Effects of dietary grape seed proanthocyanidins on growth performance, some serum biochemical parameters and body composition of tilapia (Oreochromis niloticus) fingerlings

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Abstract

The present study was performed with tilapia (Oreochromis niloticus) to evaluate the effects of diet supplementation with grape seed proanthocyanidins (GSPs) on fish growth performance, some serum parameters and body composition. Three hundred tilapia fingerlings with the initial average body weight of 8.25±0.07 g were randomly divided into five treatment groups with four replicates in each group and 15 fish in each replicate. The dietary GSPs levels of five treatment groups were 0 (control group), 200, 400, 600, and 800 mg/kg, respectively. The trial period was 49 days. Growth performance parameters were significantly improved by GSPs supplementation (P<0.05), while survival rates were similar among all groups (P>0.05). Serum parameter results showed that activities of aminotransferase aspartate in 200 and 400 mg/kg GSPs groups and alanine aminotransferase in 400 mg/kg GSPs group were lowered significantly (P<0.05). Levels of triglyceride and total cholesterol (except 200 mg/kg GSPs group) were significantly lowered, while lysozyme activity was significantly increased (P<0.05). Concentrations of GSPs (0, 200, 400, 600 and 800 mg/kg of dry matter) were added in the basal diet. All feeding ingredients were thoroughly mixed and cold pelleted using 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 used.

Introduction

Dietary flavonoids from plant foods have attracted considerable attention as a dietary additive to improve the health status and growth of aquatic animals (Kao et al., 2010; Zhai and Liu, 2013). The oligomeric proanthocyanidins are classified as flavonols and usually derived from grapeseeds or pine bark. They are also present in red wines, hops, and various flowers, leaves, fruits, berries, nuts, and beans, usually with high concentrations in skins, barks, and seeds. They contain catechin monomer, dimer and trimer, all of which are water-soluble molecules and contain a number of phenolic hydroxyls (Bagchi et al., 2002). Grape seed proanthocyanidins (GSPs) are primarily known for their antioxidant activity. The strong antioxidant effect of GSPs is due to the effective hydrogen donation, as well as effective delocalisation of an unpaired electron. The 2,3 double bond in the C-ring in conjugation with the carbonyl in the C4 improves electron delocalisation, which stabilises the antioxidant radical (Pekkarinen et al., 1999). These compounds have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions. In addition, they have been found to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems. Grape seed proanthocyanidins may be a useful component in the treatment of a number of conditions (Fine, 2000).

The safety of GSPs in oral administration was proved by some studies that determined the acute oral toxicity and the lethal dose 50 and some genotoxicity tests of the GSPs in rats (Yamakoshi et al., 2002; Lluís et al., 2011). Grape seed proanthocyanidins are used as human nutritional supplements in the United States, Australia, Japan, Korea and other countries, and grape seed extract is also used in Japan as a food additive. The dimeric procyanidins were found to be absorbed into the blood stream, and some of the products of hydrolyses of the higher oligomers and polymers were presumed to be absorbed through the intestinal membrane, and then the absorbed procyanidins and/or hydrolysates of procyanidins might display various physiological and biological functions in vivo (Yamakoshi et al., 2002).

The beneficial effects of GSPs were mostly reported in terrestrial animals (Dulundu et al., 2007; Yousef et al., 2009; El-Ashmawy et al., 2010; Boghdady, 2013; Hassan and Al-Rawi, 2013). Little information is available about dietary GSPs application on aquatic animals. The significant reduction of inflammatory responses and mortality was observed in zebrafish infected with S. aureus (Kao et al., 2010). More work is needed to confirm the beneficial effects of GSPs in aquatic animals. In this study, we mainly focus on the biological effects of GSPs on growth performance, blood biochemical parameters and whole fish body composition of tilapia.

Materials and methods

Experimental diets

Ingredients and proximate analyses of basal diet are presented in Table 1. Five experimental diets were formulated to contain various concentrations of GSPs (0, 200, 400, 600 and 800 mg/kg of dry matter). 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. 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 used.
Fish and feeding trial

Healthy tilapia (*Oreochromis niloticus*) fingerlings, purchased in the Development Center for Aquatic Animal of Zhangzhou (China), were acclimatised 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 condition, the fish were kept in twenty circular aquaria (86 cm×54 cm×54 cm). Three hundred fish with the average initial body weight (IBW) of 8.25±0.07 g were randomly divided into five treatment groups with four replicates in each group and 15 fish in each replicate. Fish were fed the diets with the GSPs levels being 0 (control group), 200, 400, 600, and 800 mg/kg, respectively. The trial continued for 49 days.

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 faeces were removed by a siphon tube. The water quality was monitored twice weekly with a multiparameter photometer (HI9804N; HANNA, Baranzate, Italy). The values of dissolved oxygen, pH, ammonia-N and nitrite-N ranged between 6 to 8 mg/L, 6.8 to 7.2 mg/L, 0 to 0.20 mg/L and 0 to 0.15 mg/L, respectively. The water temperature ranged from 22 to 28°C.

Sample collection and analysis

At the end of the trial, five fish were sampled at random from each replicate and anesthetised by dipping in 50 µL of eugenol oil suspension in water for 30 s, and then the blood sample was collected from the caudal vein of each anesthetised fish. After kept at 4°C for 30 min, the blood sample was centrifuged at 3500 g for 10 min at 4°C, and the supernatant serum was collected and stored at -80°C prior to analysis of serum lipids parameters. After collecting blood samples, the five fish were killed in an ice bath, then the fish from each replicate were pooled, weighed, minced and then dried at 70°C. The crushed fish samples were weighed, sealed in plastic bags and stored at -20°C for subsequent analysis of whole-body composition.

The analyses of aminotransferase aspartate (AST), alanine aminotransferase (ALT) and lysozyme (LZ) and levels of triglyceride (TG), total cholesterol (TC) and albumin (ALB) in serum were determined with commercial clinical investigation kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All parameters were analysed by an automated biochemical analyser (Hitachi 7020; Hitachi Ltd., Tokyo, Japan).

The analyses of proximate composition on feed ingredients, experimental diets and whole fish body were performed using the standard methods of AOAC (1995). Moisture content was determined by the drying method using an oven at 105°C. Crude protein content was determined by the Kjeldahl method after an acid digestion using Kjeltec™ 8400 Auto Sample Systems (Foss Tecator AB; Foss, Hillersed, Denmark) and crude protein was estimated by multiplying nitrogen content by 6.25. Crude lipid content was extracted by n-hexane using the Soxhlet method (Soxtec™ 2050; Foss). Ash content was determined by the combustion method using a muffle furnace at 550°C for 6 h.

Data calculation

At the beginning and at the end of the trial, body weight was measured for the fish in each aquarium after 1 day of feed deprivation. The consumption of diet was recorded. The weight gain (WG), protein efficiency ratio (PER), feed conversion ratio (FCR), and survival rate (SR) were calculated as follows: IBW (g/fish)=IBW of fish (g)/initial number of fish; FBW (g/fish)=final body weight of fish (g)/final number of fish; WG (g/fish)=final weight gain (g)-initial weight gain (g); PER(%)=100×(final body weight per fish (g)/initial body weight per fish (g))/protein intake per fish (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±standard deviation 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.

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

| Ingredients, g/kg |        |        |        |        |        |
|------------------|--------|--------|--------|--------|--------|
| Fish meal        | 50     |        |        |        |        |
| Soybean meal     | 150    |        |        |        |        |
| Rapeseed meal    | 200    |        |        |        |        |
| Cotton seed meal | 200    |        |        |        |        |
| High-gluten flour| 150    |        |        |        |        |
| Rice bran        | 200    |        |        |        |        |
| Soybean oil      | 20     |        |        |        |        |
| Monocalcium phosphate | 20 |        |        |        |        |
| Choline chloride | 2      |        |        |        |        |
| Vitamin premix⁶  | 2      |        |        |        |        |
| Mineral premix⁶  | 6      |        |        |        |        |

Nutrient level

| Crude protein, % | 33.4 |
| Crude fat, %     | 5.7  |
| Crude ash, %     |      |
| Digestible energy (calculated value), MJ/kg | 12.0 |

¹Vitamin premix (mg/kg diet): thiamin, 0.25; lactoflavin, 0.25; nic acid, 1.0; pantothenic acid calcium, 1.25; folic acid, 0.075; biotin, 0.01; hydrochloric acid pyridoxine, 0.2; cobalt amine, 0.0005; vitamin C, 5; vitamin K, 0.2; niacin, 10; vitamin E, 2; vitamin A, 0.2; choline, 20.

²Mineral premix (mg/kg diet): NaCl, 1.8; MgSO₄•7H₂O, 15; NaH₂PO₄•H₂O, 25; KH₂PO₄, 32; Ca(H₂PO₄)₂•H₂O, 20; FeSO₄•7H₂O, 2.5; calcium lactate, 1.5; ZnSO₄•7H₂O, 0.553; MnSO₄•H₂O, 0.162; CuSO₄•5H₂O, 0.01; CoCl₂•6H₂O, 0.01; KI, 0.0001.

Results

The values of growth performance parameters are presented in Table 2. The FBW, WG and PER in groups of fish fed GSPs were significantly higher than those of the control group (GSPs unsupplemented group) (P<0.05), and their FCR was significantly lower than that of the control group (P<0.05). No significant differences in FBW, WG, and FCR were found among the GSPs supplemented groups (P>0.05). There were no significant differences of SR among all the treatment groups (P>0.05).

The values of serum biochemical parameters are presented in Table 3. The AST activities were significantly different between control group and group fed 200 mg/kg or 400 mg/kg GSPs (P<0.05), no significant differ-
ences were found among other groups (P>0.05). The significant difference in ALT activity was found only between the control group and group fed 400 mg/kg GSPs (P<0.05). The TC (except 200 mg/kg GSPs group) and TG concentrations of the GSPs supplemented groups were significantly lower than those in control group (P<0.05), there were also no significant differences among all the GSPs supplemented groups (P>0.05).

The values of body composition of tilapia are presented in Table 4. No differences were found for moisture and ash levels among all the treatment groups (P>0.05). The crude protein level in GSPs supplemented groups (except 200 mg/kg GSPs supplemented group) was significantly higher than that of control group (P<0.05), there was no significant difference among the GSPs supplemented groups (P>0.05). The crude lipid levels of GSPs supplemented groups were significantly lower than that in control group (P<0.05), there were also no significant difference among the GSPs supplemented groups (P>0.05).

Table 2. Growth performance and survival rate of tilapia fingerlings fed diets with different grape seed proanthocyanidins levels.

| GSPs level, mg/kg | IBW, g/fish | FBW, g/fish | WG, g/fish | FCR | PER, % | SR, % |
|------------------|-------------|-------------|-----------|-----|--------|------|
| 0                | 8.30±0.06   | 44.50±1.93a | 36.20±2.15a | 1.48±0.04b | 240.52±4.16a | 97.93±4.15 |
| 200              | 8.27±0.07   | 54.61±5.09b | 46.33±3.14b | 1.30±0.04a | 259.36±3.83b | 95.85±4.79 |
| 400              | 8.24±0.07   | 57.31±4.35b | 49.07±4.43b | 1.31±0.05a | 258.39±6.74b | 97.93±4.15 |
| 600              | 8.24±0.07   | 54.31±4.47b | 46.06±1.71b | 1.31±0.02a | 263.23±3.36b | 100 |
| 800              | 8.22±0.08   | 52.13±4.70b | 43.90±4.22b | 1.38±0.06a | 264.64±4.63b | 95.85±4.79 |

GSPs, grape seed proanthocyanidins; IBW, initial body weight; FBW, final body weight; WG, weight gain; FCR, feed conversion ratio; PER, protein efficiency ratio; SR, survival rate. a,bValues (mean±standard deviation, n=4) in the same column with different superscripts differ significantly (P<0.05).

Table 3. Some serum biochemical parameters of tilapia fingerlings fed diets with different grape seed proanthocyanidins levels.

| GSPs level, mg/kg | AST, U/mg prot | ALT, U/mg prot | TG, mmol/L | TC, mmol/L | LZ, U/mL | ALB, g/L |
|------------------|----------------|---------------|------------|------------|----------|--------|
| 0                | 12.22±4.04b   | 46.21±4.61b   | 4.56±0.31b | 8.66±0.85b | 0.63±0.03a | 7.34±1.15 |
| 200              | 5.84±1.92a    | 38.00±6.86b   | 1.71±0.14b | 8.10±0.12b | 1.02±0.02b | 12.14±1.03 |
| 400              | 6.29±1.18a    | 28.27±4.89a   | 1.97±0.23a | 6.24±0.41a | 0.87±0.11b | 12.96±1.09 |
| 600              | 11.78±4.17b   | 33.43±8.76b   | 1.96±0.37a | 6.91±0.17a | 0.59±0.07b | 13.90±1.51 |
| 800              | 14.13±1.24b   | 46.82±7.36b   | 2.31±0.23a | 6.76±0.30a | 0.96±0.03b | 13.23±1.19 |

GSPs, grape seed proanthocyanidins; AST, aminotransferase aspartate; ALT, alanine aminotransferase; TG, triglyceride; TC, total cholesterol; LZ, lysozyme; ALB, albumin. a,bValues (mean±standard deviation, n=4) in the same column with different superscripts differ significantly (P<0.05).

Table 4. Body composition of tilapia fingerlings fed diets with different grape seed proanthocyanidins levels.

| GSPs level, mg/kg | Moisture, % | Crude protein, % | Crude lipid, % | Ash, % |
|------------------|-------------|------------------|---------------|-------|
| 0                | 74.04±0.62 | 15.03±0.17a     | 5.61±0.21b   | 4.16±0.04 |
| 200              | 74.37±0.62 | 15.43±0.13b     | 5.05±0.10b   | 4.16±0.04 |
| 400              | 74.65±0.65 | 15.63±0.45b     | 4.85±0.18b   | 4.14±0.04 |
| 600              | 74.30±0.86 | 15.74±0.23b     | 4.92±0.05b   | 4.18±0.03 |
| 800              | 73.69±0.28 | 15.86±0.12b     | 4.94±0.12b   | 4.19±0.06 |

GSPs, grape seed proanthocyanidins. a,bValues (mean±standard deviation, n=4) in the same column with different superscripts differ significantly (P<0.05).
tion effect on growth. Therefore, the optimal level of GSPs in tilapia diet should be confirmed in future studies.

Blood enzyme activity values (AST and ALT) and the levels of energetic metabolites (triglycerides and cholesterol) of fish are considered important diagnostic characters (Coz-Rakovac et al., 2005). Often their values are used in estimating the health condition of fish (Wood et al., 1990; Christofilogiannis, 1993). The TG levels are often used in fish studies as an indicator of liver disease and also considered to be major indices of the health status of teleosts (Wagner and Congleton, 2004). The TC levels can indicate disorders of lipid and lipoprotein metabolism and especially liver dysfunction. In many previous studies, it has also been commonly used as a diagnostic tool for biomonitoring the health status of farmed fish (Coz-Rakovac et al., 2005; Mensinger et al., 2005).

The serum lysozyme is used as an indicator of innate immune response in fish (Tort et al., 2003). It plays an important role in innate immunity by lysis of bacterial cell wall and thus stimulates the phagocytosis of bacteria (Ellis, 1990). The albumin is an easily available protein reserve and a protein transporter (Anderson et al., 1979). The increased albumin and globulin increased levels are considered a strong innate response in fish (Wiegerjies et al., 1996). The lower levels of AST, ALT, TC, and TG of GSPs supplemented groups in this study indicated that the tilapia might have better health status. This point was also confirmed by the higher levels of LZ and ALB, which are considered as the indicator of strong innate immune function.

The protective role of GSPs against adverse effects on health status in rats were extensively reported. The high levels of the serum biochemical parameters AST, ALT, TC, and TG induced by dietary azathioprine (El-Ashmawy et al., 2010), biliary obstruction (Dulundu et al., 2007), cisplatin (Yousef et al., 2009), doxorubicin (Boghdady, 2013), or glibberelic acid (Hassan and Al-Rawi, 2013), were lowered or normalised by dietary GSPs supplementation in above studies. From the results of those reports in rats, it was concluded that GSPs could reduce organ injury through the ability to balance the oxidant-antioxidant status.

In this study, higher protein and lower lipid levels were found in the body of tilapia fed diet containing GSPs, which was consistent with the values found in hybrid Crucian carp fed diet containing grape seed extract (Huang et al., 2012). The higher protein levels of all GSPs supplemented groups is related to the decreased crude lipid levels. The lower crude lipid in tilapia fed diets with GSPs supplementation might be due to its effects on lipid metabolism. It was found that GSPs could repress intestinal lipid absorption, chylomicron secretion by the intestine and very low density lipoprotein secretion by the liver (Osakabe and Yamagishi, 2009; Bladé et al., 2010; Ngamukote et al., 2011), inhibit intestinal lipoprotein secretion (Pal et al., 2003; Vidal et al., 2005), inhibit cellular cholesterol uptake and 5-lipoxygenase activity (Leifert and Aebwyardena, 2008), and stimulate serum ability to induce efflux of cellular cholesterol (Senault et al., 2000). The mechanism of GSPs on lipid metabolism in terrestrial animals might be similar with that in aquatic animals, which should be confirmed in the future studies.

The growth performance, serum biochemical parameters and body composition of tilapia were not improved with the increasing GSPs levels in this study. Similar results found that excess dietary GSPs could not have more beneficial effects on broiler (Wang et al., 2008; Zhou et al., 2013), piglets (Xie et al., 2012), and hybrid Crucian carp (Huang et al., 2012). The excessive intake of GSPs and other flavonoids have been suggested to exert adverse effects on the body by acting as prooxidants that generate free radicals and induce biological damage (Fine, 2000; Galati and O’Brien, 2004; Bando et al., 2007). Hence, the adequate GSPs level should be supplemented in fish diet according to the stress degree.

Conclusions

In conclusion, this study demonstrates that supplementary GSPs in tilapia fingerlings diet improve the growth performance and body composition, and confirms the beneficial effects on serum biochemical parameters observed by other authors in other species. Further studies will be necessary in order to determine the optimal supplementation level of GSPs in tilapia diet.

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