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Grape seed proanthocyanidins alleviate the negative effects of dietary cadmium on pearl gentian grouper (*Epinephelus fuscoguttatus* female x *Epinephelus lanceolatus male*)

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Abstract

The present study was conducted to evaluate whether grape seed proanthocyanidins (GSPs) could alleviate the negative effects of dietary cadmium on growth performance, body composition, activities of digestive enzymes, and antioxidant potential in the intestine of pearl gentian grouper (*Epinephelus fuscoguttatus* female x *Epinephelus lanceolatus* male). Two hundred and forty pearl gentian groupers with the initial average body weight of 31.30±0.05 g/fish were randomly divided into four treatment groups with three replicates in each group and 20 fish in each replicate. The four treatment groups were identified as control group (the basal diet), Cd group (the basal diet+300 mg/kg Cd), Cd+GSPs group I (the basal diet+300 mg/kg Cd+400 mg/kg GSPs), and Cd+GSPs group II (the basal diet+300 mg/kg Cd+800 mg/kg GSPs group), respectively. The trial lasted for 42 days. Fish exposed to 300 mg/kg dietary cadmium had lower growth performance, mineral metabolism disorders with lower calcium and phosphorus levels, higher ash level, decreased lipase and protease activities, and antioxidant potential in the intestine. Dietary GSPs supplementation could counteract those negative effects to a certain extent. The alleviation effects of Cd+GSPs group II were better than those of Cd+GSPs group I. Except for calcium and phosphorus levels in whole fish composition, 800 mg/kg dietary GSPs supplementation could not recover the adverse effects caused by Cd stress. These results suggested that GSPs might potentially mediate dietary cadmium toxicity and alleviate the negative effects on pearl gentian grouper.

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Introduction

Cadmium (Cd) is a highly toxic element and is naturally spread in feed of aquatic animals. As one of the ubiquitously distributed toxins in the aquatic system, it can disrupt growth, reproduction, the immune system, endocrine, development, and behavior (Mai et al., 2006; Cui et al., 2016; Zhai et al., 2018). The toxic effect of Cd is mediated through oxidative damage to cellular organelles by inducing the generation of reactive oxygen species (ROS), such as superoxide ion, hydroxyl radicals, and hydrogen peroxide, which may damage protein, lipid, enzymes, and DNA. Antioxidants must neutralize these ROS before entering cells (Alkhedaide et al., 2016; El-Tarras et al., 2016; Zhai et al., 2018).

In recent years, grape seed proanthocyanidins (GSPs) have attracted extensive attention as potential scavengers for ROS to alleviate oxidative stress. They are oligomeric compounds formed from catechin and epicatechin molecules, and have many other activities including antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions (Fine, 2000). In previous studies, GSPs were reported to attenuate the oxidative damage in tissue and organs, such as blood (Evcimen et al., 2020), liver (Miltonprabu et al., 2016), kidney (Nazima et al., 2015), testes (Morsi et al., 2020), brain (El-Tarras et al., 2016), pancreas (Nazima et al., 2016), and prostate (Lei et al., 2017) of rats exposed to dietary Cd. These ameliorative effects of GSPs on cadmium-induced oxidative stress were also shown in mice kidney (Chen et al., 2013) and chicken embryos (Hou et al., 2016). Similar protective effects of dietary GSPs on aquatic animals were found in tilapia (*Oreochromis niloticus*) with dietary Cd exposure (Zhai et al., 2018), American eel (*Anguilla rostrata*) exposed to high dietary histamine (Zhai et al., 2020), and greenlip abalone (*Haliotis laevigata* Donovan) under heat stress (Shiel et al., 2017).

At present, no studies regarding GSPs efficacy in attenuating Cd-induced oxidative stress have been conducted in seawater fish. The pearl gentian grouper (*Epinephelus fuscoguttatus* female x *Epinephelus lanceolatus* male), a marine carnivorous fish species, has the advantages of fast growth, high market value, and strong disease resistance. It is a new candidate which is widely cultured in China and over the world (Liu et al., 2018). This study aimed to evaluate the effects of GSPs on alleviating dietary Cd-induced growth retardation and damages of intestinal health parameters in pearl gentian grouper.

Materials and Methods

Experimental fish and cultivation

Healthy Pearl gentian grouper, purchased from Xiamen (China) Xiaodeng Fisheries Science and Technology Co., LTD, were acclimatized in a circular aquaria of 1000 L. Aerated water was provided to the circular culture system with additional aeration provided by an air pump. After adapting to experimental conditions, the fish were kept in the circular aquaria of 150 L (water capacity of 120 L) (Qingdao Zhongke Seawater Treatment Co., Ltd.). Fish were fed to satiation two times daily (at 8:00 h, and 18:00 h). Thirty minutes after feeding, uneaten pellets and feces were siphoned out. Water quality was monitored twice weekly with a multiparameter photome (HI9804N, HANNA, Baranzate, MI, Italy). Values of dissolved oxygen, pH, salinity and ammonia-N ranged between 7.0-9.2 mg/L, 7.1-7.7, 30.4-31.7 and 0-0.2 mg/L, respectively. Water temperature ranged from 24℃ to 28℃. The same source and quality of water were maintained during the adaption period and trial period.

Experimental design and diets

After adaptation to experimental conditions, two hundred and forty fish with the initial average body weight of 31.30±0.05 g/fish were randomly divided into four groups with three replicates per group and twenty fish per replicate. The four groups were fed basal diet (control group), basal diet + Cd 300 mg /kg; basal diet + Cd 300 mg /kg + GSPs 400 mg /kg, and basal diet + Cd 300 mg /kg + GSPs 800 mg /kg, respectively. The trial lasted for 42 days.

Ingredients and proximate analyses of the basal diet for pearl gentian groupers are presented in **Table 1**. The different levels of GSPs (extracted from grape seed, content >98%, purchased from Nanjing Zelang Medical Technology Co., Ltd., Nanjing, China) were

supplemented in the basal diet with 300 mg Cd/kg. All diets were mixed well and pelleted with a 2.5-mm diameter module using a laboratory pellet machine without heating. After processing, the diets were packed into small bags and stored at -20 ℃ until they were fed to the fish.

Table 1. Composition and nutrient levels of the basal diet (air-dry basis)

Ingredients	%	Nutrient levels	%
Fish meal	42.0	Dry matter	92.39
Soybean meal	27.0	Crude protein	46.92
Wheat flour	19.9	Lipid	9.88
Shrimp meal	3.0	Ash	10.22
Fish oil	2.0		
Soybean oil	2.0		
Lecithin	2.0		
Choline chloride	0.3		
$Ca(H_2PO_4)_2$	1.0		
Mineral Premix	0.5		
Vitam in premix	0.3		
Total	100.0		

Sample collection and analysis

At the end of the trial, all fish in one aquarium was weighted after 24h of fasting. Ten fish from each group were randomly anesthetized with 50 mL/L eugenol oil suspension. The fish intestines were dissected on ice and kept frozen at –80 °C for further measurements. According to the methods of Zhai et al. (2016), the activities of amylase, lipase and protease in the intestine were analyzed by UV-Vis spectrophotometer (UV-1200, Shanghai Mipoda Instrument Co., Ltd., China). The malondialdehyde (MDA) level, total antioxidant capacity (T-AOC) activity, superoxide dismutase (SOD) activity, catalase (CAT) activity, and glutathione peroxidase (GSH-Px) activity were tested by Multifunctional Microplate Reader (TECAN Infinite 200 PRO, Tecan Austria GmbH, Austria) with commercial kits according to the descriptions of Zhai et al. (2016; 2018). The level of MDA was expressed as nmol/mg protein. The values of T-AOC, GSH-Px, SOD and CAT activities were expressed as units per mg protein. The Protein concentrations of the intestinal suspension were determined by the Detergent Compatible Bradford Protein Assay Kit. The analytical kits for digestive enzymes and antioxidant parameters were provided by Nanjing Jiancheng Bioengineering Institute (China). Another three fish from each replicate were selected at random and stored at -20°C for subsequent analysis of whole fish body composition. The analyses of proximate composition on trial diets and whole fish were performed according to the methods of Zhai et al. (2014). The contents of moisture, crude protein, lipid, ash, calcium (Ca), and phosphorus (P) of whole fish were measured by standard methods (AOAC, 1995). The cadmium content of whole fish was analyzed by using Inductively Coupled Plasma Optical Emission Spectroscopy (Prodigy 7, LEEMAELABS, USA).

Data calculation

At the beginning and the end of the trial, fish weight was measured in each aquarium after 24 h of feed deprivation. The initial fish weight (IFW), final fish weight (FFW), weight gain rate (WGR), feed conversion ratio (FCR), feeding rate (FR) and survival rate (SR) were calculated as follows:

IFW (g/fish) = initial fish weight of fish per aquarium (g)/ initial number of fish.

FFW (g/fish) = final fish weight of fish per aquarium (g)/ final number of fish.

WGR (%) = 100 × [final fish weight (g/fish) - initial fish weight (g/fish)]/ initial fish weight (g/fish).

FCR = feed intake (g/aquarium)/weight gain (g/aquarium).

FR (%) = 100 × feed consumption (g/aquarium)/average fish weight (g/aquarium).

SR (%) = 100 × (final number of fish per aquarium /initial number of fish per aquarium).

Statistical analysis

The results are presented as means ± SD of four replicates. Statistical analysis was performed with SPSS 22.0 statistical software (SPSS, Chicago, IL, USA). Data from each treatment group were subjected to one-way analysis of variance (ANOVA).When overall differences were significant (P<0.05), Duncan's multiple range test was used to compare the mean values among four treatment groups. Data expressed as percentages were subjected to arcsine square root transformation before statistical analysis.

Results

Parameters of growth performance

The effects of dietary GSPs supplementation on the growth performance of pearl gentian grouper under dietary Cd stress are shown in **Table 2**. Compared with the control group, FFW, WGR, and FR of Cd group decreased significantly (P<0.05), FCR and SR were similar between those two groups (P>0.05). Compared with the Cd group, FFW, WGR, and FCR of Cd+GSPs group II improved significantly (P<0.05), and FR increased significantly in Cd+GSPs groups (P<0.05). SR values were similar among all the groups (P>0.05).

Table 2. Effects of dietary GSPs supplementation on growth performance of pearl gentian grouper exposed to dietary Cd stress

Items	Control group	Cd group	Cd+GSPs group I	Cd+GSPs group II
IFW (g/fish)	31.24±0.89	31.28±0.11	31.31±0.05	31.34±0.05
FFW (g/fish)	73.80±0.20^{c}	64.45±1.05^{a}	64.32±0.98^{a}	66.63±0.58^{b}
WGR (%)	135.69±0.64^{c}	105.91±3.36^{a}	105.51±3.12^{a}	112.88±1.86^{b}
FCR	1.00±0.04^{a}	1.00±0.03^{a}	1.04±0.02^{ab}	1.08±0.01^{b}
FR (%)	1.92±0.08^{c}	1.65±0.00^{a}	1.78±0.02^{b}	1.79±0.02^{b}
SR (%)	100.00±0.00	98.33±2.89	100.00±0.00	98.33±2.89

IFW=initial fish weight; FFW= final fish weight; WGR= weight gain rate; FCR= feed conversion ratio; FR= feeding rate; SR= survival rate.
^{abc}Values within the same row without the same superscript were significantly different at P<0.05 level.

Parameters of body composition

The effects of dietary GSPs supplementation on the body composition of pearl gentian grouper under dietary Cd stress are shown in **Table 3**.

Table 3. Effects of dietary GSPs supplementation on body composition of pearl gentian grouper exposed to dietary Cd stress

Items	Control group	Cd group	Cd+GSPs group I	Cd+GSPs group II
Moisture (%)	71.35±0.08	71.55±0.25	71.18±0.37	71.67±0.13
Crude protein (%)	17.35±0.09	17.23±0.31	17.26±0.11	17.24±0.16
Lipid (%)	6.04±0.31	5.83±0.14	5.93±0.13	5.93±0.08
ash (%)	4.32±0.14^{a}	4.54±0.09^{b}	4.51±0.14^{b}	4.45±0.11^{ab}
Ca（mg/g）	11.09±1.33^{b}	6.01±0.96^{a}	13.19±0.64^{c}	24.74±1.02^{d}
P（mg/g）	8.08±0.56^{b}	4.82±0.65^{a}	10.82±0.71^{c}	18.79±0.72^{d}
Cd（ug/g）	1.11±0.08^{a}	4.35±0.33^{d}	3.73±0.37^{c}	2.90±0.14^{b}

^{abcd}Values within the same row without the same superscript were significantly different at P<0.05 level.

There were no significant differences in contents of moisture, crude protein, and lipid among the four treatment groups (P>0.05). The ash content of the Cd group and the Cd+GSPs group I were significantly higher than that of the control group (P<0.05). The levels of Ca and P decreased significantly in Cd group compared with the control group (P<0.05). They increased significantly in Cd+GSPs groups (P<0.05), and there was a significant

difference between the Cd+GSPs group I and the Cd+GSPs group II. The Cd level in Cd group was significantly higher in the control group ($P<0.05$). Cd levels in Cd+GSPs groups decreased significantly in comparison with Cd group ($P<0.05$), and they were still significantly higher than that in the control group ($P<0.05$). A significant difference in Cd level was found between Cd+GSPs group I and Cd+GSPs group II ($P<0.05$).

Activities of digestive enzymes in intestine

The effects of dietary GSPs supplementation on digestive enzyme activities in the intestine of pearl gentian grouper under dietary Cd stress are shown in **Table 4**. Compared with control group, lipase and protease activities in the intestine of the Cd group decreased significantly ($P<0.05$). There was no significant difference in lipase activity among treatment groups exposed to dietary Cd stress ($P>0.05$). No significant difference of the protease activity was found between of the Cd+GSPs group I and the Cd+GSPs group II ($P>0.05$), while protease activity in the Cd+GSPs group II was significantly higher than that of the Cd group ($P<0.05$). There was no significant difference of amylase activity among the four treatment groups ($P>0.05$).

Table 4. Effects of dietary GSPs supplementation on digestive enzymes activities in the intestine of pearl gentian grouper exposed to Cd stress

Items	Control group	Cd group	Cd+GSPs group I	Cd+GSPs group II
Amylase (U/mg prot)	0.40±0.02	0.41±0.02	0.40±0.02	0.41±0.02
Lipase (U/mg prot)	49.45 ± 2.41^{b}	44.22 ± 0.67^{a}	44.98 ± 1.74^{a}	46.65 ± 3.20^{ab}
Protease (U/mg prot)	88.42 ± 2.00^{c}	75.88 ± 3.65^{a}	80.09 ± 2.05^{ab}	82.69 ± 1.20^{b}

^{abc}Values within the same row without the same superscript were significantly different at $P<0.05$ level.

Antioxidant parameters in intestine

The effects of GSPs on antioxidant parameters in intestine of pearl gentian grouper under Cd stress are presented in **Table 5**. MDA level in Cd group increased significantly in comparison with that of the control group ($P<0.05$), and it decreased significantly by dietary GSPs supplementation ($P<0.05$). Levels of MDA in the two Cd+GSPs groups were higher significantly than that of control group ($P<0.05$). Activities of T-AOC, SOD, CAT, and GSH-Px in Cd group decreased significantly in comparison with the control group ($P<0.05$). They were increased by dietary GSPs supplementation to certain extent. Only T-AOC activity of the Cd+GSPs groups and SOD activity of the Cd+GSPs group II were higher in comparison with those of the Cd group ($P<0.05$).

Table 5. Effects of dietary GSPs supplementation on antioxidant parameters in the intestine of pearl gentian grouper exposed to Cd stress

Items	Control group	Cd group	Cd+GSPs group I	Cd+GSPs group II
MDA（nmol/ mg prot）	4.14 ± 0.14^{a}	7.85 ± 0.14^{c}	5.70 ± 0.53^{b}	5.98 ± 0.13^{b}
T-AOC（U/mg prot）	3.31 ± 0.06^{d}	2.18 ± 0.05^{a}	2.44 ± 0.03^{b}	2.78 ± 0.03^{c}
SOD（U/mg prot）	13.00 ± 0.24^{c}	10.34 ± 0.25^{a}	10.31 ± 0.65^{a}	11.08 ± 0.85^{b}
CAT（U/mg prot）	8.90 ± 0.66^{b}	5.54 ± 0.81^{a}	5.62 ± 0.6^{a}	6.47 ± 0.12^{a}
GSH-Px（U/mg prot）	1.42 ± 0.10^{b}	1.22 ± 0.02^{a}	1.29 ± 0.08^{a}	1.31 ± 0.04^{a}

MDA= malondialdehyde; T-AOC= total antioxidant capacity; SOD= superoxide dismutase; CAT= catalase; GSH-Px= glutathione peroxidase.

^{abcd}Values within the same row without the same superscript were significantly different at $P<0.05$ level.

Discussion

In the present study, the growth performance of pearl gentian grouper was severely decreased by dietary 300 mg/kg dietary Cd exposure. Similar growth inhabitations by certain level of dietary Cd in inorganic form were confirmed in many other fish species, such as cobia (*Rachycentron canadum* L.) at 10.90 mg/kg (Liu et al., 2015), Nile tilapia(*Oreochromis niloticus*) at 25 mg/kg (Ayyat et al., 2017), rockfish (*Sebasres schlegeli*) at 25 mg /kg (Kim et al., 2004; Kang et al., 2005), yellow catfish (*Pelteobagrus fulvidraco*) at 48.57 mg/kg (Tan et al., 2010), genetic improvement of farmed tilapia (GIFT) at 100 mg/kg (Lu, 2014), crucian carp (*Carassius auratus*) at 120 mg/kg(Kim, 2009), and Parrotfish (*Oplegnathus fasciatus*) at 162 mg/kg (Okorie et al., 2014), and goldfifish (subspecies: Prussian carp *Carassius auratus gibelio* B.) at 10000 mg/kg (wet weight) (Szczerbik et al., 2006). The growth of Japanese seabass (*Lateolabrax japonicus*) was also suppressed by dietary organic Cd at 12.08 mg/kg (Mai et al., 2006). While no significant inhabitation effects of dietary inorganic Cd on growth performance were found in juvenile turbot (*Scophthalmus maximus* L.) at 50 mg/kg (Cui et al., 2016), and gibel carp (*Carassius gibelio*) at 120 mg/kg (Li et al., 2020), Atlantic salmon (*salmo salar*) at 250 mg/kg (Lundebye et al., 1999). Similarly, the growth of cobia (*Rachycentron canadum* L.) was not affected by dietary organic Cd at 10.90 mg/kg (Liu et al., 2015). The above results showed that growth inhibition effects caused by dietary Cd were different for different fish species and levels and form of Cd. For the most of previous studies, the inorganic Cd was the main form in diet, the toxicity of other form Cd should be studied in further researches. In the present trial, GSPs supplementation improved the growth performance of pearl gentian grouper in comparison to the Cd group, and could not recover to those of the control group without dietary Cd stress. The fish in our trial might be under severe stress induced by 300 mg/kg dietary Cd, and 800 mg/kg GSPs might be too low to relieve this severe stress thoroughly. In a previous study, GIFT tilapia fed 400 mg/kg dietary GSPs under 100 mg/kg Cd stress had better growth performance than the control group. Similarly, 300 mg/kg GSPs supplementation could effectively counteract the adverse effects on the growth of juvenile American eel exposed to 300 mg/kg dietary histamine. In addition, GSPs can significantly improve the growth performance of heat-stressed green-lipped abalone, and increase the survival rate (Shiel et al., 2017).

In the present trial, no significant effect of dietary Cd was found on whole fish body composition except contents of ash, Ca, and P of pearl gentian grouper. These results indicated that the dietary Cd exposure could cause abnormal mineral metabolism with decreasing levels of Ca and P and increasing levels of ash and Cd. Similar results were also shown in the studies of other fish species exposed to dietary Cd stress. The parameters of body composition were not affected by dietary Cd in turbot at 50 mg/kg (Cui et al., 2016), Japanese seabass at 12.08 mg/kg (Mai et al., 2006), and Nile tilapia fed dietary Cd at 25 mg/kg (Ayyat et al., 2017). In comparison, higher levels of moisture and ash and lower levels of crude protein and lipid were found in yellow catfish exposed to 48.75 mg/kg dietary Cd (Tan et al., 2010) and cobia exposed to 10.90 mg/kg dietary Cd (Liu et al., 2015). The Cd accumulation in the above studies increased significantly without exception. The above results indicated that response on body composition of different fish species might have different sensitivities under dietary Cd stress. In the present trial, as the level of dietary GSPs supplementation increased, there was a trend of ash content recovered to that of the control group with increased accumulation of Ca and P as well as decreased accumulation of Cd. These might contribute to the improvement of detoxification capacity of pearl gentian grouper by GSPs' antioxidant property and heavy metal chelating property (Nazima et al., 2015; Miltonprabu et al., 2016). According to the findings of Nazima et al. (2016), the chelating Cd effect could be due to the presence of functional groups involving both hydroxyl groups of ring-B and the 5-hydroxy group of ring-A of GSPs. The specific mechanism of GSPs regulating mineral metabolism disorders should be explained in future research. It was reported that the intestinal health might be improved by GSPs supplemented in the diets of GIFT tilapia (Lu, 2014) and growth retarded marbled eel (*Anguilla marmorata*) (Zhai et al., 2016). The recovery of digestive enzymes activities and antioxidant parameters in intestine of was caused by GSPs supplementation in diet of pearl gentian grouper exposed to dietary Cd stress, which

indicated that there might be an improvement of intestinal health to promote the absorption and accumulation of Ca and P.

In our study, the decreased activities of lipase and protease in Cd group were in agreement with the studies of GIFT tilapia (Lu et al., 2014) and catfish (*Silurus asotus*) (Sastry and Gupta, 1979) exposed to dietary Cd stress. There were limited relief effects of GSPs supplementation on digestive enzyme activity of pearl gentian grouper under 300 mg/kg dietary Cd stress. Only the activity of intestinal protease improved significantly by 800 mg/kg GSPs supplementation, and was still significantly lower than that of the control group without Cd stress. These results were slightly different from the study of GIFT tilapia receiving 100 mg/kg dietary Cd with 300 mg/kg GSPs supplementation returned to normal lipase and protease activities (Lu, 2014). This might be due to the high intensity of dietary Cd stress at 300 mg/kg and limited relief efficacy of GSPs supplementation at 800 mg/kg in the present trial.

In general, the toxic effects of cadmium might be likely caused by its bounding to metallothionein with less activity. The mechanism of toxicity of cadmium is related to disrupting the cellular redox state by competing with essential metals in protein-binding sites, triggering a release of Fe^{2+} and Cu^{2+} ions and increased generation of ROS (Topal et al., 2013). The redox activity of cadmium depletes antioxidants and glutathione, causes oxidative stress, enhances lipid peroxidation, and alters the lipid composition of membranes. Cadmium-induced reactive oxygen intermediates can lead to decreased DNA synthesis and strand breaks (Okorie et al., 2014; El-Tarras et al., 2016; Zhai et al., 2018). The oxidative stress might be a main approach of dietary Cd to cause the damage of tissue and organ (Alkhedaide et al., 2016; El-Tarras et al., 2016; Zhai et al., 2018). In the present trial, it was found that activities of intestinal CAT, SOD, GSH-Px and T-AOC level decreased significantly along with the increased MDA level. These results indicated that dietary Cd at 300 mg/kg could cause the oxidative stress in the intestine of pearl gentian grouper, which was consistent with the results in the intestine of GIFT tilapia exposed to dietary Cd stress (Lu, 2014). Antioxidant defenses in fish were also negatively affected by dietary cadmium exposure in the liver of yellow catfish (Tan et al., 2010) and cobia (Liu et al., 2015), liver and kidney of brown trout (*Salmo trutta fario*, L) (Topal et al., 2013). Considering the antioxidant parameters in the intestine of pearl gentian grouper exposed to dietary Cd in the present trial, only decreased MDA level and increased activities T-AOC and SOD were induced by GSPs supplementation, however, the activities of CAT and GSH-Px were not significantly affected. Besides, GSPs supplementation could not return the antioxidant parameters to normal levels. While in the study of GIFT tilapia exposed to Cd stress at 100 mg/kg, MDA level and activities of T-AOC, SOD, CAT, and GSH-Px were normalized by GSPs supplementation at 300 mg/kg (Lu, 2014). The results of antioxidant parameters in the intestine of pearl gentian grouper also confirmed that the stress induced by 300 mg/kg dietary Cd might be severe to recover to a normal status with GSPs supplementation at 800 mg/kg. Further research should be conducted to achieve the optimal supplementation level of GSPs in diet of pearl gentian grouper exposed to dietary Cd stress at 300 mg/kg.

In conclusion, 800 mg/kg GSPs supplementation might have a certain ability to reverse the decline of growth performance, levels of Ca and P, digestive enzymes activities and antioxidant potential of the intestine of pearl gentian grouper exposed to 300 mg/kg dietary Cd. The negative effects were not alleviated to a normal level due to the high-stress intensity under this trial condition. The alleviation effects of GSPs might be due to their properties of free radical scavenging, metal chelating, and antioxidant potentials.

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