Aquaculture 426–427 (2014) 112–119

Contents lists available at ScienceDirect

Aquaculture

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

Effect of dietary cornstarch levels on growth performance, enzyme activity and hepatopancreas histology of juvenile red swamp crayfish, *Procambarus clarkii* (Girard)

Xucheng Xiao [a,b], Dong Han [a,c], Xiaoming Zhu [a,*], Yunxia Yang [a], Shouqi Xie [a], Ying Huang [d]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, PR China
[b] University of Chinese Academy of Sciences, Beijing, PR China
[c] Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan 430070, PR China
[d] Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, PR China

ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form 17 January 2014
Accepted 20 January 2014
Available online 5 February 2014

Keywords:
Cornstarch
Growth
α-Amylase
Hepatopancreas histology
Procambarus clarkii

ABSTRACT

An 8-week experiment was conducted to determine the ability of juvenile red swamp crayfish *Procambarus clarkii* (Girard) to utilize different levels of cornstarch. Seven isonitrogenous practical diets were formulated with different cornstarch levels (50, 100, 150, 200, 250, 300 and 350 g kg^{-1}). The growth indices, body composition, α-amylase activity and hepatopancreas histology were examined. Results showed that the survival ranged from 71.1% to 82.2%, and was not significantly affected by dietary cornstarch levels ($P > 0.05$). Feeding rate (FR) increased with the level of cornstarch ($P < 0.05$), with no significant differences among the groups fed with cornstarch over 250 g kg^{-1} ($P > 0.05$). Weight gain (WG) and feed conversion ratio (FCR) were significantly affected by dietary cornstarch levels ($P < 0.05$), and the highest WG and lowest FCR were observed in the crayfish fed with the 200 g kg^{-1} cornstarch diet. Crude lipid and hepatopancreas lipid content increased with increasing dietary cornstarch. A saturation curve was observed in the specific activity of the α-amylase, hemolymph glucose and hepatopancreas glycogen concentration in relation to dietary cornstarch levels, with the maximum activity in crayfish fed diets containing from 200 to 350 g kg^{-1} cornstarch ($P > 0.05$). In addition, crayfish exhibited notable histological differences among the groups. The morphology of the hepatopancreas in crayfish fed with 150 g kg^{-1} and 200 g kg^{-1} was normal, with tubules tightly arranged. Different cell types in these two groups were well recognized and reasonably uniform in shape and size. It could be concluded that the optimum cornstarch level for this crayfish could be 203 g kg^{-1} when the diets contain 350 g kg^{-1} protein.

1. Introduction

Carbohydrates are the most economical source of energy in formulated aquaculture diets (Rosas et al., 2000). The concept that carbohydrates can substitute for the protein as an energy source in animal food, thereby reducing the costs associated with feed production, has already been documented (Cruz-Suarez et al., 1994). In addition, previous researches (Abdel-Rahman et al., 1979; Alava and Pascual, 1987; Andrews et al., 1972; Rosas et al., 2000) that investigated carbohydrate utilization relative to the sparing of dietary protein could consequently lead to a decrease in the amount of nitrogen waste. Therefore, knowledge of the capacity of animals to utilize carbohydrates is important.

Few studies have been conducted to determine the effect of types and levels of carbohydrates on crustaceans (Andrews et al., 1972; Guo et al., 2006; Rosas et al., 2000, 2001; Zhang et al., 2009). In *Penaeus monodon* Fabricius juveniles, survival was affected by carbohydrate levels, and trehalose promoted better growth than sucrose and glucose (Alava and Pascual, 1987). Thongrod et al. (2003) suggested that the optimal dietary cornstarch level for *Haliotis asinine* Linne was 47.81%. Rosas et al. (2000) found that 33% of dietary carbohydrates limited the growth of *Litopenaeus stylirostris* juveniles.

In most aquaculture feeds, starch has been introduced as the principal source of carbohydrate due to the fact that monosaccharides increase abnormal death rate (Abdel Rahman, 1996). Dietary monosaccharides are rapidly absorbed but poorly utilized (Gomez Diaz and Nakagawa, 1990). For these reasons, many researchers suggest incorporating more complex carbohydrates like starch to shrimp feed formulation, which undergoes enzymatic hydrolysis before assimilation, permitting glucose to be absorbed in the gut site at a slower rate (Alava and Pascual, 1987; Shiau, 1998; Shiau and Peng, 1992). Shiau and Peng (1992) demonstrated that cornstarch was better utilized

Abbreviations: FR, feeding rate; FCR, feed conversion ratio; SGR, specific growth rate; WG, weight gain.

* Corresponding author. Tel.: +86 27 68780667, +86 27 68780060; fax: +86 027 68780667.
E-mail address: xmzhu@ihb.ac.cn (X. Zhu).

0044-8486/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2014.01.029

and showed higher protein deposition in juvenile shrimp than dextrin and glucose.

In inland China, the red swamp crayfish, *Procambarus clarkii* Girard has recently become a popular cultured freshwater species due to its high market value and consumer demand. It has excellent aquaculture potential on account of its high growth rate, easy management and high yield. An annual production of 563,000 tons was recorded in 2010 (FBMA, 2011). Previous studies on *P. clarkii* have employed both fresh feedstuffs and semi-purified diets to evaluate the growth rate and recommend optimum nutrient requirements (Jover et al., 1999; Oliveira and Fabião, 1998). Jover et al. (1999) found that the carbohydrate requirement of *P. clarkii* was 36–41% when protein was 22–26%. Hubbard et al. (1986) found that best growth was observed in crayfish fed a diet with 30% crude protein and 2.5 k cal g^{-1} dietary energy. To date, very little attention has been given to carbohydrate utilization of *P. clarkii*.

The objective of the present study was to investigate the optimum dietary cornstarch levels in practical diets for *P. clarkii*, in terms of growth performance, body composition, α-amylase activity and hepatopancreas histology.

2. Materials and methods

2.1. Experimental diets

Seven isonitrogenous diets were formulated to contain 50, 100, 150, 200, 250, 300 and 350 g kg^{-1} cornstarch. Fish meal was used as the main dietary protein source. Formulation and chemical composition of the experimental diets are shown in Table 1. Oil and water were added to dry ingredients and thoroughly mixed to form a palatable mixture, and sodium alginate was used as a binder. The feeds were then made into 1-mm diameter pellets by means of an extrusion machine, oven-dried at 60 °C and stored at −4 °C until used.

2.2. Experimental animals and rearing conditions

Juvenile *P. clarkii* were obtained from the hatchery of the Institute of Fisheries Science, Hubei, China in November, 2010. Prior to the experiment, the crayfish were stocked in 10 90-L polythene tanks (water depth 15 cm). To reduce aggressive behaviors, some roof tiles (13 × 16 × 6 cm) were placed around the bottom of the tanks as shelters. Crayfish were fed to satiation twice a day at 0800 and 1700 for 2 weeks with an equal mixture of the 7 experimental diets for acclimatization.

At the beginning of the trial, healthy juvenile *P. clarkii* with an initial weight of 0.39 ± 0.00 g (mean ± S.E.) were collected and randomly allocated to 21 tanks (volume 90 L, diameter 70 cm) at a density of 15 juveniles per tank. Three tanks were randomly assigned to each of 7 treatments. 4 roof tiles were placed at four sides of the tank bottom to simulate the natural habitat. Water depth in each tank was maintained at about 15 cm and about 50%–60% tank water was replaced with aerated tap water each morning before feeding. During the experiment, the tanks were supplied with a continuous aeration to ensure that the dissolved oxygen concentration was greater than 6 mg O_2 L^{-1}. Water temperature was maintained at 19 ± 2 °C, pH between 6.8 and 7.0, ammonia-N concentration was less than 0.45 mg L^{-1} and monitored every two weeks using the appropriate methods (AOAC, 1984). The photoperiod was 12 h light:12 h dark.

During the 8 week trial, crayfish were fed experimental feeds to satiation twice a day at 0800 and 1700. Uneaten food was collected 4 h later by siphoning, dried at 70 °C and weighed. Leaching rate of uneaten food was assessed by placing a weighed amount of food in tanks without crayfish for 4 h and then collected, dried and reweighed. The leaching rate was used to calibrate the uneaten feed. Feces and newly molted shells were removed before each feeding and mortality was recorded daily.

2.3. Sample collection

At termination of the experiment, the crayfish were fasted for 24 h to clear the gastrointestinal tract of ingested food (Davis and Robinson, 1986) and then placed in a chill coma. Only intermoult crayfish, stage C or D1, were sampled from each diet treatment. Hemolymph was obtained with a syringe from three crayfish in each tank. The hepatopancreas were subsequently removed and sampled for later α-amylase activity, hepatopancreas glycogen and lipid content analysis. Another 4 crayfish in each tank were also sampled and stored at −20 °C for whole body composition analysis.

2.4. Biochemical analysis

Frozen hepatopancreas samples were homogenized with blunders using ice-cold buffer. The homogenized suspension was centrifuged at 4 °C with 664 *g* for 10 min, and subsequently used for α-amylase activity and hepatopancreas glycogen content determination. α-Amylase activity was measured according to a modified Bernfield's method (1955) using 1% glycogen as substrate diluted in a 2.5 nM $MnCl_2$, 10 mM NaCl, 10 mM, pH 7, and phosphate buffer. Hepatopancreatic glycogen was extracted according to Van Handel (1965). Total protein concentration of tissue was measured using the Bradford (1976) method with bovine serum albumin as the standard. Hemolymph glucose was determined

Table 1
Formulation and proximate composition of the experimental diets (g kg^{-1} in dry matter).

Ingredients	1	2	3	4	5	6	7
White fishmeal[a]	485	485	485	485	485	485	485
Fish oil	3	3	3	3	3	3	3
Corn starch	50	100	150	200	250	300	350
Cellulose	377	327	277	227	177	127	77
Mineral & vitamin premix[b]	10	10	10	10	10	10	10
Choline chloride	5	5	5	5	5	5	5
CaH_2PO_4	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5
Lecithin	10	10	10	10	10	10	10
Swine liver power[c]	20	20	20	20	20	20	20
Sodium alginate	30	30	30	30	30	30	30
Proximate analysis (% dry matters)							
Crude protein	353.2	354.1	348.7	359.8	358.2	351.8	348.8
Crude lipid	67.8	62.1	61.6	68.4	68.1	64.9	62.6
Ash	104.3	105.4	106.8	109.8	115.3	112.9	107.4
Gross energy (kJ g^{-1})	17.82	17.41	17.76	18.15	17.34	18.24	17.93
Carbohydrate	72.1	119.5	166.3	221.5	282.2	324.1	363.2

[a] White fishmeal was north pacific white fishmeal and purchased from American Seafoods Company, Seattle, Washington, USA.
[b] Mineral & vitamin premix was purchased from Haid Feeds Co., Ltd., Guangzhou, PR China. Product no. is (2007) 088126.VT3-2006.
[c] Swine liver power was purchased from Wuhan Coland Aquaculture Co., Ltd, Wuhan, PR China.