiological tolerability to variation in temperature and salinity [8,9].

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To increase the resilience of the cultured flounder against the challenges, proper management practices such as water sanitation, waste treatment, optimum stocking density, selection of fresh feed ingredients, replacement of farm-made feeds with formulated feeds, and optimization of feeding level should be exercised. In the current study, we mainly focused on optimization of feeding level because feeding regimen is a key factor that influences physiological performances of animals. In general, terminology of ad-libitum feeding, defined as feeding at the level that maximizes growth is commonly accepted by farmers because maximization of productivity is highly desired. Under optimal rearing conditions ad-libitum feeding may be practicable; however, given the unfavorable environmental conditions optimization of feeding level must be considered because the flounder reared under the current feeding practice may succumb to nutritionally related disease, particularly immunodeficiency problems. Not only is it important to stay away from overfeeding but also it is critical to feed animals optimally because it has been shown that immune responses are closely related to energetic status of animals [10–14]. In general, when energy reserves or food availability becomes low, animals choose to allocate less to immune defense in order to reduce the risk of starvation to death [14]. In mammals effects of dietary restriction are shown to be variable, in that dietary restriction can be advantageous with regard to autoimmune diseases [15] but is harmful in relation to defense to infections [16]. In fish there are a few studies conducted to evaluate effects of starvation on immune responses. A previous study showed that the plasma lysozyme concentration of the flounder following 21-d to 42-d starvation was significantly higher than that of the fish fed ad libitum [17]. In red sea bream (*Pagrus major*) starvation was shown to enhance survivability and overall disease resistance index against *E. tarda* infection [18]. On the other hand, alteration of liver transcriptome of innate immune response in Atlantic salmon (*Salmo salar*) in response to starvation was reported [19]. Because immune responses have effects in many physiological pathways including protein and lipid metabolism [19] which are known to be influenced by calorie restriction [20,21], findings on immune responses in response to graded feeding level will be informative, which are very scarce in literature. Therefore, we evaluated effects of graded feeding levels (i.e., food availability) ranging from lower to higher than ad-libitum feeding on growth performance and immune-related gene expression of juvenile olive flounder. Results from the current study may have implications for optimization of feeding level in order to increase the resilience of the flounder under the current, unfavorable environmental conditions.

2. Materials and methods

2.1. Fish source and feeding trial

The flounder produced in our research facility (Genetics and Breeding Research Center) were used in the current study. The larvae were reared in circular fiberglass tanks (150 cm diameter, 100 cm height, ca. 1,000 l water volume) supplied with filtered seawater along with ultraviolet (UV) light treatment. Tanks were operated in flow-through configuration. Seawater temperature ranged from 16.8 to 20.4 °C, and salinity was 34‰. Commercial feeds in different sizes and nutrient compositions were gradually switched as the larvae grew. From 18 to 23-days post hatch (dph) the Otohime A (particle size: 75–250 μm; 8% moisture, 53% crude protein, 8% crude lipid, 16% crude ash, reported by the manufacturer; Marubeni Nisshin Feed Co. LTD, Tokyo, Japan) was fed six times a day. From 23 to 28-dph the Otohime B1 (250–360 μm; 6.5% moisture, 51% crude protein, 11% crude lipid, 15% crude ash) was fed six times a day. From 29 to 37-dph the Otohime B2 (360–650 μm; 6.5% moisture, 51% crude protein, 11% crude lipid, 15% crude ash) was fed six times a day. From 38 to 53-dph the Otohime C1 (580–840 μm; 7% moisture, 51% crude protein, 11% crude lipid, 15% crude ash) was fed six times a day. As of 54-dph the Otohime C1 was fed four times a day. When the subsequent feed was switched from the current feed, the two different feeds were mixed for ease of acclimating to the larger feed (e.g., 70% Otohime A + 30% Otohime B1, 50% Otohime A + 50% Otohime B1, then 30% Otohime A + 70% Otohime B1). Two thousand and seven hundred juveniles (body weight: 0.27 ± 0.02 g; mean ± SD; total length: 31.2 ± 2.5 mm; 56-dph) were randomly distributed into 18 circular polypropylene tanks (70 cm diameter, 80 cm height, ca. 250 l water volume), resulting in 150 fish per tank. Tanks were operated in flow-through configuration with filtered seawater at a rate of 3 l/min and were equipped with an aeration apparatus. Food availability was manipulated by allocating each of the six different feeding rates (1, 4, 7, 10, 13, and 16% body weight per day; BW/d) to three replicate tanks ($N = 3$ tanks). A feeding trial lasted for two weeks. The fingerlings were hand-fed the Otohime C1 (Marubeni Nisshin Feed Co. LTD; slow-sinking pellets) four times (08:00, 11:00, 14:00, and 17:00 h) a day. Seawater inside the tank was rapidly drained once a day ca. 50% of total volume to remove fecal matter and uneaten feeds. The feeding trial was conducted indoor, and photo period during the trial was set to be 12 h:12 h (= dark:light). Seawater temperature was monitored daily using temperature data logger (HOBO® Water Temp Pro v2 (U22-001), ONSET, Bourne, MA, USA) and ranged from 16.6 to 18.3 °C. Dissolved oxygen level was measured by a DO meter (ODO Meter (O-10), Technology & Environment Corp., Seoul, Korea) and maintained at 7.1–7.2 mg/l. Ammonium concentration and pH were measured weekly using a test kit (MColortest™, EMD Millipore Corp., Billerica, MA, USA) and a pH meter (Orion Versa Star, Thermo Fisher Scientific, Chelmsford, MA, USA), respectively, and the value was < 0.2 mg/l and 8.3–8.4, respectively. The current feeding trial followed a standard operating procedure approved by the animal care and use committee of the National Institute of Fisheries Science.

2.2. Measurement

2.2.1. Growth performance

At the end of the feeding trial, all fish in each tank were weighed as a group for calculation of weight gain (WG), feed conversion ratio (FCR), and protein retention efficiency (PRE). Five fish from each tank were randomly selected and euthanized with an overdose of 2-phenoxyethanol (Sigma-Aldrich, St. Louis, MO, USA) for measuring individual total length (cm) and body weight (g) which were used for calculation of condition factor (CF). Calculation for each measurement is as follows:

$$WG\ (\%) = 100 \times (FBW - IBW) / IBW$$

$$FCR = \text{Feed weight} / (FBW - IBW)$$

$$PRE\ (\%) = 100 \times [(FBW \times FBCP) - (IBW \times IBCP)] / (\text{Feed weight} \times DCP)$$

$$CF = 100 \times FBW / TL^3$$

where FBW, IBW, FBCP, IBCP, DCP, and TL were final body weight (g), initial body weight (g), final body crude protein, initial body crude protein, dietary crude protein, and total length (cm), respectively. Protein and lipid gains in the whole fish were calculated using the equation:

$$[(FBW \times FNC) - (IBW \times INC)] / 100$$

where the FNC and INC were the final and initial nutrient compositions (%) of the whole fish, respectively. Ninety fish from each tank were randomly captured and euthanized with the 2-phenoxyethanol (Sigma-Aldrich) and stored at −20 °C for later proximate composition analysis. The proximate composition, including moisture, crude protein, crude lipid, and crude ash was analyzed through the AOAC (Association of Official Analytical Chemists) method [22]. In brief, moisture was determined by drying a sample in an oven at 105 °C for 24 h. Crude protein was determined by the Kjeldahl method (Kjeltec™ 8100, FOSS,