Aquaculture 432 (2014) 130–138

Contents lists available at ScienceDirect

Aquaculture

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

Interactive effects of dietary valine and leucine on two sizes of Japanese flounder *Paralichthys olivaceus*

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ARTICLE INFO

Article history:
Received 9 February 2014
Received in revised form 4 May 2014
Accepted 5 May 2014
Available online 13 May 2014

Keywords:
valine
leucine
Japanese flounder
interactive effect
growth performances
blood parameters

ABSTRACT

This study was carried out to investigate the interactive effects of dietary leucine and valine on the performances of two sizes Japanese flounder *Paralichthys olivaceus*. Two groups of fish with average initial weights of 0.43 ± 0.01 g and 41.2 ± 2.0 g (mean ± S.D.) were fed with diets containing two levels of leucine (1.6 and 5.0% of diet) with three levels of valine (1.2, 1.8 and 2.5% of diet, as a 2 × 3 experimental design) and the control diet for 56 days and 30 days, respectively. In trial 1, after the fish fed with test diets for 56 days, growth performance and nutrients utilization were evaluated. Interactive effects of leucine and valine were found on growth parameters (final body weight, body weight gain and special growth rate) of Japanese flounder. Not only antagonism was observed in high dietary Leu level groups but also the stimulative effect of increased dietary Val in low Leu level groups. Significant interaction was also found on feed conversion ratio of Japanese flounder fed with test diets, however, the fish whole body proximate compositions were not altered by the various diets. In trial 2, dietary Leu and Val dominated the plasma free Leu and free Val concentrations, but no antagonism was found. Interactions were also found on plasma LDH and GPT of test fish, and it was showed that for fish fed with high dietary Val level in low Leu (1.6% of diet) diet can protect the cells from oxidative stress.

1. Introduction

Branched-chain amino acid (BCAA), including valine, leucine and isoleucine, constitute 18–20% of the total amino acids present in plant and animal proteins (Li et al., 2009), which are oxidized primarily in skeletal muscle by BCAA dehydrogenase enzyme complex (Mager et al., 2003) and provide the α-amino group for the endogenous synthesis of glutamine primarily in skeletal muscle (Li et al., 2007; Newsholme and Calder, 1997). BCAA are essential amino acids and play important roles in certain biochemical reactions and the growth of monogastric and preruminant terrestrial animals (Ahmed and Khan, 2006), on the other hand, the antagonistic effects of these amino acids are investigated in poultry (Calvert et al., 1982) and mammalian (Block and Harper, 1984; Langer and Fuller, 2000), which in fish, however, have not yet been fully revealed and the results were not consistence to date (Choo et al., 1991; Hughes et al., 1984; Robinson et al., 1984; Yamamoto et al., 2004). Moreover, BCAA share the same transporter on the cell membrane (Li et al., 2007), which also indicated that it is important to balance the dietary BCAA. Above all, it is necessary to investigate the interactions of BCAA in fish diet.

Leucine (Leu) is one of the functional amino acids (Li et al., 2009). In mammalian, the role of leucine is an activator of the mTOR signaling pathway which regulates protein synthesis and degradation in cells (Meijer and Dubbelhuis, 2004). Nevertheless, Leu works with valine and isoleucine to protect and fuel the muscle, meanwhile it is also an important amino acid in producing hemoglobin, maintaining blood sugar levels and in stress resistance (Abidi and Khan, 2007). The requirement of Leu has been well established in various fish species (Wilson, 2002), including Japanese flounder, which is 3.9% of protein (Forster and Ogata, 1998). However most of them were focused on the single amino acid requirement without considering about the antagonisms between BCAA, which have not yet been fully assessed or shown consistent results in previous researches (Yamamoto et al., 2004). The utilization of Leu under various levels of other BCAA in marine fish diet is still needed to be revealed.

Valine (Val) is another branched-chain amino acid. It plays very important roles in certain biochemical reactions and in the growth of monogastric and preruminant terrestrial animals (Bae et al., 2012), as well as participants in protein and the amine neurotransmitters serotonin synthesis (Fernstrom, 2005). The previous studies on Val requirement in aqua-feed showed similar phenomenons as the studies on Leu, although the optimum requirements have been established well before (Wilson, 2002), which, in Japanese flounder is 2.5% of protein (Forster and Ogata, 1998). However, they seldomly considered about the level

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http://dx.doi.org/10.1016/j.aquaculture.2014.05.004
0044-8486/

of other BCAA in the diets (Abidi and Khan, 2004; Bae et al., 2012; Yamamoto et al., 2004). Hence, it is necessary to investigate the interactive effects between these two amino acids, in order to provide a reference for further researches on amino acid requirements, especially when BCAA is studied.

The interactive effects of Leu and Val on Japanese flounder *Paralichthys olivaceus*, an important cultured fish species in the Far East area, including China, Japan and South Korea (Ye et al., 2011), have not yet been revealed. The present study was focus on the interactive effects of Leu and Val on the growth performance, feed utilization, blood parameters and health status of Japanese flounder.

2. Materials and methods

2.1. Test diets

Seven test diets were formulated for juvenile Japanese flounder as illustrated in Table 1. Beside the control diet (fishmeal diet, as illustrated in Table 1), six semi-purified diets contained 2 levels (2 and 5% of dry diet, analyzed values) of Leu combined with 3 levels (1.2, 1.8 and 2.5% of dry diet, analyzed values) of Val were formulated: lowest level in Leu with lowest level in Val (LL–LV), lowest in Leu with intermediate in Val (LL–MV), lowest in Leu with highest in Val (LL–HV), highest in Leu with lowest in Val (HL–LV), highest in Leu with intermediate in Val (HL–MV) and highest in Leu with highest in Val (HL–HV). In these six treatments, besides other protein sources (fishmeal, casein and gelatin), crystalline amino acids (CAA) mixture was added to each diet to provide essential amino acids approximate to those in previous study (Alam et al., 2002). To produce crystalline amino acid mixture, the previous studies on amino acid requirement for Japanese flounder were referenced in associated with 50% whole body protein shown in Table 2 (Alam et al., 2000; Han et al., 2013; Kanazawa et al., 1989). The Leu and Val contents of 50% fish whole body protein were induced as the referenced optimum levels as described previously, based on which, the levels of these two amino acids in the test diets were formulated (Alam et al., 2002; Han et al., 2013). While decreasing Leu and Val, glutamic acid and aspartic acid were supplemented to each diet in order to keep the test diets isonitrogenous.

Pre-coated CAA was prepared to prevent leaching losses as described previously (Alam et al., 2002; Han et al., 2013). Briefly, CAA were weighed separately and bounded by cooked carboxymethylcellulose (CMC) at 50 °C with distilled water. Then casein and gelatin were added into the bounded CAA, after that the dough was mixed with the other dry ingredients by a mixer (Kitchen Aid, Ohio, USA) for 15 min. Meanwhile, 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 added distilled water (in total 30–35% of dry ingredients) and mixed for 15 min. The pH of the mixture was adjusted to 7.0–7.5 with 0.1 N sodium hydroxide. After all of the ingredients were thoroughly mixed, the mixture was molded into two pellet sizes (Φ = 1.2 mm and 2.8 mm, for two sizes of fish) using a single-screwed meat chopper (ROYAL Inc., Tokyo, Japan). Pellets were dried in a dry-air mechanical convection oven (DK400, Yamato Scientific, Tokyo, Japan) at 40 °C till the moisture was approximate to 15% and stored at −30 °C until use. Analyzed proximate compositions and free amino acid profile of the test diets are illustrated in Table 3.

2.2. Experimental fish and feeding protocols

2.2.1. Trial 1

Juvenile Japanese flounder (*P. olivaceus*) with mean initial body weight of 0.43 ± 0.01 g (mean ± S.D.) were obtained from a commercial hatchery (Matsumoto Suisan, Miyazaki, Japan) and transported to Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University. They were stocked in 500 L tank, and fed with commercial

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

Ingredients	Treatments[a]						
	Control	LL–VL	LL–VM	LL–VH	LH–VL	LH–VM	LH–VH
Casein[b]	0	100	100	100	100	100	100
Gelatin[c]	0	50	50	50	50	50	50
Fishmeal[d]	600	200	200	200	200	200	200
Amino acids mix[e]	0	102	102	102	102	102	102
Cod liver oil[f]	20	50	50	50	50	50	50
Soybean lechithing[b]	30	50	50	50	50	50	50
α-Starch	100	100	100	100	100	100	100
α-Cellulose	68	115	115	115	115	115	115
Carboxymethyl cellulose (CMC)	44	44	44	44	44	44	44
κ-Carrageenan[g]	25	25	25	25	25	25	25
Vitamin mix[h]	60	60	60	60	60	60	60
Mineral mix[i]	50	50	50	50	50	50	50
Stay-C[f]	3	3	3	3	3	3	3
Attractants[j]	0	10	10	10	10	10	10
Leucine[e]	0	0	0	0	25	25	25
Valine[e]	0	0	8.3	16	0	8.3	16
Aspartic acid[e]	0	19	15	11	7	3	0
Glutamic acid[e]	0	22	17.7	14	9	4.7	0
Total	1000	1000	1000	1000	1000	1000	1000

[a] The abbreviations of experimental diets are illustrated in the text.
[b] Obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan.
[c] Obtained from Nacalai Tesque, Kyoto, Japan.
[d] Obtained from Nippon Suisan Co. Ltd., Tokyo, Japan.
[e] See Table 2. Crystalline amino acid mixture (g/100 g diet): all amino acids were provided with l-form, by Nacalai Tesque, Kyoto, Japan.
[f] Obtained from Riken Vitamin, Tokyo, Japan.
[g] Obtained from 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, Cyanocobalamine (B_{12}) 0.0001, D-Biotin 0.01, Inositol 6.42, Niacine (Nicotic acid) 1.28, Ca Panthothenate 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] Taurine 0.5, betaine 0.4 and inosine-5-monophosphate 0.1, provide by Nacalai Tesque, Kyoto, Japan.