Aquaculture 435 (2015) 200–206

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary lipid levels on growth, feed utilization, body composition and antioxidants of juvenile mud crab *Scylla paramamosain* (Estampador)

Juan Zhao, Xiaobo Wen *, Shengkang Li, Dashi Zhu, Yuanyou Li

Guangdong Provincial Key Laboratory of Marine Biology, Shantou University, Shantou 515063, China

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 10 September 2014
Accepted 11 September 2014
Available online 19 September 2014

Keywords:
Mud crab
Scylla paramamosain
Dietary lipid
Growth
Antioxidant parameters

ABSTRACT

This study was carried out to determine the effects of dietary lipid levels on growth performance, feed utilization, body composition and antioxidant parameters of juvenile mud crab (*Scylla paramamosain*). Five isonitrogenic and isoenergetic experimental diets (45% crude protein) containing 0%, 3%, 6%, 9% and 12% added oils (named L0, L3, L6, L9 and L12, respectively) were fed to triplicate groups of 20 crabs (initial weight 11.53 ± 0.52 g, carapace width 3.92 ± 0.14 cm) for 8 weeks. The results showed that the survival, final body weight, carapace width, specific growth rate and molting frequency of crabs fed diet L6 were significantly higher than those of crabs fed diet L0, L3 or L12 ($P < 0.05$). The highest feed conversion ratio and the lowest protein efficiency ratio were both observed in crabs fed diet L0 ($P < 0.05$). The maximum voluntary feed intake was found in crabs fed diet L3 ($P < 0.05$). The lipid contents of whole body and hepatopancreas increased remarkably with the elevated dietary lipid levels ($P < 0.05$). The proportions of saturated fatty acid and monounsaturated fatty acid of the hepatopancreas were significantly higher in crabs fed diet L0 ($P < 0.05$). The fatty acid composition of the hepatopancreas and muscle was consistent with those in the diets. In the hepatopancreas, crabs fed diet L12 accumulated the highest concentration of malondialdehyde than other groups ($P < 0.05$), and the activities of antioxidant enzymes (superoxide dismutase, glutathione S-transferase and glutathione peroxidases) were all enhanced with the increased dietary lipid levels ($P < 0.05$). The results suggested that a proper dietary lipid level of 8.52%–11.63% (optimum 9.5%) could maintain solid growth performance and antioxidant capacity of juvenile mud crab *S. paramamosain*.

1. Introduction

Mud crabs (*Scylla* spp.) occur throughout tropical and warm temperate areas in the Indo Pacific region, supporting important inshore fisheries in many countries, because of their large size, high meat yield and delicate flavor (Keenan, 1999; Le Vay, 2001; Marichamy and Rajapackiam, 2001). In China, mud crab production had reached up to 128,983 tons in 2012, accounting for 52.89% of all crab aquaculture production (China Fishery Statistical Yearbook, 2013). *Scylla paramamosain*, one of the most popular mud crab species, has been widely cultured in the coastal areas of southern China (Ye et al., 2011). Currently, the farming of mud crab depends mainly on conventional diets such as trash fish, molluscan meat, and animal viscera. In the east of Guangdong Province, *S. paramamosain* is fed with a live food, *Potamocorbula rubromuscula*. Compared with the traditional feed, pelleted diets offer a more balanced nutrition, and have a better and steadier quality for storage. Studies on the nutritional requirement will be very important to develop cost-effective, environmentally friendly and nutritionally balanced diets for mud crabs.

Lipid is one of the most important components of diet, which serves as the source of available energy and essential fatty acids, forms the major structural components of biomembranes, acts as carriers of fat-soluble vitamins and functions as precursors of eicosanoids, hormones and enzyme cofactors for the crustacean (Higgs and Dong, 2000; Sargent et al., 1989; Watanabe, 1982). However, excessive dietary lipid could negatively affect growth performance, reduce feed consumptions and utilization of other nutrients, and even lead to fat deposition on the hepatopancreas and other tissues (Shiau and Huang, 1990). The level of dietary lipid requirement varies widely among different species, life stages and environmental conditions (D'Abramo, 1997). For most crustaceans, good growth can be obtained with a total lipid level from 2% to 12% of diet dry weight (Catacutan, 2002; Cortés-Jacinto et al., 2005; Sheen, 1997; Sheen et al., 1994; Xu et al., 2013; Zhang et al., 2013). For the growth of juvenile *Scylla serrata*, a member of the *Scylla* family, a dietary lipid level between 5.3% and 13.8% is sufficient for the nutritional needs (Sheen and Wu, 1999).

Information on the optimum dietary lipid level can be used to improve efficiency of cultivation and reduce feed costs (Peres and

* Corresponding author. Tel.: +86 754 86503435; fax: +86 754 82903435.
E-mail address: wenxbo@stu.edu.cn (X. Wen).

http://dx.doi.org/10.1016/j.aquaculture.2014.09.018
0044-8486/

Olivia-Teres, 1999; Yoshii et al., 2010). As far as we know, there is little information on the optimal lipid levels for *S. paramamosain*. Therefore, the aim of the present study was to determine the dietary lipid requirement for juvenile *S. paramamosain* through evaluating mud crabs' growth performance, feed utilization, body composition and antioxidant parameters under various dietary lipid levels.

2. Materials and methods

2.1. Experimental diets

Five isonitrogenous semi-purified diets with added oils ranging from 0 to 12% in 3% increments (0%, 3%, 6%, 9% and 12%; named L0, L3, L6, L9 and L12, respectively) were formulated. The diets were rendered isoenergetic by adjusting with tapioca starch and cellulose contents. The formulation and proximate composition of the experimental diets are provided in Table 1. White fish meal (Coastal Villages Pollock, Anchorage, AK, USA) and casein (Aoboxing Bio-tech, Beijing, China) were used as the protein sources. Mixed oil (MO) with a ratio of fish oil:safflower oil:perilla seed oil = 1:1:1 was used as the lipid source based on our previous research results (unpublished). The fatty acid composition of the experimental diets is given in Table 2. The other feed ingredients were obtained from Yuequn (Jieyang, China). Before formulation of the diets, lipid was extracted from fish meal using ethanol (1:1, w/v) in five successive treatments to minimize the endogenous lipid in diet (Sheen, 2000). All solid diet ingredients were ground and passed through an 80 mesh sieve, and then mixed thoroughly until homogenous, using the progressive enlargement method to mix the microingredients. Choline chloride (dissolved in the distilled water) and oils were subsequently added and mixed in a Hobart-type mixer for 15 min before being cold-extruded pellets through a laboratory pellet mill (SLP-45, Fishery Machinery and Instrument Research Institute, Shanghai, China) with a 1.5 mm die, and then steamed at 100 °C for 15 min. The diets were air-dried to about 10% moisture and cut into 4–6 mm length, sealed in vacuum-packed bags, and stored frozen (−20 °C) prior to use in the feeding trial.

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

	Diet				
	L0	L3	L6	L9	L12
Ingredient composition					
Defatted fish meal	33.00	33.00	33.00	33.00	33.00
Casein	20.00	20.00	20.00	20.00	20.00
Dextrin	9.00	9.00	9.00	9.00	9.00
Tapioca starch	27.00	20.25	13.50	6.75	0.00
Mixed oil[a]	0.00	3.00	6.00	9.00	12.00
Vitamin mix[b]	3.00	3.00	3.00	3.00	3.00
Mineral mix[b]	2.00	2.00	2.00	2.00	2.00
Cholesterol	0.80	0.80	0.80	0.80	0.80
Lecithin	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.70	0.70	0.70	0.70	0.70
Sodium alga acid	1.00	1.00	1.00	1.00	1.00
Squid paste	1.00	1.00	1.00	1.00	1.00
Cellulose	0.00	3.75	7.50	11.25	15.00
Proximate composition					
Moisture	10.14	9.95	9.72	9.60	9.55
Crude protein	45.93	44.30	43.72	46.08	43.64
Crude lipid	2.91	5.85	8.52	11.63	14.32
Nitrogen-free extract	39.35	35.75	31.54	24.00	22.45
Ash	10.55	11.28	11.16	11.76	10.88
Energy (kJ g^{-1})[c]	18.76	18.92	19.11	19.61	19.83

[a] Fish oil:safflower oil:perilla seed oil = 1:1:1; fish oil (Yuequn, Jieyang, Guangdong, China); safflower oil (Furun, Zibo, Shandong, China); perilla seed oil (Changbai Workshop, Shulan, Jilin, China).
[b] Xingmuwei Animal Health Product, Xiamen, Fujian, China.
[c] Computed as 21.3, 17.2 and 39.5 kJ/g of protein, carbohydrate and lipid, respectively (Cuzon and Guillaume, 1997).

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

Fatty acid[a]	Diets				
	L0	L3	L6	L9	L12
14:0	3.6	2.8	2.5	2.4	2.1
16:0	21.5	18.4	14.6	13.7	12.6
18:0	7.2	6.3	4.2	3.7	3.5
20:0	0.7	0.5	0.4	0.5	0.4
22:0	0.9	0.3	0.3	0.2	0.3
24:0	0.2	0.1	0.1	0.1	0.1
16:1n−7	8.7	5.2	4.6	3.7	3.4
18:1n−9	21.7	18.2	15.9	14.0	12.1
20:1n−9	1.5	0.9	0.6	0.4	0.3
22:1n−11	0.3	0.2	0.1	0.1	0.1
18:2n−6	7.3	23.5	28.9	29.3	30.1
20:2n−6	0.5	0.1	0.1	0.2	0.2
18:3n−3	3.7	13.8	15.8	17.6	19.5
20:3n−6	0.2	0.1	0.1	0.1	0.1
20:4n−6	1.6	0.3	0.4	0.5	0.5
20:5n−3	10.1	5.1	6.2	6.9	7.3
22:5n−3	1.6	0.3	0.4	0.5	0.5
22:6n−3	8.7	3.9	4.8	6.1	6.9
∑SFA[b]	34.1	28.4	22.1	20.6	19.0
∑MUFA[c]	32.2	24.5	21.2	18.2	15.9
∑PUFA[d]	11.5	37.4	44.8	47.1	49.8
∑HUFA[e]	22.2	9.7	11.9	14.1	15.3
∑n−3 FA[f]	24.1	23.1	27.2	31.1	34.2
∑n−6 FA[g]	9.6	24.0	29.5	30.1	30.9
n−3/n−6	2.5	1.0	0.9	1.0	1.1

[a] Data expressed as area percentage of FAME, represent mean ± SE, n = 3. Fatty acids present at ≤0.1 percentage of total fatty acids are not included.
[b] SFA: 14:0, 16:0, 18:0, 20:0, 22:0, 24:0.
[c] MUFA: 16:1n−7, 18:1n−9, 20:1n−9, 22:1n−11.
[d] PUFA: 18:2n−6, 18:3n−3, 20:2n−6.
[e] HUFA: 20:3n−6, 20:4n−6, 20:5n−3, 22:5n−3, 22:6n−3.
[f] n−3 FA: 18:3n−3, 20:5n−3, 22:5n−3, 22:6n−3.
[g] n−6 FA: 18:2n−6, 20:2n−6, 20:3n−6, 20:4n−6.

2.2. Mud crabs and experimental conditions

Juvenile mud crabs were collected from the estuarine areas around Shantou (Guangdong, China). Prior to the trial, crabs were acclimated and fed with a commercial diet (36% crude protein, 6% crude lipid, Jieyang Tongwei, Jieyang, China) for 2 weeks. At the beginning of the experiment, 300 healthy juvenile mud crabs (initial average weight 11.53 ± 0.52 g, carapace width 3.92 ± 0.14 cm) were randomly sorted into 300 polypropylene tanks (32 cm × 20 cm × 14 cm, Zhongkehai, Qingdao, China) individually, which connected to the same water through branch water pipes. There were five treatments, each with three replicates, and each replicate with 20 crabs. Five experimental diets were randomly fed to the five treatments at the same fixed rate, twice a day at 0800 and 1800 h for 8 weeks. Each tank held 5 cm height sand-filtered brackish water and was intermittently supplied with a continuous flow of water (0.2 L min^{-1}) for 8 h every day.

During the experimental period, water temperature was maintained around 25.4 to 26.7 °C, pH at 8.05 to 8.14, ammonia nitrogen lower than 0.05 mg L^{-1}, dissolved oxygen above 5.0 mg L^{-1}, and salinity from 7.8 to 8.4 g L^{-1}. The experiment was conducted in dark environment except at time for siphoning out feed residue and feces, or checking the status of crabs. Exuviae and dead crabs (if any) were removed, weighed and recorded daily. Uneaten feeds were collected and washed with double-distilled water to remove any adhering salts, oven-dried at 90 °C for 5 h, and weighted.

2.3. Samples collection and chemical analysis

At the beginning of the feeding trial, 20 juvenile mud crabs were randomly weighed and stored at −20 °C as the initial samples. At the termination of the feeding trial, all crabs in each tank were sampled for