Fish and Shellfish Immunology 80 (2018) 405–415

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Chitooligosaccharide supplementation in low-fish meal diets for Pacific white shrimp (*Litopenaeus vannamei*): Effects on growth, innate immunity, gut histology, and immune-related genes expression

Samad Rahimnejad, Xiangli Yuan, Ling Wang, Kangle Lu, Kai Song, Chunxiao Zhang*

Xiamen Key Laboratory for Feed Quality Testing and Safety Evaluation, Fisheries College, Jimei University, Xiamen, 361021, China

ARTICLE INFO

Keywords:
White shrimp
Litopenaeus vannamei
Low-fish meal diet
Chitooligosaccharide
Gut health
Immune response
Antioxidant capacity

ABSTRACT

This study evaluated the effects of supplementing chitooligosaccharide (COS) in low fish meal (FM) diets on growth, immune response, intestine and hepatopancrease histology, and expression of inflammatory and immune-related genes in Pacific white shrimp (*Litopenaeus vannamei*). A basal diet was formulated using FM and soybean meal (SM) as primary protein sources and considered as a high FM (HFM) diet, then a low FM (LFM) diet was prepared by substituting 50% of FM with SM and supplemented with 0, 0.3, 0.6, 0.9, 1.2 or 1.5 g COS kg^{-1} diet (LFM, COS3, COS6, COS9, COS12 and COS15 diets). Each diet was fed to quadruplicate groups of shrimp (0.9 g) to apparent satiation three times daily for eight weeks. At the end of the experiment no significant changes in growth and survival rate were observed among treatments ($P > 0.05$). FM replacement led to significant ($P < 0.05$) reduction of serum lysozyme activity and significant improvements were obtained by adding 0.3 or 0.6 g kg^{-1} COS to the LFM diet. A significant decrease in nitric oxide synthase activity was found in LFM group and no beneficial effects could be achieved by COS application. LFM group showed higher hepatopancrease superoxide dismutase and glutathione peroxidase activities than HFM group and further enhancements were obtained by COS application. Hepatopancrease total antioxidant capacity and alkaline phosphatase activity decreased in LFM group and COS supplementation improved their values. Expression of lysozyme, crustin, Pen3 and proPo genes were significantly up-regulated in hepatopancrease of groups received 0.3–0.9 g COS kg^{-1} diet. FM substitution enhanced the expression of HSP70 and inflammatory genes such as AIF and TNF in hepatopancrease and intestine, and COS administration at a moderate level down-regulated their expression level. Remarkable enhancement in intestinal fold height was obtained by inclusion of 0.3 or 0.6 g COS kg^{-1} diet compared to the group received LFM diet. Shrimps fed HFM and COS containing diets exhibited higher number of E-cells within their hepatopancrease tubules than the LFM group. The findings in this study clearly demonstrated that COS could enhance non-specific immune response and antioxidant activity, and ameliorate the negative impacts of high SM diets on gut and hepatopancrease health in pacific white shrimp. The optimum inclusion level of COS seems to be 0.3–0.6 g kg^{-1} of diet.

1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) has been one of the most abundantly cultured shrimp species all around the globe owing to its prominent characteristics such as rapid growth, high survival rate in intensive culture, and strong disease resistance [1,2]. In order to support the progressive expansion of shrimp farming industry, development of nutritionally adequate and cost effective feed is necessary. Dietary protein is the most important and expensive component of aquafeed. Fish meal (FM) has long been used as the main dietary protein source for aquafeed formulation due to its well-balanced amino acid profile, high digestibility and serving as a rich source of vitamins, minerals and essential n-3 fatty acids [3,4]. Generally commercial shrimp feed contains 25–50% FM [5], which makes the shrimp farming industry as one of the largest consumers of FM [4,6]. However, FM price has been considerably soared during the recent decades which stems from its limited supply and increased demand [7]. Thus, reducing the reliance on FM and its replacement with less expensive and abundantly available protein sources would be inevitable for sustainable expansion of shrimp culture. In this context, plant proteins have drawn more attention as potential replacer to FM in shrimp feed [8]. However, results of studies on different shrimp species have shown the negative

* Corresponding author. Fisheries College, Jimei University, No. 43 Yindou Road, Xiamen, 361021, China.
E-mail address: cxzhang@jmu.edu.cn (C. Zhang).

https://doi.org/10.1016/j.fsi.2018.06.025
Received 5 April 2018; Received in revised form 31 May 2018; Accepted 11 June 2018

impacts of high-plant protein diets on growth performance, feed intake, antioxidant activity, intestinal health and immune response [9,10]. Currently a growing number of studies are being conducted to identify an appropriate blend of plant proteins and other alternative feed ingredients to prevent nutritional deficiencies and provide a proper supply of essential nutrients.

Chitosan has been used as a valuable source of bioactive materials in recent decades [11], however, using chitosan as a feed ingredient is limited due to its poor solubility. Chitooligosaccharide (COS) produced by subjecting chitosan to chemical and enzymatic hydrolysis, exerts higher activity and more physiological functions than chitosan due to its lower molecular weight and enhanced solubility. The results of studies on different fish species have shown the beneficial effects of dietary COS supplementation on growth performance, nutrient digestibility and small intestinal morphology [12–14]. It is assumed that COS mediates changes in digestive enzymes activity due to its low molecular weight, thus improving apparent digestion of most nutrients and intestinal health [15]. However, to the best of our knowledge, no similar research has been performed on shrimp. Therefore, a feeding trial was performed to examine the effects of COS supplementation in low FM diets on growth performance, non-specific immune response, antioxidant capacity, gut and hepatopancrease health, and expression of inflammatory and immune-related genes in pacific white shrimp.

2. Materials and methods

2.1. Experimental diets

Totally seven isonitrogenous (39% protein) and isolipidic (8% lipid) diets were produced. A basal diet was formulated using FM (30%) and soybean meal (SM) (29%) as the major protein sources and considered as a high FM (HFM) diet, then a low FM (LFM) diet was prepared by replacing 50% of FM with SM, and supplemented with 0, 0.3, 0.6, 0.9, 1.2 or 1.5 g kg^{-1} COS (ZTHtech Co., Ltd., Beijing, China) referred to as LFM, COS3, COS6, COS9, COS12 and COS15 diets, respectively (Table 1). Squid visceral paste was used as a palatability enhancer in all diets. All dry ingredients were finely ground using a hammer mill and then passed through a 250 μm mesh. All the dry ingredients were thoroughly mixed and a mash was produced after adding fish oil, soybean oil, soybean lecithin and water. Then the pellets were produced by passing the mash through a 1.5 mm die, using multifunctional spiral extrusion machinery (CD4XITS, South China University of Technology, Guangzhou, China). The pellets were dried at 35 °C in a dry oven overnight and stored at −20 °C in airtight polyethylene bags until use. Proximate composition of the experimental diets was analyzed according to the standard methods [16].

2.2. Experimental shrimp and feeding trial

Juvenile white shrimp (specific pathogen free; SPF) were obtained from a commercial hatchery in Zhangzhou (Fujian, China) and transported to the Fisheries laboratory of Jimei University (Xiamen, China). They were stocked into three 1000-L circular fiberglass tanks, acclimatized in laboratory conditions for two weeks and fed a commercial diet (Dabeinong Technology Group Co., Ltd). After the adaptation period, 50 healthy shrimp of similar size (0.9 g) were randomly stocked into each of 28 indoor circular fiberglass tanks (300 L) in a recirculating system. The recirculating system consisted of a reservoir with a biological filter, a circulation pump and an automatic temperature control device supplied with aerated water. The experiment was performed in four replicates and shrimp were hand fed with the test diets to apparent satiation three times daily (08:00, 14:30 and 19:30) for eight weeks. Uneaten diets (if any) and fecal matter were collected and 10% of water was exchanged after each feeding. During the experimental period, water temperature, salinity and pH were measured daily and their values were in the range of 27–31 °C, 30–32 g L^{-1} and 7.5–8.1, respectively. Dissolved oxygen was kept at about 7 mg L^{-1}, total ammonia concentration was bellow 0.2 mg L^{-1} and the photoperiod was natural. Feeding was stopped 24 h prior to handling and sampling to minimize the stress on shrimp.

Table 1
Formulation and proximate composition of the experiment diets (g kg^{-1}) fed to Pacific white shrimp for 8 weeks.

Ingredients	HFM	LFM	COS3	COS6	COS9	COS12	COS15
Fish meal[a]	300	150	150	150	150	150	150
Soybean meal[b]	290	425	425	425	425	425	425
Shrimp meal	50	50	50	50	50	50	50
Squid visceral paste	20	20	20	20	20	20	20
Wheat flour	263	253	252.7	252.4	252.1	251.8	251.5
Fish oil	10	10	10	10	10	10	10
Soybean oil	15	22	22	22	22	22	22
Soybean lecithin	15	15	15	15	15	15	15
Choline chloride	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5
Calcium dihydrogen phosphate	10	20	20	20	20	20	20
Mineral premix[c]	1	1	1	1	1	1	1
Vitamin premix[d]	3	3	3	3	3	3	3
Methionine	0	3.1	3.1	3.1	3.1	3.1	3.1
Lysine	0	5.3	5.3	5.3	5.3	5.3	5.3
Vitamin C	1	1	1	1	1	1	1
Mold inhibitor[e]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sodium alginate	10	10	10	10	10	10	10
Ethoxyquin	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chitooligosaccharide	0	0	0.3	0.6	0.9	1.2	1.5
Proximate composition							
Dry matter (DM)	910	917	919	922	921	914	920
In DM:							
Protein	376	388	388	380	388	381	383
Lipid	91	90	93	88	97	87	97
Ash	70	71	77	73	73	72	73

[a] Xiamen ITG group Corp., Ltd., Xiamen, China, imported from Peru (crude protein:65.3%, crude lipid:8.65%).

[b] Soybean meal, obtained from Quanzhou Fuhai cereals and oils industry Co., Ltd. (crude protein: 46.3%, crude lipid:1.0%).

[c] Vitamin premix (mg or g kg^{-1} diet): thiamin, 10 mg; riboflavin, 8 mg; pyridoxine HCl, 10 mg; vitamin B12, 0.2 mg, vitamin K3, 10 mg; inositol, 100 mg; pantothenic acid,20 mg; niacin acid, 50 mg; folic acid, 2 mg; biotin, 2 mg; retinol acetate, 400 mg; cholecalciferol, 5 mg; alpha-tocopherol,100 mg; ethoxyquin, 150 mg; wheat middling, 1.1328 g.

[d] Mineral premix (mg or g kg^{-1} diet): Na F, 2 mg; KI,0.8 mg; Co Cl_2·6H2O (1%), 50 mg; Cu SO_4· $5H_2O$, 10 mg; $FeSO_4$· H_2O, 80 mg; $ZnSO_4$·H_2O, 50 mg; Mn SO4·H2O,25 mg; $MgSO_4$· $7H_2O$, 200 mg; Zoelite, 4.582 g.

[e] 50% calcium propionic acid and 50% fumaric acid.

2.3. Sample collection and analyses

At the end of the feeding trial, the total number and bulk weight of shrimps in each tank were determined for calculation of growth parameters and survival rate. Then, 25 shrimp from each tank (100 shrimp per dietary treatment) were randomly selected and hemolymph was withdrawn from the ventral sinus of each shrimp using sterilized 1-mL syringes, pooled in 1.5-mL eppendorfs and kept at 4 °C overnight. Then serum was separated following centrifugation at 4000 × *g* at 4 °C for 10 min and kept at −80 °C for subsequent analysis. The serum samples were used for measurement of biochemical [glucose, cholesterol, LDL-Cholesterol, HDL-Cholesterol and total protein (TP) concentrations, and alkaline phosphatase (ALP) and acid phosphatase (ACP) activities], immune [lysozyme, nitric oxide synthase (NOS) and phenoloxidase activities (PO)] and antioxidant [superoxide dismutase (SOD) activity