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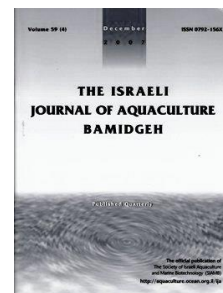
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Effects of Dietary Quercetin on the Growth Performance, Digestive Enzymes and Antioxidant potential in the Hepatopancreas of Tilapia (*Oreochromis niloticus*)

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Abstract

This trial was conducted to investigate the effects of Quercetin on the growth performance, activities of digestive enzymes, and antioxidant potential in the hepatopancreas of tilapia. Four hundred tilapia (*Oreochromis niloticus*), were randomly divided into five treatment groups with four replicates in each group/ 20 fish per replicate. The dietary Quercetin levels of the five treatment groups were 0, 200, 400, 800, and 1600 mg/kg, respectively. The trial period was 49 days. Rates of weight gain, special growth, feed conversion, and protein efficiency ratio of the Quercetin diet groups improved significantly ($P<0.05$) compared with the control group. No significant difference in survival rates were found between the control group and all Quercetin diet groups ($P>0.05$). The activity of protease, lipase, and amylase, in the stomach and intestine significantly increased when the dietary Quercetin level was above 400 mg/kg. The malondialdehyde level, and total antioxidant capacity in the hepatopancreas of Quercetin diet groups decreased significantly while catalase activity of Quercetin diet groups (apart from the group fed 1600 mg/kg Quercetin) increased significantly ($P<0.05$) with no significant differences between all Quercetin groups. Superoxide dismutase activity was not affected by Quercetin supplementation. The results of the present study suggested that Quercetin supplementation in a tilapia diet could have beneficial effects on digestive enzyme activity, antioxidant potential in the hepatopancreas, and improve growth performance.

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Introduction

Tilapia production has increased significantly on a global scale. Development of the fish culture industry and increasing demand for fish production requires intensive fish culture which often creates a highly stressful environment for fish. This results in growth inhibition, physiological disorders, or even death (Bunch and Bejerano, 1997). In order to alleviate stress, and enhance disease resistance in fish, some feed additives such as vitamin C, vitamin E, selenium (Diraman et al, 2009; Wang et al, 2009), and some oligosaccharides (Genc et al, 2007), have been tested. Herbal medicines such as flavonoids have been known to improve the overall health of animals (Kim et al., 2012; Murota and Terao, 2003). Flavonoids (or Vitamin P), the secondary metabolites of plants, are a group of phenolic compounds possessing strong antioxidant properties and influencing many other physiological conditions. They can eliminate free-radicals, lower blood lipid levels, protect organs, and inhibit the incidence of cancers (Pietta, 2000). The antioxidant capacity of flavonols is approximately fifty times higher than that of vitamin C (Dai et al, 2004).

Quercetin, one of the most widely distributed flavonols present in fruit and vegetables, can inhibit the oxidation of high density lipoprotein and low density lipoprotein, decrease the level of blood lipids (Yang et al, 2008), protect the liver and the cardiovascular system (Molina et al, 2003), and enhance immunity in terrestrial animals (Exon et al, 1998). All these biological activities of Quercetin seem to be associated with its potency as an antioxidant (Prince and Sathya, 2010). Studies on the effects of Quercetin on physiological functions have, in the main, focused on terrestrial animals; there is limited information regarding its application in aquatic animals. This research has been conducted to investigate the effects of dietary Quercetin supplementation on the growth performance, digestive enzyme activities, and antioxidant potential on tilapia (*Oreochromis niloticus*)

Materials and Methods

Fish and experimental design. Healthy male tilapia (*Oreochromis niloticus*), purchased from the Chengyi Aquaculture Company of Xiamen (China), were acclimatized in two plastic tanks (200 cm × 90 cm × 100 cm), and during the adaptation period were fed a commercial diet three times daily for 4 weeks. After adaptation to experimental conditions, four hundred fish, initial average body weight of 11.54±2.83 g, were randomly divided into five treatment groups; four replicates / group; 20 fish / replicate. The dietary Quercetin levels of the five treatment groups were 0, 200, 400, 800 and 1600 mg/kg, respectively. The trial continued for 49 days.

Preparation of diets. Ingredients and proximate analyses of the basal diet are presented in Table 1. The different levels of Quercetin (content >98%, purchased from Nanjing Zelang Medical Technology Co., Ltd., Nanjing, China) were supplemented in the basal diet. All feed ingredients were thoroughly mixed and cold pelleted with a laboratory pelleting machine using a 2.5-mm diameter module. After processing, the diets were packed into small bags and stored at -20 °C until further 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 extract	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	20		
Monocalcium phosphate	20		
Choline chloride	2		
Vitamin premix ¹	2		
Mineral premix ²	6		

¹ Vitamin premix (mg kg⁻¹ 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⁻¹ diet): NaCl, 1.0; MgSO₄•7H₂O, 15; NaH₂PO₄•2H₂O, 25; KH₂PO₄, 32; Ca(H₂PO₄)₂•H₂O, 20; FeSO₄, 2.5; calcium lactate, 3.5; ZnSO₄•7H₂O, 0.353; MnSO₄•4H₂O, 0.162; CuSO₄•5H₂O, 0.031; CoCl₂•6H₂O, 0.01; KIO₃, 0.003.

Dietary Quercetin and Tilapia Growth

Fish rearing conditions. After adaptation to experimental conditions the fish were kept in twenty circular aquaria (86 cm × 54 cm). Aerated water was supplied to the circular culture system with additional aeration provided by an air pump. The daily water exchange rate was 50%.

Fish were fed to satiation three times a day (at 8:00 h, 13:00 h and 18:00 h). Thirty minutes after the feeding, uneaten pellets and feces were siphoned out. Water quality was monitored twice weekly with a multiparameter photometer (HI9804N, HANNA, Baranzate, MI, Italy). The values of dissolved oxygen, pH, ammonia-N, and nitrite-N, ranged between 6-8 mg/l, 6.8-7.2, 0-2 mg/l and 0-0.5 mg/l, respectively. The water temperature ranged from 22°-28°C.

Data collection and calculation. At the beginning and the end of the trial, fish were batch-weighed after 1 day of feed deprivation. Consumption of each diet was recorded. Fish growth performance was evaluated on the basis of final weight, weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR). These indices were calculated as follows:

WGR = (final weight - initial weight/initial weight) × 100%;

SGR (%/day) = [Fn final wet weight (g) - In initial wet weight (g)]/Time (days);

FCR = feed intake/weight gain;

PER = body weight gain (g)/protein fed (g);

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

Sample collection. At the end of the trial, five fish were sampled at random from each replicate and anesthetized by dipping in 50 mg/l of eugenol oil suspension in water for 30s. The weight and body length of each sampled fish was measured, and the hepatopancreas, stomach, and intestines were collected and stored at -80 °C for analysis of antioxidant parameters and digestive enzymes.

Assay for activity of digestive enzymes. The stomach and intestines 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 and the supernatants with the enzyme extracts collected and stored at -80 °C until assayed.

Total soluble protein was measured using the Bradford (1976) method. Total protease activity was measured using casein hydrolysis according to the Walter (1984) method. One unit of protease activity (U) is defined as the amount of enzyme in 1 mg protein needed to catalyze the formation of 1 mg of tyrosine in 1 min. Amylase activity was determined by the starch hydrolysis method, according to the Somogy-Nelson colorimetric method, described by Bernfeld (1955). One unit of amylase activity (U) is defined as the amount of enzyme in 1 mg protein needed to catalyze 10 mg starch in 30 min. Lipase activity was measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). One unit of lipase activity (U) is defined as the amount of enzyme in 1 mg protein needed to consume 1 μmol substrate.

Assay for antioxidants. The hepatopancreas was homogenized in the same way as the intestine. The level of malondialdehyde (MDA) in the hepatopancreas was measured by thiobarbituric acid reactive species assay following the Buege and Aust (1978) method. The level of MDA was expressed as nmol/mg protein. The level of total antioxidation capacity (T-AOC) was measured by the Benzie and Strain (1996) method. One T-AOC unit is defined as 0.01 increase of absorbance value caused by 1 mg protein for 1 min at 37 °C. The superoxide dismutase (SOD) activity was assessed using tetrazolium salt to detect superoxide radicals generated by xanthine oxidase and hypoxanthine (Kakkar et al, 1984). One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical at 37 °C. The catalase (CAT) activity was measured according to the Góth (1991) method using a CAT assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). One unit of CAT is defined as the amount of enzyme needed to degrade 1 μmol H₂O₂ (hydrogen peroxide) for 1 sec at 37 °C. The values of T-AOC, SOD, and CAT activities are expressed as units per mg protein.

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 the 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 WGR, SGR, and PER, in Quercetin diet groups were significantly higher than those of the control group ($P < 0.05$), and their FCR were significantly lower than that of the control group ($P < 0.05$). No significant differences in WGR, SGR, and FCR were found among the Quercetin diet groups ($P > 0.05$). Survival rate of tilapia at the end of the experiment ranged from 92.5%-95%, and there were no significant differences among all the treatments ($P > 0.05$). Values of growth performance parameters are presented in Table 2.

Table 2. Growth and survival parameters of the tilapia fed diets with different Quercetin levels.

Quercetin level (mg/kg)	WGR (%)	SGR (%)	FCR	PER (%)	SR (%)
0	401.20±49.57 ^a	3.28±0.20 ^a	1.41±0.10 ^b	2.36±0.18 ^a	93.75±1.25
200	543.71±24.05 ^b	3.80±0.08 ^b	1.06±0.07 ^a	3.11±0.20 ^c	92.50±1.44
400	535.72±48.35 ^b	3.79±0.13 ^b	1.20±0.05 ^a	2.78±0.11 ^{bc}	94.25±1.25
800	505.05±21.73 ^b	3.67±0.07 ^b	1.16±0.10 ^a	2.87±0.26 ^{bc}	95.00±1.00
1600	523.05±22.09 ^b	3.73±0.07 ^b	1.23±0.06 ^a	2.69±0.09 ^{ab}	92.50±1.44

WGR= weight gain rate;SGR= specific growth rate;FCR= feed conversion ratio;PER= protein efficiency ratio;SR= survival rate.

^{abc}Values within the same column without the same superscript were significantly different at $P < 0.05$ level. It's the same for the following table.

Activity of digestive enzymes. Protease, lipase, and amylase activity in the stomach of the 200 mg/kg Quercetin diet group was not affected significantly ($P > 0.05$) compared to the control group. Protease activity in the intestine of these two groups (the control and the 200 mg/kg quercetin diet group) was similar ($P > 0.05$) while significant difference ($P < 0.05$) was observed in the lipase and amylase activity in the intestine. Protease, lipase, and amylase activity in the stomach and intestine of all Quercetin diet groups (except 200 mg/kg Quercetin group) was significantly higher than in the control group ($P < 0.05$). There were no significant differences of protease, lipase, and amylase activity in the stomach between the 400, 800 and 1600 mg/kg Quercetin diet groups ($P > 0.05$). Similar results were found in lipase and amylase activity in the intestine. Of all the groups, protease activity in the 1600 mg/kg Quercetin diet group was highest. Digestive enzyme activity in stomach and intestine of tilapia fed with different levels of Quercetin is shown in Table 3.

Table 3. Effect of dietary Quercetin level on digestive enzyme (U/mg protein) activity in stomach and intestine of tilapia.

Quercetin level (mg/kg)	Stomach			Intestine		
	Protease	Lipase	Amylase	Protease	Lipase	Amylase
0	8.54±0.75 ^a	24.96±1.40 ^a	1.81±0.10 ^a	50.80±6.44 ^a	33.83±5.94 ^a	2.63±0.20 ^a
200	9.08±0.83 ^a	29.90±2.00 ^a	1.74±0.07 ^a	53.25±1.59 ^a	59.22±2.43 ^b	3.34±0.22 ^b
400	13.36±0.36 ^b	39.49±3.67 ^b	2.13±0.31 ^b	67.24±3.92 ^b	60.84±3.59 ^b	4.82±0.24 ^c
800	15.36±1.57 ^b	43.45±2.64 ^b	1.92±0.33 ^b	73.95±7.47 ^{bc}	63.51±9.16 ^b	4.71±0.43 ^c
1600	13.04±1.83 ^b	38.49±3.21 ^b	1.94±0.12 ^b	80.97±5.31 ^c	56.61±7.35 ^b	4.54±0.14 ^c

Antioxidant parameters in the hepatopancreas. The results of antioxidant parameters in tilapia hepatopancreas are given in Table 4. The levels of MDA and T-AOC in Quercetin diet groups significantly decreased when compared with the control ($P < 0.05$), and no significant differences were found among all the Quercetin diet groups ($P > 0.05$). The CAT activities of Quercetin diet groups (except the 1600 mg/kg Quercetin group) were significantly higher than that of the control group ($P < 0.05$). There was no significant difference in SOD activity between the control group and the groups fed 200, 400 and 800 mg/kg Quercetin diets ($P > 0.05$), while the SOD activity in the group with 1600 mg/kg Quercetin supplementation was significantly lower than those of other groups ($P < 0.05$).

Dietary Quercetin and Tilapia Growth

Table 4. Antioxidant parameters in hepatopancreas of tilapia fed with different level of quercetin.

Quercetin level (mg/kg)	MDA (nmol/mg prot)	T-AOC (U/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)
0	0.68±0.10 ^a	0.92±0.17 ^a	3.00±0.01 ^a	18.59±1.38 ^b
200	0.48±0.08 ^b	0.42±0.06 ^b	3.10±0.08 ^a	22.47±1.94 ^a
400	0.39±0.03 ^b	0.41±0.07 ^b	3.14±0.31 ^a	22.72±1.52 ^a
800	0.48±0.07 ^b	0.51±0.10 ^b	3.23±0.23 ^a	23.40±1.90 ^a
1600	0.50±0.06 ^b	0.54±0.04 ^b	2.42±0.18 ^b	20.71±0.29 ^{ab}

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

Discussion

Supplementing Quercetin to diets significantly improved the growth performance of tilapia. A similar result in flounder showed that the weight gain of flounder fed a diet supplemented with Quercetin, for 30 and 60 days was significantly higher than the control group (Shin et al., 2010). It also indicated that 5,000 mg/kg Quercetin is more effective than 2,500 mg/kg Quercetin for growth. Research on Quercetin supplemented diets in fish diets is limited and the mechanism of Quercetin growth enhancement is not clear. From the results of previous related research, it appears that the growth promoting effect of Quercetin might be due to increased digestive enzyme activity of the intestine, increased immune system activity, and antioxidant activity in fish (Shin et al., 2010; Liu, 2012). Further research is needed to clarify the detailed mechanisms of how Quercetin promotes growth. In our study, 200 mg/kg Quercetin had a statistically significant effect on growth of tilapia. It is not clear if a Quercetin level of lower than 200 mg/kg would have significantly affected growth. The optimal level of Quercetin in tilapia diets needs further examination.

In this study it was found that apart from amylase, the activity of digestive enzymes in the stomach and intestine were significantly enhanced after feeding tilapia a Quercetin supplemented diet for 49 days. Increased activity of digestive enzymes induced by Quercetin may have promoted digestion and absorption of nutrients, and improved fish growth. Reports concerning the effects of Quercetin on digestive enzymes in fish are scarce, but isoflavone also enhanced digestive enzyme activity of *Anguilla rostrata* (Ye and Chen, 2008). The mechanism of Quercetin in promoting digestive enzyme activity is probably connected to its antioxidant properties. Quercetin supplementation may decrease oxidative damage caused by the overproduction of free radicals thereby protecting the digestive organs, such as pancreas (Coskun et al, 2005), liver (Janbaz, 2004), the gastric mucosa, and intestinal mucosa (Martín et al, 1998), from free radical damage.

MDA and T-AOC levels in Quercetin groups decreased significantly, CAT activity increased, and SOD activity remained unchanged when compared to the control group. The lower levels of MDA and T-AOC and higher activity of CAT implied that fewer free radicals were generated and antioxidant status of fish fed dietary Quercetin was improved. These observations are consistent with the report that the levels of lipid peroxidation and the activities of SOD in flounder treated with 2,500 mg/kg and 5,000 mg/kg Quercetin remarkably decreased (Shin et al., 2010). SOD and glutathione peroxidase mRNA expressions decreased, while the CAT mRNA expression increased in Quercetin-treated fish (Röhrdanz et al., 2003). Quercetin might save SOD by direct depletion of free radicals, reducing SOD activity to keep the balance of oxidation and anti-oxidation. Increased CAT activity might be due to the chelating effect of Quercetin on ferrous ions (Leopoldini et al, 2006).

Quercetin is known to be a powerful antioxidant, capable of acting as a pro-oxidant, inducing biological damage (Galati and O'Brien, 2004; Bando et al, 2007). This may explain why the activities of SOD and CAT for the highest dose of Quercetin (1600mg/kg group) were inferior to those of other Quercetin groups.

In conclusion, this study demonstrated that supplementary Quercetin in tilapia diets was beneficial to the activity of some digestive enzymes and antioxidant status in the hepatopancreas, and also improved growth performance. Further studies are necessary to determine the optimal supplementation level of Quercetin in tilapia diets.

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