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Effects of dietary lipid and protein levels on growth and physiological metabolism of *Pelteobagrus fulvidraco* larvae under recirculating aquaculture system (RAS)

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ABSTRACT

A feeding trial was conducted to investigate the effects of different dietary lipid and protein levels on both the physiological metabolism and the anti-oxidation of *Pelteobagrus fulvidraco* larvae cultured in recirculating aquaculture system (RAS). In the present study, four test diets (P52L13, P52L9, P48L13, P48L9) were formulated by two different lipid levels (13%, 9%) and two different protein levels (52% and 48%) in a factorial manner (2 × 2) to provide four different dietary protein-lipid ratios (P:L) 3.95, 5.71, 3.56 and 5.46. Each treatment was assigned in triplicate. Two days after hatching, larvae were fed with *Artemia* sp. for 1 day, and then were co-fed the *Artemia* sp.plus the tested diets for 2 days, finally fed test diets for 18 days. The results were in keeping with that of growth performance, showed that the larvae fed on P52L13 and P48L9 diets had significantly higher activities of lipoprotein lipase (LPL), glutamic-pyruvic transaminase (GPT), superoxide dismutase (SOD) and catalase (CAT) than other two groups ($P < 0.05$). The highest activities of lysozyme (LZM) and glutathione (GSH) were observed in group P52L13 and P48L9, respectively ($P < 0.05$). Concerning dietary lipid levels, the highest activities of LPL, GOT, SOD, CAT and LZM were observed for larvae fed diets containing 13% lipid ($P < 0.05$). Regarding to antioxidative factors, same trend was observed for larvae fed diets with 52% protein ($P < 0.05$). Our results clearly suggested that P52L13 and P48L9 diets could promote the metabolism of protein and lipid, and antioxidative ability of *P. fulvidraco* larvae in RAS, meanwhile, P52L13 diet is the superior.

1. Introduction

Generally, Lipid and protein are the key nutrients for growth and development of fish larvae. In fact, they are not only important for maintaining normal life activity, but also affecting the physiological metabolism, antioxidative ability and immune function of fish (Kiron, 2012; Kiron et al., 1995a; Halldorsdottir, Sveinsdottir, Freysdottir, and Kristinsson, 2014; Zhao, Wen, Li, Zhu, and Li, 2015). Furthermore, the suitable lipid level in diet can increase lipoprotein lipase (LPL) production (Zheng et al., 2010) and fatty acid synthetase (FAS) activity (Wang et al., 2011). The essential fatty acid is significant for fish phagocytes, addiment activity and antibody levels (Kiron et al., 1995b). Beside lipid, dietary protein level also significantly affects transaminase activity of fish liver. The high protein level in diet improves the activities of glutamic-pyruvic transaminase (GPT) and glutamic oxalo acetic transaminase (GOT) of many fish livers, such as *Rhamdia quelen, Oncorhynchus mykiss* and *Scophthalmus maximus* (Melo, Lundstedt, Metón, Baanante, and Moraes, 2006; Kim, Grimshaw, Kayes, and Amundson, 1992; Cowey, Brown, Adron, and Shanks, 1974). Protein is also essential material for synthesizing various enzymes and antibodies, and closely associated with immunity (Gao, Li, Zhang, Liu, and Zhou, 2012). In general, inappropriate dietary protein levels will decrease both lysozyme (LZM) and superoxide dismutase (SOD) activities of fish, while the related addiment and antibody levels will be reduced consequently (Yang and Zhou, 2006; Kiron et al., 1995a, b; Wang, Li, and Che, 2009). Therefore, appropriate dietary protein and lipid ratios (CP: CL) are playing an important role for growth and disease resistance of the aquatic animals, which will promote the nutrient utilizations (Chen, Xiao, Liang, and Zhou, 2012), enhance the metabolic enzyme and LZM activities (Jiang, 2012; Cheng et al., 2006). Recently, researches on the influence of such two nutrients on metabolism and immune are mainly concentrated on the juvenile and adult stages (Liu, 2013; Tang, 2014;

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Huang, Xu, and Chang, 2015), while only few studies that have been done to investigate the role of these important nutrients in fish larvae (Zheng et al., 2010).

The Larva is a main stage of the life cycle of the aquatic animals which represents one of the most factors in aquaculture development, e.g. high quality of the larva stage lays the foundation on fish health (Gong et al., 2014). Generally, larvae in hatcheries first fed live foods have several disadvantages, such as limited resources, deficient nutrition, disease, high cost, seasonal restrictions, etc. (Wang and Xie, 2004; Yi, 2014). Therefore, it's becoming very important to develop a palatable larval microdiets with high quality and balanced nutrients. For example, Kanazawa (1989) found that the larvae had ability to digest microdiets, but always went through high mortality and slow growth, as a result of insufficient knowledge on nutrition and palatability of diets and improper feeding techniques. Also Cahu et al. (1998) demonstrated that, freshwater larvae fed compound diets have negative effects on growth at first, but then the post-larvae could compensate growth and recover to normal level. The nutritional requirements and microdiets of marine larvae have been intensively investigated focusing on the requirements of protein (Borbam, Fracalossi, and Pezzato, 2006; Hamre, Mango, and Jenaen, 2006; Aliyu, Hashim, and Shuchien, 2010), lipid and fatty acids (Buchet, Zambonino, and Cahu, 2000; Hamza et al., 2008; Copeman, Parrish, Brown, and Harel, 2002; Furuita, Konishi, and Takeuchi, 1999; Bransdden, Battaglene, Morehead, Dunstan, and Nichols, 2005), while there are relatively few reports on fresh-water *Pelteobagrus fulvidraco* larvae (Wang et al., 2006; Wang et al., 2005; Yang, Xie, Fan, Zheng, and Lei, 2009; Zheng et al., 2010; Zhao, 2006).

The yellow catfish (*Pelteobagrus fulvidraco*) is one of the most economic important freshwater aquaculture species in China (Fish Research Laboratory of Hubei Institute of Hydrobiology, 1976). It has huge market prospects due to its delicious taste and nutrition-rich. Recently, the new variety of the yellow catfish, *P. fulvidraco* (All-male No. 1) has some special features, such as large individual and fast growth, etc. (Tian et al., 2013). Thus, it has been popularized rapidly across China, therefore the demand of larvae with high quality was increased rapidly. Although, the optimal nutrition has a great effect on the quality of larvae, healthy cultivation and survivability (Gong et al., 2014), studies on the nutrition regulation of larvae are still relatively rear, compared with both juvenile and adult fish studies. So far, there is no study on the nutritional requirements of *P. fulvidraco* larvae in the recirculating aquaculture system (RAS); as the best of our knowledge.

Many culture models have been used in *P. fulvidraco*, such as pond culture, flowing water culture, net cage culture and so on. But there are some problems in these models: food waste, water pollution, frequent fish disease and so forth, while they can be properly addressed in RAS.

In the present study, our major objective was to evaluate the effects of different dietary lipid and protein levels on the physiological metabolism and antioxidative responses on the yellow catfish (*P. fulvidraco*) larvae in RAS. Accordingly, our study will provide the fundamentals as well as the references for developing a special practical feed which have abundant and balanced nutrition for larvae reared in RAS.

2. Materials and methods

2.1. Experimental fish and culture facility

Experimental fishes were “All-male No. 1” *P. fulvidraco* larvae, provided by an intensive larvae farm (Wuhan Bairui Bio-tech Co., Ltd., Wuhan, China). The larvae with an average body weight of 4.6 ± 0.3 mg were randomly selected from the same batch, and then being assigned into 12 round tanks (diameter 0.9 m, height 0.75 m) with three replicates for each treatment (3600 fish per replicate). The larvae were firstly fed with live food (newly hatched *Artemia* sp., unenriched) for 1 day, then co-feeding *Artemia* sp. and test diets for 20 days.

Table 1
Main ingredients and chemical composition of trial diets.

Ingredients (%)	Diets			
	P52L13	P52L9	P48L13	P48L9
Fish meal[a]	27	27	23	23
Casein	20	20	20	20
Soy protein concentrate	14.34	13.65	11.28	10.62
Wheat flour	10.34	14.18	17.2	21
Plasma protein meal[b]	6	6	6	6
Lecithin	6	6	6	6
Yeast protein	5	5	5	5
Fish oil	4.8	2.72	4.8	2.72
Corn oil	2.4	1.36	2.4	1.36
Multi-vitamin[d]	0.8	0.8	0.8	0.8
Multi-mineral[e]	0.8	0.8	0.8	0.8
Binder[c]	2	2	1.5	1.5
mono calcium phosphate	0.54	0.51	1.02	1
Choline	0.2	0.2	0.2	0.2
Chemical composition(%)				
Crude protein	52.24	51.94	47.90	48.31
Ether extract	13.22	9.10	13.44	8.85
Protein lipid ratio[f]	3.95	5.71	3.56	5.46
Calcium	1.35	1.35	1.26	1.25
Total phosphorus	1.40	1.42	1.41	1.39
NaCl	0.93	0.95	0.86	0.85
Lysine[f]	3.99	3.97	3.67	3.65
Fiber	1.00	0.90	0.80	0.80

[a] High quality Peruvian fish meal (65% crude protein).
[b] Domestic high quality plasma protein meal (75% crude protein).
[c] Sodium alginates.
[d] Multi-vitamin (per kg diet): VA, 15000 IU; VC, 1000 mg; VD_3, 2500 IU; VK_3, 50 mg; VB_1, 50 mg; VB_2, 20 mg; VB_6, 30 mg; VB_{12}, 0.5 mg; VE, 300 mg; Niacin, 260 mg; Calcium pantothenate, 150 mg; Folacin 20 mg; Biotin, 2.5 mg; Inositol, 100 mg.
[e] Multi-mineral (mg/kg):Cu, 8; Zn, 250; Mn, 45; Fe, 100; I, 2.4; Co, 2; Mg, 4.
[f] The protein lipid ratio and lysine were calculated values, the rest of the nutrient levels were measured values.

2.2. Experimental diets and growth trail

Four different experimental diets were tested representing a combination of two different lipid levels (9% and 13%, presented by L9 and L13) and two different protein levels (48% and 52%, presented by P48 and P52). Each treatment was designed in triplicate. The dry ingredients were mixed thoroughly in a food mixer, then all the ingredients were mixed with the fish oil and distilled water. The moist diet was then extruded through a pelletizer to create a 3.0 mm diameter pellet. The diets were made in a feed mill (Tianjin Hai Fa Zhen Pin Industrial Development Co., Ltd. Tianjin, China). Before the experiment, we fragmented 3.0 mm diameter pellet into 0.3, 0.5, 0.8 and 1.0 mm micro-bonded particle feed to be easily consumed by *P. fulvidraco* larvae. However, the ingredients and the proximate compositions of the experimental diets were shown in Table 1.

Larvae were reared in a circle barrels with 0.48 m^3 volume. The water circulated 160 L/h for 24 h every day with 28.0 ± 1.0 °C. Dissolved oxygen ranged from 5.0 to 8.0 mg/L. The larvae were hand-fed to satiation at 6:30, 9:30, 12:30, 15:30, 18:30, 21:00 and 23:00.

2.3. Sampling and indices assays

At the end of the trail, we calculated average body weight and number of live fish in each barrel, weighed total fish weight and 100 fishes' weight. Then randomly selected 40 fishes from each barrel into 10 mL tube as measuring sample. All samples were preserved in −20 °C.