

Dietary lysine requirement of juvenile Japanese seabass, *Lateolabrax japonicus*

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Abstract

A feeding experiment was conducted to estimate the quantitative requirement of lysine in juvenile Japanese seabass by feeding six isonitrogenous and isoenergetic practical diets containing six levels of lysine ranging from 1.28% to 4.25% (dry weight) at about 0.6% increment (diets 1–6). Equal amino acid nitrogen was maintained by replacing lysine with glutamic acid. Triplicate groups of 20 fish (initial weight 5.50 ± 1.63 g) were fed to apparent satiation by hand twice daily for 10 weeks in floating sea cages. The water temperature ranged from 27 to 30 °C, the salinity from 25‰ to 28‰ and the dissolved oxygen content was approximately 7 mg l^{-1} during the experimental period. Fish fed diets with lysine from 1.28% to 4.25% (diets 1–6) all showed high survival (above 95%) and no significant differences were observed. Final weight (FW) and specific growth rate (SGR) increased with increasing dietary lysine level up to 2.46%, and thereafter, remained nearly the same. Both feed efficiency ratio (FER) and protein efficiency ratio (PER) in fish fed diets with lysine from 2.46% to 3.66% (diets 3–5) were significantly higher than those in fish fed diets with lysine of 1.28%, 1.86% and 4.25% ($P < 0.01$). The whole body crude protein and crude lipid contents were significantly affected ($P < 0.01$) by dietary lysine level, while moisture and ash showed no significant differences. The whole body protein content was positively correlated with dietary lysine levels, while lipid content was negatively correlated with it. Lysine contents of fish muscle were significantly affected by dietary lysine levels ($P < 0.05$). Fish fed the diet with 1.28% lysine showed the lowest lysine content (7.61%) in fish muscle, while fish fed the diet with 3.66% lysine had the highest value (7.83%). Other essential amino acid contents of muscle were not significantly different among the dietary treatments. Broken-line analysis on the basis of SGR, FER and PER showed that dietary lysine requirements of juvenile Japanese seabass were $2.49 \pm 0.05\%$, $2.61 \pm 0.16\%$ and $2.60 \pm 0.13\%$ dry diet ($5.80 \pm 0.12\%$, $6.07 \pm 0.37\%$ and $6.05 \pm 0.30\%$ dietary protein), respectively.

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1. Introduction

Apart from optimal protein quantity, the quality in terms of amino acid profile in the diet accounts for

maximal growth in fish (Ahmed and Khan, 2004). All fish species studied to date have been shown to require 10 indispensable amino acids in their diet for maximum growth (Wilson, 1985). An indispensable amino acid deficiency may cause reduced growth and poor diet conversion (Wilson and Halver, 1986). Therefore, to satisfy the indispensable amino acid requirements of a

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species is very important in preparing well-balanced diets. The requirements for all 10 indispensable amino acids have been established only for a few cultured fish species such as common carp (Nose, 1979), Nile tilapia (Santiago and Lovell, 1988), Indian major carp (Ahmed and Khan, 2004), catla (Ravi and Devaraj, 1991), coho salmon (Arai and Ogata, 1991), chum salmon (Akiyama and Arai, 1993), milkfish (Borlongan and Coloso, 1993), chinook salmon, channel catfish and Japanese eel (NRC, 1993).

Of all the indispensable amino acids, lysine is often the most limiting one in the ingredients used to prepare fish diets (Harris, 1980; Forster and Ogata, 1998; Small and Soares, 2000). It serves along with methionine as a precursor to carnitine, which is involved in the transportation of long chain fatty acyl groups into the mitochondria for beta oxidation (Walton et al., 1984). Investigations have been conducted to determine the requirements of several fish species for this amino acid (Ogino, 1980; Robinson et al., 1980; Akiyama et al., 1985; Arai and Ogata, 1991; Griffin et al., 1992; Gurrea et al., 2001; Tantikitti and Chimsung, 2001; Wilson, 2002), and the reported values range from 3.7% to 6.2% of dietary protein.

Japanese seabass is an economically important food fish cultured in China. It grows fast and has high market value. However, a main constraint to Japanese seabass culture is the limited supply of trash fish that is presently the main diet source for grow-out. So there is an urgent need to develop a suitable practical diet for grow-out production of Japanese seabass. One of the prerequisites for developing high efficiency diet for Japanese seabass requires complete knowledge of its nutritional requirements. A few studies have been reported on the nutrition of this seabass (Lin et al., 1994; Hu et al., 1995; Gao et al., 1998; Hong et al., 1999; Pan et al., 2000; Ai et al., 2004a, b). To our knowledge, no information is available on its essential amino acid requirements. Therefore, the present investigation was undertaken to determine the optimum dietary lysine requirement for juvenile Japanese seabass.

2. Materials and methods

2.1. Experiment diets

Six isonitrogenous and isoenergetic diets were formulated with graded levels of lysine (Table 1). Dietary lysine was quantitatively increased at the expense of glutamic acid. L-Crystalline amino acids mixture was used so that the levels of all amino acids, except lysine, would simulate the whole body amino acid pattern of Japanese seabass (fingerling with 6.26 g mean body weight). The amino acid (AA) contents of

Table 1

Composition and proximate analysis of the experimental diets (% dry weight)

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Common ingredients ^a	97.00	97.00	97.00	97.00	97.00	97.00
Lysine	0.00	0.60	1.20	1.80	2.40	3.00
Glutamic acid	3.00	2.40	1.80	1.20	0.60	0.00
<i>Proximate analysis (n=3), on dry weight basis (%)</i>						
Crude protein	43.3	42.9	43.0	43.2	43.5	43.7
Crude lipid	12.2	12.2	12.1	12.3	12.1	12.3
Ash	5.8	5.9	5.9	6.0	5.8	5.9
Moisture	8.5	8.2	8.5	8.4	8.4	8.4

^a Common ingredients (%): fish meal, 10; soybean meal, 15; zein, 30; wheat meal, 18.68; mixed amino acids, 4.87 (aspartic acid, 1.22%; glycine, 1.26%; threonine, 0.30%; arginine, 0.86%; valine, 0.43%; methionine, 0.46%; isoleucine, 0.34%); yeast meal, 3; menhaden fish oil, 5; soybean oil, 3.5; lecithin, 2.5; betaine, 0.3; antimold, 0.1; antioxidant, 0.05; mineral premix, 2{(mg or g kg⁻¹ diet): NaF, 2 mg; KI, 0.8 mg; CoCl₂·6H₂O (1%), 50 mg; CuSO₄·5H₂O, 10 mg; FeSO₄·H₂O, 80 mg; ZnSO₄·H₂O, 50 mg; MnSO₄·H₂O, 60 mg; MgSO₄·7H₂O, 1200 mg; Ca(H₂PO₄)₂·H₂O, 3000 mg; NaCl, 100 mg; zeolite, 15.447 g}; vitamin premix, 2{(mg or g kg⁻¹ diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B₁₂, 0.1 mg; vitamin K₃, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinal acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin 150 mg; wheat middling, 14.012 g}.

experimental diets are shown in Table 2. Because the experimental diets contained about 43% protein, the AA contents of the experimental diets were made comparable to those of 43% whole body protein AA contents. All the dietary AA contents were maintained nearly the same levels as the corresponding AA contents in 43% whole body protein except for lysine and glutamic acid. A satisfying increase in lysine content was obtained in the diets, although it was not identical to the quantities added initially. The final levels of lysine were 1.28%, 1.86%, 2.46%, 3.03%, 3.66% and 4.25% (dry weight), respectively, by adding crystalline L-lysine, analyzed by reverse phase high performance liquid chromatography (HPLC, HP 1100, USA). The range of dietary lysine content covered the lysine level (3.36%) in 43% crude protein from the whole body tissue of this species (Table 2).

Ingredients were ground into fine powder through 320 μm mesh. All the ingredients were thoroughly mixed with menhaden fish oil and water was added to produce a stiff dough. The dough was then pelleted with an experimental diet mill (F-26(II), South China University of Technology, China) and dried for about 12 h in a ventilated oven at 45 °C. After drying, the diets were broken up and sieved into proper pellet size. The

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