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Short communication

Effects of glutathione on the survival, growth performance and non-specific immunity of white shrimps (*Litopenaeus vannamei*)

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ABSTRACT

Reduced glutathione (GSH) is the most abundant non-enzymatic antioxidant present in mammalian cells and the main intracellular defence mechanism against oxidative stress. This study investigated the effects of GSH on survival rate, mean body gain weight, feed efficiency (FE), phenoloxidase (PO) activity, superoxide dismutase (SOD) activity, acid phosphatase (ACP), alkaline phosphatase (AKP) activity, GSH peroxidase (GPx) and susceptibility to *Vibrio alginolyticus* when Pacific white shrimps (*Litopenaeus vannamei*) were fed with GSH-containing diets. GSH was added to diets at 0.10, 0.20 and 0.30 g/kg during the eight-week breeding experiment. Oral administration of GSH had significantly increased mean body weight gain, FE, PO activity, SOD activity, ACP activity, AKP activity, GPx activity and susceptibility to *V. alginolyticus* compared with those of the control group ($p < .05$). Results indicate that GSH exerts both growth-promoting and immunostimulatory effects on Pacific white shrimps (*L. vannamei*).

1. Introduction

Litopenaeus vannamei is one of the most important economic shrimp species worldwide, and its cultivation production achieves higher yield compared with that of *Macrobrachium rosenbergii* and *Penaeus monodon* [1]. *L. vannamei* features advantages, such as tolerance to water environment stress, low nutritional requirements, fast growth and large amount of meat. However, the global expansion of shrimp farming and its production have been limited due to deteriorated pond environments, which result in frequent outbreaks and spread of shrimp diseases mainly of viral and bacterial aetiologies [2–4]. In particular, *Vibrio* species are common pathogens of vibriosis in shrimp farming and are a serious threat to aquaculture industry [5–7]. Therefore, farmers opt to use antibiotics to mitigate these challenges in aquatic animals. Nevertheless, long-term use of antibiotics leads to varying negative effects, e.g. drug residues and resistance [7–9]. In recent years, usage of antibiotics has been met with increasing opposition because of concerns on their negative long-term effects on the environment and potential harm to human consumers [10]. Long-term use of antibiotics can decrease their effectiveness because of the emergence of bacterial or viral resistant strains in shrimp, and excessive antibiotic levels can negatively affect shrimp growth and overall conditions [11]. Consequently, search for suitable antibiotic alternatives has received increasing attention.

Glutathione (GSH), L-γ-glutamyl-L-cysteinylglycine, is a tripeptide ubiquitously distributed in living cells and plays an important role in the intracellular defence mechanism against oxidative damages [12,13]. GSH performs important biological functions, including amino acid transport, DNA and protein synthesis and reduction of disulphides and other chemicals during cellular proliferation [14]. Zhang et al. reported that the addition of a suitable dose of GSH to diet improves the antioxidant capacity of muscle tissues and promotes growth of tilapia [15]. Ming et al. observed that dietary GSH increases growth performance, non-specific immunity, antioxidant capacity and expression levels of insulin-like growth factor 1 and HSP70 mRNA in grass carp (*Ctenopharyngodon idella*) [16]. Xu et al. showed that dietary GSH, as an antioxidant, improves the resistance of *L. vannamei* to ammonia exposure [17]. However, limited data elucidate the effects of GSH supplement in diet on Pacific white shrimps (*L. vannamei*).

This study aimed to evaluate the effects of dietary supplementation with GSH on breeding Pacific white shrimp (*L. vannamei*) by using indicators, such as survival rate, mean body weight gain, feed efficiency (FE), activities of phenoloxidase (PO), superoxide dismutase (SOD), acid phosphatase (ACP), alkaline phosphatase (AKP) and glutathione peroxidase (GPx) and susceptibility to *Vibrio alginolyticus*.

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Table 1
Ingredients of the experimental diets (g/kg) used for Pacific white shrimps, *Litopenaeus vannamei*, culture.

Ingredient	Glutathione diets (g/kg)			
	Control	0.10	0.20	0.30
Fish meal	430	430	430	430
Soybean meal	65	65	65	65
Yeast meal	25	25	25	25
Shrimp shell meal	70	70	70	70
Wheat flour	345	345	345	345
Cellulose	6	5.90	5.80	5.70
Glutathione	0	0.10	0.20	0.30
Gluten	25	25	25	25
Fish oil	8	8	8	8
Mineral mixture	20	20	20	20
Vitamin mixture	6	6	6	6

2. Materials and methods

2.1. Diet preparation

Table 1 lists the experimental diet formulations. GSH (99%, Sigma Chemical, St. Louis, MO, USA) was added to experimental diets at doses of 0.10, 0.20 and 0.30 g/kg with a corresponding decrease in the amounts of cellulose because unlike digestible carbohydrate proteins, lipids, minerals or vitamins, cellulose acts as dietary fiber. The ingredients were ground in a Hammer mill until they were passed through a 60-mesh screen. Experimental diets were prepared according to the methods described by Chang et al. [18]. Then, the ingredients were ground using a Hammer mill until they passed through an 80-mesh screen. The experimental diets were prepared by mixing dried ingredients with fish oil and then adding water until a stiff dough was formed. Each diet was passed through a mincer with a die to yield spaghetti-like strings, which were dried in a drying cabinet using an air blower at 35 °C to a moisture level of 10%. After drying, the pellets were stored in plastic bins at 4 °C until further use.

2.2. Shrimp culture

Shrimps were purchased from a local hatchery in Lianyungang, China and reared in a semi-intensive culture pond at Ganyu Base, School of Marine Science and Technology, Huaihai Institute of Technology (Jiangsu, China). Shrimps were acclimated to experimental conditions and fed with commercial diet for two weeks before feeding trial experiments. Water was continuously aerated by using two air stones. Laboratory conditions during the acclimation period were similar to those at the beginning of experiments.

Initially, 240 shrimps with an average body weight of 1.16 ± 0.02 g were randomly assigned to four groups and individually fed with control diet (without GSH) and GSH-containing diets at 0.10, 0.20 and 0.30 g/kg dosages. Each group consisted of 60 shrimps in a 1-ton circular fibre glass-reinforced plastic tank with 0.8 tons of water at a salinity of 3.2%. A total of 30 shrimps were subjected to susceptibility test, and the remaining 30 shrimps were subjected to immune response assay.

Shrimps were fed four times daily at a ratio of 8% of their body weight at 07:00, 12:00, 16:00 and 20:00. Each feeding amount is 30%, 20%, 20% and 30% of the total amount. Diet ingestion, moulting, growth and death of shrimps were observed and recorded throughout the experimental period. The shrimps were weighed once every three weeks, and feeding amount was adjusted according to shrimp body weight. During the experimental period, water temperature was maintained at 28 ± 1 °C, pH of 7.8–8.0 and salinity at 3.2%. Experiments were performed in triplicates.

2.3. Growth and survival rate

At the end of the eight-week feeding trial, shrimps from each aquarium were weighed. Growth was measured by the body weight gain of surviving shrimps in each aquarium and was calculated as (final body weight − initial body weight), whereas FE was calculated as [(final body wt − initial body wt)/feed intake].

2.4. Non-specific immune response assays

Each shrimp was blotted dry after the final weighing. Haemolymph was withdrawn from the ventral sinus of each shrimp with a 1 mL syringe fitted with a 25-gauge needle, which was rinsed with 50 μL of precooled (4 °C) 10% sodium citrate as anticoagulant. The following immunologic parameters were used to evaluate the stimulating effect of *V. alginolyticus* strains: PO activity, SOD activity, GPx activity, AKP activity and ACP activity.

PO activity was determined according to the methods described by Huang et al. [19]. Briefly, 200 μL of anticoagulant (4 °C) and 200 μL of shrimp haemolymph were added to a 2 mL syringe with 2.5 G needle and mixed. The solution was centrifuged at 800 × *g* at 4 °C for 10 min. In this experiment, the resulting upper layer was used as plasma. Plasma (20 mL) was strewed in spectrophotometer cuvette as an unknown sample, and anticoagulant (20 mL) was strewed in another cuvette as control. After 1 min, 880 mL $_{L}$-DOPA solutions were added to the two cuvettes. Absorption was recorded at 490 nm every 10 and 120 s. One unit of enzyme activity was defined as a linear increased in absorption of 0.001 per min per mL haemolymph.

SOD activity in supernatant of haemolymph samples was measured following the method described by Hao et al. using an assay kit (Nanjing Jiancheng Bioengineering Institute, China) [20]. The theory of assay was that SOD can inhibit superoxide-radical-dependent reactions. Diluted serum (100 μL) from each replicate was used. Result was reflected by optical density of SOD at 550 nm recorded on an ultraviolet spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit superoxide-induced oxidation by 50%.

GPx activity in supernatant of haemolymph samples was measured according to the method of Subramanian et al. [21]. GPx activity of tissue was determined spectrophotometrically at 412 nm (Libra S22 UV/Visible, Biochrom, England). Anti-oxidative enzyme detection kits were purchased from Randox Laboratories Limited (Country Antrim, UK). One unit of GPx activity was defined as the amount of enzyme that reduced GSH concentration in the reaction system at 1 mmol L^{-1} min^{-1}.

AKP and ACP activities of shrimp tissues were determined according to the methods of Subramanian et al. [21]. AKP and ACP activities of tissues were measured spectrophotometrically at 520 nm (Libra S22 UV/Visible, Biochrom, England) with alkaline AKP and ACP detection kits (RANDOX, UK), respectively. One unit of AKP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg of phenol at 37 °C in 30 min. One unit of ACP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg of phenol in 15 min at 37 °C.

2.5. Susceptibility of shrimp to V. alginolyticus

V. alginolyticus was purchased from Shanghai Ocean University, China and displayed symptoms of anorexia, inactivity, poor growth and necrotic musculature; this organism featured an LD_{50} value of 6×10^5 colony-forming units mL^{-1} (CFU) Kg^{-1} for shrimps in three days and was used as test pathogen. Stocks were cultured on tryptic soy agar medium (supplemented with 2% NaCl; Difco, Detroit, MI, USA) for 24 h at 28 °C and then transferred to 10 mL of tryptic soy broth (supplemented with 2% NaCl; Difco, Detroit, MI, USA) at 28 °C for 24 h. The cultured broth was centrifuged at 10,000 × g and 4 °C for 10 min. The pellets were resuspended in saline solution (0.85% NaCl) at 10^7 CFU for