

Effects of Chlorogenic Acid Supplementation on Growth Performance and Lipid Metabolism in Grass Carp Fed a High-Fat Diet (Postprint)

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Abstract

This study aimed to investigate the effects of dietary chlorogenic acid (CGA) supplementation in high-fat diets on growth performance and lipid metabolism in grass carp. A total of 360 healthy grass carp with an initial body weight of (7.53 ± 0.30) g were randomly divided into 4 groups. Groups C (control), 200CGA, 400CGA, and 600CGA were fed high-fat diets (crude lipid content approximately 9.00%) supplemented with CGA at levels of 0, 200, 400, and 600 mg/kg, respectively. Each group had 3 replicates with 30 fish per replicate, and the feeding trial lasted for 11 weeks. The results showed that, compared with group C: 1) The 400CGA group exhibited significantly increased final body weight, weight gain rate, and protein efficiency ($P < 0.05$), with final body weight increasing by 15.90%, weight gain rate improving by 22.96%, and protein efficiency enhancing by 17.75%, while feed conversion ratio was significantly decreased ($P < 0.05$) by 14.86%; 2) Serum total cholesterol (T-CHO) and low-density lipoprotein cholesterol (LDL-C) contents were significantly reduced in the 200CGA and 400CGA groups ($P < 0.05$), while serum high-density lipoprotein cholesterol (HDL-C) content was significantly elevated in all treatment groups ($P < 0.05$); 3) Hepatopancreatic triglyceride (TG), T-CHO, and LDL-C contents were significantly decreased in all treatment groups ($P < 0.05$), hepatopancreatic hepatic lipase (HL) and total lipase (TL) activities were significantly increased ($P < 0.05$), hepatopancreatic HDL-C content was significantly elevated in the 400CGA and 600CGA groups ($P < 0.05$), and hepatopancreatic lipoprotein lipase (LPL) activity was significantly increased in the 400CGA group ($P < 0.05$); 4) Transmission electron microscopy observations revealed that hepatopancreatic cells in the 400CGA and 600CGA groups contained smaller, fewer, and more sparsely distributed lipid droplets. These results suggest that dietary CGA supplementation at a level of 400 mg/kg in high-fat diets can promote lipid metabolism and improve growth performance in grass carp.

Full Text

Effects of Chlorogenic Acid Supplementation in High-Fat Diets on Growth Performance and Lipid Metabolism of Grass Carp (*Ctenopharyngodon idellus*)

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Abstract: This study investigated the effects of dietary chlorogenic acid (CGA) supplementation on growth performance and lipid metabolism in grass carp. A total of 360 healthy grass carp with initial body weight of (7.53 ± 0.30) g were randomly allocated into four groups: C (control), 200CGA, 400CGA, and 600CGA. Each group comprised three replicates of 30 fish, fed high-fat diets (crude lipid content approximately 9.00%) supplemented with 0, 200, 400, and 600 mg/kg CGA, respectively, for 11 weeks. Compared with the control group: (1) The 400CGA group exhibited significantly higher final body weight, weight gain rate, and protein efficiency ratio ($P < 0.05$), with increases of 15.90%, 22.96%, and 17.75%, respectively, and significantly lower feed coefficient ($P < 0.05$), which decreased by 14.86%; (2) The 200CGA and 400CGA groups showed significantly reduced serum total cholesterol (T-CHO) and low-density lipoprotein cholesterol (LDL-C) contents ($P < 0.05$), while all treatment groups had significantly elevated serum high-density lipoprotein cholesterol (HDL-C) content ($P < 0.05$); (3) All treatment groups demonstrated significantly lower hepatopancreatic triglyceride (TG), T-CHO, and LDL-C contents ($P < 0.05$), with significantly higher hepatopancreatic hepatic lipase (HL) and total lipase (TL) activities ($P < 0.05$). The 400CGA and 600CGA groups also showed significantly higher hepatopancreatic HDL-C content ($P < 0.05$), and the 400CGA group had significantly higher hepatopancreatic lipoprotein lipase (LPL) activity ($P < 0.05$); (4) Transmission electron microscopy revealed smaller, fewer, and more sparsely distributed lipid droplets in hepatopancreatic cells of the 400CGA and 600CGA groups. These results indicate that supplementation of high-fat diets with 400 mg/kg CGA can promote lipid metabolism and improve growth performance in grass carp.

Keywords: grass carp; chlorogenic acid; high-fat diet; growth performance; lipid metabolism

Fish have relatively short digestive tracts and low digestive enzyme activity, resulting in poor utilization of dietary protein and leading to excessive ammonia

nitrogen in water. This restricts fish growth and triggers diseases. To reduce nitrogen and phosphorus levels in aquaculture environments and prevent disease outbreaks, practical feeding strategies typically involve reducing dietary protein levels while moderately increasing lipid content under the premise of ensuring nutritional balance. This approach promotes fish growth while protecting the aquatic environment.

Under normal conditions, the dietary lipid requirement for grass carp ranges from 3% to 7%. However, excessive dietary lipid levels can cause abnormal fat accumulation in fish, damage hepatic and pancreatic tissues, reduce growth rates, increase feed coefficients, and ultimately decrease farming profitability. Grass carp consume large amounts of feed but have limited capacity to utilize high-fat diets, making them prone to excessive fat deposition and lipid metabolism disorders.

Chlorogenic acid (CGA), extracted from medicinal plants such as *Lonicera* and *Eucommia*, possesses antibacterial, anti-inflammatory, and antioxidant properties. Numerous studies have reported that CGA promotes growth and reduces lipid and glucose levels. While CGA has been investigated as a feed additive for Pacific white shrimp and its effects on grass carp muscle quality have been documented, its impact on lipid metabolism in fish remains unreported. Therefore, this study examined the effects of different CGA supplementation levels in high-fat diets (crude lipid content approximately 9.00%) on growth performance and lipid metabolism in grass carp, aiming to address problems of excessive fat accumulation and lipid metabolism disorders caused by high-fat diets and to provide a theoretical basis for CGA application in aquaculture and development of novel aquatic feed additives.

1.1 Experimental Diets

Since the dietary lipid requirement for grass carp is 3%-7%, this study formulated diets with 8% soybean oil (crude lipid content approximately 9.00%). CGA supplementation levels (purchased from Sigma, purity 95%) were based on previous studies by Zhang et al. and Wang et al. on common carp, soft-shelled turtle, and Pacific white shrimp: 0 (C), 200 (200CGA), 400 (400CGA), and 600 mg/kg (600CGA) CGA added to the basal diet, with microcrystalline cellulose used as filler to maintain consistent total diet weight.

Feed ingredients were ground and passed through a 60-mesh sieve. Soybean oil was added after all powder ingredients were thoroughly mixed. After oil incorporation, appropriate amounts of distilled water were added, and the mixture was processed into 1-2 mm diameter pellets using a small pelletizer. Prepared pellets were air-dried in a cool, dry location with fan assistance for 48 hours, then sealed in ziplock bags and stored at -20°C. Diet composition and nutrient levels are presented in Table 1 .

1.2 Experimental Design and Management

Grass carp were provided by the Jiangxi Institute of Fisheries. After transportation to the animal husbandry research facility of Jiangxi Academy of Agricultural Sciences, fish were disinfected with potassium permanganate and acclimated to water temperature before being stocked in an indoor recirculating aquaculture system. The system contained 20 circular tanks (100 cm diameter, 80 cm height) with temperature control. Culture water had ammonia nitrogen <0.05 mg/L, pH ~ 7.5 , and dissolved oxygen >5 mg/L. Continuous aeration was maintained throughout the 24-hour period.

After a 10-day acclimation period, 360 healthy grass carp with body weight of (7.53 ± 0.30) g were selected and randomly divided into four groups, each fed one of the experimental diets. Each group had three replicates of 30 fish per tank. Fish were fed daily at 09:00 and 16:00 at 5%-7% of body weight. Water exchange and siphoning for waste removal were performed 2-3 times weekly, with approximately one-third of the tank volume replaced each time. The formal experiment began on August 24, 2016, and ended on November 8, 2016, lasting 11 weeks.

1.3 Sample Collection

Feeding was stopped 24 hours before the experiment ended. All fish in each tank were weighed and counted. Eighteen fish per group (six per replicate) were randomly selected for measurement of body length and width. Viscera were completely removed, washed with phosphate-buffered saline (PBS), blotted dry with filter paper, and weighed. Additionally, six fish per group were randomly selected for determination of whole-body conventional nutrient composition, with samples stored at -80°C pending analysis.

For blood collection, 18 fish per group (six per replicate) were anesthetized, and caudal vein blood was drawn using 1 or 2 mL disposable syringes. Blood was allowed to coagulate naturally at 4°C overnight, then centrifuged at 3,500 r/min for 15 minutes. Serum was collected and stored at -20°C . After blood collection, fish were placed in dissecting trays, the abdominal cavity was opened, and the hepatopancreas was completely removed, washed with PBS, surface moisture removed with filter paper, and stored at -80°C .

1.4 Preparation of Hepatopancreas Homogenate

Hepatopancreas samples stored at -80°C were gradually thawed (-20°C , then 4°C). Samples were accurately weighed and placed in a 20 mL glass homogenizer. Nine volumes of ice-cold 0.68% saline solution (relative to tissue weight) were precisely added. The tissue was slowly ground for 3-5 minutes on ice. The homogenate was centrifuged at 3,000 r/min for 10 minutes in a refrigerated centrifuge. Surface lipids were removed with clean, sterilized cotton swabs, and the supernatant was carefully collected, aliquoted, and stored at -80°C .

1.5 Hepatopancreas Transmission Electron Microscopy

Hepatopancreas tissue was removed and rinsed three times with PBS. Approximately 1 mm³ sections were cut and fixed in 2.5% glutaraldehyde for over 4 hours, then rinsed twice with PBS for 1 hour each. Samples were post-fixed in 1% osmium tetroxide for 1 hour, rinsed twice with PBS for 5 minutes each, and dehydrated in ethanol. After two 10-minute washes with 1,2-epoxypropane, samples were infiltrated with a 1:1 mixture of epoxypropane and epoxy resin for 1 hour, then embedded in labeled molds. After overnight setting, samples were placed in a 60°C oven for 48 hours, sectioned (50-60 nm for TEM), stained (1% uranium acetate for 10 minutes), and photographed (nine samples per group).

1.6.1 Growth Performance Indicators

Initial body weight (IBW), final body weight (FBW), feed intake, and other data were recorded to calculate weight gain rate (WGR), feed coefficient (FC), viscerosomatic index (VSI), condition factor (CF), protein efficiency ratio (PER), and survival rate (SR) using the following formulas:

$$\text{WGR (\%)} = (\text{We} - \text{Ws}) / \text{Ws} \times 100$$

$$\text{FC} = (\text{We} - \text{Ws}) / \text{Wf}$$

$$\text{VSI (\%)} = \text{Wv} / \text{We} \times 100$$

$$\text{CF (g/cm}^3\text{)} = \text{We} / \text{L}^3$$

$$\text{PER (\%)} = (\text{We} - \text{Ws}) / \text{Wp} \times 100$$

$$\text{SR (\%)} = \text{ne} / \text{ns} \times 100$$

Where: We = FBW (g); Ws = IBW (g); t = experimental duration (d); Wf = dry feed intake (g); Wv = viscera weight (g); L = body length (cm); Wp = crude protein content in feed (%); ne and ns = final and initial fish numbers, respectively.

1.6.2 Whole-Body Conventional Nutrient Composition Determination

Whole-body moisture content was determined by oven drying at 105°C to constant weight (GB 5009.3-2010). Crude protein content was measured by the Kjeldahl method (GB 5009.5-2010). Crude lipid content was determined by Soxhlet extraction (GB 5009.4-2010). Crude ash content was measured by muffle furnace incineration (GB 5009.4-2010).

1.6.3 Lipid Metabolism Indicators

Assay kits for serum and hepatopancreatic triglyceride (TG), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and hepatopancreatic hepatic lipase (HL), lipoprotein lipase (LPL), and total lipase (TL) activities were purchased from Nanjing Jiancheng Bioengineering Institute and used according to manufacturer protocols.

1.7 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 20.0 software. When significant differences were detected, Duncan's multiple range test was performed. Significance level was set at $P < 0.05$. Results are expressed as mean \pm standard deviation.

2.1 Effects of CGA Supplementation on Growth Performance

As shown in Table 2, no significant differences were observed in final body length or viscerosomatic index among groups ($P > 0.05$). Final body weight, weight gain rate, and protein efficiency ratio increased initially then decreased with increasing CGA supplementation, peaking in the 400CGA group, which differed significantly from the control group ($P < 0.05$). Specifically, final body weight increased by 15.90%, weight gain rate by 22.96%, and protein efficiency ratio by 17.75%. Feed coefficient was collectively lower in all treatment groups compared with the control, with the 400CGA group showing a significant 14.86% reduction ($P < 0.05$). Condition factor increased with CGA supplementation, with the 600CGA group significantly higher than the control ($P < 0.05$). Survival rate was 100% in all groups.

2.2 Effects of CGA Supplementation on Whole-Body Nutrient Composition

Table 3 shows that whole-body moisture content did not differ significantly among groups ($P > 0.05$). The 400CGA group exhibited significantly higher crude protein content and significantly lower crude lipid content compared with other groups ($P < 0.05$). The control group had significantly higher crude ash content than the 400CGA group ($P < 0.05$).

2.3.1 Serum Lipid Indicators

As presented in Table 4, serum TG content did not differ significantly among groups ($P > 0.05$). Serum T-CHO content in the control group was significantly higher than in the 200CGA and 400CGA groups ($P < 0.05$) but not significantly different from the 600CGA group ($P > 0.05$). Serum HDL-C content in the control group was significantly lower than in all treatment groups ($P < 0.05$). Serum LDL-C content in the control group was significantly higher than in the 400CGA and 600CGA groups ($P < 0.05$) but not significantly different from the 200CGA group ($P > 0.05$).

2.3.2 Hepatopancreatic Lipid Indicators

Table 5 reveals that hepatopancreatic TG and T-CHO contents in the control group were significantly higher than in all treatment groups ($P < 0.05$). Hepatopancreatic HDL-C content in the 400CGA and 600CGA groups was significantly higher than in the control group ($P < 0.05$), though the difference

between the control and 200CGA groups was not significant ($P > 0.05$). Hepatopancreatic LDL-C content in the control group was significantly higher than in all treatment groups ($P < 0.05$).

2.4 Effects of CGA on Hepatopancreatic Lipid Metabolism Enzyme Activities

As shown in Table 6, hepatopancreatic HL activity in all treatment groups was significantly higher than in the control group ($P < 0.05$). The 400CGA group exhibited significantly higher hepatopancreatic LPL activity compared with the control ($P < 0.05$). Hepatopancreatic TL activity in all treatment groups was significantly higher than in the control group ($P < 0.05$).

2.5 Effects of CGA Supplementation on Lipid Deposition in Hepatopancreatic Cells

Lipids in tissue sections appear black after osmium tetroxide reduction, as shown in Figure 1 [Figure 1: see original paper]. The control group displayed dense, numerous, and large lipid droplets, indicating substantial lipid deposition in hepatopancreatic cells. In contrast, treatment groups showed markedly smaller and more sparsely distributed lipid droplets, with reduced lipid deposition, particularly in the 400CGA and 600CGA groups. Further comparison revealed that the 400CGA group had fewer, smaller, and more sparsely distributed lipid droplets than the 600CGA group, indicating superior lipid metabolism at the 400 mg/kg supplementation level.

3.1 Effects of CGA Supplementation on Growth Performance

As a lipid-soluble compound, CGA is absorbed in its original form in the stomach and small intestine, entering the bloodstream to exert antioxidant and lipid-lowering effects. The growth-promoting, antioxidant, lipid-lowering, and glucose-reducing effects of CGA in livestock and poultry have received considerable attention, and preliminary studies have explored CGA as a novel feed additive in aquaculture.

Xiao reported that 200 mg/kg dietary CGA improved growth performance in soft-shelled turtles. Zhang demonstrated that 200 mg/kg CGA significantly increased weight gain in common carp and promoted intestinal development, which may explain the improved growth performance. In this study, compared with the control group, 400 mg/kg CGA supplementation significantly increased final body weight, weight gain rate, and protein efficiency ratio by 15.90%, 22.96%, and 17.75%, respectively, while decreasing feed coefficient by 14.86%. Condition factor increased gradually with CGA supplementation, with 600 mg/kg CGA producing a significant increase. These results indicate that 400 mg/kg CGA supplementation enhanced growth performance in grass carp. The optimal supplementation levels differ from previous studies, possibly due

to variations in fish species, culture duration, dietary nutrient composition, and CGA sources.

3.2 Effects of CGA Supplementation on Whole-Body Nutrient Composition

This study found that CGA supplementation significantly affected whole-body crude lipid, crude protein, and crude ash contents. The 400CGA group showed significantly higher crude protein content than other groups, suggesting that 400 mg/kg CGA promoted protein utilization. This differs from findings in spotted scat. Studies on tilapia, flounder, and European sea bass have reported that excess dietary lipid deposits in fish tissues when not metabolized. In this study, the significantly lower whole-body crude lipid content in the 400CGA group indicates that 400 mg/kg CGA effectively reduced lipid deposition.

CGA supplementation also decreased whole-body crude ash content, with the control group significantly higher than the 400CGA group. This may be because the control group, lacking CGA to assist lipid metabolism, accumulated more lipid, which promoted absorption and deposition of fat-soluble vitamins A, D, E, and K and energy substances. These vitamins participate in various physiological and biochemical reactions, potentially increasing inorganic salt deposition and ash content, though this hypothesis requires further verification. The significant effects of 400 mg/kg CGA on whole-body crude lipid and protein contents demonstrate its ability to enhance the nutritional value of grass carp.

3.3.1 Serum and Hepatopancreatic Lipid Metabolism

Shimoda reported that oral CGA reduced visceral fat accumulation and lipid content in rats. De Sotillo found that intravenous CGA administration significantly decreased TG content in rat liver and plasma. Frank demonstrated that dietary CGA reduced hepatic T-CHO content in rats. Zhang showed that oral administration of 20 mg/mL CGA significantly decreased TG content in mouse liver and skeletal muscle. Wang reported that honeysuckle extract CGA effectively reduced serum and hepatic T-CHO content.

This study found that CGA supplementation effectively reduced serum T-CHO and LDL-C contents while increasing serum HDL-C content, indicating beneficial regulation of serum lipid metabolism. The 400CGA group showed significant changes in all three parameters, suggesting that 400 mg/kg CGA optimally regulates serum lipid metabolism. CGA also demonstrated clear lipid-lowering effects in the hepatopancreas. Under these experimental conditions, the control group had significantly higher hepatopancreatic TG and LDL-C contents and significantly lower HDL-C content compared with treatment groups, confirming that CGA promotes hepatopancreatic lipid metabolism. These findings align with Shimoda and De Sotillo, corroborating CGA's regulatory role in lipid metabolism.

Both 400 and 600 mg/kg CGA enhanced lipid metabolism capacity. Comparing lipid metabolism indicators between these groups and the control revealed that 400 mg/kg CGA produced optimal regulatory effects. In serum, the 400CGA group showed significant changes in T-CHO, HDL-C, and LDL-C contents. In hepatopancreas, compared with the control, the 400CGA group reduced TG, T-CHO, and LDL-C by 64.73%, 47.05%, and 57.95%, respectively, while increasing HDL-C by 74.95%. The 600CGA group reduced TG, T-CHO, and LDL-C by 60.07%, 41.16%, and 45.35%, respectively, while increasing HDL-C by 76.04%. Thus, 400 mg/kg CGA demonstrated stronger regulatory effects on hepatopancreatic lipid metabolism than 600 mg/kg. Collectively, 400 mg/kg CGA provided optimal regulation of serum and hepatopancreatic lipid metabolism.

3.3.2 Hepatopancreatic Lipid Metabolism Enzymes

LPL primarily hydrolyzes TG in chylomicrons, very low-density lipoproteins, and intermediate-density lipoproteins into free fatty acids and glycerol for tissue storage and oxidative utilization. HL mainly hydrolyzes TG in chylomicron remnants and participates in HDL remodeling, VLDL metabolism, and reverse cholesterol transport regulation. This study demonstrated that CGA supplementation effectively increased hepatopancreatic lipid metabolism enzyme activities, with significant differences observed in the 400CGA group compared with the control. Specifically, the 400CGA group increased LPL, TL, and HL activities by 57.95%, 74.95%, and 86.24%, respectively. The significant reduction in hepatopancreatic TG and T-CHO contents in the 400CGA group may result from elevated LPL and HL activities promoting TG hydrolysis into free fatty acids. Therefore, 400 mg/kg CGA supplementation enhanced lipid metabolism capacity in grass carp hepatopancreas under high-fat conditions.

3.3.3 Effects of CGA Supplementation on Lipid Deposition in Hepatopancreatic Cells

Osmium tetroxide reacts with lipids to form black osmium-lipid complexes that are insoluble in organic solvents like xylene used in fixation, enabling precise visualization of lipid droplet size and quantity. Transmission electron microscopy of osmium-stained hepatopancreatic sections revealed numerous, large lipid droplets densely distributed in hepatocytes of the control group, indicating substantial lipid deposition and severe metabolic burden from long-term high-fat feeding. Failure to timely degrade these lipids can cause nutritional fatty liver disease and impair normal liver function.

In contrast, treatment groups showed smaller, more sparsely distributed lipid droplets, demonstrating that CGA supplementation reduced hepatopancreatic lipid content and fat deposition, particularly in the 400CGA and 600CGA groups. Further observation indicated that the 400CGA group had fewer, smaller, and more sparsely distributed lipid droplets than the 600CGA group, confirming superior lipid metabolism at 400 mg/kg supplementation.

These results demonstrate that 400 mg/kg CGA in high-fat diets promotes hepatopancreatic lipid metabolism.

Conclusion

Under the conditions of this study, supplementation of high-fat diets with 400 mg/kg CGA is recommended for juvenile grass carp to enhance lipid metabolism capacity and promote growth.

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