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The effects of dietary leucine on the growth performances, body composition, metabolic abilities and innate immune responses in black carp *Mylopharyngodon piceus*

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ABSTRACT

The present study was focused on the growth, body composition, metabolic abilities and innate immune responses in juvenile black carp *Mylopharyngodon piceus* fed with six levels of dietary leucine (7.3, 12.4, 16.2, 21.9, 28.3 and 34.5 g kg^{-1}) for 9 weeks. Results showed that the highest weight gain (WG) and the lowest feed conversion ratio (FCR) was obtained at 23.5 and 23.9 g kg^{-1} dietary leucine using second-order polynomial model, respectively. Adequate dietary leucine content (21.9 and 28.3 g kg^{-1}) could significantly up-regulate the expression levels of neuropeptide Y (NPY) and ghrelin (GRL) in the brain of black carp juveniles. The protein efficiency ratio (PER), feed efficiency ratio (FER) and protein deposition ratio (PDR) were also significantly increased by adequate dietary leucine content (21.9 and 28.3 g kg^{-1}) ($p < 0.05$). Adequate dietary leucine content (21.9 and 28.3 g kg^{-1}) could significantly up-regulate the activities of metabolic enzymes, such as α amylase, trypsin, chymotrypsin and elastase in the liver of Black carp ($p < 0.05$). However, the activities of alanine transaminase (ALT), aspartate aminotransferase (AST) and leucine aminopeptidase (LAP) were significantly reduced in the fish serum by adequate dietary leucine content (21.9 and 28.3 g kg^{-1}) compared with leucine-deficient diet (7.3 and 12.4 g kg^{-1}). In addition, 21.9 and 28.3 g kg^{-1} dietary leucine could significantly increase complement component 3 (C3) and C4 contents, lysozyme (LYZ) activities in the serum compared with the leucine-deficient diet (7.3 and 12.4 g kg^{-1}) ($p < 0.05$). Furthermore, optimal dietary leucine could also significantly up-regulate the mRNA expression levels of LYZ, interferon α (IFN-α), hepcidin (HEPC), natural resistance-associated macrophage protein (NRAMP), C3 and C9 in the blood of juvenile black carp compared with the leucine-deficient diets (7.3 and 12.4 g kg^{-1}) ($p < 0.05$). In conclusion, these results suggest that adequate dietary leucine (21.9 and 28.3 g kg^{-1}) could increase growth performances, improve metabolic abilities and then enhance non-specific immunities in black carp juveniles.

1. Introduction

It is well-known that protein quantity and dietary amino acid (AA) content are major factors that influence animal's growth, reproduction, health and feed costs [1,2]. AA are not only building blocks for tissue proteins, but also essential substrates for the synthesis of molecular-weight substances with important physiological roles [3,4]. According to the growth or nitrogen balance, AA are classified as essential AA (EAA) or nonessential AA (NEAA) for animal [3–5]. Although dietary requirements of AA depend on animal species, developmental stage, physiological status, intestine microbiota and environmental factors [4], it is still necessary to determine the dietary EAA requirements of different fish species

Abbreviations: AA, amino acid; EAA, essential amino acid; NEAA, nonessential amino acid; BCAA, branched-chain amino acid; HMB, β-Hydroxy-β-methyl-butyrate; MS 222, tricane methanesulphonate; IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, special growth ratio; FCR, feed conversion ratio; PER, protein efficiency ratio; FER, feed efficiency ratio; PDR, protein deposition ratio; ALT, alanine transaminase; AST, aspartate aminotransferase; LAP, leucine aminopeptidase; NPY, Neuropeptide Y; GRL, Ghrelin; LYZ, lysozyme; IFN-α, Interferon α; HEPC, hepcidin; NRAMP, natural resistance associated macrophage protein; C3, complement component 3; C9, complement component 9.

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due to the important effects of EAA on animal growth, feed costs and nitrogen pollution [5,6].

Among these EAA, leucine is an essential functional AA for normal growth and better health in the nutrition of human and animals [7,8]. As one of the branched-chain amino acids (BCAA) in high-quality protein resources, leucine could act important roles in stimulating protein synthesis and inhibiting protein degradation [3,8–10]. Optimal level of dietary leucine could increase the secretion of growth hormone and regulating relative gene expression for the animal growth [5,7,8,11,12], although such studies are limited in fish. However, leucine deficiency or overload might blunt animal growth performance and reduce diet conversion [13–15]. Generally, fish growth rate is dependent on digestive and absorptive ability of nutrients including leucine, which correlates with the activities of digestive enzymes [16–18]. Although many studies have indicated that dietary AA have an important role in digestive and absorptive enzymes [17–19], few studies have been conducted to investigate the effects of dietary leucine on fish liver enzyme activities. In addition, leucine and its metabolite, β-Hydroxy-β-methyl-butyrate (HMB), also play important roles in animal immune function. During the last decade, lots of animal studies have found that dietary supplementation with leucine and/or HMB could increase the number of liver-associated lymphocytes and NK cells, and then reduce mortality in animal [20,21]. Appropriate leucine supplementation could increase the immune parameters including the expression levels of antioxidant enzymes, β-microglobulin, immunoglobulin-M and toll-like receptor-22 in the head kidney of *Labeo rohita* fingerling [22]. In addition, optimal dietary leucine also regulates the intestinal immune status, immune-related signaling molecules and tight junction transcript abundance in the intestine of grass carp (*Ctenopharyngodon idella*) [23]. On the contrary, inadequate intake of leucine might induce immune impairment [24,25], such as enhancing the susceptibility of pathogens, reducing the contents of antibody, transferrin and complement component 3 (C3) in animal serum [26]. However, little information could be available about dietary leucine on the immune indexes in the blood of fish [23,25–28].

Black carp (*Mylopharyngodon piceus*), an important carnivorous species [29,30], is one of the main freshwater-cultured species in China, although it has been widely cultured in Southeast Asia, Europe and America and used to control aquatic snails [31–34]. However, Black carp culture has suffered a series of problems from artificial feed since there was only a few information about nutrients' requirements [29,30,35,36]. In addition, there is no research on the leucine requirement of black carp, even its effects on the metabolic abilities and innate immune functions in black carp up to date. Therefore, the first aim of the present study was to get information of the requirements of leucine since it is very important and urgent for black carp culture. The second aim was to explore the metabolic and non-specific immune regulatory mechanisms of dietary leucine by evaluating the expression levels of relative genes, activities of metabolic and immune enzymes in black carp.

2. Materials and methods

2.1. Diet preparation

Six isonitrogenous (average crude protein: 399.6 g kg^{-1}) and isolipidic (average crude lipid: 54.9 g kg^{-1}) purified diets were formulated to contain graded levels of dietary leucine (Table 1). Casein and gelatin were used as protein sources, fish oil and lecithin oil were used as the lipid source, and dextrin was used as the sources of dietary leucine. L-crystalline amino acid mixture was used to suite for the levels of all amino acids, except for leucine, of the whole body amino acid pattern of Black carp. The AA contents of the experimental diets are shown in Table 2. Dietary leucine was quantitatively increased at the expense of glutamic acid. The

Table 1
Ingredient and proximate composition of the experimental diets (on dry weight basis).

Ingredient (g Kg^{-1})	Composition of the Experimental diets					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Casein [a]	50.0	50.0	50.0	50.0	50.0	50.0
Gelatin [b]	50.0	50.0	50.0	50.0	50.0	50.0
Dextrin [b]	290.0	290.0	290.0	290.0	290.0	290.0
Fish oil [c]	48.0	48.0	48.0	48.0	48.0	48.0
EAA mixture [d]	279.1	279.1	279.1	279.1	279.1	279.1
Microcrystalline cellulose [b]	179.9	179.9	179.9	179.9	179.9	179.9
Carboxymethyl cellulose [b]	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mixture [e]	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin mixture [f]	5.0	5.0	5.0	5.0	5.0	5.0
$Ca(H_2PO_4)_2$ [b]	10.0	10.0	10.0	10.0	10.0	10.0
Attractant [g]	5.0	5.0	5.0	5.0	5.0	5.0
lecithin oil [c]	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride [b]	3.0	3.0	3.0	3.0	3.0	3.0
L-leucine [b]	0.0	6.0	12.0	18.0	24.0	30.0
L-glutamic acid [b]	30.0	24.0	18.0	12.0	6.0	0.0
Proximate composition (g Kg^{-1} of dry matter)						
Crude protein	396.5	395.2	401.7	397.5	402.8	403.6
Crude lipid	55.3	53.7	56.2	55.9	54.6	53.5
Ash	31.4	32.3	31.7	31.1	31.8	32.4
L-leucine	7.3	12.4	16.2	21.9	28.3	34.5
Gross energy (MJ kg^{-1})	17.19	17.07	17.26	17.28	17.25	17.14

[a] Casein, obtained from Sigma Chemical, St. Louis, MO, USA, crude protein 88.52%.
[b] Gelatin, obtained from Sinopharm Chemical Reagent Co., LTD, Shanghai, China, crude protein 87.17%.
[c] Zhejaing Yixing Feed Group Co. Ltd., China.
[d] Amino acid premix (g Kg^{-1} diet): L-Arginine, 16.4; L-histidine, 5.5; L-isoleucine, 10.5; L-lysine, 19.1; L-methionine, 5.0; L-phenylalanine, 11.0; L-threonine, 9.2; L-valine, 14.3; tryptophan, 2.2; L-alanine; 25.1; L-aspartic acid, 44.9; L-cystine 4.7; L-glumatic acid, 71.0; L-glycine, 22.5; L-serine, 9.0; L-tyrosine, 8.7. Amino acids obtained from Sinopharm Chemical Reagent Co., LTD (Shanghai, China).
[e] Mineral mixture (mg or g Kg^{-1} diet): NaCl, 100 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 65 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; KI, 0.8 mg; Na_2SeO_3, 1.22 mg; zoelite, 18.45 g.
[f] Vitamin premix (mg or g Kg^{-1} diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0.2 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 80 mg; folic acid, 20 mg; biotin, 10 mg; retinal acetate, 5 mg; cholecalciferol, 3 mg; α-tocopherol, 300 mg; ascorbic acid, 1 g; microcrystalline cellulose, 2.41 g.
[g] Attractant composition: taurine: betain-HCl: glycine = 1:3:3.

Table 2
Amino acid (AA) compositions of the main ingredients in the experimental diets and in the mixtures (% dry matter).

Amino acids (%)	Dietary leucine content (g Kg^{-1})					
	7.3	12.4	16.2	21.9	28.3	34.5
Essential amino acids (EAA)						
L-arginine	21.76	21.65	20.65	22.25	20.32	21.66
L-histidine	7.08	6.96	7.18	7.26	6.85	7.15
L-isoleucine	13.47	13.51	13.81	13.75	13.32	13.54
L-leucine	7.31	12.37	16.24	21.91	28.32	34.50
L-lysine	24.08	23.72	24.28	24.17	24.24	24.58
L-methionine	6.72	6.76	6.62	6.70	6.77	6.72
L-phenylalanine	13.94	13.67	14.24	14.19	13.76	13.63
L-threonine	12.05	11.98	12.15	12.25	12.35	12.31
L-valine	18.46	18.55	18.32	18.40	18.57	18.11
Non-essential amino acids (NEAA)						
L-alanine	29.96	29.86	30.39	30.88	29.88	29.48
L-aspartic acid	52.39	52.57	53.01	51.64	51.47	51.94
L-cystine	4.84	4.63	4.96	4.80	4.91	4.89
L-glutamic acid	108.04	101.71	95.68	90.23	86.36	81.00
L-glycine	39.69	39.92	40.50	40.23	40.76	39.62
L-serine	13.09	12.84	13.34	13.52	13.50	13.38
L-tyrosine	11.33	11.69	11.59	11.90	11.28	11.24
L-proline	9.41	9.69	9.41	9.70	9.47	9.50