Aquaculture 414–415 (2013) 173–182

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Interactive effects of dietary arginine and histidine on the performances of Japanese flounder *Paralichthys olivaceus* juveniles

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ARTICLE INFO

Article history:
Received 8 April 2013
Received in revised form 28 June 2013
Accepted 2 July 2013
Available online 8 July 2013

Keywords:
Arginine
Histidine
Japanese flounder
Interactive effect
Growth performance
Blood parameters

ABSTRACT

A 60-day feeding trial was carried out to investigate the interactive effects of arginine and histidine on the performances of Japanese flounder juveniles. Fish were fed experimental diets containing six different rations of arginine (Arg) and histidine (His), 2 levels of Arg such as 1.74 (AL) and 2.75 g/100 g diet (AH), combined with 3 levels of His such as 1.01(HL), 1.50 (HM) and 1.88 g/100 g diet (HL), respectively. The results indicated that the growth performances (final body weight, body weight gain and special growth rate) were highest in fish fed AH–HM combined group. Those parameters were significantly higher than other groups except AH–HL group. On the other hand, the interactions of arginine and histidine were found on the growth parameters. In addition, the significantly better nutrient utilizations (feed conversion ratio, protein efficiency ratio and protein retention) were also observed in higher Arg supplemented groups. There were no significant effects on the hematocrit, hemoglobin, glucose, total cholesterol, total bilirubin and mucus bactericidal activity of experimental fish among treatments. Japanese flounder fed the diet containing low dietary arginine together with low dietary histidine showed higher oxidative stress and lower lysozyme activities. The dietary arginine and histidine levels significantly affected the concentrations of fish muscle free amino acids. It would be concluded that dietary Arg and His functioned interactively, and the flounders fed the diet containing 2.70 g arginine with 1.56 g histidine/100 g diet showed the better growth performance and physiological status than other groups.

1. Introduction

One of the main problems of fishmeal substitution in aqua-feed is the imbalanced amino acid profiles, which may cause symptoms of disease or sub-healthy status, especially lack of some functional amino acids. Therefore, many studies were hammered at searching the appropriate rations of amino acids in aquafeeds and investigating the relationship between amino acids in the fish nutrition research (Alam et al., 2002b, 2005; Li et al., 2009; Teshima et al., 2002). Some functional amino acids are important regulators of key metabolism pathways that are necessary for maintenance, growth, reproduction, and immunity in organisms, therefore maximizing efficiency of food utilization, enhancing protein accretion, reducing adiposity, and improving health (Wu, 2009). Arginine (Arg) and histidine (His) are two alkali amino acids among the ten indispensable amino acids in feed for optimal growth of fish (Alam et al., 2002b; Li et al., 2009; Teshima et al., 2002; Wilson, 2002). Arg has been considered to be one of the limiting amino acid in plant-protein based diet of penaeid shrimp *Penaeus monodon* (Millamena et al., 1998), meanwhile, some of the physiological functions of Arg have been reported previously, such as protein synthesis (Alam et al., 2002c), catabolism into NO, and so on. Those functions are also related to an important cytotoxic function in macrophages and inflammation mediation in fish (Trichet, 2010). Polyamines, which are synthesized from Arg, has a function to enhance the growth of Atlantic salmon *Salmo salar* (Berge et al., 2002), increase the insulin like growth factor in Brown trout *Salmo trutta* (Baños et al., 1999), *Oncorhynchus tchawytscha* and *Oncorhynchus kisutch* (Plisetskaya et al., 1991). On the other hand, His in white muscle of several fish species was proved to be utilized as a direct energy source, which could protect the other amino acid under starved conditions (Abe and Ohmama, 1987). Free His was also considered as an intracellular buffer as fish move vigorously, resulting in accumulation of acidic end products during the burst swimming (Abe et al., 1986; Shiau et al., 1997), enrolling of histamine production, which take part in allergic and inflammatory reactions (Ahmed and Khan, 2005). Furthermore, according to the relationship between His and taurine, it was suggested that His may have a compensation effect for osmoregulation in milkfish *Chanos chanos* (Shiau et al., 1997). His and its related imidazole derivatives were illustrated to improve the quality of fish filet (Førde-Skjærvik et al., 2006; Li et al., 2009; Ogata, 2002).

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0044-8486/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.aquaculture.2013.07.001

Arg has been proved to have interactions or intracellular transformations with some other amino acids(Wu, 2009). For example, lysine inhibits the arginase activity in cells and influences the arginine supplementation (Wu and Morris, 1998) and citrulline and ornithine has been reported to perform as the premier of arginine in rainbow trout (*Salmo gairdneri*) (Chiu et al., 1986). However, there are very limited studies on His concerning the participation in the metabolic pathway of other amino acids, except the investigation of growth effects on free amino acids in milkfish (*C. chanos*), in which it was found that the Arg concentration decreased significantly in white muscle, meanwhile the His showed an opposite result (Shiau et al., 1997). Same terminal degraded product of Arg and His was also reported in fish, previously (De Silva and Anderson, 1995), which implied there may be an interaction, directly or indirectly, between the two amino acids in fish. As a matter of fact, no data on the interaction of these two indispensable amino acids was available, particularly on marine fish species.

Japanese flounder *Paralichthys olivaceus* is an important cultured fish species in the Far East area, including China, Japan and South Korea (Kawamura and Hosoya, 1997; Ye et al., 2011a). Requirements of several essential amino acids were investigated on the Japanese flounder, such as lysine (Forster and Ogata, 1998), methionine (Alam et al., 2000), arginine (Alam et al., 2002a). Meanwhile, some primary exploration on the proper amino acid profiles in Japanese flounder diets was illustrated in some previous studies (Alam et al., 2002c). However no data was available of amino acids interactive effects or proper ration of the two alkaline amino acids on Japanese flounder. Under the circumstances, the present study was focus on the interactive effects of Arg and His on the growth index, immune parameters and stress test.

2. Materials and methods

2.1. Experimental diets

Six semi-purified low fishmeal diets containing various Arg and His rations were formulated as illustrated in Table 1. Lowest level in Arg with lowest level of His (AL–HL), lowest in Arg with intermediate in His (AL–HM), lowest in Arg with highest in His (AL–HH), highest in Arg with lowest in His (AH–HL), highest in Arg with intermediate in His (AH–HM) and highest in Arg with highest in His (AH–HH) were assigned among test diets, respectively. Besides 20% fishmeal, the protein sources mainly included casein, gelatin as well as crystalline amino acids which were added to provide the amino acid patterns similar to those of juvenile Japanese flounder whole body protein except for Arg and His (Alam et al., 2002a). While increasing the Arg and His, the aspartic acid and alanine in the diets decreased proportionally in order to keep isonitrogenous conditions (Buentello and Gatlin, 2000). To produce crystalline amino acids mixture, the previous studies on amino acids requirement for Japanese flounder were referenced in associated with 50% whole body protein shown in Table 2 (Alam et al., 2000, 2002a; Kanazawa et al., 1989).

The preparation of pre-coated crystalline amino acids (CAA) and experimental diets followed the previous study (Alam et al., 2002a) with a slight modification. Briefly, CAA mixtures were weighted separately and pre-coated by carboxymethyl-cellulose (CMC) that cooked at 50 °C with distilled water in order to prevent leaching losses. Then casein and gelatin were added into the bound CAA, and all the pastes were mixed with the other dry ingredients, which were mixed and stirring consistently in a Kitchen Aid multi-function mixer (Kitchen Aid, Ohio, USA) for 15 min. Then, the gelatinized κ-carrageenan was added to the mixture to improve water stability of the diets. Pollack liver oil and soybean lecithin were added into the mixer and mixed for another 15 min, then the distilled water (in total 30–35% of dry ingredients) was added and mixed for 15 min. The pH of the diets was adjusted to 7.0–7.5 with 0.4 N sodium hydroxide as described previously (Alam et al., 2002c). After all ingredients were mixed well in the mixer (Kitchen Aid, Ohio, USA), the mixture was molded into two pellet sizes ($\Phi = 1.5$ mm and 2.2 mm) using a single-screwed meat chopper (ROYAL Inc., Tokyo, Japan). Pellets were dried in dry-air mechanical convection oven at 40 °C (DK400, Yamato Scientific, Tokyo, Japan) for 60 min and stored in a −30 °C freezer until use.

Table 1
Compositions of the experimental diets (g/kg, dry matter basis).

Ingredients	Diets[a]					
	AL–HL	AL–HM	AL–HH	AH–HL	AH–HM	AH–HH
Casein[b]	100.0	100.0	100.0	100.0	100.0	100.0
Gelatin[c]	50.0	50.0	50.0	50.0	50.0	50.0
Fishmeal[d]	200.0	200.0	200.0	200.0	200.0	200.0
Crystalline amino acids mixture[e]	111.6	111.6	111.6	111.6	111.6	111.6
Pollack liver oil[f]	50.0	50.0	50.0	50.0	50.0	50.0
Soybean lecithin[b]	50.0	50.0	50.0	50.0	50.0	50.0
α-Starch	100.0	100.0	100.0	100.0	100.0	100.0
α-Cellulose	135.0	135.0	135.0	135.0	135.0	135.0
Carboxymethyl cellulose (CMC)	44.0	44.0	44.0	44.0	44.0	44.0
κ-Carrageenan[g]	25.0	25.0	25.0	25.0	25.0	25.0
Vitamin mixture[h]	50.0	50.0	50.0	50.0	50.0	50.0
Mineral mixture[i].	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin C[j]	3.0	3.0	3.0	3.0	3.0	3.0
Attractants[k]	10.0	10.0	10.0	10.0	10.0	10.0
l-Arginine[c]	0.0	0.0	0.0	17.6	17.6	17.6
l-Histidine[c]	0.0	4.2	9.6	0.0	4.2	9.6
Aspartic acid[c]	19.3	16.3	13.5	8.5	6.0	2.6
Alanine[c]	12.1	10.9	8.3	5.3	3.6	1.6
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

[a] AL–HL: lowest in both arginine & histidine, AL–HM: lowest in arginine and intermediate level in histidine, AL–HH: lowest in arginine and highest in histidine, AH–HL: highest in arginine and lowest in histidine, AH–HM: highest in arginine and intermediate in histidine, AH–HH: highest in both arginine & histidine.
[b] Wako Pure Chemical Industries, Ltd., Osaka, Japan.
[c] Nacalai Tesque, Kyoto, Japan.
[d] Nippon Suisan Co. Ltd., Tokyo, Japan. With proximate composition (% dry matter basis): moisture,8.2; crude protein. 72.0; crude lipid, 14.7 and ash, 11.6.
[e] Crystalline amino acid mixture: l-form, Nacalai Tesque, Kyoto, Japan. See Table 2.
[f] Riken Vitamin, Tokyo, Japan.
[g] Sigma-Aldrich, St. Louis, MO, USA.
[h] Vitamin mixture (g/kg diet): Vitamin D_3 0.03, Menadione $NaHSO_3 \cdot 3H_2O$ (K_3) 0.15, dl-α-Tocopherol Acetate (E) 0.64, Thiamine-Nitrate (B_1) 0.10, Riboflavin (B_2) 0.32, Pyridoxine-HCl (B_6) 0.08, Cyanocobalamin (B_{12}) 0.0001, d-Biotin 0.01, Inositol 6.42, Niacin (Nicotic acid) 1.28, Ca Pantothenate 0.45, Folic acid 0.02, Choline chloride 13.12, ρ-Aminobenzoic acid 0.64, β-carotene 0.30, Cellulose 6.43.
[i] Mineral mixture (g/kg diet): $MgSO_4$ 5.07, Na_2HPO_4 3.23, K_2HPO_4 8.87, Fe Citrate 1.10, Ca Lactate 12.09, $Al(OH)_3$ 0.01, $ZnSO_4$ 0.13, $CuSO_4$ 0.004, $MnSO_4$ 0.03, $Ca(IO_3)_2$ 0.01, $CoSO_4$ 0.04.
[j] Stay-C 35.
[k] Taurine 0.5, betaine 0.4 and inosine-5-monophosphate 0.1, provide by Nacalai Tesque, Kyoto, Japan.

2.2. Experimental fish and feeding protocol

Juvenile Japanese flounder (*P. olivaceus*), with mean initial body weight of 1.00 ± 0.01 g (mean ± S.D.), were purchased from a commercial hatchery (Matsumoto Suisan, Miyazaki, Japan). and transported to Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University. They were stocked in one 500 L tank, acclimating to the laboratory conditions for one week and fed on a commercial diet (Higashimaru Co. Ltd, Kagoshima, Japan) during this period. Eighteen fish per tank with the triplicate tanks per treatment were stocked in eighteen 100 L black polyethylene tanks with flow-through sea water (2.0 L/min/tank), where each tank was equipped with continuous aeration. The tanks were maintained under a 12/12 h light/dark regime by fluorescent lamp. Fish in each tank were fed twice (08:30 and 16:30 h) a day manually under the apparent satiation for 60 days.