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Comparison of leucine requirement in olive flounder (*Paralichthys olivaceus*) by free or synthetic dipeptide forms of leucine

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ABSTRACT

We report a promising dietary model for amino acid (AA) requirement studies in fishes. An eight-week feeding trial was carried out to compare the efficacy of free leucine (Leu) and dipeptide Leucinyl-Glycine (Leu-Gly) in diets for olive flounder. Growth performance and whole-body AA compositions were examined. Triplicate groups of fish (average weight, 0.27 ± 0.001 g) (23 fish per replicate) were fed seven isonitrogenous and isocaloric experimental diets containing 6 (basal), 9, 12 and 15 g Leu/kg diet in the two different forms. The basal diet contained 6 g Leu/kg from fish meal and the other six diets were prepared by adding 3 g incremental levels of Leu or Leu-Gly. Growth rate was significantly ($P<0.05$) increased in the groups fed Leu-Gly in comparison to free Leu while no significant differences were observed by the supplementation level. Whole-body accumulations of most indispensable and dispensable AA were higher in the fish fed Leu-Gly, however, no significant differences were detected. A second-order polynomial regression analysis on the basis of weight gain revealed better utilization efficiency for Leu-Gly. Higher weight gain (0.63 g) was obtained in fish fed Leu-Gly than fish fed Leu (0.55 g). The results demonstrated that dipeptide Leu has higher efficiency in protein synthesis than free Leu and further confirmed that AA availability could be better in fish when fed dipeptide than free forms.

1. Introduction

The nutritive value of dietary proteins for fish is influenced by their amino acid (AA) composition and availability (Wilson and Cowey, 1985; Wilson and Poe, 1985). The quantitative indispensable AA (IDAA) requirements of various fish species have been studied to achieve optimum growth and feed utilization, cost-effective diet formulation, and desirable carcass quality (NRC, 2011). Diets with balanced IDAA composition are of great value in fish feeding especially in early life stages (Ostaszewska et al., 2010). AA is not only used for protein synthesis but also as energy source by fish larvae and other life stages (Rønnestad and Conceição, 2005).

Dabrowski et al. (2003) reported that a synthetic dipeptide-based diet can support growth of rainbow trout in the early stages whereas a free AA-based diet failed. Luo et al. (2005) found that weight gain of marine grouper fed an intact protein-based control diet was significantly higher than that of fish fed crystalline AA-based diets in a methionine study, and

Abbreviations: AA, amino acid; Leu, leucine; Leu-Gly, Leucinyl-Glycine; IDAA, indispensable amino acid; DAA, dispensable amino acid; BCAA, branched-chain amino acid.

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concluded that crystalline AA is likely to be utilized at a lower efficiency than AA derived from intact protein. It is generally thought that the inefficiency of free AA in fish is due to its faster uptake and subsequent catabolism compared to those from intact protein (Murai et al., 1987; Cowey and Walton, 1988; Rønnestad et al., 2000; Dabrowski et al., 2003 2007). Another explanation was proposed by higher leaching loss of free AA than bound AA in aquatic environments prior to ingestion (Zarate and Lovell, 1997). Therefore, microencapsulation or coating techniques of nutrients including crystalline AA have been under development to reduce the rate of the nutrient uptake by slowing the rate of release of AA/nutrients (Villamar and Langdon, 1993; De la Higuera et al., 1998) and the leaching rates of free AA from feeds during exposure to water (Segovia-Quintero and Reigh, 2004).

Leucine (Leu) is essential for the optimal growth and health of fish because it is abundantly needed for protein synthesis in muscle tissues. It is a ketogenic AA and is specific among branched-chain AA (BCAA) in its ability to stimulate insulin release from the islet cells of the pancreas (Panten et al., 1974). Leu deficiency can cause severe biochemical malfunctions resulting in growth retardation. Leu was proposed as one of the limiting AA that affects growth rate of turbot larvae (Conceicao et al., 1997). This might be true for other flatfishes and the requirement has been reported for only a limited number of cultured fish species including Chinook salmon (Chance et al., 1964), common carp (Nose, 1979), channel catfish (Wilson et al., 1980), Japanese eel (Nose, 1979), Nile tilapia (Santiago and Lovell, 1988), chum salmon (Akiyama, 1987), lake trout (Hughes et al., 1983) and Indian major carp (Abidi and Khan, 2007).

Olive flounder, *Paralichthys olivaceus*, is one of the most important marine cultured species in Korea, Japan and China. In the past, emphasis was given to verify protein requirements (Lee et al., 2002; Kim et al., 2002, 2003, 2005, 2010) but information on AA requirements for the fish is still in its infancy. The quantitative AA requirement of olive flounder has been studied only for lysine (Forster and Ogata, 1998), methionine (Alam et al., 2000, 2001) and arginine (Alam et al., 2002).

The IDAA requirements of fish are usually determined based on the growth rates of fish fed graded levels of the targeted AA in a free form. Dabrowski et al. (2010) hypothesized that the AA requirements in fishes might have been over-estimated in most previous studies which had used a free form of AA. Therefore, we conducted a feeding trial in this study with different forms of Leu (free or dipeptide) with olive flounder to prove the hypothesis and provide a promising AA source for re-evaluation of the previous results on dietary AA requirements for fishes.

2. Materials and methods

2.1. Experimental design and diets

A semi-purified basal diet was formulated to contain 6 g Leu/kg from fish meal. Fish meal was included to increase palatability of the experimental semi-purified diets. A mixture of synthetic L-AA without Leu was prepared according to Dabrowski et al. (2003) and used as main protein source. Six additional diets were prepared by adding incremental levels (3 g/kg) of two different forms of Leu to the control diet to be 9, 12, and 15 g/kg diet, respectively. Leucinyl-Glycine (Leu-Gly) was used as the Leu source in a dipeptide form and crystalline L-Leu was used as a free form. The dipeptide, Leu-Gly, was supplemented into the basal diets based on the molecular weight. Diets were kept isonitrogenous (44% crude protein) and isocaloric (4.48 kcal/g gross energy) by decreasing glycine while increasing the two different forms of Leu levels. All dietary ingredients were well mixed, pelletized and freeze-dried. The dried diets were then prepared as crumble types and sieved to make proper sizes. The size of the diets was gradually increased over the course of feeding trial as fish grew. The compositions and proximate analysis of the seven experimental diets are given in Table 1. AA concentrations of the experimental diets are provided in Table 2. Leu concentration in the diets was confirmed with its intended levels.

2.2. Feeding trial

Olive flounder (*P. olivaceus*) at the early stages were transported from a private hatchery (Udo Fisheries, Jeju, Korea) to a semi-recirculation marine culture system for feeding study, Jeju National University, South Korea. The fish were fed a microparticulate diet (Love Larva No. 1-4, Maruha, Shimonoseki, Japan) for one week to be acclimated to the experimental facilities and conditions. The conditioned experimental fish averaging at 0.27 ± 0.001 g were then randomly distributed into twenty-one 20 L tanks (23 fishes/tank) in line with the semi-recirculation system. The water flow was kept at a rate of approximately 1 L/min and water temperature was maintained at 20–21 °C during the feeding trial by a thermometer (OKE-6422H, I'm TECH, Busan, Korea). Dissolved oxygen levels were maintained at a proper level over 8.0 mg/l by aeration and monitored throughout the feeding study. Triplicate groups of fish were hand-fed the experimental diets to apparent satiation for eight weeks. Fish were initially fed six times per day until week 5 and then fed four times per day from week 6. Growth was measured every two weeks. Feeding was stopped 24 h prior to weighing to minimize stress of the fish. Experimental protocols followed the guidelines approved by the Animal Care and Use Committee of Jeju National University (Jeju, South Korea).

2.3. Chemical analysis

At the end of feeding trial, all fishes were sampled for whole-body AA analysis. Diets and whole-body samples were freeze-dried and finely ground using a grinder. Crude protein was determined by Kjeldahl method using an Auto Kjeldahl system (Kejltec System 2300, Sweden) (AOAC, 1990; method no. 984.13). Crude lipid was determined by ether-extraction