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Short communication

Reduced glutathione supplementation in practical diet improves the growth, anti-oxidative capacity, disease resistance and gut morphology of shrimp *Litopenaeus vannamei*

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ABSTRACT

The present study was conducted to investigate the effects of reduced glutathione (GSH) supplementation in practical diet on growth performance, anti-oxidative response, disease resistance and intestine morphology of shrimp *Litopenaeus vannamei*. Two control diets based on the commercial formulation were designed with high level (27%) and low level (22%) of fish meal, respectively. Based on the control diet with low level of fish meal, 75, 150 and 225 mg/kg of GSH were added, respectively, to make the other three experimental diets. The five formulated diets were named as C1, C2, G1, G2 and G3, respectively. The shrimp (initial body weight: 0.30 ± 0.02 g) were fed with the five experimental diets for 8 weeks followed by a challenge test with *Vibrio parahaemolyticus*. The results showed that the specific growth rate (SGR) of shrimp in the C2 group was significantly lower than that in C1. The SGRs in G1 and G2 had no significant difference with those in C1 and C2. However, the SGR in G3 was significantly lower than that in C1. The serum GSH concentration in C2 was significantly lower than the other groups, but the malondialdehyde concentration was significantly higher. The supplementation of dietary GSH significantly improved the total anti-oxidative capacity and activities of glutathione S-transferase and glutathione peroxidase in serum. The villus height of intestine in the GSH supplemented groups had no significant difference with C1, but was significantly higher than C2. The jejunum wall thickness of intestine in G2 and G3 was significantly higher than those in the other groups. After the challenge test, the cumulative mortalities in G1 and G2 were significantly lower than C1 and C2. However, there was no significant difference in cumulative mortalities among G3, C1 and C2. In conclusion, based on the present experimental conditions, 75–150 mg/kg of GSH was suggested to be supplemented into the practical diet to improve the growth, anti-oxidative capacity, disease resistance and gut health of shrimp *L. vannamei*.

1. Introduction

The shrimp *Litopenaeus vannamei* is a commercially important crustacean species that is being widely farmed in the world. The production of the farmed *L. vannamei* in China in 2016 was 1.67 million metric tons [1]. Under the current intensive culture, however, stresses such as nutrition deficiency, high density, environmental factors changes, always lead to metabolism disorders, disease resistance decreased and the disease broke out. More and more researches focus on how to improve the health and the growth of shrimp through the way of nutrition and feeds [2].

Reduced glutathione (GSH) is a natural tripeptide composed of γ-glutamyl, cysteine and glycine, which can be transformed into oxidized glutathione in vivo to maintain normal physiological function [3,4]. Studies in terrestrial animals have shown that GSH has the function of protecting the animal's intestinal mucosa, regulating the immune system, promoting DNA, protein synthesis, promoting cell growth and proliferation [5–7]. In the studies in fish, Zhou et al. [8]pointed out that 320 mg/kg of dietary GSH can improve the growth and protein efficiency ratio, and decrease the feed conversion ratio of tilapia *Oreochromis niloticus*. Zambonino et al. [9] confirmed that dietary GSH increased the growth and survival, and reduced the probability of

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occurrence of skeletal malformations in sea bass *Dicentrarchus labrax*. Yuan et al. [10] found that dietary GSH can promote the growth and development, enhance the nutrient metabolism, and improve the whole-body protein content of grass carp *Ctenopharyngodon idellus*. Furthermore, it can also enhance the DNA synthesis by promoting the synthesis of IGF-1 in liver of *C. idellus* [11]. In Japanese flounder, it was found that dietary GSH can increase the growth, activity of the glutathione peroxidase and the total anti-oxidative capacity in serum, and decrease the serum malondialdehyde concentration [12].

Little published data on the application of GSH in diet for shrimp is available. The reports were only found on supplementation of GSH in purified diet with casein and gelatin as the protein sources on the growth and metabolism responses of shrimp. After an 8-week feeding trial with purified diets supplemented with 60–300 mg/kg of GSH, it was found that the optimal dietary GSH level was 174.13 mg/kg to improve the weight gain rate of *L. vannamei*. A higher content of dietary GSH (240 mg/kg) significantly decreased the growth [13]. Xu et al. [14]found that 120–240 mg/kg of dietary GSH improved the resistance of *L. vannamei* to water ammonia exposure. However, the published data were based on the purified diets. Further studies based on the practical diet are needed for the application of GSH in shrimp feeds, especially for the commercial shrimp feeds with high supplementation of plant protein sources, such as soybean meal. The aim of the present study is to investigate the effects of GSH supplementation in practical diets with low level of fish meal on the growth, survival, immune response, disease resistance and gut health of *L. vannamei*. It is helpful for making alternative diets with reduced fish meal level added with reduced GSH for shrimp.

2. Materials and methods

2.1. Experimental diets

The experimental diets used fish meal, soybean meal and peanut meal as the main protein source, and fish oil, soybean oil and soybean lecithin as the main lipid source. Diet formulation and its proximate composition are given in Table 1. Five experimental diets were formulated to be isolipidic (about 8% crude lipid) and isonitrogenous (about 37% crude protein). Two control diets based on the commercial formulation were designed with high level (27%) and low level (22%) of fish meal, respectively. Based on the control diet with low level of fish meal, 75, 150 and 225 mg/kg of GSH were added, respectively, to make the other three experimental diets. The five experimental diets were named as C1, C2, G1, G2 and G3, respectively. The GSH with 99.99% purity was provided by Shandong Jincheng Pharmaceutical Group Co., Ltd. (Zibo, Shandong, China).

All the dietary ingredients were ground into fine powder and sieved through an 80-mesh sieve. All powder ingredients were blended using the progressive enlargement method and mixed thoroughly with fish oil, soybean oil and soybean lecithin. And then, cold water was added till a stiff dough was produced. The dough was extruded through a granulator (EL220, Shandong Haiyang). The diameters of the diet particles were 1.0 mm and 1.5 mm. After having being dried (50 °C, 12 h), the diets were stored at −20 °C.

2.2. Feeding trial

The feeding trial was carried out in Zhuhai Baijiao Tongwei Special Aquatic Research Institute, Guangdong Province, China. The shrimps were purchased from Hainan Haiyi Aquatic Seedlings Co., Ltd. Prior to the initiation of this feeding trial, the shrimps were acclimatized to the culture system and the practical diet for 3 weeks in the outdoor cement pool (1.5 m^3). Then healthy shrimps with similar size (initial mean body weight: 0.30 ± 0.02 g) were randomly distributed into indoor tanks (300L). Each group had 5 tanks, and each tank was used as a replicate. There were 30 shrimps per tank.

Table 1
Formulation and proximate composition of the experimental diets (dry matter, %).

Ingredients(%)	C1	C2	G1	G2	G3
Fish meal	27	22	22	22	22
Soybean meal	5.02	14.04	14.04	14.04	14.04
Shrimp shell meal	5	5	5	5	5
Squid visceral meal	5	5	5	5	5
Peanut meal	4	2	2	2	2
High-gluten flour	20	20	20	20	20
Wheat starch	14.32	13.35	13.35	13.35	13.35
Wheat gluten	6.89	6.77	6.77	6.77	6.77
Microcrystalline cellulose	3.88	2.52	2.5125	2.505	2.4975
GSH[a]	0	0	0.0075	0.015	0.0225
Fish oil	1.88	2.36	2.36	2.36	2.36
Soybean oil	0.36	0.31	0.31	0.31	0.31
Soybean lecithin	1.5	1.5	1.5	1.5	1.5
Cholesterol	0.2	0.2	0.2	0.2	0.2
Molt hormone	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.1	0.1	0.1	0.1	0.1
Mold inhibitor	0.1	0.1	0.1	0.1	0.1
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
$Ca(H_2PO_4)_2$	1.5	1.5	1.5	1.5	1.5
Vitamin C	0.1	0.1	0.1	0.1	0.1
Vitamin premix[b]	1.5	1.5	1.5	1.5	1.5
Mineral premix[c]	1.5	1.5	1.5	1.5	1.5
Proximate analysis (% diet)					
Crude protein	36.93	36.98	36.74	36.21	36.24
Crude lipid	7.88	8.00	7.99	7.75	7.93

[a] Glutathione (GSH, 99.99% purity): Shandong Jincheng Pharmaceutical Group Co., Ltd. (Zibo, Shandong, China).

[b] Vitamin premix (IU or g kg^{-1}diet): thiamin, 0.5 g; riboflavin, 0.7 g; pyridoxine HCl, 0.6 g; vitamin B12, 0.002 g; vitamin K3, 0.5 g; vitamin A, 450,000 IU; vitamin D3, 150,000 IU; vitamin E, 5 g; niacin acid, 3.5 g; folic acid, 0.15 g; biotin, 0.060 g; inositol, 8 g.

[c] Mineral premix (g kg^{-1} diet): $MgSO_4H_2O$, 25; $CuSO_4.5H_2O$, 2; $FeSO_4H_2O$, 2; $ZnSO_4H_2O$, 10; $MnSO_4H_2O$, 3; $CoCl_2.6H_2O$, 0.08; $Ca(IO_3)_2$, 0.1; Na_2SeO_3, 0.01.

During the feeding trial, shrimps were fed by hand four times daily at 07:00, 11:00, 16:00 and 21:00, respectively, for 8 weeks. The shrimps were initially fed 8 and 10% of the initial stocked weight in week 1 and week 2, respectively. From the week 3, the amount of the feeds offered to shrimp was adjusted weekly according to the daily checking of uneaten feed. The uneaten feed, feces and molts were removed by siphoning the aquaria prior to the morning feeding. During the feeding trial, the water temperature was 26–28 °C, dissolved oxygen was not less than 7.0 mg/L, pH was 7.8–8.2, and the total ammonia nitrogen level was less than 0.03 mg/L.

2.3. Sample collection

At the termination of the 8-week feeding trial, the shrimps were not fed for 24 h before sampling. All the shrimps were counted and weighed for calculation of the growth rate and survival. Six to eight shrimps per tank were randomly selected and frozen at −20 °C for determination of the whole-body composition. Another six to eight shrimps per tank were randomly chosen for the hepatopancreas somatic indices and the relevant immune indicators. For each shrimp, the hemolymph was withdrawn from the ventral sinus with sterile syringe and stored in 1.5 ml centrifuge tube. The hemolymph was allowed to clot at 4 °C for 12 h. After being centrifuged at 8000 × g for 10 min at 4 °C, the serum was collected and frozen at −80 °C until assayed. The midgut of 5 shrimp was stored in Bonn's solution for the analysis of intestinal morphology.

2.4. Challenge test

After the feeding trial, the remaining shrimps were used for the challenge test. Three tanks were used for the three replicates for each group. There were 15 shrimps per tank. Shrimps were challenged with

Table 2
Effects of dietary glutathione on the growth performance of shrimp.

Group	SR(%)	SGR(%)	FI(% day^{-1})	FCR	PER	HSI (%)
C1	95.35 ± 1.01	6.43 ± 0.05^{b}	3.97 ± 0.09	1.12 ± 0.03	2.41 ± 0.06	3.31 ± 0.01^{a}
C2	89.24 ± 3.08	6.20 ± 0.08^{a}	3.89 ± 1.63	1.14 ± 0.05	2.38 ± 0.18	3.31 ± 0.06^{a}
G1	92.32 ± 1.01	6.25 ± 0.04^{ab}	3.70 ± 0.14	1.07 ± 0.04	2.55 ± 0.16	3.62 ± 0.07^{b}
G2	93.26 ± 2.12	6.25 ± 0.08^{ab}	4.07 ± 0.19	1.17 ± 0.05	2.36 ± 0.19	3.56 ± 0.03^{b}
G3	86.07 ± 5.30	6.17 ± 0.06^{a}	4.11 ± 0.23	1.18 ± 0.06	2.40 ± 0.24	3.84 ± 0.02^{c}

SR: survival rate; SGR: specific growth rate; FI: feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; HSI: hepatopancreas somatic indices.
Values are mean ± SE of five replicates.
Values within the same column with different letters are significantly different ($P < 0.05$).

Vibrio parahaemolyticus. The concentration of *V. parahaemolyticus* was adjusted to 1.0×10^{6} CFU ml^{-1}. Shrimps were intraperitoneally injected with 100 μl of bacterial suspension, which corresponds to the LD_{50} of this bacterial suspension. The mortality of shrimp was recorded twice daily at morning and evening. Cumulative mortality rate was calculated. The challenge test lasted for 8 days.

2.5. Calculation and sample analysis

The following parameters were calculated.

Survival rate (SR,%) = 100 × (final amount of shrimps)/(initial amount of shrimps)

Specific growth rate (SGR,% day^{-1}) = 100 × (Ln final weight - Ln initial weight)/days

Feed intake (FI,% day^{-1}) = 100 × feed fed/[days × (initial weight + final weight)/2]

Feed conversion ratio (FCR) = dry feed intake/(final wet weight - initial wet weight)

Protein efficiency ratio (PER) = (final weight-initial weight)/(dry feed intake × dietary protein content)

Hepatopancreas somatic indices (HSI) = 100 × (hepatopancreas somatic weight/body weight)

Cumulative mortality rate (%) = 100 × (final death of shrimps)/(initial injected shrimps)

The proximate composition of diets, feed ingredients and shrimp samples were analyzed using the standard methods of AOAC (1995). Samples of diets and shrimps were dried to a constant weight at 105 °C to determine dry weight. Crude protein was calculated from the determination of the total nitrogen (N × 6.25) using the Kjeldahl method (2300-Autoanalyzer, FOSS, Denmark). Crude lipid was determined by gravimetric analysis following ether extraction of the lipids according to the Soxhlet method (36680-analyzer, BUCHI, Switzerland).

The activities of glutathione S-transferase (GST), glutathione peroxidase (GPX), the total anti-oxidative capacity (T-AOC), malondialdehyde (MDA) and GSH in serum were measured by the methods used in the previous study of Wang et al. [12] with the specific analytical procedures and commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China).

Five shrimps per tank were randomly chosen for the total haemocyte count (THC). For each shrimp, the hemolymph was withdrawn from the ventral sinus with sterile syringes to 0.2 ml anticoagulant solution (30 mmol l^{-1} trisodium citrate, 10 mmol l^{-1} EDTA,0.34 mmol l^{-1} sodium chloride, 0.12 mmol l^{-1} glucose, adjust pH to 7.55 and osmotic pressure to 780 m Osm kg^{-1}). The proportion of hemolymph and anticoagulant solution was 1:1. The hemolymph with anticoagulant solution was placed in a hemocytometer to count the total hemocyte number under an optical microscope.

The analysis of intestinal morphology was conducted with the method of Guo et al. [15]. At the termination of the feeding trial, the whole intestine was sampled from five shrimps per tank, injected with Bouin's fixative solution and then transferred into 70% ethanol after 24 h. Following fixation, the intestine was processed and stained with hematoxylin–eosin (H&E) using standard histological techniques and examined for jejunum wall thickness and villus height under a light microscope (Olympus, DP72). The electronic images were further analyzed using Image J software for assessing the dimensions of both jejunum wall thickness and villus height in different groups.

Results are presented as mean ± SE (standard error of means). Data were subjected to one-way analysis of variance (ANOVA) using SPSS 17.0 for windows. When overall differences are significant ($P < 0.05$), Duncan's test was used to compare the means among individual groups.

3. Results

3.1. Survival and growth performance

As indicated in Table 2, there were no significant differences in survival, which ranged from 86.07% to 96.35%. The group C1 had the significantly higher SGR than that of groups C2 and G3 ($P < 0.05$). The SGRs in groups of G1 and G2 had no significant difference with those in C1 and C2. There were no significant differences in FI, FCR and PER among all the groups. The group G3 had the significantly higher HSI (3.84%) than that of others ($P < 0.05$). There was no significant difference in HSI between C1 and C2, which was significantly lower than those in groups of G1, G2 and G3.

3.2. Body compositions

The whole-body compositions of the shrimp are presented in Table 3. There was no significant difference in the content of moisture (77.26–77.75%) among all the groups ($P > 0.05$). The group G3 had the significantly highest content of protein (77.85%) among all the groups ($P < 0.05$). The group G1 had significantly lower content of lipid than the groups of C1, C2 and G3 ($P < 0.05$), and had no significant difference with the group G2.

Table 3
Effects of dietary glutathione on the whole-body compositions of shrimp (% of dry weight basis).

Group	Moisture (%)	Protein (%)	Lipid (%)
C1	77.29 ± 0.55	75.46 ± 0.16^{a}	6.38 ± 0.29^{b}
C2	77.26 ± 0.12	74.49 ± 0.20^{a}	6.55 ± 0.33^{b}
G1	77.75 ± 0.23	75.19 ± 0.41^{a}	4.42 ± 0.11^{a}
G2	77.31 ± 0.25	75.15 ± 0.52^{a}	5.42 ± 0.57^{ab}
G3	77.65 ± 0.14	77.85 ± 1.34^{b}	6.36 ± 0.21^{b}

Values are mean ± SE of five replicates.
Values within the same column with different letters are significantly different ($P < 0.05$).

Table 4
Effects of dietary glutathione on the hemolymph parameters of shrimp.

Group	THC (10^6/ml)	GST (U/ml)	GPX(U)	GSH (mg/L)	T-AOC (U/ml)	MDA (nmol/ml)
C1	7.61 ± 0.451^{a}	153.77 ± 2.40^{ab}	567.55 ± 26.7^{a}	2.96 ± 0.11^{b}	12.83 ± 0.43^{b}	23.38 ± 0.45^{a}
C2	10.5 ± 0.284^{b}	143.84 ± 5.28^{a}	823.08 ± 12.58^{bc}	2.16 ± 0.11^{a}	8.14 ± 0.34^{a}	40.13 ± 0.59^{c}
G1	12.9 ± 0.220^{c}	159.54 ± 4.16^{b}	1261.54 ± 121.82^{d}	2.96 ± 0.11^{b}	11.86 ± 0.48^{b}	23.50 ± 2.02^{a}
G2	13.5 ± 0.451^{c}	183.32 ± 3.26^{c}	1006.59 ± 46.31^{c}	3.92 ± 0.16^{c}	12.21 ± 0.14^{b}	32.01 ± 0.82^{b}
G3	7.26 ± 0.563^{a}	182.52 ± 3.00^{c}	697.8 ± 12.06^{ab}	3.52 ± 0.30^{bc}	26.57 ± 0.18^{c}	31.92 ± 0.39^{b}

THC: the total haemocyte count; GST: glutathione S-transferase; GPX: glutathione peroxidase; GSH: glutathione; T-AOC: the total anti-oxidative capacity; MDA: malondialdehyde.
Values are mean ± SE of five replicates.
Values within the same column with different letters are significantly different ($P < 0.05$).

3.3. Serum anti-oxidative parameters

The serum anti-oxidative parameters of shrimp are presented in Table 4. The total haemocyte count (THC) in the groups of G1 and G2 was significantly higher than those in C1, C2 and G3 ($P < 0.05$). Supplementation of GSH in diet significantly increased the activity of glutathione-S-transferase (GST), glutathione peroxidase (GPX) and the content of GSH. The activity of GST in G1 and G2 was significantly higher than that C2 ($P < 0.05$). The activity of GPX in G1 was significantly higher than those in the other groups ($P < 0.05$). Group C2 had the significantly lowest GSH and T-AOC among all the groups ($P < 0.05$). Supplementation of GSH in diet significantly increased the content of GSH and T-AOC. The content of MDA in C2 was significantly higher than that in the other groups. Content of MDA in G2 and G3 was significantly higher than that in group C1. There was no significant difference in MDA between G1 group and C1 group.

3.4. Intestinal morphology

The intestinal morphology is presented as Fig. 1. The jejunum wall thickness and villus height are presented in Fig. 2. Among all the groups, C2 had the significantly lowest jejunum wall thickness (350.70 μm) and villus height (249.21 μm) ($P < 0.05$). There was no significant difference in the thickness of the jejunum wall between the groups of G2 (449.53 μm) and G3 (469.91 μm) ($P > 0.05$), which was significantly higher than those in C1 (414.98 μm) and G1 (395.52 μm) ($P < 0.05$). There was no significant difference in villus height between G1 (516.92 μm) and G3 (524.56 μm) groups ($P > 0.05$), which was significantly higher than those in the other groups (C1: 365.97 μm; C2: 249.21 μm; G2: 344.90 μm) ($P < 0.05$).

3.5. The challenge test

As indicated in Fig. 3, the supplementation of GSH in diet significantly affected the cumulative mortality of shrimp during the challenge test ($P < 0.05$). There was no significant difference in the cumulative mortality between C1 (73.33 ± 5.77%) and C2 (70.00 ± 3.85%) ($P > 0.05$). However, they were significantly higher than those in G1 (40.00 ± 1.93%) and G2 (53.33 ± 1.92%) ($P < 0.05$). The cumulative mortality in group G3 (63.33 ± 5.77%) was significantly higher than G1 ($P < 0.05$), and had no significant difference with C1 and C2 ($P > 0.05$).

4. Discussion

4.1. Supplementation of GSH in practical diet on the growth performance

It was found, in the present study, that the SGR in C2 with 22% of dietary fish meal (FM) was significantly lower than that in C1 with 27% FM. However, based on the C2, 75–150 mg/kg of GSH supplementation in practical diets can significantly improve the SGR, and make the SGRs has no significant differences with that in C1. It was confirmed in the present study that supplementation of GSH in the low fish meal practical diet can improve the growth of shrimp. This is in agreement with the previous study in tilapia [8] and Japanese flounder [12]. In the study of tilapia, it was found that 80–320 mg/kg of GSH supplementation in purified diet with casein and gelatin as the dietary protein sources improved the growth. However, higher dietary GSH (400 mg/kg) significantly decreased the growth. Based on the weight gain, the optimal dietary GSH content was determined as 355.13 mg/kg [8]. In the study of Japanese flounder, it was found that 100–400 mg/kg of GSH supplementation in purified diet with casein and gelatin as the dietary protein sources improved the growth. However, higher dietary GSH (700 mg/kg) significantly decreased the growth. Based on the weight gain, the optimal dietary GSH content was determined as 368.92 mg/kg [12]. In regard to the effects of dietary GSH on shrimp, it was found that 60–180 mg/kg of GSH supplementation in purified diet with casein and gelatin as the dietary protein sources improved the growth. However, higher dietary GSH (240 mg/kg) significantly decreased the growth. Based on the weight gain, the optimal dietary GSH content was determined as 174.13 mg/kg [13]. In the present study, it

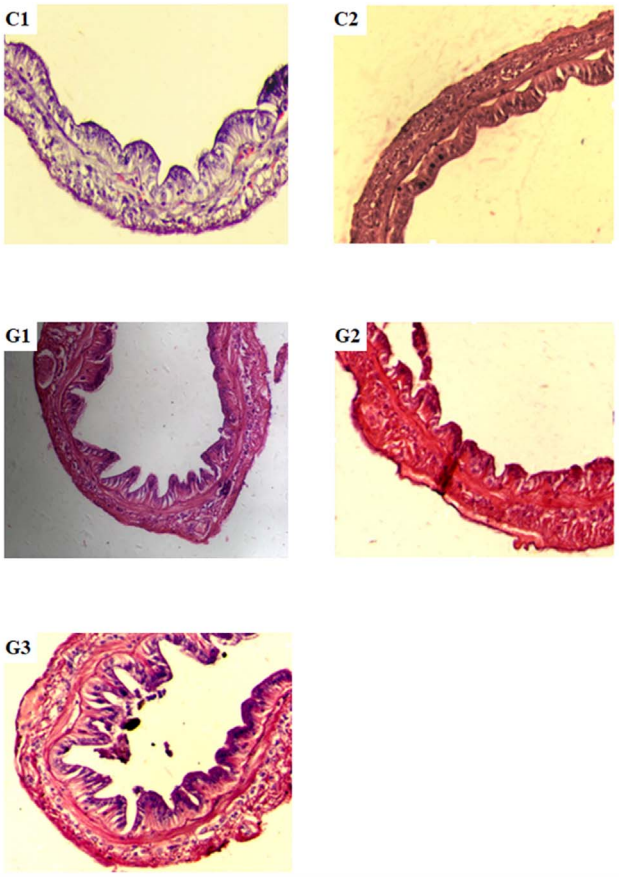

Fig. 1. Effects of dietary glutathione on the intestinal morphology of shrimp (× 20).

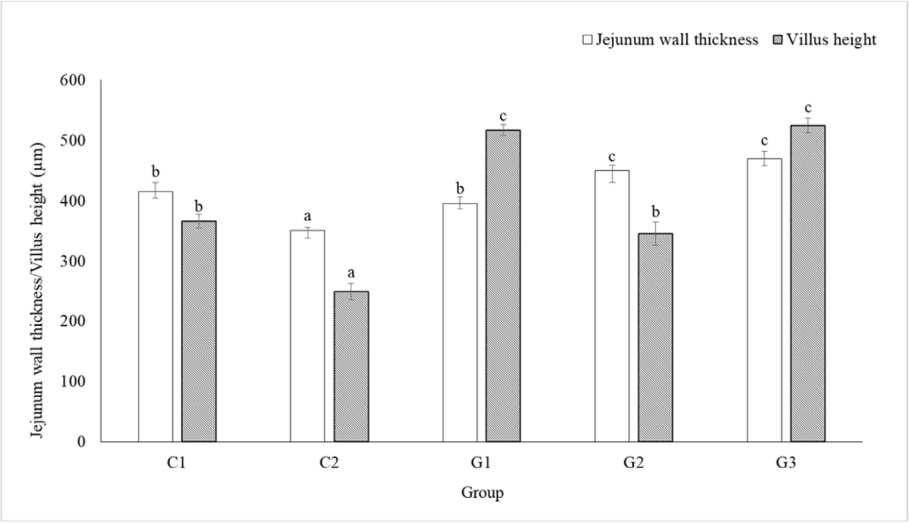

Fig. 2. Effects of dietary glutathione on the jejunum wall thickness and villus height of intestine of shrimp.

was also found that higher dietary GSH (225 mg/kg) depressed the growth of shrimp, and the SGR significantly lower than that in C1 with higher dietary fish meal (27%). It was suggested that the growth improvement in shrimp by dietary GSH is dose dependent. The dietary supplementation of GSH must be controlled within the appropriate range.

4.2. Supplementation of GSH in practical diet on the health

The GSH is a necessary substrate for the decomposition of peroxides by GPX and is an important indicator of the anti-oxidative capacity of the body. Results from the previous studies on the effect of dietary GSH supplementation on the deposition of GSH in body were not consistent. Jiao et al. [16] found that dietary GSH could increase the GSH deposition in muscle and liver of tilapia. The GSH content in the hepatopancreas in shrimp [13]and liver in Japanese flounder [12] increased significantly and then decreased with the increase of dietary GSH content. Also in the study of tilapia, Liang et al. [17]found that there were no significant effects of dietary GSH on the content of GSH in muscle, liver and serum. It was suggested, in the present study, that serum GSH content in C2 group with 22% of dietary fish meal significantly lower than that in C1 with 27% of dietary fish meal. Based on the C2, supplementation of GSH diet significantly increased the serum GSH content, activities of GST, GPX and T-AOC in serum, and significantly decreased the serum MDA content. The GSH can eliminate the H_2O_2 in the body and the intermediates LOO- or LOOH in the process of lipid peroxidation under the catalysis of GST. It can prevent the oxidation of the divalent iron in the hemoglobin and remove the high activity free radicals, which itself is oxidized to form oxidized glutathione (GSSG). The GST can exist in body as a protective mechanism against oxidative damage. The MDA as a lipid peroxide causes oxidative stress and cell damage. It can be seen that the supplementation of a suitable range of GSH in diet can effectively enhance the anti-oxidative capacity of shrimp.

In the present study, it was confirmed that supplementation of GSH in diet (75 and 150 mg/kg) increased the total haemocyte count. Crustaceans rely mainly on cellular and humoral immunity to resist adverse external stress, reduce infection, and eliminate disease [18]. Among them, the cellular immunity is mainly performed by the haemocyte, which has the function of phagocytosis, killing and discharge the foreign body of shrimp [19]. Compared to the groups without dietary GSH supplementation, in the present study, the groups supplemented with 75 and 150 mg/kg of GSH had the significantly low cumulative mortality of shrimp after the challenge test. However, the cumulative mortality and the THC in the group with 225 mg/kg of dietary GSH supplementation had no significant difference with those in the groups without GSH supplementation. It can be seen that the supplementation of appropriate content of GSH in diet can enhance the immune response of shrimp and improve its disease resistance. However, this enhancement has an obvious dose-dependent character, and over dose of GSH has a negative effect on immune and disease resistance of shrimp.

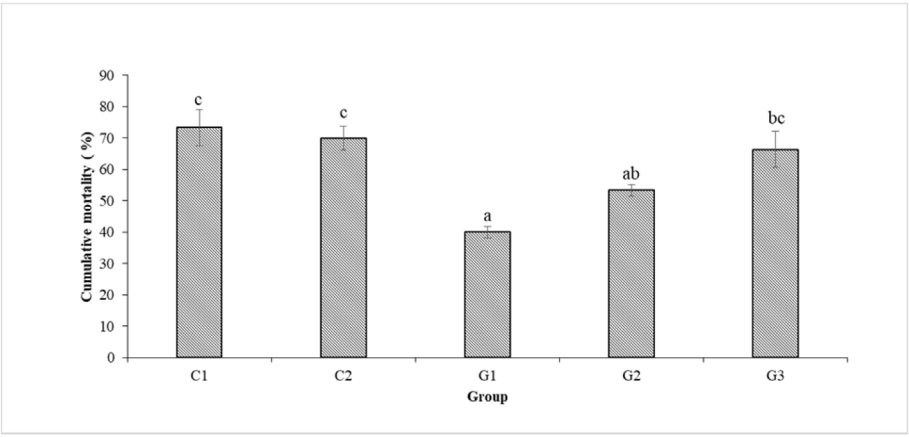

Fig. 3. Effects of dietary GSH on the cumulative mortality of shrimp after challenge test.

5. Conclusion

In order to meet the demand for the growth, intestinal health and disease resistance of *L. vannamei*, according to the results of the present study, it is suggested that the dosage of GSH in practical diet with 22% of fish meal is 75 mg/kg, and not higher than 150 mg/kg.

Acknowledgment

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