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Effects of dietary arginine supplementation on growth performance, flesh quality, muscle antioxidant capacity and antioxidant-related signalling molecule expression in young grass carp (*Ctenopharyngodon idella*)

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ABSTRACT

Growth performance, flesh quality, antioxidant status and antioxidant-related signalling molecule expression in the muscle of young grass carp, which were fed graded levels of arginine (6.9–24.5 g/kg diet) for eight weeks, were investigated. Muscle protein, lipid and nitric oxide contents, shear force, hydroxyproline concentration, and pH were significantly improved by appropriate arginine. Cooking loss, lactate content, cathepsins activities, malondialdehyde and protein carbonyl contents exhibited an opposite tendency. Additionally, optimum arginine significantly enhanced glutathione content and the activities and gene expression of copper/zinc superoxide dismutase, catalase and glutathione peroxidase in muscle. Moreover, the expression levels of glutamate–cysteine ligase, target of rapamycin, ribosome protein S6 kinase 1, casein kinase 2 and NF-E2-related factor 2 in muscle were significantly elevated by appropriate arginine. However, optimum arginine significantly decreased Kelch-like ECH-associated protein 1 mRNA levels in muscle. In conclusion, arginine improved the flesh quality and muscle antioxidant capacity and regulated antioxidant-related signalling molecule expression.

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1. Introduction

Fish growth primarily depends on the growth of muscle which is the major edible portion for consumers (Periago et al., 2005). Hence, it is important to investigate flesh quality. Flesh quality traits primarily involve the water-holding capacity (WHC), pH and firmness (Brinker & Reiter, 2011). Nutrients play an important role in the improvement of flesh quality (Khan, Qureshi, Nasir, Rasool, & Iqbal, 2011). A previous study in our laboratory indicated that myo-inositol deficiency resulted in Jian carp (*Cyprinus carpio* var. Jian) muscle lesions, which decreased flesh quality (Jiang et al., 2010). However, Buckley, Morrissey, and Gray (1995) reported that optimum Vitamin E supplementation was shown to be effective in increasing pig muscle WHC, thereby improving meat quality. A recent study from our laboratory indicated that optimum zinc (Zn) supplementation improved grass carp (*Ctenopharyngodon idella*) muscle WHC and pH (Wu et al., 2014). Arginine (Arg) has been demonstrated to be an important nutrient for fish (Zhou, Zeng, Wang, Xie, & Zheng, 2012). However, few studies have focused on the effects of dietary Arg on flesh quality in fish. In pig, Arg improved muscle pH (Ma et al., 2010) and WHC (Tan et al., 2009). This data suggested that Arg might also influence flesh quality in fish, which is valuable to investigate.

Metabolic processes, as well as other processes occurring in muscle tissue, cause the formation of reactive oxygen species (ROS) (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004). A high level of ROS can interact with both lipids and protein and induce oxidative stress (Tokur & Korkmaz, 2007). Importantly, fish muscle tissue is more sensitive to oxidative stress due to excessively high levels of polyunsaturated fatty acids (Martinez-Alvarez, Morales, &

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Sanz, 2005; Periago et al., 2005). Oxidative stress is a major cause of decreasing flesh quality (Buckley et al., 1995). Tokur and Korkmaz (2007) reported that decreased flesh quality might be related to the destruction of muscle structural integrity, which resulted from oxidative damage in fish. Nutrients could improve flesh quality by reducing oxidative damage. A previous study in our laboratory demonstrated that dietary Zn improved grass carp flesh quality through attenuating muscle oxidative damage (Wu et al., 2014).However, there is no report concerning the effects of Arg on lipid peroxidation and protein oxidation in fish, which requires further investigation. To prevent oxidative damage, fish have developed antioxidant systems (Martinez-Alvarez et al., 2005). In general, fish antioxidant systems are composed of non-enzymatic compounds (GSH) and antioxidant enzymes (including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) (Martinez-Alvarez et al., 2005). These antioxidant enzymes play an important role in eliminating ROS (Chen, Zhou, Li, & Wu, 2013). To our knowledge, few studies have evaluated the effects of Arg on antioxidant enzyme activity in fish. In rat muscle tissue, it was shown that Arg significantly enhanced the activities of copper/zinc superoxide dismutase (SOD1), CAT and GPx (Petrović et al., 2008). Lambertucci, Levada-Pires, Rossoni, Curi, and Pithon-Curi (2007) reported that the changes in antioxidant enzyme activities were partly dependent on antioxidant enzyme gene transcription in rat muscle tissue. However, few studies have examined the effects of Arg on antioxidant enzyme gene expression in fish. In rat brown adipose tissue, Arg elevated mRNA levels of CAT and GPx (Otašević et al., 2011). The above data indicated that Arg could affect antioxidant enzyme activity and gene expression in fish. This possibility requires further investigation.

Antioxidant enzyme gene expression is partly regulated by a wide variety of transcription factors. Chen, Zou, Li, and Wu (2013) reported that NF-E2-related nuclear factor 2 (Nrf2) is an important transcription factor that can bind to the antioxidant-responsive element (ARE) and induce transcriptional of antioxidant enzyme genes, such as SOD, CAT and GPx, in mouse liver. However, Kelch-like ECH-associated protein 1 (Keap1) was identified as a Nrf2-binding protein, which depresses Nrf2 translocation to the nucleus (Ma, 2013). To our knowledge, few studies have investigated the effects of Arg on Nrf2 and Keap1 gene expression in fish. A recent study from our laboratory cloned the cDNA of Nrf2 (GenBank accession number KF733814 and GenBank accession number JX462955) and Keap1 (GenBank accession number KF811013 and GenBank accession number JX470752) of grass carp and of Jian carp for the first time and showed that some nutrients, such as choline and myo-inositol, affected Nrf2 and Keap1 expression in the intestine of Jian carp (unpublished data). In rats, Arg significantly up-regulated the mRNA level of Nrf2 in left ventricular myocardial tissue (Ramprasath et al., 2012). The effects of Arg on Nrf2 expression may be related to its metabolite. Nitric oxide (NO) is the major metabolite of Arg and acts as an important signalling molecule (Ramprasath et al., 2012). *In vitro*, NO up-regulated Nrf2 gene expression in rat vascular smooth muscle cells (Liu et al., 2007). Notably, these data indicated that Arg might regulate Nrf2 expression in fish through NO. Furthermore, several cellular signalling molecules, such as target of rapamycin (TOR), could coordinately regulate Nrf2 expression. *In vitro*, mTOR was reportedly involved in the up-regulation of Nrf2 expression in human hepatic carcinoma cells (Shay, Michels, Li, Kong, & Hagen, 2012). Recently, our laboratory cloned the cDNA of TOR (GenBank accession number FJ899680 and GenBank accession number JX854449) of Jian carp and of grass carp for the first time and showed that the TOR gene is expressed in fish muscle. Moreover, casein kinase 2 (CK2) is a highly conserved serine/threonine kinase, and further studies have shown that CK2 could regulate mTOR and its downstream target, ribosome protein S6 kinase (S6K), in human glioblastoma cells (Olsen, Svenstrup, & Guerra, 2012). Recently, the cDNA of CK2 (GenBank accession number KF914143) was cloned from grass carp for the first time in our laboratory. A study in mice has shown that polyamines, which are major metabolites of Arg, enhanced CK2 activity *in vitro* and *in vivo* (Shore, Soler, & Gilmour, 1997). These data indicated that a possible correlation exists between Arg and the gene expression of antioxidant-related signalling molecules involved in the Nrf2 signalling pathway of the muscle in fish. This possibility is worth investigating.

The present study was conducted to investigate the beneficial effects of dietary Arg on fish growth and on flesh quality through enhancing the muscle antioxidant capacity. In a further study, we determine the relative expression of antioxidant enzyme genes and antioxidant-related signalling molecules of fish, which could provide partial theoretical evidence for the effects of Arg on fish flesh quality and growth. Grass carp is an important economic freshwater species that is widely cultured (Li, Tang et al., 2013). The dietary Arg requirement was evaluated in juvenile grass carp (Wang S., 2006). However, the requirement of Arg may be varied at different growth stage in fish. In grass carp, lysine requirement at the juvenile stage was higher (54.4 g/kg protein) (Wang, 2006) than that at sub-adult stage (38.9 g/kg protein) (Li, Tang et al., 2013). To date, no study has been conducted to evaluate the dietary Arg requirement in young grass carp. Hence, it is valuable to investigate the dietary Arg requirement of young grass carp.

2. Materials and methods

2.1. Experimental diets and design

The isonitrogenous (300 g/kg protein) and isolipidic (41.7 g/kg lipid) diets were formulated according to Ren et al. (2013). The basal diet was constituted from the following ingredients (g/100 g diet): fish meal (7.80), casein (3.00), gelatine (3.99), crystal amino acid mix (18.86), arginine premix (5.00), fish oil (2.20), soybean oil (1.89), α-starch (28.00), corn starch (13.34), vitamin premix (1.00), trace mineral premix (2.00), cellulose (10.00), $Ca(H_2PO_4)_2$ (2.27), choline chloride (0.60), ethoxyquin (0.05). The dietary protein level was fixed at 30%, which supported the optimal growth of grass carp (Khan, Jafri, & Chadha, 2004). Crystalline amino acids were used to simulate the amino acid profile of whole chicken egg protein, except for arginine, according to Li and Tang (2013). A complete description of the ingredients and the composition of the basal diets were prepared according to Li et al. (2014). Different concentrations of L-arginine were added to a basal diet mixture to constitute the six levels of 6.0 (basal diet), 10.0, 14.0, 18.0, 22.0 and 26.0 g arginine/kg diet. Final arginine concentrations of the six experimental diets were measured using high-performance liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) to be 6.9, 10.4, 14.1, 17.6, 21.4 and 24.5 g arginine/kg diets, respectively. All ingredients were mixed, pelleted, and stored at −20 °C until use, as described by Lee et al. (2011).

2.2. Feeding management

The procedures used in this study were approved by the University of Sichuan Agricultural Animal Care Advisory Committee. Grass carp were obtained from Bai Long Lake (Sichuan, China). Before beginning the experiment, grass carp were fed with the base diet for 2 weeks to acclimate to the experimental diet and conditions according to Zhang et al. (2009). After the acclimatisation period, 540 grass carp with an average initial weight of 278.82 ± 0.68 g were randomly distributed into 18 experimental cages ($1.4 \times 1.4 \times 1.4\ m^3$), each of which was equipped with a 100 cm diameter disc of 1-mm gauze in the bottom to collect the

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