Regulatory Effects of Fermented Mulberry Leaves on Serum Lipid and Glucose Levels in Hyperlipidemic Tilapia (Postprint)

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Abstract

This experiment employed a high-fat diet to induce a hyperlipidemic tilapia model to investigate the regulatory effects of fermented mulberry leaves on blood lipid and glucose levels in hyperlipidemic tilapia. A total of 450 male GIFT tilapia with an initial average weight of 45 g were randomly divided into 5 groups, with 3 replicates per group and 30 fish per replicate. The normal control group was fed a basal diet, while the other four groups were fed, after establishment of the hyperlipidemia model, a basal diet (model control group), diets containing 7.5% and 15.0% fermented mulberry leaves (low-dose and high-dose fermented mulberry leaf groups, respectively), and a diet containing 0.5% ginkgo flavonoids (ginkgo flavonoid group). The experimental period lasted 8 weeks. The results showed that after 5 and 8 weeks of feeding, the body weight of tilapia in the model control group was significantly lower than that in the normal control, low-dose fermented mulberry leaf, high-dose fermented mulberry leaf, and ginkgo flavonoid groups ($P<0.05$), while the body weight of tilapia in the high-dose fermented mulberry leaf group showed no significant difference from the normal control group ($P>0.05$). Serum total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-C) contents, and TCHO/high-density lipoprotein cholesterol (HDL-C) ratio in the low-dose fermented mulberry leaf, high-dose fermented mulberry leaf, and ginkgo flavonoid groups were significantly lower than those in the model control group ($P<0.05$), whereas the serum TCHO, triglyceride (TG), LDL-C contents, and TCHO/HDL-C ratio in the high-dose fermented mulberry leaf group showed no significant difference from the normal control group ($P>0.05$). Compared with the model control group, serum HDL-C content in the high-dose fermented mulberry leaf and ginkgo flavonoid groups was significantly increased ($P<0.05$). Compared with the model control group, serum superoxide dismutase (SOD) and catalase (CAT) activities as well as

CAT/SOD ratio in the normal control, low-dose fermented mulberry leaf, high-dose fermented mulberry leaf, and ginkgo flavonoid groups were significantly elevated (P<0.05), while serum malondialdehyde (MDA) content, hepatic lipid content, and hepatosomatic index (HSI) were significantly reduced (P<0.05); furthermore, these aforementioned parameters showed no significant difference between the high-dose fermented mulberry leaf group and the normal control group (P>0.05). The high-fat diet significantly elevated blood glucose levels in the model control, low-dose fermented mulberry leaf, high-dose fermented mulberry leaf, and ginkgo flavonoid groups at week 0 (P<0.05); after 8 weeks of feeding, blood glucose levels in the high-dose fermented mulberry leaf and ginkgo flavonoid groups were significantly lower than those in the model control group (P<0.05) and showed no significant difference from the normal control group (P>0.05). In conclusion, fermented mulberry leaves can dose-dependently reduce blood lipid and glucose levels, enhance antioxidant capacity, and prevent the development of hyperlipidemia in hyperlipidemic tilapia.

Full Text

Effects of Fermented Mulberry Leaves on Serum Lipid and Blood Glucose Levels in Hyperlipidemic Tilapia (Oreochromis niloticus)

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Abstract: This study established a hyperlipidemic tilapia model using a high-fat diet to investigate the regulatory effects of fermented mulberry leaves on serum lipid and blood glucose levels in hyperlipidemic tilapia. A total of 450 male GIFT tilapia with an initial average body weight of 45 g were randomly divided into five groups, with three replicates per group and 30 fish per replicate. The normal control group was fed a basal diet, while the other four groups were fed the basal diet (model control group), diets containing 7.5% and 15.0% fermented mulberry leaves (low-dose and high-dose fermented mulberry leaf groups, respectively), or a diet containing 0.5% ginkgo flavone (ginkgo flavone group) after the hyperlipidemia model was established. The experimental period lasted for eight weeks. The results showed that after five and eight weeks of feeding, the body weight of tilapia in the model control group was significantly lower than that in the normal control group, low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group (P<0.05), while no significant difference was observed between the high-dose fermented mulberry leaf group and the normal control group (P>0.05). The serum total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-C) contents, and TCHO/high-density lipoprotein cholesterol (HDL-C) ratio in the low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group were significantly lower than those in the model control group (P<0.05), whereas the serum TCHO, triglyceride (TG), LDL-C contents, and TCHO/HDL-C ratio in the high-dose fermented mulberry leaf group showed no significant differences compared with the normal control group (P>0.05). Compared with the model control group, the serum HDL-C content was significantly elevated in both the high-dose fermented mulberry leaf group and the ginkgo flavone group (P<0.05). Additionally, compared with the model control group, the serum superoxide dismutase (SOD) and catalase (CAT) activities and CAT/SOD ratio were significantly increased (P<0.05), while serum malondialdehyde (MDA) content, hepatopancreas lipid content, and hepatosomatic index (HSI) were significantly decreased (P<0.05) in the normal control group, low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group; however, these indices showed no significant differences between the high-dose fermented mulberry leaf group and the normal control group (P>0.05). The high-fat diet significantly elevated blood glucose levels in the model control group, low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group at week 0 (P<0.05). After eight weeks of feeding, blood glucose levels in the high-dose fermented mulberry leaf group and ginkgo flavone group were significantly lower than those in the model control group (P<0.05) and did not differ significantly from the normal control group (P>0.05). These findings indicate that fermented mulberry leaves can dose-dependently reduce serum lipid and blood glucose levels, enhance antioxidant capacity, and prevent the development of hyperlipidemia in hyperlipidemic tilapia.

Keywords: fermented mulberry leaves; tilapia (Oreochromis niloticus); hyperlipidemia; antioxidant

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Hyperlipidemia is a crucial factor contributing to fatty liver disease in fish and has attracted widespread attention. Studies have shown that dietary factors or nutritional components can also disrupt lipid metabolism in farmed fish, inducing nutritional fatty liver disease that subsequently affects survival, growth, and product quality. Mulberry leaves, which serve both medicinal and dietary purposes, are abundant resources in China and have garnered interest due to their rich content of functional compounds with hypoglycemic and hypolipidemic effects, including polysaccharides, flavonoids, and alkaloids. Animal experiments have demonstrated that dietary supplementation with mulberry leaves or their extracts can exert hypoglycemic and hypolipidemic effects through multiple pathways. Our previous research has also confirmed that mulberry leaves can effectively regulate lipid metabolism in fish. To further validate the lipid-lowering efficacy of mulberry leaves, this study induced hyperlipidemia in tilapia (Oreochromis niloticus) using a high-fat diet and subsequently examined the effects of fermented mulberry leaves on hyperlipidemia and hyperglycemia, providing fundamental data for the development and utilization of mulberry leaves in aquafeeds.

1.1 Experimental Diets

A basal diet was formulated using fish meal, soybean meal, rapeseed meal, and cottonseed meal as protein sources and soybean oil as the lipid source. Three isonitrogenous and isoenergetic experimental diets were prepared by substituting wheat flour with 7.5% and 15.0% fermented mulberry leaves (produced via solid-state fermentation using lactic acid bacteria, yeast, Bacillus, and nitrogen-fixing bacteria; containing 32.5% dry matter, 24.7% crude protein, 10.3% crude lipid, and 8.7% ash) and 0.5% ginkgo flavone (product of Shenzhen Neptune Pharmaceutical Co., Ltd., approval number: National Medicine Standard Z20050178, specification: 9.6 mg total flavonol glycosides and 2.4 mg terpene lactones per tablet), respectively. The composition and nutrient levels of the experimental diets are presented in Table 1 . All dietary ingredients were passed through a 245 m sieve and mixed using the progressive expansion method to produce pellet diets with diameters of 2.5 and 4.0 mm, which were air-dried and stored at 4 °C until use.

1.2 Establishment of the Hyperlipidemic Tilapia Model

A total of 450 male GIFT tilapia with an initial average body weight of 45 g were randomly divided into five groups (one normal control group and four model groups), with three replicates per group and 30 fish per replicate. The normal control group was fed the basal diet, while the model groups were fed a high-fat diet (composition: 10% lard, 2% cholesterol, and 88% basal diet) for two weeks to induce hyperlipidemia before the formal experiment began. After the hyperlipidemia model was established, two model groups were fed experimental diets containing 7.5% and 15.0% fermented mulberry leaves, respectively; one model group was fed the basal diet; and one model group served as the model control and was fed the experimental diet containing 0.5% ginkgo flavone. Tilapia were reared in indoor freshwater recirculating aquaria (effective volume: 300 L) for eight weeks at a daily feeding rate of 4%-6% body weight, with three feedings daily at 08:30, 13:30, and 18:30. The water source was aerated tap water, and during the experimental period, water temperature was maintained at (26.7±2.1) °C, dissolved oxygen concentration >6.7 mg/L, pH at 7.3±0.5, ammonia nitrogen <0.57 mg/L, and nitrite nitrogen <0.09 mg/L.

1.3 Sample Preparation and Analysis

Body weight was measured at the start of the experiment (week 0) and at weeks 2, 5, and 8. Blood samples were collected every 10 days to monitor dynamic changes in whole blood glucose levels. At the end of the feeding trial, fish were fasted for 24 hours and weighed. Four fish from each replicate were randomly selected, anesthetized with MS-222, and dissected to obtain viscera and hepatopancreas for determination of hepatosomatic index (HSI) and hepatopancreas lipid content. An additional three fish from each replicate were randomly selected for blood collection from the caudal vein. Blood was centrifuged at 4,000×g for 10 min at 4 °C to obtain serum, which was stored at -20 °C until analysis.

Serum indices, including total cholesterol (TCHO), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were measured using an automatic biochemical analyzer (Hitachi 7100). Serum superoxide dismutase (SOD) and catalase (CAT) activities and malondialdehyde (MDA) content were determined using assay kits (Nanjing Jiancheng Bioengineering Institute). Blood glucose levels were measured using a Johnson & Johnson blood glucose meter. Protein content was determined using the Coomassie brilliant blue method.

1.4 Data Processing and Analysis

All data are expressed as means ± standard error. One-way ANOVA was performed using SPSS 22.0, and Tukey' s multiple comparison test was applied when significant differences were detected.

2.1 Effects of Fermented Mulberry Leaves on Body Weight of Hyperlipidemic Tilapia

As shown in Table 2 , no significant differences in body weight were observed among all groups at the start of the experiment or after two weeks of feeding ($P>0.05$). However, after five and eight weeks, the body weight of tilapia in the model control group was significantly lower than that in the normal control group, low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group ($P<0.05$), while no significant difference was found between the high-dose fermented mulberry leaf group and the normal control group ($P>0.05$). All groups exhibited varying degrees of weight gain by the end of the feeding trial.

2.2 Effects of Fermented Mulberry Leaves on Serum Lipid Indices of Hyperlipidemic Tilapia

Table 3 shows that after eight weeks of feeding, the serum TCHO and LDL-C contents and TCHO/HDL-C ratio in the low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group were significantly lower than those in the model control group ($P<0.05$). However, the serum TCHO, TG, LDL-C contents, and TCHO/HDL-C ratio in the high-dose fermented mulberry leaf group showed no significant differences compared with the normal control group ($P>0.05$). Additionally, the serum HDL-C content was significantly elevated in both the high-dose fermented mulberry leaf group and the ginkgo flavone group compared with the model control group ($P<0.05$).

2.3 Effects of Fermented Mulberry Leaves on Serum Antioxidant Indices, Hepatopancreas Lipid Content, and Hepatosomatic Index

As presented in Table 4 , compared with the model control group, the serum SOD and CAT activities and CAT/SOD ratio were significantly increased ($P<0.05$), while serum MDA content, hepatopancreas lipid content, and HSI were significantly decreased ($P<0.05$) in the normal control group, low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group. Notably, these indices showed no significant differences between the high-dose fermented mulberry leaf group and the normal control group ($P>0.05$).

2.4 Effects of Fermented Mulberry Leaves on Blood Glucose Levels of Hyperlipidemic Tilapia

Table 5 reveals that the high-fat diet significantly elevated blood glucose levels in the model control group, low-dose fermented mulberry leaf group, high-dose fermented mulberry leaf group, and ginkgo flavone group compared with the normal control group at week 0 ($P<0.05$). After two and four weeks, blood glucose levels in these groups remained significantly lower than in the normal control group ($P<0.05$). However, after eight weeks, blood glucose levels in the high-dose fermented mulberry leaf group and ginkgo flavone group were significantly lower than those in the model control group ($P<0.05$) and did not differ significantly from the normal control group ($P>0.05$).

Abnormal lipid metabolism, particularly elevated serum TCHO, TG, and LDL-C contents and reduced HDL-C content, plays a significant role in the pathogenesis of fatty liver disease in fish. The present results demonstrate that fermented mulberry leaves reduced serum TCHO and TG contents while increasing serum HDL-C content and decreasing LDL-C content and the TCHO/HDL-C ratio in hyperlipidemic tilapia in a dose-dependent manner. These findings suggest that fermented mulberry leaves possess lipid-lowering activity in hyperlipidemic tilapia, possibly by enhancing reverse cholesterol transport. Previous studies have shown that ginkgo flavone regulates lipid levels by increasing plasma HDL-C content and decreasing plasma cholesterol content. Our results confirm that ginkgo flavone can reduce serum lipid levels in hyperlipidemic tilapia, suggesting that fermented mulberry leaves may exert their lipid-regulating effects by influencing lipid-related indices. However, the underlying mechanisms of mulberry leaf-induced lipid regulation in fish require further investigation.

Under normal conditions, SOD primarily scavenges oxygen free radicals to mitigate or prevent damage to the organism caused by free radicals and lipid peroxides, while CAT converts hydrogen peroxide (H O) produced during SOD-mediated reactive oxygen species scavenging into water (H O), thereby protecting the organism from injury. Our study found that preventive administration of fermented mulberry leaves enhanced serum SOD and CAT activities and the CAT/SOD ratio while reducing serum MDA content in hyperlipidemic tilapia.

Previous research has indicated that flavonoids in mulberry leaves lower blood glucose levels by improving antioxidant capacity, promoting insulin secretion, and accelerating glucose oxidation and decomposition. Our findings further support this hypothesis, suggesting that dietary fermented mulberry leaves may improve lipid metabolism and reduce blood glucose and lipid levels by enhancing antioxidant capacity and inhibiting lipid peroxidation. Mulberry leaves contain the alkaloid 1-deoxynojirimycin (DNJ) and flavonoids that can inhibit disaccharidase activity on the intestinal brush border membrane, slowing carbohydrate digestion and absorption, while also regulating key enzyme activities in hepatic glucose metabolism, thereby modulating the metabolism and transformation of the three major nutrients. These mechanisms involve the c-Jun N-terminal kinase (JNK) signaling pathway. Given that fish have limited capacity to utilize carbohydrates, improving their tolerance to dietary carbohydrates holds significant practical importance. The specific mechanisms through which mulberry leaves exert hypoglycemic effects in aquatic animals warrant further investigation.

In conclusion, fermented mulberry leaves can dose-dependently reduce serum lipid and blood glucose levels, enhance antioxidant capacity, and prevent the development of hyperlipidemia in hyperlipidemic tilapia.

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