Aquaculture Reports 10 (2018) 8–16

Contents lists available at ScienceDirect

Aquaculture Reports

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

Metabolic and immune effects of orange-spotted grouper, *Epinephelus coioides* induced by dietary arginine

Fenglu Han, Shuyan Chi*, Beiping Tan, Xiaohui Dong, Qihui Yang, Hongyu Liu, Shuang Zhang, Di Liu, Yuanfa He

Laboratory of Aquaculture Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang, China

ARTICLE INFO

Keywords:
Epinephelus coioides
Arginine
Growth performance
Metabolism
Immunity

ABSTRACT

Arginine (Arg) as an important substrate for protein metabolism and endocrine influences on fish health directly. To evaluate the effect of dietary Arg on these endocrine and immunity of juvenile orange-spotted grouper (*Epinephelus coioides*), an experiment adding Arg with 0.00, 0.30, 0.60, 0.90, 1.20, 1.50, and 1.80% in seven diets was conducted. Dietary Arg levels reached 2.13 (Diet 1), 2.42 (Diet 2), 2.71 (Diet 3), 2.95 (Diet 4), 3.20 (Diet 5), 3.48 (Diet 6) and 3.74% (Diet 7), respectively. Each treatment was randomly assigned to triplicate groups of 30 fish (7.52 ± 0.02 g) each tank. The present results indicated that different Arg levels of diets were no significant effects on the fish survival rate of each groups ($P > 0.05$) after 8 weeks feeding experiment. The highest weight gain and specific growth rate were found in Diet 4, and significantly higher than the other groups ($P < 0.05$). Hepatic arginase activities were significantly higher in fish fed Diet 6 and Diet 7 compared to the activities of the fish fed first three Diets ($P < 0.05$). Hepatic ornithine decarboxylase activities of fish fed Diet 1 and Diet 2 were significantly decreased compared to the fish fed Diet 4–Diet 7 ($P < 0.05$). With ascending dietary Arg, the serum Insulin (INS) and insulin-like growth factor I (IGF-I) levels significantly increased. Gene expression of tissue toll-like receptors 22 in the three tissues were found first up-regulated and then down-regulated with the Arg increasing. Gene expression of major histocompatibility complex II in liver and kidney significantly increased in fish fed Diet 4 and Diet 6, and decreased thereafter. However, gene expression of hepcidin decreased with dietary Arg increased. The present study showed alterations in serum INS and IGF-I levels in response to Arg-induced growth activation. Additionally, the results indicate that Arg has beneficial effects on regulating mRNA expression of immune-associated genes and improving humoral and cellular immunity of fish.

1. Introduction

As one of the versatile essential amino acids (AA) for fish, arginine (Arg) takes part in several growth-related processes and promotes diverse physiological effects including hormone secretion, muscle growth and immune cell activation. For example, poor survival rates of channel catfish *Ictalurus punctatus* would be improved after supplement Arg in the Arg-deficient diet (Buentello and Gatlin, 2000). Arg possessing a direct secretory-promoting effect on fish hormones, though the focus was primarily on insulinotropic potential (Mommsen et al., 2001; Vega-Rubín de Celis et al., 2004), can stimulate AA uptake and protein synthesis and then affect fish growth, such as brown trout *Salmo trutta* (Baños et al., 1999), barfin flounder *Verasper moseri* (Andoh, 2007), largemouth bass *Micropterus salmoides* (Sink and Lochmann, 2007). By injecting Arg, not only higher plasma insulin (INS) level was founded in barfin flounder than that injecting glucose (Andoh, 2007), but also insulin peptide-1, glucagon and glucagon-like peptide-1 in rainbow trout *Oncorhynchus mykiss* plasma were dramatically increased (Mommsen et al., 2001). While the secretion mechanism of INS differs with insulin-like growth factor-I (IGF-I), many functions of the two hormones overlap. Previous studies reported various effects of IGF-I on growth, metabolism, development, reproduction (Cruz et al., 2006; Woll and Podrabsky, 2017; Montrezor and Urbinati, 2017), and immune response (Franz et al., 2016) in fish. Based on what Andoh (2007) and Pohlenz et al. (2013) reported, the growth promoting effects of Arg were partially ascribed to its ability to activate the production of IGF-I, which accelerated efficiency of nutrient utilization.

Arg also participates in regulating crucial enzymes such as arginase, nitric oxide synthase (NOS) and ornithine decarboxylase (ODC) (Zhou et al., 2011a), after that the relative productions impact on some metabolic pathways involving protein synthesis, urea cycle and metabolism of glutamic acid, proline, glucose and fatty acids (Hird, 1986;

* Corresponding author at: Laboratory of Aquaculture Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China.
E-mail address: chishuyan77@163.com (S. Chi).

https://doi.org/10.1016/j.aqrep.2018.03.002
Received 28 November 2017; Received in revised form 2 March 2018; Accepted 6 March 2018

Flynn et al., 2002). Nevertheless, definitive elucidations on the role of dietary Arg in modulating health growth and urea cycle via arginase and ODC in marine fish were insufficient. Dietary Arg degradation via hepatic arginase is a major pathway for ureagenesis in juvenile sea bass liver (Tulli et al., 2007). Arginase catalyzes the final step of ornithine-urea cycle leading to a conversion of L-arginine to urea and L-ornithine, the precursor for prolines and polyamines. Polyamines which produced through ODC are not only necessary for regulating several ion channels and cell proliferation (Pegg, 2014; Morris, 2009), but also for playing an important role in tissue repair, wound healing and neural development (Satriano, 2004; Lange et al., 2004), for instance, polyamines exerted anti-inflammatory properties in both salmon liver cells and head kidney immune cells (Holen et al., 2014).

By affecting gene expression or by regulating nutrient availability for immune cells through endocrine control, Arg may act via NO and polyamines to exert positive effects on the immune system, whatever *in vivo* and *in vitro* experiments of channel catfish (Andersen et al., 2016; Pohlenz et al., 2012; Buentello et al., 2007). Favorable influences of Arg-enriched diet on the resistance of Jian carp *Cyprinus carpio var. Jianto* infected with *Aeromonas hydrophila* (Chen et al., 2015) and sea bass *Dicentrarchus labrax* infected with *Vibrio anguillarum* (Azeredo et al., 2015) have been demonstrated. Comparing to diet meeting Arg requirement level, however, higher diet Arg level was thought a general inhibitory effect of Arg on the immune defenses and disease resistance of European seabass (*Dicentrarchus labrax*) due to interleukins and immune-cell marker transcripts being down-regulated in intestine (Azeredo et al., 2015).

Orange-spotted grouper (*Epinephelus coioides*), the major species being maricultured in China and Southeast Asian countries, are high-priced and popular seafood fish (Zhou et al., 2011b). Previous study has indicated that dietary Arg supplementation can enhance growth of orange-spotted grouper (Luo et al., 2007). It is not very clearly how Arg would regulate the growth and immune in marine fish through endocrine and through genes expression of the crucial pathway. Moreover, the rapidly developing aquaculture industry is facing the challenges, for instance the outbreak of diseases caused by viruses, bacteria and parasites (Chua et al., 1994) and the use of the plant protein ingredients in feed (Hedrera et al., 2013; Gu et al., 2016). Therefore, we undertook a series of studies to preliminarily explore the effects of dietary Arg levels on circulating hormone levels and tissue toll-like receptors 22 (TLR22), major histocompatibility complex (MHC) II and hepcidin gene expression in grouper.

2. Materials and methods

2.1. Experimental diets and procedure

Formulation and proximate composition of the experimental diets are provided in Table 1. The experimental diets were supplemented with L-Arg at 0.0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5% and 1.8%. Diets were maintained as isonitrogenous by adjusting the levels of a 50:50 glycine-aspartate mix. The Arg concentrations of the experimental diets were 2.13% (control, un-supplemented Arg), 2.42%, 2.71%, 2.95%, 3.20%, 3.48% and 3.74%, respectively. The amino acid composition of each diet was presented in Table 2.

The Animal Care and Use Committee of Guangdong Ocean University approved all experimental procedures. Juvenile groupers were obtained from the Hao Li Hatchery (Zhanjiang, China). Six hundred and thirty groupers (mean initial weight of 7.52 ± 0.02 g) were randomly distributed into 21 glass aquaria (1 m^3), with 30 juveniles in each aquarium. Each experimental diet was randomly assigned to aquaria in triplicate. Fish were fed twice per day at 8:00 and 17:00 h. During the experimental period, temperatures ranged from 26 to 30 °C, pH was 7.7–8.0, ammonia nitrogen was lower than 0.05 mg/ L, and dissolved oxygen was not less than 6.0 mg L^{-1}. The feeding trial lasted for 8 weeks.

2.2. Sample collection

At the end of the 8-week feeding experiment, approximately 24 h after the last feeding, all fish were counted and individually weighed. Fish per tank were randomly sampled and anaesthetized with benzocaine (30 mg/ L). Dorsal muscle and liver samples were dissected and immediately frozen in liquid nitrogen and stored in a freezer at −80 °C freezer prior to enzymes analysis. Then the intestine, liver and kidney of 3 fish from each cage were sampled and respectively pooled in a centrifuge tube with RNAlater solution (Ambion, Austin, TX, USA) and frozen at −80 °C until the analysis of gene expression. Six fish from each tank were randomly selected and blood samples were drawn from the caudal vein with 1-mL syringe. Blood samples were immediately centrifuged (2700 × *g* for 10 min) at 4 °C and the plasma was separated and stored at −80 °C for later plasma hormone level analyses.

2.3. Serum INS and IGF-I concentration

Serum INS levels were measured by time-resolved fluoroimmunoassay (TR-FIA), according to Andoh (Andoh, 2007). This assay uses anti-barfin flounder insulin-II antiserum and B1 monobiotinylated barfin flounder insulin-I, which was synthesized chemically (Peptide Institute, Osaka, Japan). Insulin-I was used at known concentrations as a standard. The cross-reactivities of insulin-I and -II are identical in this TR-FIA (Andoh, 2007). No significant cross-reactivity was observed in the fractions collected during reversed-phase chromatography of the serum, except for those containing insulin, suggesting that the antiserum does not recognize preproinsulin, proinsulin, or their intermediates.

Serum IGF-I levels were measured by TR-FIA using anti-barramundi IGF-I anti-serum (GroPep Pty Ltd., Adelaide, Australia) and tri-biotinylated flounder IGF-I as the labeled ligand (Baños et al., 1999). Flounder IGF-I was also purchased from GroPep and used at known concentrations as a standard. All measurements were made within 3 days after sampling.

2.4. The arginase and ODC activity

The enzyme activity of arginase and ODC in dorsal muscle and liver were determined by enzyme-linked immuno sorbent assay (Jiang lai Biotechnology Co., Ltd., Shanghai, China). The specific activity (one unit, U) of arginase and ODC were expressed as μmol urea g^{-1} protein per min and μmol mg^{-1} protein per hour, respectively.

2.5. Real-time quantitative PCR

Total RNA isolation was conducted with TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and 2 μL total RNA was used to synthesize cDNA using the PrimeScript® RT reagent Kit with gDNA Eraser (TaKaRa). Realtime quantitative PCR analysis of TLR22, MHC-II, Hepcidin and housekeeping gene (β-actin) was performed according to standard protocols in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, CA. USA). The gene-specific primers used in this study are listed in Table 3. Target gene mRNA concentration was normalized to the mRNA concentration of the reference gene β-actin, a housekeeping gene of orange-spotted grouper.

2.6. Calculations and statistical analysis

The following variables were calculated:

Weight gain (WG, %) = 100 × (final total weight − initial total weight)/initial total weight

Specific growth rate (SGR, %/ day) = 100 × (Ln final individual