Fish & Shellfish Immunology 67 (2017) 359–367

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

α-lipoic acid ameliorates n-3 highly-unsaturated fatty acids induced lipid peroxidation via regulating antioxidant defenses in grass carp (*Ctenopharyngodon idellus*)

Xiao-chen Shi [a], Ai Jin [a], Jian Sun [a], Zhou Yang [a], Jing-jing Tian [a], Hong Ji [a, *], Hai-bo Yu [a], Yang Li [a], Ji-shu Zhou [a], Zhen-yu Du [b], Li-qiao Chen [b]

[a] College of Animal Science and Technology, Northwest A&F University, Yang ling 712100, China
[b] Laboratory of Aquaculture Nutrition and Environmental Health, School of Life Sciences, East China Normal University, Shanghai, China

ARTICLE INFO

Article history:
Received 30 April 2017
Received in revised form
8 June 2017
Accepted 12 June 2017
Available online 13 June 2017

Keywords:
α-lipoic acid
n-3 highly-unsaturated fatty acids
Lipid peroxidation
NF-E2-related nuclear factor 2
Kelch-like-ECH-associated protein 1

ABSTRACT

This study evaluated the protective effect of α-lipoic acid (LA) on n-3 highly unsaturated fatty acids (HUFAs)-induced lipid peroxidation in grass carp. The result indicated that diets with n-3 HUFAs increased the production of malondialdehyde (MDA) ($P < 0.05$), thereby inducing lipid peroxidation in liver and muscle of grass carp. Meanwhile, compared with control group, the hepatosomatic index (HSI) and kidney index (KI) of grass carp were markedly increased in n-3 HUFAs-only group. However, diets with LA remarkably inhibited the n-3 HUFAs-induced increase of HSI, KI, and MDA level in serum, liver and muscle ($P < 0.05$). Interestingly, LA also significantly elevated the ratio of total n-3 HUFAs in fatty acid composition of muscle and liver ($P < 0.05$). Furthermore, LA significantly promoted the activity of antioxidant enzymes in serum, muscle and liver of grass carp ($P < 0.05$), including superoxide dismutase (SOD), catalase (CAT), and glutathione s-transferase (GST). The further results showed that LA significantly elevated mRNA expression of antioxidant enzymes with promoting the mRNA expression of NF-E2-related nuclear factor 2 (Nrf2) and decreasing Kelch-like-ECH-associated protein 1 (Keap1) mRNA level. From the above, these results suggested that LA could attenuate n-3 HUFAs-induced lipid peroxidation, remit the toxicity of the lipid peroxidant, and protect n-3 HUFAs against lipid peroxidation to promote its deposition in fish, likely strengthening the activity of antioxidant enzymes through regulating mRNA expressions of antioxidant enzyme genes via mediating Nrf2-Keap1 signaling pathways.

1. Introduction

Dietary n-3 long-chain highly unsaturated fatty acids (HUFAs), including eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), play a prominent physiological roles on promoting growth performance, reducing lipid accumulation and constituting the structure of cell membrane of aquatic animals [1–4], and therefore the fish oil rich in n-3 HUFAs has been used the superior lipid source in aquatic diets [3]. However, tissue lipid n-3 HUFAs content is a critical factors in lipid peroxidation due to their high number of double bonds, and as animals [5], particularly fish, tissues contain large quantities of n-3 HUFAs, they may be more at risk from oxygen free radicals attack than mammals [6,7]. The previous study reported that the excess n-3 HUFAs supplementation in fortification may exert the decreased feed intake, poor growth performance, hepatic pathology and higher blood lipid peroxidate in Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and grass carp, which might be due to lipid peroxidation [2,8,9]. In fish, n-3 HUFAs play an important physiological role in normal growth and the development of membrane structure and function, so lipid peroxidation *in vivo* caused by oxygen radicals is a principal cause of some disorder, including a decrease bio-membrane fluidity, a rise on the penetration of bio-membrane, and the inactivation of enzymes on membrane [1,10].

To maintain health and prevent lipid peroxidation-induced damnify, there must be effective antioxidant systems and antioxidant operating in fish. The 1, 2-dithiolane-3-pentanoic acid, known

* Corresponding author.
E-mail address: jihong@nwsuaf.edu.cn (H. Ji).

http://dx.doi.org/10.1016/j.fsi.2017.06.036
1050-4648/

as α-lipoic acid (LA), has a redox active disulfide group. The reduced form of LA, known as dihydrolipoic acid (DHLA), interacts with reactive oxygen and reactive nitrogen species (RONS), and both forms act as antioxidants [11,12]. As a "universal antioxidant", LA could directly clear up the reactive oxygen species (ROS). In addition, the lipoic acid can increase the ratio of glutathione (GSH) and GSSH via restoring the GSSH [13], reprocess endogenous non-enzymatic antioxidant, such as ascorbic acid and α-tocophery [14], as well as increase the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione s-transferase (GST) [15]. In the aquatic organisms, the lipoic acid is used to improve the antioxidant and detoxification status [13]. The study with lipoic acid in pacu (*Piaractus mesopotamicus*) suggested that it remits the ascorbic acid deficiency [16]. In addition, the LA-supplemented diets enhances the GCL activity in liver and muscle, and decreases the reactive oxygen species (ROS) concentration in brain [17]. Zhang, Chen [18] found that wrinkles abalone (*Haliotis discus hannai*) had a favorable growth and antioxidant capacity with LA-supplemented diets ranging from 200 to 800 mg/kg. Furthermore, it was reported that diets with LA relieved lipid peroxidant in muscle and promoted the antioxidant status in brain of Plata pompano (*Trachinotus marginatus*) [19].

As mentioned above, it is suggested that LA-treatment promotes the antioxidant capacity in several aquatic organisms in terms of activities of antioxidant enzyme (e.g. CAT, SOD and GST) and non-enzymatic antioxidants (e.g. GSH) [13]. However, to date, no study has addressed the antioxidant effect of LA on molecular mechanism in fish. The antioxidant enzyme and compounds play an indispensable role on clearing reactive oxygen species (ROS) in aquatic organisms [20–24]. ROS, as a cellular signal molecule, plays a pivotal role in regulating many metabolic pathway including inflammation and obesity [25], whereas excess or inadequate removal of ROS might cause the oxidant stress and lipid peroxidant [26]. The NF-E2-related factor 2 (Nrf2) is a critical role in regulating the cellular antioxidant defenses when cells are under the oxidant stress [27], and it is a pivotal transcription factor that identified the antioxidant response elements (ARE) inducing the transcription of antioxidant enzyme genes, including SOD, CAT, and GST [13]. The nuclear migration of Nrf2 is depressed by the Kelch-like-ECH-associated protein 1 (Keap1) as an Nrf2-binding protein [27,28].

So our present study was designed to explore whether LA could attenuate n-3 HUFAs-induced lipid peroxidation via modulating Nrf2-Keap1 pathway, and elevating the antioxidant defenses to protect n-3 HUFAs against oxygen free radicals in fish. The present study may provide some evidence for the effect of lipoic acid on ameliorating lipid peroxidation in fish.

2. Materials and methods

2.1. Feed ingredients and experimental diets

The formulation and proximate composition of the experimental diets are shown in Table 1. Four isonitrogenous (33.0% crude protein) and isolipidic (5.0% crude lipid) experimental diets (control, HUFA, HUFA + LA1,HUFA + LA2) were formulated with the same macronutrient content and considering two levels of α-lipoic acid (600 mg kg^{-1}, 1200 mg kg^{-1}) with the appropriate supplementation of n-3 HUFAs according to Ji, Li [2]. α-Lipoic acid (>99% purity) were obtained from Suzhou Fushilai Pharmaceutical Co., Ltd. (Changshu, Jiangsu Province, China). All ingredients were purchased from Hua-qin Husbandry and Technology Co., Ltd. (Yangling, Shaanxi Province, China). Fish meal, soybean meal, cottonseed meal and other ingredients were used as diets protein sources. Soybean oil (Kerry Oils & Grains Co., Ltd, Shenzhen, Guangzhou Province, China), lard oil (Kangle market, Yangling, Shaanxi Province, China) and linseed oil (Hoval Seasons Bio-Sci Co., Ltd, Changchun, Jilin Province, China) were added to satisfy the essential fatty acid requirements [1.0% linoleic acid (LNA) and 1.0% alpha-linoleic acid (ALA)] of grass carp [29]. The refined fish oil purchased from Marine biological products Co., Ltd (Xuancheng, Anhui Province, China) was replaced lard oil in diets to provide the appropriate content of n-3 HUFAs. Gas chromatography analysis revealed that the actual dietary n-3 HUFAs levels were 0.05%, 0.49%, 0.50% and 0.49% of dry matter for the HUFA-free (control) group and the HUFA groups (HUFA, HUFA + LA1, HUFA + LA2), respectively (Table 2). The dough was pelleted to proper size (2.5 mm pellet diameter) and the pellets were dried in a cool and well-

Table 1
Formulation and chemical composition of the experimental diets (g/kg dry matter).

Ingredient (g/kg)	Group			
	Control	HUFA	HUFA + LA1	HUFA + LA2
Fish meal	60	60	60	60
Soybean meal	291	291	291	291
Cottonseed meal	291	291	291	291
Wheat flour	286	286	286	286
Lord oil	17	0	0	0
linseed oil	15	15	15	15
Fish oil	0	17	17	17
Soybean oil	3	3	3	3
$Ca(H_2PO_4)_2$	15	15	15	15
Mixture[a]	10	10	10	10
α-Lipoic acid[b]	0	0	0.6	1.2
Bentonite	12	12	11.4	10.8
Total	1000	1000	1000	1000
Proximate composition				
Moisture (%)	7.65	7.54	7.70	7.43
Crude protein (%, N%*6.25)	38.79	38.03	38.48	38.67
Lipid (%)	5.56	5.58	5.36	5.74
Ash (%)	6.97	7.06	7.00	6.86

[a] Contained 1% vitamin and 1% mineral; Ingredients including/1 kg: VA 67 IU, VD 16.2 IU, VE 7.4 g, VK3 340 mg, VB1 670 mg, VB2 1000 mg, VB6 800 mg, VB12 1.4 mg, VC 10 g, D-pantothenic acid 2.65 g, folic acid 330 mg, nicotinamide 5.35 g, choline chloride 35 g, biotin 34 mg, inositol 8 g, Fe 14 g, Cu 350 mg, Zn 4 g, Mn 1.4 mg, Mg 10 g, Co 30 mg, I 40 mg, Se 35 mg.
[b] Supplied by Suzhou Fushilai Pharmaceutical Co., Ltd. (Changshu, Jiangsu Province, China): >99% purity.

Table 2
Fatty acid composition of the experimental diets (% total fatty acid).

Fatty acid	Groups			
	Control	HUFA	HUFA + LA1	HUFA + LA2
C14:0	1.27	1.06	1.02	1.02
C16:0	20.16	13.16	13.80	13.65
C18:0	10.76	6.35	6.61	6.38
∑SFA	32.19	20.57	20.43	20.65
C16:1n-7	1.21	2.61	2.94	2.74
C18:1n-9	25.21	22.76	22.49	22.78
∑MUFA	26.42	25.37	25.43	25.52
C18:2n-6	24.00	24.10	24.03	23.95
C18:3n-6	0.21	0.94	0.48	0.52
C20:4n-6	1.05	0.66	0.62	0.52
C22:4n-6	ns	ns	ns	ns
∑n-6 PUFA	25.26	25.70	25.63	24.99
C18:3n-3	16.18	19.12	18.78	19.06
C20:5n-3	0.63	5.36	5.65	5.57
C22:6n-3	0.49	3.40	3.10	3.22
∑n-3 HUFA	1.12	8.76	8.75	8.79
∑n-3 PUFA	17.30	27.88	27.53	27.85
∑PUFA	41.42	53.58	53.16	53.04
n-3/n-6 PUFA	0.68	1.10	1.07	1.11

SFA saturated fatty acid, MUFA monounsaturated fatty acid, HUFA highlyunsaturated fatty acid, PUFA polyunsaturated fatty acid.