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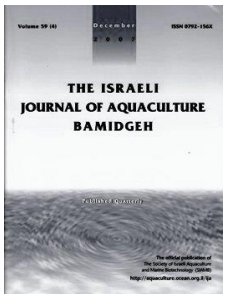

Effect of Grape Seed Proanthocyanidins on Alleviating Dietary Cadmium (Cd) Induced Growth Retardation and Oxidative Stress in Hepatopancreas of Juvenile Tilapia (*Oreochromis niloticus*)

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Keywords: grape seed proanthocyanidins; tilapia; growth; oxidative stress; dietary cadmium

Abstract

This trial was conducted to evaluate grape seed proanthocyanidins (GSPs) on alleviating the effects of dietary cadmium (Cd) induced growth retardation and oxidative stress in hepatopancreas of juvenile tilapia (*Oreochromis niloticus*). Two hundred and forty fish were randomly divided into four groups with four replicates in each group and 15 fish in each replicate. The four groups were: control group (fed with a basal diet), Cd group (fed with a basal diet+100 Cd mg/kg), Cd+ GSPs group I (fed with a basal diet+100 mg Cd/kg+400 mg GSPs/kg), and Cd+ GSPs group II (fed with a basal diet+100 mg Cd/kg+800 mg GSPs/kg). The trial period was 49 days. Final body weight and weight gain rate of Cd group were significantly affected ($P<0.05$) compared with the control group. There was a significant difference in final body weight, weight gain rate, and feed conversion rate between the Cd (control) group and the two GSPs groups ($P<0.05$). Feeding rate and survival rate of all groups was similar ($P>0.05$). Levels of malondialdehyde, glutathione, and total antioxidation capacity and activities of superoxide dismutase, catalase, and glutathione peroxidase in hepatopancreas of the Cd group were affected significantly ($P<0.05$). No significant differences in malondialdehyde level and antioxidant potential parameters (except glutathione peroxidase in Cd+GSPs group II) were found between control group and all GSPs supplemented groups ($P>0.05$). Results indicate that dietary GSPs supplementation may alleviate dietary Cd-induced growth retardation and oxidative stress in hepatopancreas of tilapia.

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Introduction

Cadmium (Cd) is one of the most abundant and ubiquitously distributed toxins in the aquatic system. It can disrupt growth, reproduction, the immune system, endocrine, development, and behavior (Kim et al., 2004; Cui et al., 2016). A major Cd source in aquaculture feed is squid viscera meal (SVM). Another source of Cd in aquafeed are mineral ingredients that are contaminated with inorganic Cd (Mai et al., 2006; Liu et al., 2015). Fish have two storage sites for Cd: gills and intestine (Mai et al., 2006). Many studies have concentrated on Cd distribution in the tissues of fish exposed to waterborne Cd. However, dietary Cd exposure in fish is often ignored, and some researchers have suggested that dietary Cd exposure is a major cause of Cd accumulation in fish (Berntssen et al., 2001; Tan et al., 2010). Accumulation of Cd in fish has been linked to oxidative damage and other chronic effects (Hartl, 2013).

At present, mitigating dietary heavy metals toxicity in fish depends mainly on the addition of elevated calcium in fish diets (Baldisserotto et al., 2005). Recent research findings have suggested that administration of naturally occurring non-enzymatic antioxidants like carotenoids, flavonoids, minerals, vitamins etc. can prevent or subdue various toxic effects of heavy metals in particular oxidative stress. Among all the natural antioxidants, grape seed proanthocyanidins (GSPs) have attracted considerable attention for their antioxidant activity in aquatic animals (Lange et al., 2014; Zhai et al., 2014; Duong et al., 2016; Shiel et al., 2017). GSPs have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions (Fine, 2000). The dimeric procyanidins of GSPs are absorbed into the bloodstream, and some of the products of hydrolytes of the higher oligomers and polymers are also presumed to be absorbed through the intestinal membrane. The absorbed procyanidins and/or hydrolytes of procyanidins might display various physiological and biological functions in *vivo* (Yamakoshi et al., 2002). Many studies suggest that GSPs can act as an effective protector in Cd induced oxidative stress mediated hepatic toxicity in terrestrial animals (Nazimabashir et al., 2014; Miltonprabu et al., 2016). Similarly, positive health benefits from dietary GSPs supplementation have also been identified in tilapia (*Oreochromis niloticus*) (Zhai et al., 2014), zebrafish (*Danio rerio*) (Kao et al., 2010), and greenlip abalone (*Haliotis laevigata* Donovan) (Lange et al., 2014; Duong et al., 2016; Shiel et al., 2017). Studies regarding GSPs efficacy in attenuating Cd-induced oxidative stress in fish have not been undertaken. The aim of this study was to evaluate the effects of GSPs on alleviating dietary Cd-induced growth retardation and oxidative stress in tilapia. In this study, we also evaluated the potential of GSPs in alleviating dietary Cd impairment to fish, effects of dietary GSPs supplementation on growth performance and antioxidant potential of tilapia exposed to 100 mg/kg dietary Cd were investigated.

Materials and Methods

Experimental fish and cultivation

Healthy tilapia, purchased from the Development Center for Aquatic Animals of Zhangzhou (China), were acclimatized in two plastic tanks (200 cm × 90 cm × 100 cm), and during the adaptation period were fed a commercial diet at 08:00, 13:00, and 18:00 daily for 4 weeks. After adaptation to experimental conditions the fish were kept in sixteen circular aquaria (86 cm × 54 cm × 54 cm). Aerated water was supplied to the circular culture system with additional aeration provided by an air pump. The daily water exchanged was 50%. Fish were fed to satiation three times daily (at 8:00 h, 13:00 h and 18:00 h). Thirty minutes after the feeding, uneaten pellets and feces were siphoned out. The water quality was monitored twice weekly with a multiparameter photome (HI9804N, HANNA, Baranzate, MI, Italy). Values of dissolved oxygen, pH, and ammonia-N ranged between 7-9 mg/L, 7.1-7.5 and 0-0.2 mg/L, respectively. Water temperature ranged from 22°C-28°C. The same water source and water quality were maintained for the adaption period and trial period. In order to avoid waterborne Cd contamination, the uneaten feed and feces were siphoned out daily every two hours.

Experimental design and diets

After adaptation to experimental conditions, two hundred and forty fish with initial average body weight of 8.14 ± 0.05g were randomly divided into four groups with four replicates per group and 15 fish per replicate. The four groups were fed: a basal diet (control diet); basal diet with 100 mg Cd/kg added; basal diet supplemented with 100 mg Cd/kg and 400 mg GSPs/kg; and basal diet supplemented with 100 mg Cd/kg and 800 mg GSPs/kg, respectively. The Cd-enriched diets were made according to Lu (2014) by adding Cadmium Chloride into the commercial basal diet. The measured dietary Cd concentrations in four groups were 0.15±0.02, 100.12±0.14, 100.21±0.11, 100.13±0.09 mg/kg, respectively. The Cd concentration was determined by atomic absorption spectrophotometry with a graphite furnace and an acetylene-air flame (Solaar M6, Thermo Electron, USA). The trial lasted for 49 days.

Ingredients and proximate analyses of the basal diet 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 100 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 °C until they were fed to the fish.

Table 1. Ingredients and proximate analyses of basal diet for tilapia

Ingredients	*g/kg*	*Nutrient level*	
Fish meal	50	Crude protein (%)	33.4
Soybean meal	150	Crude fat (%)	5.7
Rapeseed extraction	200	Crude ash (%)	12.0
Cotton Seed meal	200	Digestible energy ((MJ/kg)	12.8
High-gluten flour	150		
Rice bran	200		
Soybean oil	10		
Monocalcium phosphate	10		
Choline chloride	2		
Vitamin premix[1]	2		
Mineral premix[2]	6		

[1] Vitamin premix (mg/kg diet): thiamin, 0.25; lactoflavin, 0.25; Nick acid, 1.0; pantothenic acid calcium, 1.25; folic acid, 0.075; biotin, 0.03; hydrochloric acid pyridoxine, 0.2; cobalt amine, 0.0005; vitamin C, 5; vitamin K, 0.2; inositol, 10; vitamin E, 2; vitamin A, 0.2; choline, 20.

[2] Mineral premix (mg/kg diet): NaCl, 1.0; $MgSO_4 \bullet 7H_2O$, 15; $NaH_2PO_4 \bullet 2H_2O$, 25; KH_2PO_4, 32; $Ca(H_2PO_4)_2 \bullet H_2O$, 20; $FeSO_4$, 2.5; calcium lactate, 3.5; $ZnSO_4 \bullet 7H_2O$, 0.353; $MnSO_4 \bullet 4H_2O$, 0.162; $CuSO_4 \bullet 5H_2O$, 0.031; $CoCl_2 \bullet 6H_2O$, 0.01; KIO_3, 0.003.

Data Calculation

At the beginning and at the end of the trial, body weight of fish in each aquarium was measured after 1 day of feed deprivation. Diet consumption was recorded. The initial body weight (IBW) and final body weight (FBW) of fish, weight gain rate (WGR), feed conversion ratio (FCR), and survival rate (SR) were calculated as follows:

IBW (g/fish) = initial body weight of fish (g)/ initial number of fish;

FBW (g/fish) = final body weight of fish (g)/ final number of fish;

WGR (%) = 100× [final wet weight (g) - initial wet weight (g)]/ initial wet weight (g);

FCR = feed intake (g)/weight gain (g);

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

Sample collection and analysis

At the end of the trial, six fish were sampled at random from each replicate and anesthetized by dipping in 50 mg/L of eugenol oil suspension in water for 30s. Then the body weight of the fish was measured. The hepatopancreas collected and stored at -80°C for analysis of antioxidative parameters. The hepatopancreas from each replicate were pooled and homogenized in 10 volumes (v/w) of ice-cold normal saline (0.68%). The homogenates were centrifuged at 10,000 g for 15 min at 4°C to collect the supernatants, and the enzyme extracts were stored at -80˚C until assayed. Total protein content of

supernatants, malondialdehyde level (MDA), total antioxidant capacity level(T-AOC), glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD) activity, and catalase (CAT) activity were measured according to the methods of Zhai et al. (2015). The content of reduced glutathione (GSH) in hepatopancreas was determined by a commercial ELISA kit (Shanghai Yansheng Industrial Co., LTD) according to the methods of Lu (2014). 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 GSH level was expressed as mg per g protein of wet tissue, respectively.

Statistical analysis

Statistical analysis was performed with SPSS 18.0 statistical software (SPSS, Chicago, IL, USA). The results are presented as means ± SD of four replicates. Data from each group were subjected to one-way analysis of variance (ANOVA). Duncan's multiple range test was used to compare the mean values among the groups when overall differences were significant ($P<0.05$). Data expressed as percentages or ratios were subjected to arcsine transformation prior to statistical analysis.

Results

Growth performance and Survival

Parameters of growth performance and survival of tilapia are shown in table 2. Compared with control group, the FBW and WGR of Cd group were significantly affected ($P<0.05$) by dietary Cd exposure. FCR was similar between those two groups ($P>0.05$). The differences in FBW, WGR, and FCR of Cd+GSPs groups were significantly different from those of control group and Cd group ($P<0.05$), and no significant difference was found between Cd+GSPs group I and Cd+GSPs group II ($P>0.05$). SR values were similar among all the groups ($P>0.05$).

Table 2. Effects of dietary GSPs on growth and survival parameters of tilapia under dietary cadmium stress

Item	*Groups*			
	Control	*Cd*	*Cd+GSPs I*	*Cd+GSPs II*
IBW g/fish	8.13±0.02	8.14±0.04	8.16±0.07	8.10±0.03
FBW g/fish	68.07±2.35^{b}	55.73±0.93^{a}	73.27±1.92^{c}	74.93±2.23^{c}
WGR(%)	736.86±28.89^{b}	583.40±13.01^{a}	796.04±28.04^{c}	826.37±26.94^{c}
FCR	1.33±0.04^{b}	1.37±0.01^{b}	1.22±0.05^{a}	1.30±0.07^{a}
FR(%)	4.14±0.04	4.18±0.01	4.04±0.15	4.18±0.04
SR(%)	100	100	100	100

IBW= initial body weight; FBW= final body weight; WGR= weight gain rate; SGR=specific growth rate; FCR= feed conversion ratio; FR= feeding rate; SR= survival rate.

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

MDA levels and antioxidant potential in hepatopancreas

MDA levels and antioxidant potential in hepatopancreas of tilapia are shown in table 3. Compared with control group, the activities of SOD, CAT, and GSH-Px, and T-AOC and GSH levels in Cd group decreased significantly ($P<0.05$). The MDA levels increased significantly ($P<0.05$). Significant differences in MDA levels and antioxidant potential were found between Cd group and Cd+GSPs groups ($P<0.05$). No significant differences in MDA levels and antioxidant potential parameters (except GSH-Px activity and GSH level of control group and Cd+GSPs group I) were found between the control group and Cd+GSPs groups ($P>0.05$). MDA levels and antioxidant potential parameters were similar between Cd+GSPs group I and Cd+GSPs group II ($P>0.05$).

Table 3. Effects of dietary GSPs on MDA levels and antioxidant potential in hepatopancreas of tilapia

Item	*Groups*			
	Control	*Cd*	*Cd+GSPs I*	*Cd+GSPs II*
MDA (nmol/ mg	0.29±0.04[a]	0.70±0.03[b]	0.29±0.02[a]	0.29±0.03[a]
T-AOC(U/mg prot)	1.16±0.10[b]	0.65±0.15[a]	1.05±0.18[b]	1.04±0.09[b]
SOD(U/mg prot)	169.86±6.84[b]	134.89±13.74[a]	177.55±14.46[b]	160.78±6.50[b]
CAT(U/mg prot)	23.71±1.34[b]	11.56±2.80[a]	21.20±4.50[b]	28.04±4.12[b]
GSH-Px (U/mg prot)	67.99±7.51[c]	40.32±6.66[a]	47.48±9.06[ab]	60.10±3.15[bc]
GSH(mg/g prot)	4.81±0.33[b]	3.62±0.15[a]	5.48±0.34[bc]	6.54±0.59[c]

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

[abc]Values within the same column without the same superscript were significantly different at $P < 0.05$ level.

Discussion

In the present study, growth performance of tilapia was significantly affected by dietary Cd exposure. These results are consistent with previous studies in juvenile tilapia exposed to dietary Cd at 100 mg/kg (Lu, 2014) or 200 mg/kg (Jia et al., 2012), juvenile rockfish (*Sebasres schlegeli*) with dietary Cd at 0.5 to 125 mg /kg (Kang et al., 2005) or both 25 and 125 mg /kg (Kim et al., 2004), juvenile *Pelteobagrus fulvidraco* fed the diet with Cd level being 48.57 and 474.7mg/kg (Tan et al., 2010), Japanese seabass (*Lateolabrax japonicus*) with exposure to dietary Cd from SVM at 12.08 mg/kg, juvenile cobia (*Rachycentron canadum* L.) fed diet with Cd level being 10.0mg/kg (Liu et al.,2015). This indicates that different fish species have different tolerance to Cd stress. The growth performance of tilapia exposed to dietary Cd with GSPs supplementation was significantly improved in comparison with the Cd group and the control group. This suggests that GSPs supplementation alleviates growth retardation induced by dietary Cd. Similarly, positive health benefits from dietary GSPs supplementation have also been identified in several aquatic animal species. It was reported that dietary 200mg/kg GSPs had a positive effect on growth and body composition, and ameliorated serum biochemistry parameters of tilapia, *Oreochromis niloticus* (Zhai et al., 2014). Significant reductions in inflammatory responses and mortality in zebrafish, *Danio rerio*, infected with *Staphylococcus aureus* pre-incubated with grape seed extract was reported (Kao et al. 2010). When fed a commercial diet containing 5% grape seed extract, greenlip abalone (*Haliotis laevigata* Donovan) exposed to high summer water temperature stress were reported to have shown improved survival and feed intake in (Lange et al., 2014; Duong et al., 2016; Shiel et al., 2017).

To protect cells against damage caused by free radicals, organisms have developed several defense mechanisms. These include antioxidant enzymes such as SOD, CAT GSH-Px, and non enzymatic antioxidants including glutathione, flavonoids, vitamin A, vitamin E, vitamin C, and ubiquinone (Urso et al., 2003). Antioxidant enzymes are recognized as the primary cellular defense against free radical-mediated oxidative stress. The level of these enzymes is a pertinent indirect approach to evaluate the antioxidant-prooxidant conditions. GSH is an antioxidant that defends against exogenous toxic injury by augmenting the defense via scavenging of free radicals. Its concentration in tissues can be used as a marker of oxidative stress in freshwater fish under the effect of a particular toxicant (Sukhovskaya et al., 2017). In this study, dietary Cd exposure significantly elevated MDA content in the hepatopancreas tilapias compared to the control group. The high level of MDA suggests that the lipid peroxidation in the hepatopancreas enhances ROS produced by heavy metals exposure (Zhai et al., 2015). The levels T-AOC and GSH and CAT, SOD and GSH-Px activities in the present study decreased under dietary Cd exposure. It was found that when the stress by heavy metals exposure is severe and prolonged, the antioxidant enzymes are depleted, and their concentrations decline (Annabi et al., 2007). In this study, most parameters related to antioxidant potential in

hepatopancreas of Cd+GSPs groups were better than those of control group, which suggests that GSPs alleviate oxidative stress in tilapia exposed to dietary Cd. Another study also found in found that dietary 400 mg/kg GSPs could significantly decrease the levels of free radicals in hepatopancreas of GIFT tilapia exposed to 100 mg/kg Cd in diets (Lu 2014). The protective effects of GSPs have also been found in the oxidative stress-mediated hepatic injury and dysfunction in rats (Nazimabashir et al., 2014; Miltonprabu et al., 2016). Therefore, GSPs might act as the exogenous antioxidant and be taken through diet to maintain homeostasis between free radicals and antioxidants and thus prevent heavy metal toxicity. The strong antioxidant effect of GSPs is due to effective hydrogen donation, as well as effective delocalization of an unpaired electron. The 2,3 double bond in the C-ring in conjugation with the carbonyl in the C4 improves electron delocalization, which stabilizes the antioxidant radical (Pekkarinen et al., 1999).

In summary, the present study showed that GSPs alleviated growth retardation and could act oxidative stress of tilapia exposed by dietary Cd. The results suggest that GSPs as an effective protector from oxidative stress-induced by dietary heavy metals in fish.

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