Fish and Shellfish Immunology 78 (2018) 69–78

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Effects of dietary arginine on antioxidant status and immunity involved in AMPK-NO signaling pathway in juvenile blunt snout bream

Hualiang Liang[a], Ke Ji[a], Xianping Ge[a,b,*], Mingchun Ren[a,b,**], Bo Liu[a,b], Bingwen Xi[a,b], Liangkun Pan[b]

[a] Wuxi Fisheries College, , Wuxi 214081, China
[b] Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China

ARTICLE INFO

Keywords:
Blunt snout bream *(Megalobrama amblycephala)*
Arginine
Nitric oxide (NO)
Adenosine monophosphate activated protein kinase (AMPK)

ABSTRACT

The present study assessed the effects of dietary arginine on antioxidant status and immunity involved in AMPK-NO signaling pathway in juvenile blunt snout bream. Fish were fed six practical diets with graded arginine levels ranging from 0.87% to 2.70% for 8 weeks. The results showed that compared with the control group (0.87% dietary arginine level), significantly higher mRNA levels of adenosine monophosphate activated protein kinase (AMPK) and nitric oxide synthetase (NOS), activities of total nitric oxide synthetase (T-NOS) and nitric oxide synthetase (iNOS), and plasma nitric oxide (NO) contents were observed in fish fed with 1.62%–2.70% dietary arginine levels. Significantly higher levels of NOS and iNOS were observed in fish fed with 1.62%–2.70% dietary arginine levels in enzyme-linked immune sorbent assay. At dietary arginine levels of 1.22%–2.70%, the mRNA levels of iNOS were significantly improved. Dietary arginine also significantly influenced plasma interleukin 8 (IL-8) and tumour necrosis factor-α (TNF-α) contents. Furthermore, dietary arginine significantly affected the activity and mRNA level of glutathione peroxidase (GPx), the mRNA levels of pro-inflammatory factor including IL-8 and TNF-α and plasma malondialdehyde (MDA) content. However, total superoxide dismutase (T-SOD) activity, plasma complement component 3 (C3) content, plasma immunoglobulin M (IgM) content, plasma interleukin 1β (IL-1β) content and the mRNA levels of copperzinc superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (Mn-SOD) and IL-1β were not significantly affected by dietary arginine. After *Aeromonas hydrophila* challenge, the death rate was significantly lowered in fish fed with 1.62%–1.96% dietary arginine levels. Furthermore, the mRNA levels of AMPK, NOS and iNOS, plasma NO content and the activities of T-NOS and iNOS showed an upward trend with increasing dietary arginine levels. Significantly higher levels of NOS and iNOS were observed in fish fed with 1.62%–2.70% dietary arginine levels in enzyme-linked immune sorbent assay. At dietary arginine levels of 1.96%–2.31%, T-SOD activities were significantly improved. Significantly higher GPx activities were observed in fish fed with 1.22%–2.70% dietary arginine levels. At dietary arginine levels of 1.22%–2.31%, the plasma TNF-α and IL-8 contents were significantly decreased. Significantly lower plasma IL-1β contents were observed in fish fed 1.62%–1.96% dietary arginine levels. Dietary arginine significantly influenced the mRNA levels of antioxidant and pro-inflammatory genes including Cu/Zn-SOD, Mn-SOD, GPx, IL-8, TNF-α and IL-1β. Significantly higher plasma C3 contents and significantly lower plasma MDA contents were observed in fish fed with 1.62%–1.96% arginine levels. Furthermore, plasma IgM contents were significantly improved at dietary arginine levels of 1.62%–2.31%. However, high dietary arginine group (2.70%) significantly improved the mRNA levels of pro-inflammatory genes including IL-8, TNF-α and IL-1β and plasma MDA, IL-8, TNF-α and IL-1β contents as compared with optimal dietary arginine levels (1.62% and 1.96%). The present results indicate that optimal arginine level (1.62% and 1.96%) could improve antioxidant capacity, immune response and weaken tissues inflammatory involved in arginine-AMPK-NO signaling pathway, while high arginine level resulted in excessive NO production, leading to increase oxidative stress damage and inflammatory response in juvenile blunt snout bream.

* Corresponding author. Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, China.
** Corresponding author. Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, China.
E-mail addresses: gexp@ffrc.cn (X. Ge), renmc@ffrc.cn (M. Ren).

https://doi.org/10.1016/j.fsi.2018.04.028
Received 22 January 2018; Received in revised form 16 April 2018; Accepted 17 April 2018
Available online 18 April 2018

1. Introduction

Head kidney is an important immune organ in teleost fish, and its role is equivalent to mammalian bone marrow [1]. Head kidney contains a large number of T and B lymphocytes, macrophages and granulocytes that are the basis upon which specific and non-specific immunity is acquired [2]. Therefore, maintaining head kidney immune homeostasis is of vital importance for fish. Nutrients have been demonstrated to play an important role in the improvement of fish immunity [3]. In recent years, amino acid supplementation has been shown to modulate fish immune response, such as valine, threonine and tryptophan [4–6]. Arginine is an essential amino acid in all fish species studied so far and the most versatile amino acid for fish [7]. The fish growth rate is often related to the diseases resistance [8]. Available evidences showed that arginine supplementation could improve immune response and survival rates in marine shrimp (*Penaeus monodon*) and channel catfish (*Ictalurus punctatus*) [9,10]. Moreover, a study has demonstrated that an arginine-enriched diet has a positive effect on the resistance of channel catfish to infection with *Edwardsiella ictaluri* [11].

Previous studies pointed out that nitric oxide (NO) is positive relevant to animal and human immunity [12,13]. L-Arginine (L-Arg) is the sole substrate of nitric oxide (NO) synthase required for the production of NO [12] and hypoargininemia can lead to impaired systemic NO production [14]. In mammals, L-Arginine could stimulate NO production in porcine trophectoderm cells and mice [12,15]. In aquatic animals, dietary arginine could increase NO production such as channel catfish [16], gibel carp (*Carassis auratus gibelio* var. CAS III) [17], Senegalese sole (*Solea senegalensis* Kaup, 1858) [18] and grass carp (*Ctenopharyngodon idella*) [19]. Studies showed that addition of arginine to the diet due to the enhanced release of nitric oxide (NO) from macrophages [20,21], which can decrease release of liver pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) [22] and interleukin 1β (IL-1β) [23]. Furthermore, arginine can also regulate body antioxidative ability such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [22], which is regulated by NO [24,25]. Furthermore, in mammal, researches pointed out the existence of a positive feedback interaction between adenosine monophosphate activated protein kinase (AMPK) and nitric oxide synthetase (NOS) [26–28]. However, few studies have focused on the effect of arginine on antioxidative ability and inflammatory involved in AMPK-NO signaling pathway in fish.

Blunt snout bream (Cyprinidae, *Megalobrama amblycephala*) is a herbivorous freshwater fish species native to China and is an important freshwater fish species in China [29]. It is also found in North America (northern Canada to southern Mexico), Africa and Eurasia [30]. The dietary arginine requirement of juvenile blunt snout bream has been determined, and found arginine deficiency or excess resulted in growth depression in blunt snout bream [31]. However, there is no information concerning the effect of dietary arginine on antioxidant status and immunity of head kidney in blunt snout bream. Therefore, the aim of this study was to investigate the effects of dietary arginine on antioxidant status and immunity for juvenile blunt snout bream involved in AMPK-NO signaling pathway.

2. Materials and methods

2.1. Diet preparation and experimental procedure

Six practical diets (33.0% crude protein, 7.0% crude lipid) were formulated to contain graded arginine levels using fish meal, rapeseed meal and corn gluten as protein sources, soybean oil and lecithin as a lipid source (Table 1). Dietary arginine levels were 0.87% (control), 1.22%, 1.62%, 1.96%, 2.31% and 2.70%. An amino acid profile of the experimental diets was formulated to simulate the whole body amino acid pattern of blunt snout bream expected for arginine (L-arginine 99%; Shanghai Feeer Technology Development Co. Ltd. China). Dietary arginine was replaced with equal proportions of glycine (Table 1).

Table 1
Composition of the basal diet (% dry matter).

Ingredients			
Fish meal[a]	5	Vitamin and mineral premix[c]	1.5
Rapeseed meal[a]	5	Monocalcium phosphate	3
Corn starch	12.1	Vitamin C	0.05
Corn gluten[a]	22	Microcrystalline cellulose	10
Soybean oil	3	Ethoxy quinoline	0.01
Soybean lecithin	2	Bentonite	3
Amino acid mix[b]	9.24	L-arginine	*
Choline chloride	0.1	Glycine	2-*
Wheat meal[a]	22		

The feed formulation references Liang et al. [31].

[a] Rapeseed meal obtained from Wuxi Tongwei feedstuffs Co., Ltd, Wuxi, China, crude protein 37.5%, crude lipid 1.4%; Corn gluten, obtained from Wuxi Tongwei feedstuffs Co., Ltd, Wuxi, China, crude protein 55.9%, crude lipid 3.3%; fish meal, obtained from Wuxi Tongwei feedstuffs Co., Ltd, Wuxi, China, crude protein 61.4%, crude lipid 9.3%; wheat meal obtained from Wuxi Tongwei feedstuffs Co., Ltd, Wuxi, China, crude protein 11.8%, crude lipid 1.2%.

[b] Amino acid premix (g/100 g diet): L-histidine, 0.22; L-isoleucine, 0.50; L-lysine, 1.57; L -phenylalanine, 0.2; L-threonine, 0.53; L-valine, 0.44; L-aspartic acid, 1.18; serine, 0.31; glycine, 1.55; alanine; 0.39; L -tyrosine, 0.07; tryptophan, 0.12; glumatic acid, 0.14; proline 0.10. Amino acids obtained fromFeeer Co., LTD (Shanghai, China).

[c] Vitamin and mineral mix (IU or mg/kg of diet): Vitamin A, 900 000 IU; Vitamin D, 250 000 IU; Vitamin E, 4500 mg; Vitamin K 3, 220 mg; VitaminB 1, 320 mg; Vitamin B 2, 1090 mg; Vitamin B 5, 2000 mg; Vitamin B 6, 5000 mg; Vitamin B 12, 116 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60 000 mg; Biotin, 50 mg; Niacin acid, 2500 mg; provided by Tongwei Feed Group Co. (Jiangsu, China). Supplied as L-form (99%, Shanghai Feeer Technology Development Co. Ltd., Shanghai, China). Mineral mix (g/kg of diet): calciumbiphosphate, 20 g; sodiumchloride, 2.6; potassium chloride, 5 g; magnesium sulphate, 2 g; ferrous sulphate, 0.9 g; zinc sulphate, 0.06 g; cupric sulphate, 0.02; manganese sulphate, 0.03 g; sodium selenate, 0.02 g; cobalt chloride, 0.05 g; potassium iodide, 0.004; and zeolite was used as a carrier.

Juvenile blunt snout bream were obtained from the breeding farm of the Freshwater Fisheries Research Centre (FFRC) of the Chinese Academy of Fishery Sciences. Prior to the feeding trial, all fish were selected based on health and similarities in size and then cultured in floating net cages (1 m × 1 m × 1 m). The fish were fed a 33% protein and 7% lipid commercial diet (Wuxi Tongwei feedstuffs Co. Ltd., Wuxi China) for two weeks for adapting to the experimental condition. Fish (20.0 ± 0.03 g, 11.12 ± 0.05 cm) were fasted for 24 h, weighed, and then randomly distributed to 18 net cages with 20 fish in each cage for farm pond culture. Each experimental diet was randomly assigned to triplicate cages for 8 weeks. Fish were hand-fed to apparent satiation three times daily at 8:00, 12:00 and 16:00. During the experimental period, the water temperature ranged from 26 to 28 °C, pH values ranged from 7.3 to 7.8, dissolved oxygen concentrations ranged from 6.0 to 7.5 mg/L, ammonia nitrogen levels ranged from 0.005 to 0.009 mg/L and hydrogen sulfide levels ranged from 0.005 to 0.008 mg/L. The handling of the experimental animal (blunt snout bream) was based on the Ministry of Agriculture, China and international animal welfare laws, guidelines and policies [32].

2.2. Aeromonas hydrophila challenge test

At the end of the feeding trial, 17 fish (88.51 ± 3.13 g; 15.87 ± 0.16 cm) obtained from each cage were moved to a tank and allowed to acclimate for 7 days. *Aeromonas hydrophila* (*A. hydrophila*) was cultured according to the method described by our previous experimental [33]. Briefly, *A. hydrophila* was inoculated aseptically in nutrient broth and incubated for 24 h at 160 rpm on a shaker. Then the