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Effects of different levels of *Yucca schidigera* extract on the growth and nonspecific immunity of Pacific white shrimp (*Litopenaeus vannamei*) and on culture water quality

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ABSTRACT

A total of 6000 shrimp (initial body weight of 0.82 ± 0.02 g) were randomly distributed into twelve concrete pools (4.5 m × 3.5 m × 0.5 m) with 500 shrimp per pool and 3 pools per diet, respectively. Four kinds of isonitrogen and isoenergetic diets were formulated with 0, 0.1%, 0.2% and 0.3% *Yucca schidigera* extract (YSE), respectively. The experiment lasted 100 d. The results showed as follows: when supplemented with 0.2% dietary YSE, the shrimp weight gain rate significantly increased ($p < 0.05$), but no significant change was found in feed conversion ratio, specific growth rate, and survival rate ($p > 0.05$). Addition of 0.2% dietary YSE significantly increased the shrimp serum protein content, as well as the activities of acid phosphatase, alkaline phosphatase, nitric oxide synthase, glutamic–pyruvic transaminase, phenol oxidase, and hepatopancreas proteases ($p < 0.05$), but did not significantly affect the activities of total superoxide dismutases (T-SOD) and aspartate transaminase ($p > 0.05$). In the late culture period (5^{th} week to 14^{th} week) the total amount of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and reactive phosphorus in the culture water significantly decreased ($p < 0.05$). These results indicated that addition of 0.2% YSE in the diet was beneficial to the growth and nonspecific immunity of white shrimp and water quality.

1. Introduction

Yucca schidigera, a plant native to southwest USA and Mexico, has aroused interest in both livestock and poultry husbandry operations because its extract is capable of conserving ammonia (Headon and Walsh, 1993). YSE is composed of steroidal saponins, polysaccharides, and polyphenols, which have great absorption capacity for harmful volatile compounds, such as ammonia and hydrogen sulfide. YSE is used to reduce the abundance of these volatiles in animal houses and thus improve animal breeding environment and animal performance. YSE has been extensively studied and applied in livestock production (Colina and Chang, 2001; Liang et al., 2009; Preston et al., 1987; Wallace et al., 1994; Zhao et al., 2003).

The other function of YSE is to play an important role in improving nonspecific immunity in terrestrial animal. The major active components of the YSE used as additives are the steroidal saponins in animal feed (Cheeke, 1998). The steroidal saponins form complexes with cell walls of cellulolytic and amylolytic bacteria, which disrupt membrane function and cell growth of some bacterial genera, thereby reducing their numbers in the rumen (Wang et al., 2000 a,b). YSE can be applied in aquaculture in the same way as livestock husbandry, but few studies have been conducted on its use in the production of shrimp and other aquatic animal (Santacruz-Reyes and Chien, 2009, 2010; Tidwell et al., 1992). Feed and feeding are two of the most important aspects to consider in shrimp culture. Unconsumed feed is probably the main source of pollution in aquaculture ponds and discharge-receiving ecosystems (Martinez–Cordova et al., 2002). Inadequate feeding practices such as overfeeding and overfertilization can maximize the problem because of the high accumulation of organic matter (Martinez et al., 1998). Degradation of organic matter produces several kinds of metabolites, particularly nitrogenous metabolites, such as ammonia and nitrite. Ammonia is also the main product in crustacean excretion (Claybrook, 1983).

In natural environments and aquaculture systems, ammonia can be found as a cation (NH^{4+}) or free (NH_3). Alcaraz et al. (1999) studied the acute toxicity of ammonia and nitrite in white shrimp *Penaeus setiferus* post-larvae. Therefore, examining the effects of dietary YSE on the growth and nonspecific immunity of *Litopenaeus vannamei* and water culture quality has great academic significance and practical value.

2. Materials and methods

2.1. Experimental design

Fish meal, soybean meal and peanut meal were used as dietary sources of protein, whereas fish oil and soybean oil were used as lipid sources. Mineral premix and vitamin premix were also given.

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YSE was supplemented to the basal diet at three levels (1 g/kg, 2 g/kg, and 3 g/kg feed). The basal diet was used as control.

Diets with addition of 0 (control Diet 1), 0.10% (Diet 2), 0.20% (Diet 3), and 0.30% (Diet 4) YSE (Shanghai GenTech) to the basal diet were prepared (Table 1) and granulated by Zhanjiang Dongteng Aquatic Feed Company.

2.2. Animals

L. vannamei was provided by Zhanjiang Yuehai Feed Company in Guangdong, China. A total of 6000 healthy, physically uniformed animals, weighing approximately 0.82 g were selected. Shrimps were randomly divided into four groups and fed for 100 days with Diet 1, Diet 2, Diet 3, and Diet 4. The experiment was performed in triplicate; 500 shrimps were used in each replication. All shrimps were cultivated in 12 concrete pools (4.5 m × 3.5 m × 0.5 m).

2.3. Feeding

Triplicate groups of shrimp were fed with the reference and test diets by hand until visual satiety four times a day at 0700, 1130, 1700, and 2130 h. Total feed provided was 5% to 7% of body weight per day. Body weight was recorded weekly from 30 randomly picked shrimps with feeding network from 4 angles of each cement pool.

Feeding amount was adjusted according to the changes in body weight. At the end of the trial, animal number, body weight, dietary amount, and feed conversion ratio were obtained.

Seawater sediment was initially filtered prior to the trials. The filtrated water was kept at continuous oxygenation throughout the experimental period. Shrimp diet ingestion, molting, and growth were observed, and the amount of released diet, water temperature, pH, salinity, dissolved oxygen, and other water quality parameters were recorded daily. Water temperature and salinity were maintained within the ranges of 25.0 °C to 28.5 °C and from 26.5 ppt to 28.0 ppt, respectively.

$$\text{Weight gain rate}(WGR\%) = (\text{final body weight} - \text{initial weight}) \times 100/\text{initial body weight}$$

$$\text{Feed conversion ratio}(FCR) = \text{feed intake}/(\text{final body weight} - \text{initial weight})$$

$$\text{Specific growth rate}(SGR) = 100 \times [(\ln \text{final body weight g}) - (\ln \text{initial body weight g})]/\text{feeding time}$$

$$\text{Survival rate}\,(SR\%) = \text{shrimp number at the end of the test} \times 100/\text{the number at the beginning of the test}$$

Table 1
Composition and nutrients of test diets (%).

Ingredients (%)	Diets			
	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	30.00	30.00	30.00	30.00
Soybean meal	20.00	20.00	20.00	20.00
Peanut meal	12.00	12.00	12.00	12.00
Shrimp shell powder	6.00	6.00	6.00	6.00
Brewer's yeast	6.00	6.00	6.00	6.00
High gluten flour	20.77	20.67	20.57	20.47
Lecithin	1.50	1.50	1.50	1.50
Fish oil + soybean oil (1:1)	1.50	1.50	1.50	1.50
Mineral premix [a]	0.50	0.50	0.50	0.50
Vitamin premix [b]	0.20	0.20	0.20	0.20
Choline	0.50	0.50	0.50	0.50
VC phosphate	0.03	0.03	0.03	0.03
Calcium phosphate	1.00	1.00	1.00	1.00
YSE	0.00	0.10	0.20	0.30
Total	100.00	100.00	100.00	100.00
Proximate composition				
Crude proteins (%)	39.58	39.55	39.52	39.56
Crude lipid (%)	6.82	6.76	6.80	6.79
Energy (MJ/kg)	19.58	19.55	19.60	19.54

[a] , Mineral premix consisted of (g/kg premix): 13.71 g ferric citrate; 28.28 g $ZnSO_4 \cdot 7\,H_2O$; 0.12 g $MgSO_4 \cdot 7\,H_2O$; 12.43 g $MnSO_4 \cdot H_2O$; 19.84 g $CuSO_4 \cdot 5\,H_2O$; $CoCl_2 \cdot 6\,H_2O$ 4.07 g; 0.03 g KIO_4; 15.325 g KCl; 0.02 g Na_2SeO_3; 906.18 g cellulose.

[b] , Vitamin premix consisted of (g/kg premix): 25.50 g thiamine; 25.0 g riboflavin; 50.0 g vitamin B6; 0.1 g vitamin B12; 5.0 g vitamin K; 99.0 g vitamin E; 10.0 g cellulose A, 50 g vitamin D; 101.0 g nicotinic acid; 61.0 g D-calcium pantothenate; 25.0 g biotin; 6.25 g folic acid; 153.06 g inositol; 383.44 g cellulose.

2.4. Sample collection

Feeding was stopped 24 h before the end of the trial. At the end of the trial, shrimp number, weight gain and survival rates were obtained. From each pool, ten shrimps were randomly selected and stored in −20 °C for composition analysis; another twenty shrimps with hepatopancreas removed were weighed and deep frozen in liquid nitrogen, and then stored in −80 °C to calculate weight ratio of liver over body, and to measure liver enzyme activity. Blood samples were drawn from the pericardial cavity 20 shrimps from each pool; samples were placed in 1.5 ml eppendorf tubes, placed in 4 °C overnight, and centrifuged the following day. Supernatants were collected for later use. From the shrimps without hepatopancreas, muscles were also harvested and stored at −20 °C.

2.5. Chemical assays

2.5.1. Shrimp body composition

Moisture, crude protein, crude lipid, and crude ash content were measured according to AOAC (1995). In a typical procedure, moisture content was obtained by placing the shrimps in a 105 °C oven until constant weight was reached; crude protein content was measured by Kjeltec 2300-Auto-analyzer (Foss Tecator, Sweden); crude lipid determination was performed using an automatic analyzer (Soxtec System 2050, Foss Tecator, Sweden); crude ash determination was conducted by weighing the crude ash residue after burning the shrimp sample in a muffle furnace at 550 °C for 12 h.

2.5.2. Serum enzyme activity assay

The activities of serum enzymes superoxide dismutases (SOD), acid phosphatase (ACP), alkaline phosphatase (AKP), nitric oxide synthase (NOS), phenol peroxidase (PO), aspartate transaminase (AST), and glutamic–pyruvic transaminase (GTP) were measured. SOD activity was determined using the improved pyrogallol oxidation method (Yang et al., 2006), whereas AKP activity was measured using alkaline phosphatase test kits (Jiancheng Bioengineering Institute, Nanjing, China). One unit is defined as the amount of enzyme in 1 g of tissue that produced 1 mg phenol per 15 min at 37 °C. Activity determination for NOS, PO, GOP, and GTP was measured using test kits made by Jiancheng Bioengineering Institute (Nanjing, China). Total protein, glucose, cholesterol, and triglyceride levels in serum were determined using a fully automatic biochemistry analyzer (LX-20 type, Beckman Coulter Inc. of the United States).

2.5.3. Activities of digestive enzymes in hepatopancreas

Protease, lipase, and amylase were determined using enzyme assay kits and Uquant microplate reader (Jiancheng Inst., Nanjing, China). One unit is defined as the amount of enzyme that hydrolyzed casein and produced 1 μg tyrosine per minute at 37 °C. Determination of amylase activity was conducted using Iodine-Starch kit (Jiancheng Inst., Nanjing, China). One unit is defined as the amount of enzyme