Fish & Shellfish Immunology 51 (2016) 365–372

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Effects of Ala-Gln feeding strategies on growth, metabolism, and crowding stress resistance of juvenile *Cyprinus carpio* var. Jian

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ARTICLE INFO

Article history:
Received 17 January 2016
Received in revised form
21 February 2016
Accepted 29 February 2016
Available online 2 March 2016

Keywords:
Ala-Gln
Feeding strategy
Cyprinus carpio var. Jian
Crowding stress

ABSTRACT

The present study was conducted to evaluate the effects of different L-alanyl-L-glutamine (Ala-Gln) feeding strategies on the growth performance, metabolism and crowding stress resistance related parameters in juvenile Jian carp (*Cyprinus carpio* var. Jian) under crowded condition (80 g/L). Juvenile Jian carp (initial weight 26.1 ± 0.6 g) were distributed into five groups which fed with graded concentrations (0% or 1.0%) of Ala-Gln for eight weeks. Control group (I, 0/0) fed with control diet (0% Ala-Gln) throughout the feeding trial. The other four groups employed different control and experimental diet feeding strategies ranging from two weeks control diet fed and two weeks experimental diet (1% Ala-Gln) fed (II, 0/2) to eight weeks experimental diet fed (V, 4/4). Results revealed that Mean weight gain (MEG) under all different feeding strategies of Ala-Gln were significantly higher than that of the control group ($p < 0.05$), and MEG of group II (201.90%) was even higher than that of group IV (184.70%). Liver glycogen and blood total protein of groups II, III and V were significantly higher than that in groups I and IV ($p < 0.05$). The highest level of serum thyroxine (10.07 ng/ml), insulin-like growth factor-I (52.40 ng/ml) and insulin (9.73 μ IU/mL) were observed in group V. However, diet supplemented with Ala-Gln did not affect the levels of serum glucose, cortisol and catecholamine in fish. The mRNA expression of GR1a, GR1b and GR2 were also significantly changed in Ala-Gln supplementation groups compared with control group ($p < 0.05$). After fish intraperitoneally injected with virulent *Aeromonas hydrophila*, the fish survival rates were significantly increased in all Ala-Gln supplementation groups compared with control group ($p < 0.05$). Results from the present experiment showed the importance of dietary supplementation of Ala-Gln in benefaction of the growth performance, metabolism and crowding stress resistance in Jian carp breeding. The optimal feeding strategy was alternatively fed with control diet and then experimental diet at an interval of two weeks for juvenile Jian carp under crowded condition.

1. Introduction

Intensively farmed fish in commercial aquaculture are usually exposed to a variety of stressors such as crowding, capture, vaccination, confinement and transport [1]. When fish are subjected to adverse stressors, some endocrine and physiological alterations occur, often resulting in changes in the ability of the fish to grow, reproduce and survive [2]. Crowding is generally accepted as one of the most common stressors to be found in fish aquaculture [3]. Several studies have revealed that, under crowding condition, the pituitary-inter-renal axis gives rise to the secretion of catecholamine and cortisol [4,5], increases plasma glucose levels by changing glucolytic and gluconeogenic enzyme activities [2], triggers haematological, metabolic and immunological changes [6,7], eventually leading to growth retardation and death [1]. In order to combat or diminish the adverse effects of crowding in aquaculture, many measures have been adopted. These include aeration [8], water exchange [9], water quality improvement [10], and inclusion in fish diet of some anti-stress compounds such as amino acids [11,12], highly unsaturated fatty acids [2], vitamins [13], nucleotide [4] and medicinal plant extract [14] that counteract the immunosuppressive stress effects.

Glutamine (Gln), a conditionally essential amino acid [15], plays

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http://dx.doi.org/10.1016/j.fsi.2016.02.034
1050-4648/

an important role in gastrointestinal structure protection, redox status maintenance, immunity enhancing and growth promoting for fish [16,17]. Previous research has showed that dietary Gln could improve food intake, activities of digestive enzyme in intestine, and growth performance of Jian carp [18]. Meanwhile, Gln has been found to improve immune responses of several fish species such as hybrid striped bass (Morone chrysops × *Morone saxatilis*), red drum (*Sciaenops ocellatus*), Jian carp (*Cyprinus carpio* var. Jian) and channel catfish (*Ictalurus punctatus*) [19–21]. However, current fish feed ingredients contain little free Gln because of concern over its degradation during a prolonged period of storage to form pyroglutamate (a potentially toxic substance for the brain) [22]. Based on the above-mentioned problems for free Gln, using chemical and biotechnological methods, the stable and highly soluble peptide L-alanyl-L-glutamine (Ala-Gln) can be synthesized as an effective alternative to Gln for food and feed additive. Ala-Gln supplemented parenteral nutrition was clinically safe, had better nitrogen balance, and maintained intestinal permeability in postoperative patients [23]. Addition of Ala-Gln in food can effectively prevent intestinal oxidative injury and inflammatory disease in neonates [24]. Replacement of Gln with Ala-Gln during *in vitro* maturation, numbers of early cleaved embryos for pig was significantly increased after activation [25]. Meanwhile, replacement of Gln with Ala-Gln during *in vitro* culture significantly increased total cell numbers in blastocysts [25]. Nevertheless, few reports concern the crowding stress resistance functions of dietary Ala-Gln in fish and it was almost scarcely reported in freshwater fish. A previous study from our laboratory found that dietary Ala-Gln supplementation improved with the increase of feed intake and protein productive ratio, and decreased the coefficient of mass variation for juvenile Jian carp (*Cyprinus carpio* var. Jian). In addition, we also found that optimum Ala-Gln level for Jian carp in the stocking densities of 20, 40 and 80 g/L should respectively be 0.30–0.60%, 0.60–0.88% and 0.90–1.08% [26]. As a nutritional additive of Ala-Gln, it must also be taken into account that the same nutritional additive dose may have an immunostimulant or no effect on the immune system, depending on parameters such as time, administration route and others [27]. However, to date, no study has reported the effects of Ala-Gln feeding strategies on growth performance, metabolism, and crowding stress resistance of fish.

Taken together, this study expands on our previous report, which indicated that optimum Ala-Gln level for Jian carp in the stocking densities of 80 g/L should be 0.90–1.08% [26]. In this study, the effects of various Ala-Gln feeding strategies on survival, growth performance, and activities of digestive enzyme, enzymatic antioxidative status and crowding stress resistance of juvenile Jian carp were investigated in a recirculating aquaculture system, which may provide a possible mechanism in antioxidant roles and crowding stress resistance improving effects of dietary Ala-Gln on juvenile Jian carp.

2. Materials and methods

2.1. Diets

The formulation and nutrient content of the basal was diet is shown in Table 1. Fish meal (Pesquera Lota Protein Ltd., Lota, Chile), Ala-Gln (Sangao Biochemical Co. Ltd., Sichuan, China) and alanine (Sangao Biochemical Co. Ltd., Sichuan, China) were used as dietary protein sources. Fish oil (Corpesca SA, Mejillones, Chile) and corn oil (Beidahuang Corn Industry Co. Ltd., Jilin, China) were used as dietary lipid sources. The basal diet was formulated to contain 350 g/kg crude protein, 50 g/kg crude lipid and 17 MJ/kg gross energy, the reported optimum for the growth of Jian carp [26,28,29]. The experimental diet was obtained by supplementing 1.0% of Ala-Gln, which the content has been proven to be beneficial for Jian carp reared in high-density [26]. The control diet was supplemented 1.0% of alanine to adjust the dietary nitrogen level. Diets were produced to be 1.0 mm pellets and the dried pellets were stored in sealed bags at −20 °C until used. The crude protein, crude lipid, crude ash and gross energy of the experimental and control diets were analyzed according our previous study [26].

2.2. Feeding strategies

An 8-week feeding trial was conducted in this study. A total of five different feeding strategies were used, denoted as I(0/0), II(0/2), III(4/0), IV(0/4) and V(4/4), respectively. Group I (control group) was always fed with control diet throughout the feeding trial; group II fed with control diet for two weeks and then experimental diet for 2 weeks, then this was repeated to cover 8 weeks; groups III fed with the experimental diet at the former 4 weeks, and substituted the control diet for the experimental one at the latter 4 weeks; groups IV fed with control and experimental diets at the former and latter 4 weeks, respectively; group V incessantly fed with the experimental diet for 8 weeks.

2.3. Fish and management

Hatchery-reared juvenile Jian carp obtained from the Lin-Jiang Hatchery (Jilin, China) were adapted to experimental conditions for 2 weeks prior to the experiment. During that period, fish (density less than 20 g/L) were stocked in a recirculating aquaculture system (flow rate: 1.0 L/min) equipped with a biofilter for the removal of nitrogenous waste, and fed with control diet. After the acclimatization, 1500 healthy fish with an initial weight of 26.1 ± 0.6 g were randomly assigned to each of 15 glass aquariums, and made the final density as 80 g/L. Water flow rate in each aquarium was maintained at 1.0 L/min, and the water was drained through biofilters to remove solid substances and reduce ammonia concentration. The water temperature and pH were 25 ± 2 °C and 7.1 ± 0.1, respectively. Dissolved oxygen was higher than 6.0 mg/L. The experimental units were under a natural light and dark cycle (approximately 13:11 h light: dark). For the feeding trial, each experimental diet was randomly assigned to aquaria in triplicate. Water quality was monitored weekly for pH, nitrite, ammonia, temperature and dissolved oxygen and kept within optimal levels for juvenile Jian carp. Fish were hand fed their assigned diet two times (09:00 and 17:00) a day (2.5% body weight). Before the feeding, the weight of feed given to fish was measured. Thirty minutes after the feeding, uneaten feed were removed by siphoning, and then dried and weighted to calculate the feed intake. Weight measurement and sampling were conducted every 2 weeks.

2.4. Sample collection and analyses

2.4.1. Sample collection

Five representative fish per aquarium were randomly sampled every 2 weeks from the beginning of the feeding trail. Prior to all sample collection, fish were euthanized via MS-222 (300 mg/L, Kangting Biotechnology Co., Ltd, Shanghai, China) overdose, and then the whole-body weights were recorded. Blood samples (approximately 1.0 mL) were drawn from the caudal vein with a sterile syringe (1 mL, 27-gauge needle) 12 h after the final feeding. Serum was separated by centrifugation at 3800*g* for 10 min after placing at 4 °C for 24 h, and then stored at −80 °C until analysis. Fish were dissected for the sampling of liver and head-kidney. All the tissue samples were flash-frozen in liquid nitrogen and stored at −80 °C until use.