Aquaculture 372–375 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Aquaculture

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

Comparison of effect of chitin, chitosan, chitosan oligosaccharide and N-acetyl-D-glucosamine on growth performance, antioxidant defenses and oxidative stress status of *Penaeus monodon*

Jin Niu [a,*], Hei-Zhao Lin [a], Shi-Gui Jiang [a], Xu Chen [a], Kai-Chang Wu [a], Yong-Jian Liu [b], Sheng Wang [c], Li-Xia Tian [b,**]

[a] Key Laboratory of Aquatic Product Processing, Ministry of Agriculture, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou 510300, PR China
[b] Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou, 510275, PR China
[c] Guangdong Haid Research Center of Husbandry and Aquaculture,511400, PR China

ARTICLE INFO

Article history:
Received 9 July 2012
Received in revised form 12 October 2012
Accepted 15 October 2012
Available online 25 October 2012

Keywords:
Penaeus monodon
Chitin
Chitosan
Chitosan oligosaccharide
N-acetyl-D-glucosamine

ABSTRACT

Two trials were conducted to evaluate the effects of chitin and its derivatives (chitosan, chitosan oligosaccharides and N-acetyl-D-glucosamine) on shrimp, *Penaeus monodon*, first on growth performance, secondly on antioxidant defenses and oxidative stress status of digestive gland. In the first experiment (trial 1), Shrimp (mean initial wet weight 1.49 g) were fed with five diets (control diet; chitin diet; chitosan diet; chitosan oligosaccharides diet and N-acetyl-D-glucosamine diet) in triplicate for 70 days. Growth performance (final body wet weight, FBW; weight gain, WG; biomass gain, BG) of shrimp fed chitosan diet was higher ($P<0.05$) than that of shrimp fed the other diets. Survival of shrimp in chitosan diet groups was higher ($P<0.05$) than that of shrimp in control, chitosan oligosaccharides and N-acetyl-D-glucosamine diet groups, but no statistical difference was found in survival of shrimp in chitin and chitosan diet groups. The total antioxidant status (TAS) and glutathione peroxidase (GSH-Px) activity of shrimp fed chitin, chitosan and chitosan oligosaccharides diets were higher ($P<0.05$) than those of shrimp fed control and N-acetyl-D-glucosamine diets. The superoxide dismutase (SOD) activity of shrimp fed control diet was higher ($P<0.05$) than that of shrimp fed other diets. Digestive gland malondialdehyde (MDA) and carbonyl protein contents of shrimp fed chitin and chitosan diets were lower that those of shrimp fed other diets ($P<0.05$). A low dissolved oxygen (DO) stress test was conducted for 7-day after the rearing trial (trial 2). The antioxidant response was characterized by higher TAS and higher antioxidant enzyme activities (SOD, PO, GSH-Px) and higher oxidative stress levels (MDA and carbonyl protein contents) in the digestive gland compared to levels found in trial 1. Survival of shrimp fed chitin and chitosan diets was higher ($P<0.05$) than that of shrimp fed other diets after trial 2. The glutathione (GSH) content, TAS, PO and GSH-Px activities of shrimp fed chitosan diet were higher ($P<0.05$) than those of shrimp fed other diets. The SOD activity of shrimp fed chitosan diet was lower ($P<0.05$) than that of shrimp fed control, chitosan oligosaccharides and N-acetyl-D-glucosamine diets, but no difference ($P>0.05$) was found between shrimp fed chitin and chitosan diets. Moreover, the oxidative stress levels (MDA and carbonyl protein contents) recorded in the digestive gland with shrimp submitted to the chitin and chitosan diets were lower. In conclusion, results suggested that dietary intake containing chitin or chitosan could enhance the growth performance of *P. monodon* and improve its resistance to DO stress.

1. Introduction

Marine shrimp, like other aquatic animals, are constantly threatened by micro-organisms such as fungi, bacteria and protozoa, which can greatly affect their health and well-being. Until recently, disease management strategies were based mainly on chemotherapy (Aoki, 1992). In most cases, water is disinfected before use and antibiotics are used to protect cultured species from microbial infections (Vadstein, 1997). Such practices have led to promote the spread of antibiotic-resistant pathogens in both cultured species and in the environment (Kesarcodi-Watson et al., 2008). Moreover, the emergence of drug resistance in pathogens, problems associated with drug residues in cultured shrimp, and awareness toward environmental pollution problems emanating from the use of chemotherapeutants have led to greater focus on alternative methods of disease management (Tonguthai and Chanratchakool,

Abbreviations: BG, biomass gain; DO, dissolved oxygen; FBW, final body wet weight; FCR, feed conversion ratio; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; PO, phenoloxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TAS, total antioxidant status; WG, weight gain.

* Corresponding author. Tel.: +86 20 84457012; fax: +86 20 84451442.
** Corresponding author. Tel.: +86 20 84110789; fax: +86 20 84115896.
E-mail addresses: gzniujin2003@163.com (J. Niu), edls@mail.sysu.edu.cn (L.-X. Tian).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2012.10.021

1992). Except for pathogen pressure, reared shrimp are also subject to temperature, dissolved oxygen, salinity changes and other environmental perturbations which can severely affect their physiological state (Le Moullac and Haffner, 2000; Niu et al., 2011; Wang and Chen, 2005). An emerging field of study in physiology of aquatic species is therefore increasingly focusing on "oxidative stress", especially since many researchers have reported the effect of environmental perturbations on oxidative stress. Such studies include salinity stress (Chien et al., 2003), thermal stress (Chien et al., 2003), NH3 stress (Pan et al., 2003), dissolved oxygen depletion stress (Niu et al., 2011) being linked to changes to physiological states of shrimps.

Oxidative stress results from either increased exposure or production by the organism of reactive oxygen species (ROS) or from a decrease in the antioxidant defenses due to exposure, resulting in oxidative damage to lipids, protein and nucleic acid. Lipid peroxidation is a ubiquitous phenomenon in the body under the influence of oxidative stress (Ozturk et al., 2003). ROS have been implicated as major initiators of tissue damage and can up regulate enzyme activity, signal transcription, and gene expression (Massafra et al., 2000). ROS production is also implicated in the immune response mechanism to both prokaryotic and eukaryotic pathogens (Adema et al., 1991). The antioxidant defense system and immune system are closely linked to responses due pathogens and other stress-related issues that might lead to respiratory burst (Holmblad and Söderhäll, 1999). It has even been declared that the antioxidant and oxidative responses could become useful parameters for assessing the immune response in cultured organisms exposed to given environmental perturbations (Campa-Cordova et al., 2005).

It is known that use of immunostimulants increase the non-specific immunity and survival in fish culture (Anderson, 1992). The immune stimulatory effects of immunostimulants like chitin, chitosan and other polysaccharides have been widely studied in fish (Cuesta et al., 2003; Dautremepuits et al., 2004), but few have focused on crustacean, let alone the comparative effect of chitin and its derivatives (chitosan, chitosan oligosaccharides and N-acetyl-D-glucosamine) on growth performance and antioxidant defenses of penaeid shrimp. Chitin is a natural polymer found abundantly in the shells of crustaceans, insects, and in fungi. Chitosan is a linear homopolymer of b-(1,4)-2-amino-2-deoxy-D-glucose and is prepared by the alkaline deacetylation of chitin obtained from shrimp and crab shells and has many applications in medicine, agriculture and aquaculture. Degradation of O-glycosidic linkages of chitosan by different methods leads to the production of chitosan oligosaccharides varying in the degree of polymerization as well as number and sequences of glucosamine and N-acetyl-D-glucosamine units (Chen et al., 2010).

The aim of the present study was to evaluate and compare, firstly, the possible effect of dietary chitin and its derivatives (chitosan, chitosan oligosaccharides and N-acetyl-D-glucosamine) supplementation on the growth performance of *Penaeus monodon*. Secondly, the possible effect of dietary chitin and its derivatives on the antioxidant defenses and oxidative stress status in *P. monodon*, this effect was evaluated in two different situations: normal rearing shrimp and shrimp under low DO stress. We also looked at oxidative damage to lipids and proteins to evaluate oxidative stress status by measuring respectively MDA, which is a commonly used indicator to evaluate lipid peroxydation, and carbonyl proteins which is recently being used as biomarker of oxidative damage to protein in fish (Parvez and Raisuddin, 2005).

2. Materials and methods

2.1. Experimental diets

In this study, two trials (trial 1 and trial 2) were conducted. The formulation and proximate composition of the five experimental diets are presented in Tables 1 and 2. Chitin (C7170), chitosan (C3646), chitosan oligosaccharides (523682) and N-acetyl-D-glucosamine (A4106) were purchased from Sigma (St Louis, Mo, USA) and added to the basal diet all at inclusion of 0.4%. The method of diet preparation was the same as described by Niu et al. (2011). Briefly, all the dry ingredients of the experimental diets were weighed, combined and thoroughly mixed to homogeneity in a Hobart-type mixer. Oil was then added and thoroughly mixed for 5 min. Deionized water (40% dry ingredients mixture) was added and mixed for another 5 min. The wet mixture was placed in a monoscrew extruder (Institute of Chemical Engineering, South China University of Technology, Guangzhou, P.R. China) and extruded through a 1.2-mm die. The resulting pellets were dried at 25 °C with the aid of an air conditioner and an electrical fan. All the diets were stored at −20 °C until used.

Table 1
Composition of the basal diet.

Ingredients	%
White fish meal[a]	31
Soybean meal[b]	19
Peanut bran[b]	17.30
Wheat flour[b]	20
Beer yeast[b]	5
Soybean lecithin[b]	1
Soybean oil[b]	1
Fish oil[c]	1
Choline chloride (50%)	0.6
Monocalcium phosphate	1
Vitamin premix[d]	1
Mineral premix[d]	1
Ascorbic Phosphate ester	0.1
Sodium alginate	1

[a] Imported from N.E.L.T.O.Australia Pty Ltd.
[b] Zhuhai Shihai Feed Corporation Ltd, Zhuhai, China.
[c] Imported from New Zealand (Bakels Edible Oils Ltd, Mt Macnganui).
[d] Vitamin premix (g kg^{-1}): h-Carotene, 3 M.I.U.; Cholecalciferol, 0.6 M.I.U.; Thiamin, 3.6; Riboflavin, 7.2; Pyridoxine, 6.6; Cyanocobalamine, 0.02; a-Tocopherol, 16.5; Menadione, 2.4; Niacin, 14.4; Pantothenic acid, 4; Biotin, 0.02; Folic acid, 1.2; Inositol, 30; Ascorbic acid, 100; cellulose was used as a carrier (according to Bautista-Teruela et al. (2003)).
[d] Mineral premix (g kg^{-1}): P, 120; Ca, 120; Mg, 15; Fe, 1.5; Zn, 4.2; Cu, 2.1; K, 75; Co, 0.11; Mn, 1.6; Se, 0.01; Mo, 0.005; Al, 0.025; I, 0.4; cellulose was used as a carrier (according to Bautista-Teruela et al. (2003)).

2.2. Shrimp and experimental set up

In trial 1, juvenile *P. monodon* were obtained from a semi-intensive culture pond near Hongsha Bay, Sanya, Hainan province, China. Shrimp were acclimated to the experimental conditions and fed the basal diet for 2 weeks before the experiment started. A total of 450 shrimp with an initial body weight of 1.49 g were distributed randomly into 15 fiberglass tanks (500 l, 0.5 m^2 bottom, 3 tanks per diet, 30 shrimp per tank). Water exchange in each tank was adjusted to approximately

Table 2
Formulation and proximate composition of each diet (% dry matter).

Basal diet	100	99.6	99.6	99.6	99.6
Chitin	0	0.4	0	0	0
Chitosan	0	0	0.4	0	0
Chitosan oligosaccharide	0	0	0	0.4	0
N-Acetylglucosamine	0	0	0	0	0.4
Proximate composition					
Moisture	9.43	9.33	9.46	9.35	9.47
Crude protein	43.07	43.02	43.23	43.36	43.35
Crude lipid	7.17	7.20	7.24	7.20	7.24
Ash	9.16	9.11	9.14	9.16	9.13