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Effects of dietary phosphatidylcholine (PC) levels on the growth, molt performance and fatty acid composition of juvenile swimming crab, *Portunus trituberculatus*

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ABSTRACT

A 50-day feeding trial was conducted to investigate the effects of dietary phosphatidylcholine (PC) levels on the growth performance, molt and body composition of the juvenile swimming crab, *Portunus trituberculatus*. Four isonitrogenous purified casein-based diets containing three PC levels and one commercial crude soybean lecithin (SL) level were formulated. A PC-deficient diet was designed as a control diet. Each diet was given to triplicate groups of 15 *P. trituberculatus* (initial weight, 25.4 ± 0.1 g/crab). The occurrence of molt death syndrome (MDS) was lowest in crabs fed the diet with 20 g/kg PC. The growth of crabs showed a significant positive correlation with dietary PC level, increasing linearly with PC level ($P < 0.05$), while growth performance was not significantly affected by SL supplementation. The highest growth performance was obtained in crabs fed the diet with 20 g/kg PC. Whole body triglyceride and cholesterol levels also increased linearly with increasing dietary PC level ($P < 0.05$). Serum triglyceride levels were significantly increased with SL supplementation ($P < 0.05$). Our results show that the n-3/n-6 ratios and 22:6n-3 concentrations in both hepatopancreas and muscle increased significantly with SL addition ($P < 0.05$). This study shows that PC supplementation in a casein-based diet had a significant growth-promoting effect on *P. trituberculatus*. Moreover, dietary SL had a greater effect on muscle and hepatopancreas fatty acid profiles than purified PC supplementation in diets.

1. Introduction

The swimming crab, *Portunus trituberculatus*, is distributed along the coasts of China, Korea and Japan (Hamasaki et al., 2006; Wu et al., 2010) and has recently become one of the most important marine species in China. Over the last several decades, much progress has been made in hatchery, grow-out techniques and accelerated growth for this species (Wu et al., 2010). In 2012, the area for aquatic breeding for this species was approximately 29,036 ha, yielding an output of 400,348 tons according to the Chinese Fishery Statistical Yearbook (2013). However, the per unit output of *P. trituberculatus* is poor

Abbreviations: PC, phosphatidylcholine; SL, soybean lecithin; PL, phospholipids; MDS, molt death syndrome; MF, molting frequency; SMF, successful molting frequency; IBW, initial body wet weight; FBW, final body wet weight; DFI, daily feed intake; FCR, feed conversion ratio; WG, weight gain; SGR, specific growth rate; PER, protein efficiency ratio; HSI, hepatosomatic index; SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acids.

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due to high mortality. Many of these deaths are due to the presence of calcium deposits embedded on and in the inner surface of the exuvial exoskeleton, which is known as molt death syndrome (MDS) (Bowser and Renée, 1981). Previous studies have demonstrated that the occurrence of MDS was related to lipid nutrition, including phospholipids (PL) (Bowser and Renée, 1981; Gong et al., 2004), cholesterol (Gong et al., 2004) and n-3 highly unsaturated fatty acids (n-3HUFA) (Dan and Hamasaki, 2011).

Several nutritional studies have also demonstrated that PL are important essential nutrients to marine crustaceans and finfish and have beneficial effects on growth (Azarm et al., 2013; Seoka et al., 2008), survival (Hansen et al., 2011; Niu et al., 2008), dietary nutrients utilization (Saleh et al., 2013; Uyan et al., 2009), skeletal/organ development (Geurden et al., 1998; Wang et al., 2012), molting performance (Bowser and Renée, 1981) and immunity (Li et al., 2014a). It has also been suggested that the beneficial effects of PL cannot be replicated by supplementation of choline, inositol, phosphorous and fatty acids (Coutteau et al., 1997; Tocher et al., 2008). Moreover, de novo synthesis of PL is so low that it cannot meet the metabolic requirements of crustaceans and finfish (Kanazawa, 1985; Tocher et al., 2008), and PL has been widely used in crustacean and fish diets. However, due to the high cost of purified PL, most feeding trials reported in the literature have been performed with variable or low purity PL (Kanazawa et al., 1979; Pascual, 1986; Teshima et al., 1986). It has also been reported that different PL classes may play slightly different roles and thus have different effects in different species (Tocher et al., 2008). As a result, it is difficult to draw conclusions with regard to the qualitative and quantitative requirements for PL in crustaceans and finfish.

Previous studies have suggested that phosphatidylcholine (PC) is the main growth-promoting fraction in PL (Coutteau et al., 1997; Kenari et al., 2011), which may be explained by the specific role of PC as the major constituent of polar lipids in membranes and its specific function for the synthesis and secretion of lipoproteins (Coutteau et al., 2000). Several studies have evaluated precise PL requirements using highly purified PC sources, including 900 g/kg PC in *Salmo trutta caspius* (Kenari et al., 2011), 950 g/kg PC in *Penaeus japonicus* and 800 g/kg PC in *Penaeus penicillatus* (Chen and Jenn, 1991) and *Penaeus monodon* (Chen, 1993). Larval growth of Japanese flounder, *Paralichthys olivaceus*, was improved by purified PC and not by purified phosphatidylinositol (PI) or phosphatidylethanolamine (PE) (Kanazawa, 1993). Camara et al. (1997) also suggested that other phospholipids (mainly PI and PE) in soybean lecithin are not required for postlarval *P. japonicus* when diets contain an adequate source of PC.

Some studies have already been conducted on lipid nutrition requirements of *P. trituberculatus*. Previous studies have suggested that 42–138 g/kg dietary lipids are appropriate for swimming crab and that soybean oil and linseed oil could substitute for fish oil in fish meal-based diets for this species (Han et al., 2013a, 2015). Another study suggested that 6 g/kg cholesterol could meet the requirements for the swimming crab (Han et al., 2013b). Our previous work showed that 10 g/kg PL (soybean lecithin or egg yolk lecithin) supplemented in diets could satisfy the requirement of juvenile *P. trituberculatus* (Li et al., 2014a,b). However, all of these studies employed commercial crude PL preparations consisting of mixtures of different PL classes. Considering the importance of PC for the crustaceans, the aim of the present study was to determine the influence of pure PL (980 g/ kg PC) on the growth performance, molting and fatty acid composition of *P. trituberculatus.*

2. Materials and methods

2.1. Experimental diets

Four isonitrogenous purified casein-based diets containing three phosphatidylcholine (PC, 980 g/kg) levels (5 g/kg, 10 g/kg and 20 g/kg of dry basis) and one commercial crude soybean lecithin level were formulated (designated as PC 5, PC 10, PC 20 and SL15). A PC-deficient diet was designed as a control diet. Casein was used as the protein source. Fish oil and soybean oil were used as lipid sources. Corn starch was used as the major dietary carbohydrate source. Ingredients, proximate composition and gross energy of the diets are presented in Table 1. The fatty acid compositions of the five experimental diets are given in Table 2.

All dry ingredients were ground through 250-μm mesh and mixed well in a feed mixer. Micro components were mixed with the progressive enlargement method. Oils were added to the diets and mixed for 15 min. Distilled water was then added to the mixture to produce a homogeneous dough. The experimental diets were extruded through a 2-mm and 3-mm die using a laboratory pellet machine (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China). All diets were dried at 50 °C and stored in a freezer at −20 °C until use.

2.2. Crabs and feeding trial

Juvenile crabs were obtained from the Zhejiang province Key Lab of Mariculture and Enhancement (Zhoushan, China) from one spawned batch. Prior to the feeding trial, all crabs were acclimated to indoor culture conditions for 7 days. There were three replicates (15 crabs per replicate) for each diet treatment. A total of 225 juveniles (25.4 ± 0.1 g/crab) were then randomly allocated into 225 plastic baskets ($\Phi 30 \times \Phi 40 \times H\ 40$ cm). The crabs were fed with a daily ration of 6%–8% of body weight divided into two meals per day (08:00 and 16:00 h). Mortality and molts were checked and recorded daily. Molt death syndrome (MDS) was determined according to the description of Bowser and Renée (1981), as shown in Fig. 1. The water temperature was 26.67 ± 0.78 °C, and the salinity was 27.11 ± 0.98 g/L. The experiment was conducted under a natural indoor photoperiod.