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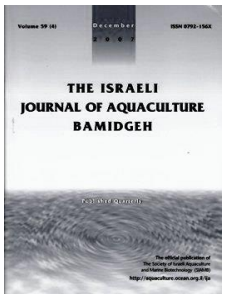

Effects of Quercetin on Alleviating Dietary Lead (Pb)-Induced Growth Retardation and Oxidative Stress in Juvenile Tilapia (*Oreochromis niloticus*)

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Key words: Quercetin, tilapia, growth, oxidative stress, dietary lead

Abstract

This trial spanning 28 days, was conducted to investigate the effects of quercetin on alleviating dietary lead (Pb)-induced growth retardation and oxidative stress in juvenile tilapia (*Oreochromis niloticus*). Four hundred fish were randomly divided into four treatments with four replicates in each group, 25 fish in each replicate. The four treatments were: control treatment (fed with a basal diet), Pb treatment (fed with a basal diet+800 mg Pb/kg), Pb+Q1 treatment (fed with a basal diet+800 mg Pb/kg+800 mg quercetin/kg), and Pb+Q2 treatment (fed with a basal diet+800 mg Pb/kg+1600 mg quercetin/kg). Compared with the control treatment, final body weight, weight gain rate and feed conversion rate of Pb treatment were significantly affected ($P<0.05$). The significant differences in growth performances (except feed conversion rate of Pb+Q1 treatment) were found between Pb treatment and all quercetin supplementation treatments ($P<0.05$). There were no significant differences in growth performance between the control treatment, Pb+Q1 treatment and Pb+Q2 treatment ($P>0.05$). Survival rate of all treatments was similar ($P>0.05$). Malondialdehyde level, total antioxidation capacity level, and activity of superoxide dismutase, catalase and glutathione peroxidase in hepatopancreas of Pb treatment were significantly affected ($P<0.05$). No significant differences in malondialdehyde level and antioxidant potential parameters (except catalase in Pb+Q2 treatment) were found between the control treatment and all quercetin supplementation treatments ($P>0.05$).Results indicated that dietary quercetin supplementation could ameliorate the harmful effects of dietary Pb exposure on growth and effectively normalize antioxidant status in hepatopancreas of tilapia.

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Introduction

Lead (Pb) is a non-nutrient metal and a serious pollutant in the aquatic environment. The potential threat of Pb to aquatic creatures comes mainly from waterborne and diet born Pb. In the past, most attention was paid to the toxicity and mitigating methods of waterborne Pb in aquatic organisms (Weber, 1993; Lorenz et al., 2001).There is growing interest in dietary Pb toxicity to aquatic organisms due to the increasing occurrence of serious dietary Pb contamination in aquaculture (Dai et al., 2009a; Dai et al., 2012; Luszczek-Trojnar et al., 2012). Pb in diets taken up in fish via the gastrointestinal tract leads to Pb accumulation and impairs the function of most body tissues and organs, leading to toxic effects such as physiological, biochemical, behavioral, and genotoxic dysfunction (Dai et al., 2012; Luszczek-Trojnar et al., 2012; Luszczek-Trojnar et al., 2014). Toxicity produced by Pb may be due to the fact that it induces oxidative damage in tissues (Dai et al., 2010; Ling and Hong, 2010). Elevated calcium in diets mitigates Pb toxicity (Alves and Wood, 2006). There is a growing need to develop effective and economical technologies to alleviate Pb toxicity to fish (Dai et al., 2010; Dai et al., 2012). Research findings suggest that administration of various antioxidants can reduce or prevent various toxic effects of Pb and generation of oxidative stress (Saglam et al., 2013). Naturally occurring non-enzymatic antioxidants such as carotenoids, flavonoids, minerals, vitamins etc., and the role they play in neutralizing free radicals generated in the body under dietary Pb stress conditions, have been researched (Flora et al., 2012).

Among all the antioxidants, quercetin, a typical catechol type of flavonoid, is distributed ubiquitously in fruit and vegetables. It is of interest due to its potential as a free-radical scavenger, modulator of enzymatic activity, inhibitor of cellular proliferation, as well as its potential antibiotic, antiallergic, antidiarrheal, antiulcer, and antiinflammatory characteristics (Ross and Kasum, 2002; Kim er al., 2012). Quercetin is known to act as an effective antioxidant by chelating heavy metal ions and/or scavenging free radicals.

These biological effects of quercetin seem to be associated with its potency as an antioxidant (Terao, 2009). Quercetin is absorbed quickly and is deposited in aglycone form and acts as an antioxidant in tilapia (*Oreochromis niloticus*) (Park et al., 2009). The improvement of antioxidant potential in hepatopancreas of tilapia was confirmed (Zhai and Liu, 2014). Little information is available about its effects on dietary Pb-induced impairment to tilapia. The importance of tilapia in global aquaculture has increased over the past several decades (Sarker et al., 2012). Tilapia is a suitable species to study the effects of quercetin on alleviating dietary lead-induced oxidative damage and growth retardation in fish. In this study, we investigated the potential of quercetin for alleviating dietary Pb damage to fish, and the effects of dietary quercetin supplementation on growth performance and antioxidant potential in tilapia (*Oreochromis niloticus*) exposed to 800 mg/kg dietary Pb.

Materials and Methods

Materials. Healthy male juvenile tilapia (*Oreochromis niloticus*) were purchased from Chengyi Aquaculture Company of Xiamen (China). Quercetin (content>98%) was obtained from Nanjing Zelang Medical Technology Co., LTD of Nanjing (China).

Experimental design, diets and fish rearing conditions. Fish were acclimated to laboratory conditions in two plastic tanks (200 cm × 90 cm × 100 cm) at 26±1 °C under a natural photoperiod for 4 weeks. Aerated water was supplied to the circular culture system with additional aeration provided by an air pump. 50% of the water was changed daily. The tilapia were fed a commercial basal diet from FWUSOW industry Corporation Limited (Xiamen, China) to satiation three times daily. 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). The values of dissolved oxygen, pH and ammonia-N ranged between 6 to 7.5 mg/l, 6.7to 7.3 and 0 to 0.2 mg/l, respectively. In order to avoid waterborne pb contamination, the feces after first clearance were removed daily every 2 hours with a sihon tube. Waterborne Pb was not detected throughout the experiment.

After acclimation, 400 fish with an initial average body weight of 14.44±3.82 g and initial body length of 8.20±0.65cm were randomly divided into four treatments in four replicate circular aquaria (86 cm × 54 cm × 54 cm) for each treatment, 25 fish in each replicate, and reared under the same conditions as those during the acclimation period for 4 weeks. The four treatment diets fed to the fish were: basal diet (control diet), basal diet added with 800 mg Pb/kg, basal diet supplemented with 800 mg Pb/kg and 800 mg Quercetin/kg and basal diet supplemented with 800 mg Pb/kg and 1600 mg Quercetin/kg, respectively. Pb-enriched diets were formulated by adding lead nitrate to the commercial basal diet (Alves et al., 2006). The measured dietary Pb concentrations in the four diets were 3.45±0.02, 805.69±5.34, 802.98±6.71, 806.53±5.79 mg/kg, respectively. Pb concentration was determined by atomic absorption spectrophotometry with a graphite furnace and an acetylene-air flame (Solaar M6, Thermo Electron, USA).

Ingredients and proximate analyses of the basal diet are presented in Table 1. All diets were mixed well and pelleted using a laboratory pellet machine with a 2.5-mm diameter module without heating. After processing, the diets were packed into small bags and stored at -20 °C until use.

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 (computed value, 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.

Sample collection and analysis. At the end of the trial, five fish from each replicate were sampled at random and anaesthetized by dipping in 50 mg/l of eugenol oil suspension in water for 30s. The body weight of the fish was measured; the hepatopancreata were collected and stored at -80 °C for analysis of antioxidative parameters. The hepatopancreata from each replication 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 supernatant was assayed (Bradford, 1976). The level of malondialdehyde (MDA) in hepatopancreas was measured (Buege and Aust, 1978). The level of MDA was expressed as nmol/mg protein. The level of total antioxidant capacity (T-AOC) was measured (Benzie and Strain, 1996). Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity were measured (Kakkar et al. 1984). The catalase (CAT) activity was measured (Góth, 1991). The values of T-AOC, GSH-Px, SOD and CAT activities were expressed as units per mg protein.

Data Calculation. At the beginning and at end of the trial, body weight and length of the fish in each aquarium were 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).

Statistical analysis. Statistical analysis was performed with SPSS 11.5 statistical software (SPSS, Chicago, IL, USA). The results are presented as means ± SD of four replicates. 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 mean values among the treatment groups. Data expressed as percentages or ratios were subjected to arcsine transformation prior to statistical analysis.

Results

Growth performance and Survival. The values for growth performance and survival of tilapia are shown in table 2. Compared with control treatment, the FBW, WGR and FCR of Pb treatment were significantly affected ($P<0.05$) by the Pb ingestion. These parameters except FCR were similar between Pb treatment and Pb+Q1 treatment ($P>0.05$), while a significant difference was found between Pb treatment and Pb+Q2 treatment ($P<0.05$. No significant difference was found between Pb+Q1 treatment and Pb+Q2 treatment ($P>0.05$). The SR values were similar among all the treatments ($P>0.05$).

Table 2. Effects of dietary quercetin on growth and survival parameters of tilapia under dietary lead stress

Item	*Treatments*			
	Control	*Pb*	*Pb+Q1*	*Pb+Q2*
IBW(g/fish)	14.16±0.42	14.16±0.39	14.13±0.36	14.27±0.41
FBW(g/fish)	$35.76±1.65^{b}$	$30.25±1.20^{a}$	$33.27±1.16^{ab}$	$35.46±1.23^{b}$
WGR(%)	$150.29±12.48^{c}$	$110.61±8.76^{a}$	$130.35±10.57^{ab}$	$148.57±14.07^{bc}$
FCR	$1.48±0.09^{a}$	$1.81±0.05^{b}$	$1.59±0.07^{a}$	$1.58±0.10^{a}$
SR(%)	100	100	100	100

IBW= initial body weight; FBW= final body weight; WGR= weight gain rate; FCR= feed conversion ratio; SR= survival rate.
^{abc} Values within the same column with different superscripts were significantly different ($P<0.05$).

MDA levels and antioxidant potential in hepatopancreas. The MDA levels and antioxidant potential in hepatopancreas of tilapia are shown in table 3. Compared with control treatment, the MDA level and SOD and GSH-Px activity of tilapia exposed to dietary Pb were significantly increased ($P<0.05$), and the T-AOC level and CAT activity were significantly decreased ($P<0.05$). No significant differences of MDA levels and antioxidant potential were found between the control treatment and quercetin supplemented treatments ($P>0.05$). Significant differences of MDA levels and antioxidant potential were found between Pb treatment and quercetin supplemented treatments ($P<0.05$). The MDA levels and antioxidant potential apart from CAT activity were similar between Pb+Q1 treatment and Pb+Q2 treatments ($P>0.05$).

Table 3. Effects of dietary Quercetin on MDA levels and antioxidant potential in hepatopancreas of tilapia under dietary lead stress

Item	*Treatments*			
	Control	*Pb*	*Pb+Q1*	*Pb+Q2*
MDA (nmol/mg protein)	$0.22±0.09^{a}$	$0.48±0.05^{b}$	$0.23±0.07^{a}$	$0.21±0.10^{a}$
T-AOC (U/mg protein)	$2.65±0.12^{b}$	$1.69±0.22^{a}$	$2.53±0.31^{b}$	$2.68±0.14^{b}$
SOD (U/mg protein)	$140.29±12.48^{a}$	$165.61±8.76^{b}$	$145.35±10.57^{a}$	$138.57±14.07^{a}$
CAT (U/mg protein)	$41.49±0.58^{b}$	$30.25±1.20^{a}$	$42.40±1.31^{b}$	$49.74±1.09^{c}$
GSH-Px (U/mg protein)	$13.52±1.52^{a}$	$18.16±1.69^{b}$	$14.13±1.56^{a}$	$13.27±1.61^{a}$

MDA= malondialdehyde; T-AOC= total antioxidation capacity; SOD= superoxide dismutase; CAT= catalase; GSH-Px =glutathione peroxidase. ^{abc} Values within the same column with different superscripts were significantly different ($P<0.05$).

Discussion

In the present study, growth performance of tilapia was significantly affected by dietary Pb. These results were not consistent with results reported by Dai et al. (2009b), where tilapia were fed with dietary Pb 800mg/kg. The IBW of tilapia was about 32 g per fish (Dai et al. (2009b), while IBW of tilapia in our study was about 14 g per fish. This suggests that fish size might have an effect on resistance to Pb stress. Growth performance of freshwater rainbow trout (*Oncorhynchus mykiss*) was not affected when fish were fed 520 mg Pb/kg for 21days (Alves et al., 2006). This suggests that different species may react differently to Pb stress. Growth performance of tilapia exposed to dietary Pb with Quercetin supplementation was significantly better in comparison with the Pb treatment and similar to the control indicating that quercetin supplementation reduces growth retardation induced by dietary Pb. In previous research, the growth promoting effect of quercetin was demonstrated through the increase of digestive enzyme activity of intestine, level of immunity, and antioxidant ability in fish (Shin et al., 2010; Zhai and Liu, 2014).

Enzymatic antioxidants like SOD, CAT, GSH-Px are produced endogenously in cells, whereas non-enzymatic antioxidants like carotenoids, flavonoids, vitamins, minerals, etc. are constituents of many fruits, vegetables, nuts, grains and meat (Ling and Hong, 2010; Flora et al., 2012). The alteration of antioxidant enzymes reflects antioxidant status. In this study, dietary Pb exposure significantly elevated MDA content in the hepatopancreas as compared to the control group. Pb exposure may have increased MDA levels in fish (Dai et al., 2010; Ling and Hong, 2010). The high level of MDA suggests that the lipid peroxidation in the hepatopancreas enhances the reactive oxygen radical (ROS) caused by lead exposure (Atli and Canli, 2010;Dai et al., 2010; Ling and Hong, 2010). The T-AOC level and CAT activity in present study decreased under dietary Pb exposure, whereas compensation of the SOD and GSH-Px activity increased. Similar alteration of SOD and GSH-Px were found in the hepatopancreas and red and white muscle of tilapia with cadmium exposure (Almeida et al., 2002). SOD and GSH-Px behave differently under oxidative stress. When the stress from Pb exposure is moderate, SOD/GSH-Px activity is stimulated, but if stress is prolonged, the enzyme becomes less effective and its concentration declines (Campana et al., 2003; Annabi et al., 2007). In this study, dietary Pb exposure increased GSH-Px and SOD activity, due to the short duration of Pb exposure.

In the present study, no significant differences of antioxidant potential in the hepatopancreas were found between the control and the Pb+Quercetin treatments. This suggests that quercetin alleviates oxidative stress of tilapia exposed to dietary Pb and possibly acts as an exogenous antioxidant, helping to maintain a balance between free radicals and antioxidants; it also prevents heavy metal toxicity as well as other deleterious effects (Flora et al., 2012). Quercetin is absorbed quickly and this flavonoid is deposited mainly in aglycone form in the hepatopancreas after absorption in tilapia (Park et al., 2009). The catechol structure of quercetin, which possesses two hydroxyl groups at neighboring positions, is remarkably superior to other flavonoids in terms of electron donating ability. Therefore, quercetin can exert a powerful radical scavenging influence. The mechanism for the antioxidative action of dietary Quercetin can be explained by the chelating action of lead by forming a coordinate covalent bond with the lead ions through its ortho-phenolic groups located on the quercetin B ring (Bravo and Anacona, 2001).

The present study showed that quercetin mitigated growth retardation and oxidative stress of tilapia exposed to dietary Pb. This suggests that quercetin could act as an active, in vivo antioxidant in tilapia exposed to dietary Pb.

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