Lat. Am. J. Aquat. Res., 39(3): 534-543, 2011
DOI: 10.3856/vol39-issue3-fulltext-13

Research Article

Enhancement of superoxide dismutase and catalase activity in juvenile brown shrimp, *Farfantepenaeus californiensis* (Holmes, 1900), fed β-1.3 glucan vitamin E, and β-carotene and infected with white spot syndrome virus

Rosario Pacheco[1], Felipe Ascencio[1], Martha Zarain[2], Gracia Gómez[1] & Ángel Campa[1]
[1]Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195
Colonia Playa Palo de Santa Rita, La Paz, Baja California Sur, 23096, México
[2]Centro de Ciencias de Sinaloa, Avenida de las Américas 2771 Norte
Colonia Villa Universidad, Culiacán, Sinaloa 80010, México

ABSTRACT. The effect of dietary β-1.3-glucan, vitamin E, and β-carotene supplements in juvenile brown shrimp, *Farfantepenaeus californiensis*, inoculated with white spot syndrome virus (WSSV) was evaluated. Groups of 30 organisms (weighing 1 ± 0.5 g) were cultured in 60 L fiberglass tanks and fed daily with β-1.3-glucan (0.1%), vitamin E (0.01%), and β-carotene (0.01%) for 23 days; the specimens were then inoculated with WSSV. The antioxidant activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) were determined in the hepatopancreas and muscle at 0, 1, 6, 12, 24, and 48 h after inoculation. Shrimp fed with β-1.3-glucan, vitamin E, and β-carotene significantly increased SOD activity in the hepatopancreas and muscle at 12 and 24 h post-infection, respectively. Shrimp fed with vitamin E and β-1.3-glucan registered an increment in SOD activity from 12 to 48 h post-infection. Shrimp fed with β-carotene increased SOD activity before infection with WSSV, and shrimp fed with β-1.3-glucan and vitamin E increased CAT activity, also before infection. The CAT activity response in shrimp muscle increased with respect to the control group for all treatments tested from 1 to 6 h after inoculation with WSSV. The highest antioxidant response was registered in shrimp fed with vitamin E. Juvenile shrimp fed with vitamin E and later inoculated with WSSV registered 100% mortality at 72 h, but shrimp fed with β-1.3-glucan and β-carotene showed greater resistance to WSSV, with mortality at 144 h post-infection. This study demonstrated the capacity of juvenile *Farfantepenaeus californiensis* fed β-1.3-glucan, vitamin E, or β-carotene to increase the antioxidant response before and after viral infection.

Keywords: *Farfantepenaeus californiensis*, shrimp, WSSV, superoxide dismutase, catalase.

Incremento de la actividad superóxido dismutasa y catalasa en juveniles de camarón café *Farfantepenaeus californiensis* (Holmes, 1900) alimentados con β-1,3 glucano vitamina E y β-caroteno e infectados con el virus de la mancha blanca

RESUMEN. Se evaluó el efecto de β-1,3-glucano, vitamina E y β-caroteno en la dieta de juveniles de camarón café *Farfantepenaeus californiensis* inoculados con virus del síndrome de la mancha blanca (WSSV). Se colocaron grupos de 30 camarones (peso 1 ± 0,5 g) en contenedores de fibra de vidrio de 60 L y se alimentaron diariamente durante 23 días con β-1,3-glucano (0,1%), vitamina E (0,01%), y β-caroteno (0,01%) y posteriormente se inocularon con WSSV. Se determinó la actividad antioxidante de la enzima superóxido dismutasa (SOD) y catalasa (CAT) en hepatopáncreas y músculo a las 0, 1, 6, 12, 24 y 48 h después de la infección. Los grupos de camarones alimentados con los tratamientos incrementaron la actividad SOD en el hepatopáncreas y músculo a las 12 y 24 h después de la infección, respectivamente. Los juveniles tratados con vitamina E y β-1,3-glucano mantuvieron un incremento en la actividad SOD desde las 12 a 48 h post-infección. Los camarones alimentados con β-caroteno incrementaron la actividad de SOD antes de la infección con WSSV y los que fueron alimentados con β-1,3-glucano y vitamina E incrementaron la actividad CAT también antes de la infección. La actividad CAT en músculo se incrementó respecto al grupo control, con todos los grupos de camarones tratados desde 1 hasta 6 h posteriores a la inoculación con WSSV. La actividad antioxidante más alta se registró en los camarones alimentados con vitamina E. Los juveniles alimentados con vitamina E y posteriormente inoculados con WSSV, registraron 100% de mortalidad a las 72 h, pero los que fueron alimentados con β-1,3-glucano y β-caroteno resistieron la infección hasta las 144 h. Los resultados de

este estudio mostraron la capacidad de juveniles de *Farfantepenaeus californiensis* alimentados con β-1,3-glucano, vitamina E o β-caroteno, de incrementar la respuesta antioxidante antes y durante una infección viral.

Palabras clave: *Farfantepenaeus californiensis*, camarón, WSSV, superóxido dismutasa, catalasa.

Corresponding author: Ángel Campa (angcamp04@cibnor.mx)

INTRODUCTION

White spot syndrome virus (WSSV) infection is considered one of the most devastating diseases and is responsible for severe economic losses in shrimp culture industry worldwide (Lightner, 1996). WSSV is highly pathogenic and has a broad host range within decapods crustaceans, it has been found in at least 18 cultured or wild penaeid shrimp species (Durand *et al*., 1997; Lightner *et al*., 1998) as well as copepods, marine crabs, prawn and freshwater crabs, rotifer, polychaete worms and some aquatic insect larvae species (Lo *et al*., 1996b; Flegel, 1997; Ramírez-Douriet *et al*., 2005; Escobedo-Bonilla *et al*., 2008). The brown shrimp, *F. californiensis*, is a commercially important fishery in México, and is being studied as an alternative in tropical shrimp culture or as an alternate culture during the winter season in Mexican northern regions, with extreme climate changes (Martínez-Córdova *et al*., 1998).

Investigations on the antioxidant system in shrimp have shown that WSSV infection causes oxidative stress by forming excessive amounts of reactive oxygen species (ROS) which are involved in phagocytosis activation (Schwarz, 1996; Mohankumar & Ramasamy, 2006). The phagocytic process is the main cellular defense reaction, and together with humoral components, constitutes the first line of defense of crustaceans against infectious microorganism like bacteria, fungi and viruses (Soderhäll & Cerenius, 1992; Lee & Söderhäll, 2001). This process consists of chemotaxis, adherence, ingestion, pathogen destruction, and exocytosis (Vargas-Albores & Yepiz-Plascencia, 1998). Phagocytic cells destroy the internalized organisms by two routes: (1) an aerobic process which will be described in detail herein subsequently, and (2) other anaerobic process that is attributed to the action of diverse microbicidal enzymes, such as lysozyme and low molecular weight AMP (Nappi & Ottaviani, 2000). The aerobic process uses NADPH or NADH as an electron donor, and reduces an oxygen electron to form a radical superoxide ion. This radical in turn changes to hydrogen peroxide (H_2O_2) spontaneously or by the action of the superoxide dismutase (SOD), which can easily diffuse through the cell membranes into the cytoplasm where the antioxidant catalase (CAT) reduce the hydrogen peroxide producing a new oxygen molecule and thereby detoxify ROS (Mohankumar & Ramasamy, 2006; Aguirre-Guzmán *et al*., 2009).

The principal ROS molecules are: hydroxyl radical (OH^-), superoxide anion (O_2^-), transition metals such as iron and copper, nitric oxide (NO) and peroxynitrite ($ONOO^-$) (Dormandy, 1983). Although, the ROS are crucial to normal biological processes, they can cause direct cellular injury by including lipid and protein peroxidation and damage to nucleic acid (Richard *et al*., 1990). Thus, there is a critical balance in cell between ROS generation and antioxidant defense systems to protect themselves against free radical toxicity and to maintain the cellular homeostasis (Sies, 1991; Winston & Di Giulio, 1991; Livingstone, 2001). These systems also include both enzymatic and nonenzymatic components, where the enzymatic system is comprised of superoxide dismutase (SOD), and CAT acting on O_2^- and H_2O_2, respectively (Halliwell & Gutteridge, 1996). The nonenzymatic system includes small water-soluble molecules such as vitamin C, as well as lipid-soluble molecules such as carotenoids and vitamin E (Packer, 1991; Liu *et al*., 2007). The shrimp antioxidant system can be activated by exposure to different immunos-timulants as sodium alginate (Cheng *et al*., 2005), zymosan A (Zhang *et al*., 2008), lipopoly-saccharides (LPS), fucoidan, heat-killed *Vibrio penaeicida* (Campa-Córdova *et al*., 2005).

The uses of immunostimulants, such as β-glucan, have been successfully applied to enhance resistance of fishes and crustaceans against bacterial and viral infections (Chang *et al*., 2000, 2003). Previous studies have been demonstrated that shrimp fed with glucan diets increased activity of SOD than those of a glucan-free group before challenge with pathogenic WSSV and bacteria (Chang *et al*., 2003; Campa-Córdova *et al*., 2005). However, these studies only considered SOD and did not consider other antioxidants enzymes such as CAT. The antioxidant activity is also reinforced by several molecules of nutritional interest that are of a chemical structure compatible with the antioxidant properties found *in vivo*, such as pigments and vitamins, which are also capable of modulating

the cellular response to ROS (Muñiz-Rodríguez, 2009). Vitamin E is considered the most important antioxidant in extracellular fluids, since it is capable of protecting polyunsaturated fatty acids from peroxidation and it has the ability to scavenge oxygen-derived free radicals (Wang *et al*., 2006; Liu *et al*., 2007). Likewise, carotenoids also improve antioxidant defense system (SOD and CAT), protecting the body against free radicals (Flores-Leyva, 2006). Additional benefits of carotenoids include provitamin A activity, as well as enhancing immune response, reproduction, growth, maturation, photoprotection, and defense against hypoxic conditions common in pond cultures of prawns (Lorenz, 1998).

The antioxidant effects of vitamins and carotenoids have poorly been evaluated in shrimp. In addition, antioxidant activity with immunostimulants, vitamins, and carotenoids in brown shrimp (*F. californiensis*) has not been previously evaluated, although it has been shown to be beneficial in other species of shrimp. Thus, the present work was designed to evaluate the effects of dietary β-1.3-glucan, vitamin E and β-carotene supplementation on the antioxidant activity in muscle and hepatopancreas of *F. californiensis* infected with WSSV.

MATERIALS AND METHODS

Experimental animals

Juvenile brown shrimp (*F. californiensis*) (1 ± 0.5 g) were captured in Bahía de La Paz, Baja California Sur, México. Before the experiments, the animals were acclimated during 15 days in an aerated 1500 L fiberglass tank containing filtered (0.2 μm) seawater, salinity at 35 g L^{-1}, pH 7.8, and the temperature was maintained at 25°C. Tank water was changed at the rate of 50% daily. Shrimp were fed *ad libitum* daily with commercial feed (PIASA, 35% of protein level).

Diet preparation

The composition of the experimental diet is described in Table 1. The diets were prepared at the Aquatic Nutrition Laboratory of the Centro de Investigaciones Biológicas del Noroeste (CIBNOR), the formulation was similar to that described by Villarreal *et al*. (2004) for *F. californiensis*. The following were the supplemental compounds used to formulate three experimental diets: β-1.3-glucan from *Laminaria digitata* (Sigma, Cat. Nº L-9634); dl-α-tocopheryl acetate (vitamin E) (Sigma, Cat. Nº T-3376) and β-carotene (Sigma, Cat. Nº C-9750). The immunostimulants were supplemented separately to the test diets at doses of 100 mg kg^{-1} vitamin E (Fernández-Giménez *et al*., 2004), 100 mg kg^{-1} β-carotene (Flores-Leyva, 2006) and 10 g kg^{-1} β-1.3-glucan (Chang *et al*., 2003). All ingredients were thoroughly mixed with cod liver oil and water was added to produce pellet of 2 mm in diameter. Feed was dried at 30°C during 3 h. After drying, the diet was stored at -20°C in dark plastic bags during the experiment.

Proximal analysis of the experimental diets

The different diets were determined in triplicate moisture (AOAC, 1995, Nº 930.15), crude protein (AOAC, 1995, Nº 976.05), ether extract (AOAC, 1995, N° 920.39), crude fiber (AOAC, 1995, Nº 962.09), ash (AOAC, 1995, Nº 942.05) and gross energy (cal g^{-1}) in an adiabatic calorimeter (Parr 1261).

Preparation of the WSSV stock solution

Viral extract was prepared following the protocol described by Huang *et al.* (2001), using shrimp tail muscle that tested positive for WSSV by PCR. Infected tissue was homogenized in TN buffer (Tris-HCl 10 mM, NaCl 400 mM, pH 7.4, 1:5 w/v) and centrifuged at 5,500 x g for 20 min. The supernatant was passed through a 0.45 μm-pore-size filter and then passed through a 0.2 μm-pore size syringe filter (Acrodisc, Pall, Port Washington, NY) to generate aliquots that were stored at -80°C until used. The results of preliminary experiments indicated that the optimal inoculums level of the WSSV stock solution to induce 100% mortality within 72 h in juvenile *F. californiensis* through intramuscular injection was 20 μL per shrimp.

Experimental protocol

Groups of 30 shrimp were kept in 60 L fiberglass containers. The experiment was designed with four treatments in triplicate: (1) shrimp fed with basal diet without additives (control group); (2) shrimp fed with β-1.3-glucan (10 g kg^{-1}); (3) shrimp fed with vitamin E (100 mg kg^{-1}); (4) shrimp fed with β-carotene (100 mg kg^{-1}). Shrimp were fed twice daily at 8:00 and 17:00 h during 23 days. At the end of the experimental feeding, shrimp from all treatments were injected intramuscularly with 20 μL virus inoculum per shrimp. Four randomly chosen shrimp per treatment were sampled at 0 (before inoculation), 1, 6, 12, 24 and 48 h post-inoculation and stored at -80°C until biochemical analysis. The experimental diets were continued after the inoculation until the end of the experiment, and mortality percentage was determined (López *et al*., 2003). Pleopods of sampled shrimp were excised and placed in 1.5 mL microcentrifuge tubes and stored at -20°C until WSSV diagnosis by PCR.

Table 1. Nutrimental composition of experimental diets.

Tabla 1. Composición nutrimental de las dietas experimentales.

Ingredient (g/100 g diet)	Control	β-1.3-glucan	β-carotene	Vitamin E
Fish meal[a]	29.5	29.5	29.5	29.5
Wheat meal[b]	40.25	40.25	40.25	40.25
Soybean meal[b]	20.0	20.0	20.0	20.0
Cod fish oilb	2.46	2.46	2.46	2.46
Soybean lecithin[b]	1.5	1.5	1.5	1.5
Alginic acid[c]	2	2	2	2
Vitamin mix[d]	2.1	2.1	2.1	2.1
Dibasic sodium Fosfate[c]	1.2	1.2	1.2	1.2
Mineral mix[d]	0.5	0.5	0.5	0.5
Cholesterol[c]	0.5	0.5	0.5	0.5
β-1.3-glucan[c]		0.1		
β-carotene[c]			0.01	
Vitamin E[c]				0.01

[a]Tuna by-product meal (Productos Pesqueros de La Paz, BCS., México).

[b]Wheat meal, soybean meal and cod fish oil (Promotora Industrial de Acuasistemas, S.A. de C.V. La Paz, BCS., México)

[c]From Sigma-aldrich, USA.

[d]Vitamin mix (composition mg kg^{-1}): A (retinol): 0.516, B1 (thiamin): 150, B2(riboflavin): 100, pantothenic acid: 100, niacinamide (nicotinic acid): 300, B6 (pyridoxine): 50, folic acid: 20, B12 (cyanocobalamin): 0.1, biotin: 1.0, C (ascorbic acid): 0.09, D3 (colecalciferol): 0.06, E (tocoferol): 400, K (menadione): 20, choline: 0.06

[d]Mineral mix (composition g kg^{1}): NaHPO4 = 0.8295, KI = 0.0175, MgSO4•7H2O = 0.500, CuCl2• 2H2O = 0.00175, ZnSO4 •7H2O = 0.0315,CoCl2 •2H2O50.000875, MnCl2•4H2O = 0.00805, KCl = 0.50

WSSV detection by PCR

The PCR reactions were performed according to the technique described by Lo *et al*. (1996a), and following the recommendations of the manufacturer of commercial kit IQ-2000 (Farming Intelligene Tech Corp, USA).

Antioxidant activity

Frozen hepatopancreas and muscle were thawed, dissected, and 100 mg fragment of each tissue were homogenized with a pestle in 1.5 mL microcentrifuge tubes containing 1mL phosphate buffer (50 mM, pH 7.0). The homogenate was centrifuged at 13,000 x g for 10 min at 4°C. Supernatant was removed and stored at -80ºC.

Catalase activity was measured by following the kinetic of reduction of hydrogen peroxide at 240 nm using the extinction coefficient 0.04 mm cm^{-1} (Downs *et al*., 2001). The kinetic was determined measuring the absorbance at 240 nm in a BioMate 3 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc. USA). Relative enzyme activity (RCAT) was expressed as the ratio of the specific activity of treated shrimp to that of controls and was used as an index of CAT activity.

Superoxide dismutase activity was determined according the method described by Beauchamp & Fridovich (1971) using nitro blue tetrazolium (NBT) in the presence of riboflavin. The absorbance numbers were input into an in-house generated computer program described elsewhere (Vázquez-Juárez *et al*., 1993) to calculate specific activity (units per milligram of protein). Relative enzyme activity (RSOD) was expressed as the ratio of the specific activity of treated shrimp to that of controls and was used as an index of SOD activity.

Statistical analysis

One-way analysis of variance (ANOVA) using the *F* test was applied to analyze the differences among treatments and the control. When ANOVA differences occurred, Tukey *post-hoc* test was used to identify the nature of these differences. Values were significantly different at $P < 0.05$.

RESULTS

Proximal analysis of the experimental diets

Table 2 shows the proximal analysis (dry basis) of all treatments. The chemical composition of the six diets was similar, varying only in the inclusion levels of experimental food additives.

Activity of antioxidant enzymes

All shrimp fed with the treatments during 23 days showed a significant ($P < 0.05$) increase in SOD activity in hepatopancreas at 12 h post-infection (Fig. 1a). RSOD increased significantly ($P < 0.05$) in muscle at 24 h post-infection with all treatments tested (Fig. 1b). From 12 to 48 h, shrimp exposed to vitamin E and β-1.3-glucan induced significant ($P < 0.05$) RSOD increase in muscle over the control. Only shrimp fed with β-carotene increased significantly ($P < 0.05$) SOD activity in muscle than the control group at 0 h (before infection).

CAT activity in hepatopancreas increased signify-cantly over the control in shrimp fed with vitamin E and β-1.3-glucan before infection (Fig. 2a). Shrimp fed with β-1.3-glucan show high and significant level of RCAT in hepatopancreas at 1 h after infection.

Significant increase in RCAT occurred in muscle with all doses tested over the control at 1 and 6 h post-infection (Fig. 2b). Shrimp exposed to β-carotene and vitamin E increased significantly RCAT from 1 to 12 h post-infection. The highest increase of antioxidant response was registered in shrimp exposed to vitamin E (3.5 times than the control) at 12 h post-challenge (Fig. 2b). Table 3 resume SOD and CAT values in hepatopancreas and muscle of juvenile *F. californiensis*.

Shrimp mortality after WSSV infection

All shrimp fed with the treatments were susceptible to WSSV infection. Clinical signs of infection were presented as reduction in feed intake and lethargy at 6 h post-challenge. PCR results were positive to all infected shrimp. Groups of shrimp fed with vitamin E, β-carotene, and control, showed earliest mortalities at 12 h and β-1.3-glucan at 48 h after the challenge with WSSV (Fig. 3). Feeding juvenile shrimp with β-1.3-glucan and β-carotene showed longest resistance to WSSV infection reaching 90 and 100% of mortality at 144 h, respectively. Organisms fed with vitamin E and the control reached 100% mortality at 72 h after WSSV infection (Fig. 3).

DISCUSSION

Some authors have reported that the administration of β-glucans enhance the antioxidant and immune response in shrimp (Campa-Córdova *et al*., 2002; Zhang *et al*., 2005; Wang *et al*., 2008). Accumulation of SODs in response to oxidative stress caused by biological agents is one of the main antioxidant defense pathways. Increased levels of SOD have been linked to induce oxidative stress (Fridovich, 1995). Campa-Córdova *et al*. (2005) exposed juvenile shrimp (*Litopenaeus vannamei*) to β1.6-glucan by immersion and posterior inoculation with *Vibrio penaeicida*. They reported increased SOD activity in muscle 2.5 times than the control at 48 h post-infection, similar to our study (Fig. 1b). Glucans are capable of enhancing resistance against various pathogens, including white spot syndrome virus (WSSV) infection in tiger shrimp (Chang *et al*., 1999), as well as *Vibrio damsela* infection in post-larvae of black tiger shrimp (Su *et al*., 1995; Liao *et al*., 1996). Oral administration of β-glucans has been reported to increase the resistance of *Penaeus monodon* against to WSSV infection (Chang *et al*., 2003). However, dose-time interaction effect of glucans has not been investigated so far (Sahoo *et al*., 2008). Sritunyalucksana *et al*. (1999) found that *P. monodon* fed with a diet containing 10 g kg^{-1} of β1.3-glucan for 20 days increased resistance to WSSV. In this study, shrimp fed with β1.3-glucan or β-carotene

Table 2. Proximate analysis of nutrimental composition of experimental diets.

Tabla 2. Análisis proximal de la composición nutricional de las dietas experimentales.

Sample	Moisture (%)	Protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	NFE[1] (%)	Gross energy (cal/g^{-1})
Control	7.5 ± 0.05	36.5 ± 0.34	8.3 ± 0.20	0.5 ± 0.07	9.0 ± 0.18	45.71	4535.9 ± 13
β-carotene	7.1 ± 0.15	36.3 ± 0.18	8.3 ± 0.21	0.6 ± 0.10	9.0 ± 0.06	45.78	4435.5 ± 25
β-glucan	6.9 ± 0.05	36.2 ± 0.42	8.3 ± 0.22	1.0 ± 0.21	9.0 ± 0.03	45.62	4619.4 ± 17
Vitamin E	6.8 ± 0.24	36.6 ± 0.16	8.3 ± 0.25	0.7 ± 0.08	9.5 ± 0.72	45.16	4507 ± 15

[1]NFE= nitrogen free extract.

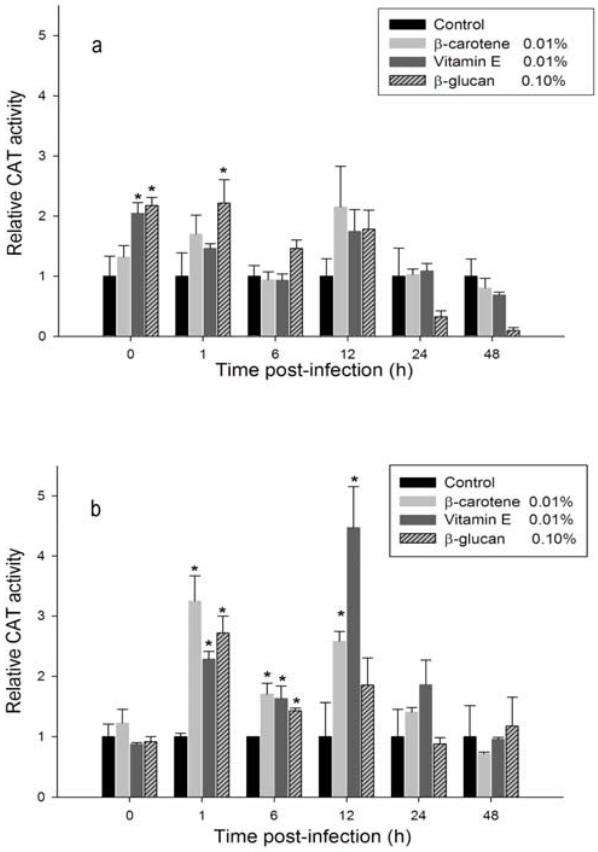

Figure 1. a) Relative SOD activity in hepatopancreas, and b) muscle of juvenile *F. californiensis* infected with WSSV. Error bars: mean ± standard deviation. *Significantly different than control ($P < 0.05$).

Figura 1. a) Actividad relativa de SOD en hepatopancreas, y b) músculo de juveniles de *F. californiensis* infectados con WSSV. Barras de error: media ± desviación estándar. *Significativamente diferente al grupo control ($P < 0,05$).

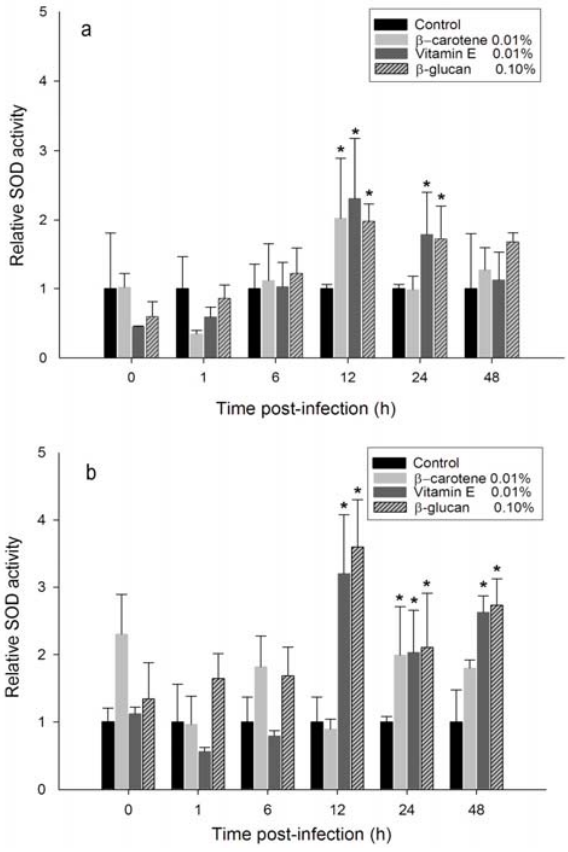

Figure 2. a) Relative CAT activity in hepatopancreas, and b) muscle of juvenile *F. californiensis* infected with WSSV. Error bars: mean ± standard deviation. *Significantly different than control ($P < 0.05$).

Figura 2. a) Actividad relativa de CAT en hepatopancreas, y b) músculo de juveniles de *F. californiensis* infectados con WSSV. Barras de error: media ± desviación estándar. *Significativamente diferente al grupo control ($P < 0,05$).

showed significant ($P < 0.05$) lowest values of mortality between 24 and 120 h post-infection (Fig. 3). Shrimp fed with vitamin E increased antioxidant response during WSSV infection, but showed low survival (Fig. 3). Fernández-Giménez *et al*. (2004) recommended 100 mg kg^{-1} of vitamin E to brown shrimp *F. californiensis* to improve growth in ponds. However, Lee & Shiau (2004) concluded that 179 mg kg^{-1} improve growth and immune response in shrimp.

The capacity of shrimp hepatopancreas or muscle to generate antioxidant activity following challenge with WSSV registered indexes from 1.8 to 4.5 times higher than the control. In addition, juvenile shrimp reached maximum antioxidant activity in muscle (Fig. 2b, Table 3). Thus, particular physiological conditions in tissue may cause differences in the capacity of the antioxidant response (Downs *et al*., 2001). In this study, catalase activity was higher in muscle than in hepatopancreas. In contrast, Tavares-Sánchez *et al*. (2004) did not find gene expression of catalase in muscle of the shrimp *L. vannamei*. However, Yang *et al*. (2010) reported higher catalase activity in muscle than in hepatopancreas of *L. vannamei* fed with the marine yeast *Rhodosporidium paludigenum*.

In this study, significant increases in the antioxidant of juvenile *F. californiensis* fed with β-glucan, vitamin E, or β-carotene were observed, but the antioxidant activities among treatments were different. Shrimp exposed to vitamin E registered a higher response (4.5-fold higher that of control) than

Table 3. Antioxidant enzyme activity in hepatopancreas and muscle of *F. californiensis* fed with different diets and inoculated with WSSV.

Tabla 3. Actividad de enzimas antioxidantes en hepatopáncreas y músculo de *F. californiensis* alimentado con diferentes dietas e inoculado con WSSV.

Enzyme	Treatment	Tissues	Time after inoculation with WSSV (h)					
			0	1	6	12	24	48
CAT	Control	Hepatopancreas	1 ± 0.33	1 ± 0.39	1 ± 0.18	1 ± 0.29	1 ± 0.47	1 ± 0.29
		Muscle	1 ± 0.21	1 ± 0.06	1 ± 0.01	1 ± 0.56	1 ± 0.45	1 ± 0.51
	β-carotene	Hepatopancreas	1.32 ± 0.19	1.7 ± 0.32	0.94 ± 0.13	2.15 ± 0.68	1.03 ± 0.1	0.8 ± 0.16
		Muscle	1.22 ± 0.23	3.24 ± 0.42*	1.7 ± 0.18*	2.58 ± 0.17*	1.4 ± 0.08	0.71 ± 0.03
	Vitamin E	Hepatopancreas	2.05 ± 0.17*	1.46 ± 0.08	0.93 ± 0.10	1.75 ± 0.36	1.09 ± 0.12	0.68 ± 0.05
		Muscle	0.87 ± 0.03	2.28 ± 0.13*	1.63 ± 0.21*	4.47 ± 0.68*	1.86 ± 0.41	0.96 ± 0.03
	β-glucan	Hepatopancreas	2.18 ± 0.14*	2.22 ± 0.39*	1.46 ± 0.14	1.78 ± 0.32	0.32 ± 0.10	0.1 ± 0.04
		Muscle	0.92 ± 0.08	2.72 ± 0.28*	1.43 ± 0.04*	1.85 ± 0.45	0.88 ± 0.10	1.18 ± 0.47
SOD	Control	Hepatopancreas	1 ± 0.81	1 ± 0.47	1 ± 0.37	1 ± 0.06	1 ± 0.06	1 ± 0.81
		Muscle	1 ± 0.21	1 ± 0.56	1 ± 0.37	1 ± 0.37	1 ± 0.08	1 ± 0.48
	β-carotene	Hepatopancreas	1.02 ± 0.21	0.35 ± 0.05	1.13 ± 0.53	2.03 ± 0.86*	0.98 ± 0.21	1.28 ± 0.32
		Muscle	2.3 ± 0.59*	0.96 ± 0.42	1.82 ± 0.46	0.89 ± 0.15	2 ± 0.72*	1.8 ± 0.12
	Vitamin E	Hepatopancreas	0.45 ± 0.01	0.59 ± 0.15	1.03 ± 0.36	2.31 ± 0.87*	1.79 ± 0.61*	1.14 ± 0.40
		Muscle	1.12 ± 0.10	0.56 ± 0.6	0.79 ± 0.8	3.2 ± 0.88*	2.03 ± 0.63*	2.63 ± 0.25*
	β-glucan	Hepatopancreas	0.6 ± 21	0.86 ± 0.19	1.23 ± 0.37	1.98 ± 0.25*	1.73 ± 0.47*	1.69 ± 0.13
		Muscle	1.34 ± 0.54	1.64 ± 0.37	1.69 ± 0.43	3.6 ± 0.70*	2.11 ± 0.80*	2.74 ± 0.39*

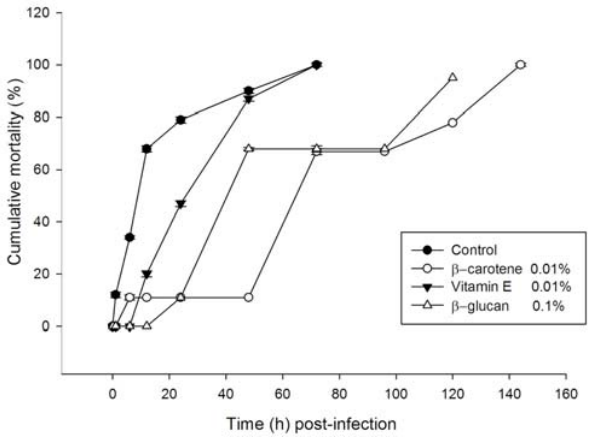

Figure 3. Mortality (%) of juvenile *F. californiensis* infected with WSSV.

Figura 3. Mortalidad (%) de juveniles de *F. californiensis* infectados con WSSV.

shrimp exposed to β-glucan, or β-carotene. The molecular structure, including the molecular weight, and dietary levels of β-glucan, vitamin E, or β-carotene have important role in the biological response (Azad *et al*., 2007; Sukumaran *et al*., 2010).

The highest SOD activity in hepatopancreas of juvenile shrimp fed with all treatments was recorded at 12 h post-infection (Fig. 1a), and increased CAT activity with all treatments was recorded in muscle at 1 h post-infection (Fig. 2b). Increased levels of antioxidant activity in cells is related to a rapid detoxifying response and also reflected the important role of SOD and CAT removing excessive reactive oxygen species from cells (Moreno *et al*., 2005).

Increased SOD and CAT activity was registered before WSSV infection (0 h). Shrimp fed with β-carotene showed significant SOD increase in muscle at day 23 before challenge, and decreased to basal values at 1 h post-challenge. Feeding juvenile shrimp with β-1.3-glucan and vitamin E during 23 days, showed increased CAT activity in hepatopancreas at 0 h and decreased at 6 h post-infection to basal values. The increase in the antioxidant activity is resulted from upregulated expression of SOD and CAT mRNA (Liu *et al*., 2007) and protection from oxidative stress and potential pathogens (Lorenzon *et al*., 2002). However, decreased antioxidant response might

indicate an accumulation of superoxide anion radical and consequent oxidative stress in cells and susceptibility to pathogens (Mercier *et al*., 2006; Mohankumar & Ramasamy, 2006).

This study showed activation of antioxidant defenses generated by feeding β-glucan, vitamin E, and β-carotene in juvenile shrimp *F. californiensis* during WSSV infection. Additional studies would further the understanding of SOD, CAT, and other antioxidant enzymes in cultivated marine species. Also, assays focusing on appropriate doses and sampling in various tissues help us understand physiological responses by feed additives.

ACKNOWLEDGMENTS

We thank Alejandro Ramos of CIBNOR for providing statistical analyses. Ira Fogel of CIBNOR provided many editorial comments and changes. Funding was provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT Grant 101552 to A.C.). R.P.M. is the recipient of a CONACYT doctoral fellowship.

REFERENCES

AOAC. 1995. Official methods of analysis of the Association of Official Analytical Chemists. Vol. I. Washington, 1234 pp.

Aguirre-Guzmán, G., J.G. Sánchez-Martínez, A. Campa-Córdova, A. Luna-González & F. Ascencio. 2009. Penaeid shrimp immune system. Thai J. Vet. Med., 39: 205-215.

Azad, I.S., J.S. Dayal, M. Poornima & S.A Ali. 2007. Supra dietary levels of vitamins C and E enhance antibody production and immune memory in juvenile milkfish, *Chanos chanos* (Forskal) to formalin-killed *Vibrio vulnificus*. Fish Shell. Immunol., 23: 154-163.

Beauchamp, C. & I. Fridovich. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-286.

Campa-Córdova, A.I., N.Y. Hernández-Saavedra, R. De Philippis & F. Ascencio. 2002. Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp (*Litopenaeus vannamei*) as a response to β-glucan and sulfated polysaccharide. Fish Shell. Immunol., 12: 353-366.

Campa-Córdova, A.I., N.Y. Hernández-Saavedra, G. Aguirre-Guzmán & F. Ascencio. 2005. Immunomodulatory response of superoxide dismutase in juvenile American white shrimp (*Litopenaeus vannamei*) exposed to immunostimulants. Cienc. Mar., 31: 661-669.

Chang, C.F., M.S. Su, H.Y. Chen, C.F. Lo, G.H. Kou & I.C. Liao. 1999. Effect of dietary beta-1.3-glucan on resistance to white spot syndrome virus (WSSV) in postlarval and juvenile *Penaeus monodon*. Dis. Aquat. Org., 36: 163-168.

Chang, C.F., M.S. Su, H.Y. Chen & I.C. Liao. 2000. Immunomodulation by dietary by dietary β-1.3-glucan in the brooders of the black tiger shrimp *Penaeus monodon*. Fish Shell. Immunol., 10: 505-14.

Chang, C.F., M.S. Su, H.Y. Chen & I.C. Liao. 2003. Dietary β-1.3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. Fish Shell. Immunol., 15: 297-310.

Cheng, W., C.H. Liu, C.M. Kuo & J.C. Chen. 2005. Dietary administration of sodium alginate enhances the immune ability of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish Shell. Immunol., 18: 1-12.

Dormandy, T.L. 1983. An approach to free radical. Lancet, 29: 1010 -1014.

Downs, C., J. Faiths & C. Woodley. 2001. Assessing the health of grass shrimp (*Palaeomonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. Mar. Biotechnol., 3: 380-397.

Durand, S., D.V. Lightner, R.M. Redman & R. Bonami. 1997. Ultraestructure and morphogenesis of White Spot Syndrome Baculovirus (WSSV). Dis. Aquat. Org., 29: 205-211.

Escobedo-Bonilla, C.M., V. Alday-Sanz, M. Wille, P. Sorgeloos, M.B. Pensaert & H.J. Nauwynck. 2008. A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. J. Fish Dis., 31: 1-18.

Fernández-Giménez, A., J. Fenucci & A. Petriella. 2004. The effect of vitamin E on growth, survival and hepatopancreas structure of the Argentine red shrimp *Pleoticus muelleri* Bate (Crustacea, Penaeidea). Aquacult. Res., 35: 1172-1178.

Flegel, T.W. 1997. Special topic review: major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. World J. Microbiol. Biotechnol., 13: 433-442.

Flores-Leyva, L. 2006. Evaluación de pigmentos carotenoides como aditivos alimentarios para la prevención de infecciones producidas por el virus del Síndrome de la Mancha Blanca (WSSV) y la bacteria *Vibrio harveyi* en camarón blanco *Litopenaeus vannamei*. Tesis de Maestría. Centro de Investigaciones Biológicas del Noroeste, S.C. La Paz, BCS, México, 45 pp.

Fridovich, I. 1995. Superoxide radical and superoxide dismutases. Annu. Rev. Biochem., 64: 97-112.

Halliwell, B. & J.M.C. Gutteridge. 1996. Oxygen radicals and nervous system. Trends Neurosci., 8: 22-26.

Huang, C.H., L.R. Zhang, J.H. Zhang, L.C. Xiao, Q.J. Wu, D.H. Chen & J.K.K. Li. 2001. Purification and characterization of White Spot Syndrome Virus (WSSV) produced in an alternate host: Crayfish, *Cambarus clarkia*. Virus Res., 76: 115-125.

Lee, M.H. & S.Y. Shiau. 2004. Vitamin E requirements of juvenile grass shrimp, *Penaeus monodon*, and effects on non-specific immune responses. Fish Shell. Immunol., 16: 475-485.

Lee, S.Y. & K. Söderhäll. 2001. Characterization of a pattern recognition protein, a masquerade-like protein, in the freshwater crayfish. *Pacifastacus leniuculus*. J. Immunol., 166: 7319-7326.

Liao, I.C., M.S. Su, C.F. Chang, B.Y. Her & T. Kojima. 1996. Enhancement of the resistance of grass prawn *Penaeus monodon* against *Vibrio damsela* infection by beta-1.3-glucan. J. Fish. Soc. Taiwan, 23: 109-116.

Lightner, D.V. 1996. A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, 304 pp.

Lightner, D.V., K.W. Hasson, B.L. White & R.M. Redman. 1998. Experimental infection of wes-tern hemisphere penaeid shrimp with Asian white spot syndrome virus and Asian yellow head virus. J. Aquat. Anim. Health., 10: 271-281.

Liu, Y., W.N. Wang, A.L. Wang, J.M. Wang & R.Y. Sun. 2007. Effects of dietary vitamin E supplementation on antioxidant enzyme activities in *Litopenaeus vannamei* (Boone, 1931) exposed to acute salinity changes. Aquaculture, 265: 351-358.

Livingstone, D.R. 2001. Contaminated-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Poll. Bull., 42: 656-665.

Lo, C.F., J.H. Leu, C.H. Ho, C.H. Chen, S.E. Peng, Y.T. Chen, C.M. Chou, P.Y. Yeh, C.J. Huang, H.Y. Chou, C.H. Wang & G.H. Kou. 1996a. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Dis. Aquat. Org., 25: 133-141.

Lo, C.F., C.H. Ho, S.E. Peng, C.H. Chen, H.C. Hsu, Y.L. Chiu, C.F. Chang, K.F. Liu, M.S. Su, C.H. Wang & G.H. Kou. 1996b. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. Dis. Aquat. Org., 27: 215-225.

López, N., G. Cuzon, G. Gaxiola, G. Taboada, M. Valenzuela, C. Pascual, A. Sánchez & C. Rosas. 2003. Physiological, nutritional, and immunological role of dietary β-1.3-glucan and ascorbic acid 2-monophosphate in *Litopenaeus vannamei* juveniles. Aquaculture, 224: 223-243.

Lorenz, T. 1998. A review of the carotenoid, astaxanthin, as a pigment and vitamin source for cultured *Penaeus* prawn. NatuRosea Tech. Bull., Cyanotech Corporation, 51: 1-7.

Lorenzon, S., P. Pasqual & E.A. Ferrero. 2002. Different bacterial lipopolysaccharides as toxicants and stressors in the shrimp *Palaemon elegans*. Fish Shell. Immunol., 13: 27-45.

Martínez-Córdova, L.R., M.A. Porchas-Cornejo, H. Villarreal-Colmenares & J.A. Calderón-Pérez. 1998. Evaluation of three feeding practices on the winter culture of yellowleg shrimp, *Penaeus californiensis* (Holmes), in low water exchange ponds. Aquacult. Res., 29: 573-578.

Mercier, L., E. Palacios, A.I. Campa-Córdova, D. Tovar-Ramírez, R. Hernández-Herrera & I.S. Racotta. 2006. Metabolic and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* exposed to a repeated handling stress. Aquaculture, 258: 633-640.

Mohankumar, K. & P. Ramasamy. 2006. White spot syndrome virus infection decreases the activity of antioxidant enzymes in *Fenneropenaeus indicus*. Virus Res., 115: 69-75.

Moreno, I., S. Pichardo, A. Jos, L. Gómez-Amores, A. Mate, C.M. Vázquez & A.M. Camean. 2005. Antioxidant enzyme activity and lipid peroxidation in liver and kidney of rats exposed to microcystin-LR administered intraperitoneally. Toxicon, 45: 395-402.

Muñiz-Rodríguez, P. 2009. Contribución a la salud de los alimentos con compuestos antioxidantes. Electron. J. Biomed., 1: 6-9.

Nappi, A.J. & E. Ottaviani. 2000. Cytotoxicity and cytotoxic molecules in invertebrates. BioEssays, 22: 469-480.

Packer, L. 1991. Interactions among antioxidants in health and disease: vitamin E and its redox cycle. Proc. Soc. Exp. Biol Med., 200: 271-276.

Ramírez-Douriet, C., R. De Silva-Dávila, J. Méndez-Lozano, D. Escobedo-Urias, I. Leyva-Arana & M. López-Meyer. 2005. White spot syndrome virus detection in zooplankton of coastal lagoons and shrimp commercial ponds in Sinaloa, Mexico. In: American Fisheries Society (eds.). 135th Annu. Meet. Am. Fish. Soc. The Anchorage, Alaska, 53 pp.

Richard, C., F. Lemonnier, M. Thibault, M. Cuturier & P. Auzepy. 1990. Vitamin E deficiency and lipoperoxidation during adult respiratory distress syndrome. Am. J. Respir. Crit. Care Med., 18: 4-9.

Sahoo, P.K., A. Das, S. Mohanty, B.R. Mohanty, B.R. Pillai & J. Mohanty. 2008. Dietary β-1.3-glucan improves the immunity and disease resistance of

freshwater prawn *Macrobrachium rosenbergii* challenged with *Aeromonas hydrophila*. Aquacult. Res., 39: 1574-1578.

Sahul-Hameed, A.S., G. Balasubramanian, S. Syed Musthaq & K. Yoganandhan. 2003. Experimental infection of twenty species of Indian marine crabs with white spot syndrome virus (WSS). Dis. Aquat. Org., 57: 157-161.

Schwarz, K.B. 1996. Oxidative stress during viral infection: a review. Free Radic. Biol. Med., 21: 641-649.

Sies, H. 1991. Oxidative stress: oxidants and antioxidants. Exp. Physiol., 82: 291- 295.

Söderhäll, K. & L. Cerenius. 1992. Crustacean immunity. Annu. Rev. Fish Dis., 2: 3-23.

Sritunyalucksana, K., P. Sithisarn, B. Withayachumnarnkul & T.W. Flegel. 1999. Activation of prophenoloxidase, agglutinin and antibacterial activity in haemolymph of the black tiger prawn, *Penaeus monodon*, by immunostimulants. Fish. Shell. Immunol., 9: 21-30.

Su, M.S., K.F. Liu, C.F. Chang & I.C. Liao. 1995. Enhancement of grass prawn *Penaeus monodon* post larvae viability by beta-1.3-glucan from *Schizophyllum commune*. J. Fish. Res., Taiwan, 3: 125-132.

Sukumaran, V., D.W. Lowman, T.P. Sajeevan & R. Philip. 2010. Marine yeast glucans confer better protection than that of baker's yeast in *Penaeus monodon* against white spot syndrome virus infection. Aquat. Res., 41: 1799-1805.

Tavares-Sánchez, O.L., G.A. Gómez-Anduro, X. Felipe-Ortega, M.A. Islas-Osuna, R.R. Sotelo-Mundo, C. Barillas-Muryc & G. Yepiz-Plascencia. 2004. Catalase from the white shrimp *Litopenaeus vannamei*: molecular cloning and protein detection. Comp. Biochem. Phys. B, 138: 332-337.

Vargas-Albores, F. & G. Yepiz-Plascencia. 1998. Shrimp immunity: review. Trends Comp. Biochem. Physiol., 5: 195-210.

Received: 10 June 2011; Accepted: 9 October 2011

Vázquez-Juárez, R., F. Vargas-Albores F. & J.L. Ochoa. 1993. A computer program to calculate superoxide dismutase activity in crude extracts. J. Microbiol. Meth., 17: 239-244.

Villarreal, H., A. Hernández-Llamas, M. Rivera, A. Millán & S. Rocha. 2004. Effect of substitution of shrimp meal, fish meal and soy meal with red crab *Pleuroncodes planipes* (Stimpson) meal in polluted diets for post-larvae and juvenile *Farfantepenaeus californiensis* (Holmes, 1900). Aquat. Res., 35: 178-183.

Wang, W.N., Y. Wang & A.L. Wang. 2006. Effect of supplemental L- ascorbyl-2-polyphosphate (APP) in enriched live food on the immune response of *Penaeus vannamei* exposed to ammonia-N. Aquaculture, 256: 552-557.

Wang, Y.C., P.S. Chang & H.Y. Chen. 2008. Differential time-series expression of immune-related genes of Pacific white shrimp *Litopenaeus vannamei* in response to dietary inclusion of β-1.3-glucan. Fish Shell. Immunol., 24: 113-121.

Winston, G.W. & R.T. Di Giulio. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol., 19: 137-161.

Yang, S.P., Z.H. Wu, J.C. Jian & X.Z. Zhang. 2010. Effect of marine red yeast *Rhodosporidium paludigenum* on growth and antioxidant competence of *Litopenaeus vannamei*. Aquaculture, 309: 62-65

Zhang, Z.F., M.Y. Shao & K.H. Kang. 2005. Changes of enzyme activity and hematopoiesis in Chinese prawn *Fenneropenaeus chinensis* (Osbeck) induced by white spot syndrome virus and zymosan A. Aquacult. Res., 36: 674-681.

Zhang, Q., F. Li, X. Zhang, B. Dong, J. Zhang, Y. Xie & J. Xiang. 2008. cDNA cloning, characte-rization and expression analysis of the antioxidant enzyme gene, catalase of Chinese shrimp *Fenneropenaeus chinensis*. Fish Shell. Immunol., 24: 584-591.