Feeding tea polysaccharides affects lipid metabolism, antioxidant capacity and immunity of common carp (Cyprinus carpio L.)

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Tea polysaccharides play a role in lipid metabolism, antioxidant capacity and immunity of mammals. To investigate the functions of tea polysaccharides on fish, the common carp (Cyprinus carpio L.) was selected as the animal model in this study. In our study, the common carp (45±0.71g) were randomly divided into four groups and were fed fodder with 50% carbohydrate. The common carp were orally administrated with 0 mg/kg BW (control group), 200 mg/kg BW (low-dose group), 400 mg/kg BW (medium-dose group) and 800 mg/kg BW (high-dose group) tea polysaccharide for two week. At the end of experiment, the serum glucose, TG, MDA contents and antioxidase activities were measured by commercial kits. The serum immune factors levels were tested by ELISA. The genes expression levels related to antioxidant capacity, metabolism and immunity were measured by real-time PCR. The results showed that the glucose, TG and MDA contents in serum were significantly decreased by tea polysaccharides treatment. The serum activities of SOD were significantly increased by low-dose tea polysaccharides treatment. The serum activities of GPX were significantly increased by medium-dose tea polysaccharides treatment. The serum level of TGFβ was significantly increased, and the serum level of IL-12 was markedly decreased. In the hepatopancreas, the expression of acc1, fas, srebp1c, lpl, gys and ppar were significantly reduced, and the expression of pygl, cat, mnsod, ho-1 and gr were significantly up-regulated in the tea polysaccharides group. In the intestine, the expression of zo-1, occ and gip was significantly up-regulated in the high-dose treatment group. Moreover, the expression of glu2 and slgt1 were significantly down regulated. In the spleen, the expression of il-12, tnfα and il-6 were significantly decreased, and the

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Introduction

Aquaculture provides an essential source of edible protein for humans. With the rapid development of aquaculture, the demand of fishmeal is increasing, which induces the escalating price of fishmeal (1). The study showed that dietary carbohydrate and lipid inclusion at optimal levels could promote protein utilization, prevent lipid oxidation, and save fishmeal protein in the aquatic animal feed (2). However, overloaded dietary carbohydrate induced low feed intake, poor growth performance (3), metabolism dysfunction, impairment of antioxidant capacity and sub-health status of fish (4, 5). Moreover, overloaded dietary carbohydrate will reduce the immunity, and increase the infection with disease of cultured fish (6). In addition, with the expansion of farming scale and environmental degradation, diseases in farming occur frequently, which causes a number of deaths of cultured fish (7). Although the antibiotic can relieve the morbidity of fish, antibiotic resistance has become a severe problem worldwide (8). The frequent occurrence of antibiotic-resistant bacteria in the aquaculture sites is due to the abuse of antibiotics in aquaculture (9). Therefore, the antibiotics were prohibited in aquaculture by the Chinese government because of the various negative effects after July 2020. For these reasons, it is an urgent problem that the new antibiotic substitute has been exploited for use in aquaculture. As the environmental friendly substance, plant extracts are low toxicity, safety, and minimal environmental impacts (10). In aquatic animals, plant extracts play important role in enhancing the immune function, and promote antibacterial, antiviral, antiparasitic activities of the immune system (10). In addition, plant extracts have been as immunostimulant to prevent diseases of aquatic animals in recent years (11).

As the important economic agricultural product, tea possesses multiple beneficial effects, including antioxidant capacity, reduction of cholesterol, protect against cardiovascular disease, anti-microbial, and anti-cancer (12–14). The beneficial effects of tea attribute to its variety of bioactive compounds, including polysaccharides, polyphenols, alkaloids, volatile oils, amino acids, etc (14–18). The tea polysaccharides attracted attention for its bioactivities, such as antioxidant, anti-cancer, anti-radiation, hypoglycemic activities and anti-HIV (14, 15, 19, 20). Tea polysaccharides were mostly heteropolysaccharides, in which a protein via N- or O-covalently linkages carries one or more carbohydrate chains attached to a polypeptide backbone (14, 15). The bioactivity study indicated that the tea polysaccharides suppressed the formation and accumulation of fat, and promoted its decomposition to prevent obesity of rats (Rattus norvegicus) (21). For example, a report of polysaccharides from green tea of Huangshan Maofeng (HMTP) showed that HMTP could protect against liver injury by CCl₄-induced, and inhibit lipid peroxidation and the increase antioxidant activity in mice (Mus musculus) (22). Furthermore, the tea polysaccharides could significantly reduce blood glucose levels, and increase the capacity of glucose tolerance in mice (23, 24).

Common carp is one of the most widely cultivated freshwater fish species all over the world, which is the fourth most cultured fish species in aquaculture (25). The production of common carp accounts for 7.7% of the total fish production in 2018 in the world (26). In addition, the production of common carp reaches 4,411,900 metric tons in 2019 (25). To meet the needs of human for fish, the intensive high-density and high nutrition farming model was rapidly developed. With the farming model and environmental degradation, diseases in fish farming occur frequently, which causes huge losses to the aquaculture industry. As a safe and environmental friendly plant extracts, tea polysaccharides have antioxidant capacity, anti-microbial actions and increases immunity in mammals. But the beneficial effects of tea polysaccharides in fish have never been reported. To assess the biological functions of tea polysaccharides on fish, the common carp was employed as a model in this study. The effects of tea polysaccharides on the immunity, metabolism and antioxidant capacity were evaluated in common carp in our study.

Materials and methods

Materials and chemicals

Tea polysaccharides was produced in meilunbio (Dalian, China). Glucose test kit was purchased from Rsbio (Shanghai, China). Triglyceride (TG) test kit was purchased from Dongou (Zhejiang, China). Superoxide dismutase (SOD), glutathione oxidase (GPX), total antioxidant capacity (T-AOC) and methane
dicarboxylic aldehyde (MDA) test kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Animals experiment

The experiment process was referred to the previous studies (27, 28). The common carp with an initial body weight (45 ± 0.71g) were obtained from Yanjin Fishery (Yanjin, Henan). Approximately 120 healthy individuals were randomly divided into four groups (30 fish in each group). Before the experiment, fish were acclimated to indoor tanks (diameter: 52 cm, water high: 62 cm) at room temperature with recirculating water under a cyclical light-dark photoperiod (12 h: 12 h) for two weeks. Then, the fish were fed with a high carbohydrate diet or the same diet with 200 mg/kg BW/day (low-dose group), 400 mg/kg BW/day (medium-dose group) and 800 mg/kg BW/day (high-dose group) tea polysaccharides by gavage for two weeks. The formulations and compositions of each diet were listed in Table 1. All ingredients were weighted individually before mixed thoroughly in a feed drum mixer for 30 min. Then dissolved water was added and mixed to form a loosely shaped dough. The mixture was transformed into pellets using a single screw extruder (Fishery Machinery and Instrument Research Institute, China Academy of Fishery Science, Shanghai, China); the pellets were then air-dried at room temperature and stored at −20°C.

Biochemical analysis

At the end of the experiment, all fish were anesthetized by MS222 (Sigma, USA), and the blood samples were collected from the caudal vein. After still standing at 4°C at least 30 min, the serum was isolated by centrifugation at 7500 g for 10 min. The serum was stored at −80°C for the detection of immune factors and biochemical analysis. And then, the fish were decapitated. The hepatopancreas, foregut and spleen samples were immediately collected, and snap-frozen in liquid nitrogen and stored at −80°C until RNA extraction. Parts of the hepatopancreas tissue were removed for glycogen contents measure via the commercial kit (Jiancheng, China). All animal experiments were approved by the Animal Care Committee of Henan Normal University.

Serum samples analysis

In the serum, the content of glucose, TG and MDA of all groups was determined by commercial kits (Jiancheng, China). The enzyme activities of SOD, GPX and T-AOC were determined by commercial kits (Jiancheng, China). The experiments were performed according to the manufacturer’s protocol. The levels of IL-1β, IL-6, IL-10, IL-12, tumor necrosis factor α (TNFα) and transforming growth factor β (TGFβ) were measured by ELISA assay referred to pervious study (28).

Common carp spleen cells isolation and treatment

The common carp spleen cells were isolated by collagenase IV/DNase II digestion method. The experimental method of isolation was referred to previous study (29). The isolated spleen cells were cultured in the 24 wells plate with 1 mL DMEM/F12 medium contained 10% fetal bovine serum (FBS) with the density of 1×10^6 cells/well. After overnight cultured, the cell medium was replaced to fresh DMEM/F12 without FBS. Before treatment, the cells were cultured for 1 h in the DMEM/F12 without FBS. Then, the cells were treated with LPS (25 μg/mL), tea polysaccharides (400 μg/mL), LPS (25 μg/mL) + tea polysaccharides (400 μg/mL) for 12 h. By the end of the study, the cells were lysed by RNAiso Plus for RNA extraction.

RNA extract, cDNA synthesis and real-time PCR

The total RNA of the hepatopancreas, gut and spleen were extracted by RNAiso Plus (Takara, Japan). The total RNA concentration was measured by UV spectrophotometer (Nanodrop 2000, Thermo). 1 μg of total RNA was digested

| Ingredients                        | Percent of total |
|-----------------------------------|------------------|
| Casein                            | 33               |
| Fish meal                         | 10               |
| Fish oil                          | 2.5              |
| soybean oil                       | 1.5              |
| Mineral premix^a                   | 1                |
| Vitamin premix^b                   | 1                |
| Sodium carboxymethylcellulose     | 0.5              |
| Glucose                           | 50               |
| Choline chloride                  | 0.5              |
| Total                             | 100              |

Proximate composition

|                     | Percent (dry matter) |
|---------------------|-----------------------|
| Crude protein (%)   | 34.75                 |
| Crude lipid (%)     | 4.28                  |
| Ash (%)             | 2.46                  |

^aVitamin premix (mg or IU/kg diet): vitamin A, 6000 IU; vitamin D3, 2000 IU; vitamin E, 50 mg; ascorbic acid, 200 mg; pantothenic acid, 35 mg; nicotinic acid, 30 mg; thiamine, 15 mg; riboflavin, 15 mg; pyridoxine HCl, 6 mg; cyanocobalamin, 0.03 mg; menadione, 5 mg; inositol, 200 mg; folic acid, 3 mg; biotin, 0.2 mg.

^bMineral premix (mg or g/kg diet): magnesium, 100 mg; iron, 150 mg; zinc, 80 mg; manganese, 20 mg; copper, 4 mg; iodine, 0.4 mg; cobalt, 0.1 mg; selenium, 0.1 mg.
with gDNA Eraser at 42°C for 2 min to eliminate genomic DNA. Then, the first-strand cDNA was synthesized using PrimeScript RT reagent kit (PrimeScript RT reagent kit with gDNA Eraser, Takara). The synthesized first-strand cDNA was used as template for real-time PCR and the primers were shown in Table 2.

The genes expression levels were evaluated by real-time PCR. Real-time PCR was performed using SYBR green qPCR mix (Bimake, China) on the LightCycler 480 II Sequence Detection System (Roche, Switzerland) according to the manufacturer’s instructions. The real-time PCR reaction was in a total volume of 10 μl and the following conditions were used: 95°C for 5 min; and 40 cycles of 95°C for 15 s, 56°C for 15 s, and 72°C for 30 s. 18S rRNA are used as the internal reference, and remained stable in various treatments throughout the study. The genes relative expression levels were normalized to 18S rRNA. The results were calculated by the comparative Ct method (30).

Statistical analysis

All data are shown as mean ± standard error of the mean (S.E.M). Statistical analysis was performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Fisher’s Least Significance Difference (LSD) test was used to identify the significant difference. A probability value of $P < 0.05$ was considered significant.

Results

Serum content of glucose and triglyceride, the activity of antioxidant enzyme and glycogen content in hepatopancreas

In our present study, the result showed that the content of glucose and TG in serum was significantly decreased in the tea polysaccharides treatment groups compared to that of control group (Table 3). Moreover, the activity of SOD enzyme significantly increase by low-dose tea polysaccharides treatment (Table 3). The activity of GPX enzyme significantly increase by medium-dose tea polysaccharides treatment (Table 3). However, the contents of MDA were dramatically reduced in the tea polysaccharides groups compared to that of control group (Table 3). As shown in the Figure 1A, the contents of glycogen in hepatopancreas were markedly decreased in the high-dose group compared to that in the control group.

| Gene | Accession no. | Forward (5′→3′) | Reverse (5′→3′) |
|------|---------------|-----------------|-----------------|
| occ1 | KF975606      | ATGTTGTCCTCTCCGTGTAAG | TCCGTAAGAACCTCGTGTAAGA |
| zo-1 | KY290394      | AGGGATGTTCTCCGCCTAACC | CTTCAAGCTCTACTACCTC |
| sglt-1 | JN867793.1  | CGCACCTCAACCCCTCAT | CATTGCGCTCAGTTCGGAATCC |
| mnsad | XM_019111527.1 | TTCCTGTGAGCGCCCTTGT | TGGTGCTGTTGCTTGATGAG |
| cat | JF411604.1 | TGGCTGTATACCTCCCT | TGGTGCTGTTGCTTG |
| gr | XM_019102099.1 | CTAAGAAGAGGAGGACAGATTG | CAGAAGGGTCGAGGTGCAT |
| mnsad | XM_019088899.1 | GACCGAGGTGCCTTACCAT | CTTATGGCGCTAGAAGGAATC |
| IL-6 | XM_019073058.1 | CTTCACTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| il-1β | AB010701.1 | CTTCTGCTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| il-12 | JX524550.1 | CTTCACTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| tgb1 | AF139467.1 | CTTCTGCTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| accl | XM_01906370.1 | CTTCACTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| fas | QA66045.1 | CTTCACTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| srebplc | KY763985 | GAGCCCGCTGAGAACAATCT | CCTCTGGCGCTTATCCCA |
| glut2 | XM_019072653.1 | GAGCAGCTTGGTGGGAAATCAT | GCTTTAGGTCGTCGTCG |
| pgyl | XM_019125106.1 | TGGTGACAGGCAGCTTTCCT | ACCTGCGAACCTGGGCTT |
| g5p | GPWU0103253.1 | AGTTATGCGCGGCTTAC | TTTTTGGCCGCTGCTGCTTACAT |
| g6s | XM_019090903.1 | TTTTGGCCGCTGCTGCTTACAT | ATATGACGCTCAGTGGCAG |
| pl | FJ716101.1 | CGTCTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| pparγ | XM_019069405.1 | TGGCAAGGATTCTCCGCG | AAGAACTGGGCGTGGGCTG |
| ha-1 | JX527180.1 | CGCTGCTGCTGCTTACAT | CTTATGGCGCTAGAAGGAATC |
| 18s | FJ710827.1 | GAGAAGGGATGCTCGCG | AAGAACTGGGCGTGGGCTG |
| β-actin | M25013 | CGTGAACATCAAGGAGAAG | CTTATGGCGCTAGAAGGAATC |
The contents of immune factors in serum

In Table 4, the results showed that the contents of IL-1β and TNFα in serum were significantly reduced in the tea polysaccharides treatment groups compared to that of the control group. The content of IL-12 in serum was markedly decreased in the high-dose tea polysaccharides treatment group. However, the content of TGFβ in serum was dramatically elevated in the high-dose tea polysaccharides treatment group compared to that of the control group.

Effect of tea polysaccharides on expression of the genes related to metabolism in hepatopancreas

The results showed that the expression of fatty acid synthesis gene acc1, fas and pparγ in hepatopancreas was significantly inhibited in the tea polysaccharides treatment groups compared to that of control group (Figures 1B, C). The srebp1c expression level was markedly decreased by the low- and medium-dose tea polysaccharides treatment (Figure 1D). The lpl expression level was markedly decreased by the medium- and high-dose tea polysaccharides treatment (Figure 1F). Moreover, the pygl expression level was significantly increased in the high-dose tea polysaccharides treatment group (Figure 1G). However, the gys expression level was significantly decreased in the high-dose tea polysaccharides treatment group (Figure 1H).

Effect of tea polysaccharides and LPS on expression of the genes related to immunity in common carp spleen cells

The genes expression of tnfa, il-1β, il-6 and il-12 were significantly increased in common carp spleen cells by

Effect of tea polysaccharides and LPS on expression of the genes related to immunity in common carp spleen cells

The genes expression of tnfa, il-1β, il-6 and il-12 were significantly increased in common carp spleen cells by

Effect of tea polysaccharides on expression of the genes related to antioxidant in hepatopancreas

In the Figure 3, the results showed that the ho-1 expression level in hepatopancreas was significantly increased in the high-dose tea polysaccharides treatment group compared to that of control group (Figure 3A). In the low and medium-dose tea polysaccharides treatment groups, the expression of gr and mnsod were markedly promoted compared to that of control group (Figures 3B, C). In addition, the cat expression level was significantly up-regulated in hepatopancreas by the medium-dose tea polysaccharides treatment (Figure 3D).

Effect of tea polysaccharides on expression of the genes related to immunity in spleen

The effect of tea polysaccharides on expression of immune-related genes was detected in the spleen tissue of common carp. The results showed that the mRNA levels of tnfα and il-12 were significantly inhibited in the medium- and high-dose tea polysaccharides treatment groups (Figures 4A, B). The il-6 expression level was significantly decreased in the tea polysaccharides treatment groups (Figure 4C). Moreover, the expression of il-10 was markedly promoted in the low- and medium-dose tea polysaccharides treatment groups (Figure 4D). The expression of anti-inflammatory factor tgfβ was significantly increased by the high-dose tea polysaccharides treatment (Figure 4E).

### TABLE 3 Effects of tea polysaccharides on the plasma components of common carp.

| Component | 0mg/kg BW | 200mg/kg BW | 400mg/kg BW | 800mg/kg BW |
|-----------|-----------|-------------|-------------|-------------|
| Glucose (mM) | 2.81 ± 0.22a | 1.44 ± 0.20a | 1.96 ± 0.16a | 2.15 ± 0.27ab |
| TG (mM) | 3.13 ± 0.33a | 2.28 ± 0.18b | 2.10 ± 0.18b | 2.31 ± 0.29ab |
| SOD (U/mL) | 57.52 ± 3.02b | 110.56 ± 5.37a | 80.53 ± 13.80b | 65.19 ± 9.26b |
| T-AOC (U/mL) | 4.07 ± 0.42 | 4.90 ± 0.44 | 4.17 ± 0.38 | 4.75 ± 0.45 |
| MDA (nM) | 10.72 ± 1.27a | 7.42 ± 0.66b | 7.61 ± 0.44b | 7.47 ± 0.34b |
| GPX (U/mL) | 342.74 ± 37.47b | 382.64 ± 34.15ab | 431.60 ± 18.98a | 324.34 ± 24.84b |

All data are shown as mean ± S.E.M. (n = 7-8). Significant differences (P<0.05) were indicated by different letters.
treatment with LPS compared to those in control group (Figures 5A–D). By tea polysaccharides treatment, the mRNA levels of \( \text{tnf} \alpha, \text{il-1} \beta, \text{il-6} \) and \( \text{il-12} \) were inhibited in spleen cells. Moreover, the promoted mRNA levels of \( \text{tnf} \alpha, \text{il-1} \beta, \text{il-6} \) and \( \text{il-12} \) in spleen cells were alleviated in the LPS and tea polysaccharides group (Figures 5A–D). The \( \text{il-10} \) and \( \text{tgf}\beta \) expression were markedly decreased in common carp spleen cells by treatment with LPS (Figures 5E, F). The \( \text{il-10} \) expression was significantly increased by treatment with tea polysaccharides (Figure 5E). Furthermore, the inhibited mRNA levels of \( \text{il-10} \) and \( \text{tgf}\beta \) were alleviated in the LPS and tea polysaccharides treatment group (Figures 5E, F).

**Discussion**

As a group of heteropolysaccharides extracted from tea, tea polysaccharides reveals multiple beneficial bioactivity in previous studies (14, 15). In our study, the serum glucose levels were significantly decreased in the tea polysaccharides treatment groups. The result was similar to that in previous studies. In mice, the blood glucose level was significantly decreased after injection with tea polysaccharides (31). Furthermore, the blood glucose content of alloxan-induced diabetic mice was markedly reduced after four-week oral administration of puerh tea polysaccharides (PTPS) (32), and was suppressed increase after six days oral administration of green tea polysaccharides (GTPS) (33). In addition, the serum glucose levels were decreased by daily oral administration of tea polysaccharides in diabetic and non-diabetic mice (23, 34, 35). These results indicate tea polysaccharides can reduce blood glucose level in mammals and fish. Previous study suggested that the reduction in intestinal glucose transport by tea polysaccharides was mainly mediated by the biochemical inhibition of transport activity (36). In our study, the results also showed that the tea polysaccharides can decrease the \( \text{sglt1} \) and \( \text{gult2} \) genes expression in the foregut. We speculate that tea polysaccharides reduce the serum glucose by lowing the glucose transport in gut of common carp.

The antioxidant activity of tea polysaccharides was reported in previous studies (14, 15). In our study, the enzyme activities of
serum SOD and GPX were promoted in the tea polysaccharides groups. However, the contents of MDA were significantly reduced in the tea polysaccharides groups. A report showed that the serum level of T-SOD was significantly promoted in rats, and the elevated serum content of MDA was attenuated in the green tea polysaccharides treated group (37). In addition, pretreatment with Keemun black tea polysaccharides (KBTP), the hepatic T-SOD and GSH levels were reduced, and the MDA content was decreased in CCl₄-intoxicated mice (38). Moreover, the study showed that the contents of MDA were reduced, and the SOD, catalase and GPX activities were increased in the plasma, liver and heart of mice after treatment with crude tea polysaccharides for 30 days (39). Furthermore, compared to MC (HFD without additional treatment) group, the content of MDA was significantly decreased and the GPX and CAT activities were increased in the Chinese Liupao tea polysaccharides (CLTPS) treatment groups (40). It is indicated that the antioxidant activity of tea polysaccharides can implement in the carbohydrate-, lipid-, toxicant-induced or normal physiological status.

It is a crucial activity that tea polysaccharides promote immunity (14, 15, 36, 41). Tea polysaccharides activate immune cells to secrete various biological reaction mediators, such as cytokines, free radicals, and lyases (36). In the present study, the contents of IL-1β, TNFα and IL-12 were significantly decreased, and the level of TGFβ was markedly promoted in the serum of common carp after tea polysaccharides treatment. Furthermore, the contents of IL-2, IL-6, IL-10 and IFN-γ were significantly reduced in the colitis-associated cancer (CAC) mice by treatment with tea polysaccharides, and the levels of IL-10 were markedly increased in tea polysaccharides groups compared to that in Azoxymethane/Dextran sulfate sodium (AOM/DSS) group (42). Moreover, the serum IL-6 and TNFα levels were decreased, and the serum IL-2, IL-4 and IL-10 levels were increased in gastric cancer mice after tea polysaccharides treatment (43). In addition, the study of Yuan and his colleagues showed that IL-6 and TNFα levels were significantly decreased in mouse splenocytes treated with tea polysaccharides compared with that in native control (NC) group (44). Based on those results, it is indicated that improving the activity of antioxidant enzymes of tea polysaccharides was not only in mammals but also in the fish. In addition, the expression of il-1β, infα, il-6 and il-12 were significantly inhibited, and the expression of il-10 and tgfβ were dramatically increased in the spleen of common carp in the tea polysaccharides groups. In the common carp spleen cells, the tea polysaccharides could relieved LPS-induced immune related genes expression. The previous study also showed that the expression levels of il-6 and tnfα were reduced in white adipose tissue of rats in the tea polysaccharides treatment groups (37). Based on the above study, it is manifested that tea polysaccharides plays immunocompetence by regulating the activity of antioxidant enzymes and expression of immune factors.
Tea polysaccharides also have a fat-lowering effect (36). In our study, the serum TG contents were also significantly reduced in the tea polysaccharides groups. The activity of reducing TG levels of tea polysaccharides was reported in previous studies. For instance, the black tea polysaccharides significantly reduced the TG content in serum and liver of rats compared to that in model control (Wu et al., 2016). The HMTP can decrease the CCl₄-elevated level of serum TG (22), and administration of GTPS or black tea polysaccharides (BTPS) in mice before the CCl₄ injection can resist the CCl₄-induced increases in the level

![Effects of tea polysaccharides on genes expression related to antioxidant capacity in hepatopancreas. (A) ho-1; (B) gr; (C) mnsod; (D) cat. At end of the experiment, fish were killed and the hepatopancreas was collected for RNA extraction and real-time PCR. All data are shown as mean ± S.E.M. (n = 10–12). Significant differences were indicated by asterisks, *, P < 0.05; **, P < 0.01; ***, P < 0.001.](image)

![Effects of tea polysaccharides on genes expression related to immunity in spleen. (A) tnfα; (B) il-12; (C) il-6; (D) il-1β; (E) il-10; (F) tgfβ. At end of the experiment, fish were killed and the hepatopancreas was collected for RNA extraction and real-time PCR. All data are shown as mean ± S.E.M. (n = 10–12). Significant differences were indicated by asterisks, *, P < 0.05; **, P < 0.01; ***, P < 0.001.](image)
The expression of *ppar* genes expression was affected by kuding tea treatment, and furthermore, the results revealed that the lipogenic-related genes expression were decreased in the foregut in the tea polysaccharides treatment group. The decreased expression of *ppar* and lipogenic genes were inhibited in the liver of mice (47). From above results, it is indicated that the fat-lowering activity of tea polysaccharides is exerted by regulating the expression of lipid metabolism related genes in mammals and fish.

In the present study, the expression of occ and zo-1 were significantly increased in the foregut of common carp in the tea polysaccharides groups. The expression of *gip* was increased, and *sgitl1* and *glut2* expression were decreased in the foregut in the tea polysaccharides groups. *SGLT1* and *GLUT2* are the important glucose transporter in the intestines. In a previous study, the intestinal glucose transport was significantly decreased by treatment with green tea extract (GTE), water soluble polysaccharides derived from green tea (WSP), and GTE +WSP. The expression of *sglt1* was markedly decreased in the Caco-2 cells by treatment with wheat starch + GTE + WSP (49).

Moreover, the protein expression of PI3Kp85, p-Akt and GLUT4 in diabetic mice were increased in the liver by orally gavage with tea polysaccharides, and the serum glucose level was accompanied decreased (34). In addition, the expression of *gys* was inhibited and the hepatopancreas glycogen content was decreased in the tea polysaccharides treatment group. The expression of *pygl* was significantly increased in the tea polysaccharides treatment group. The decreased hepatopancreas glycogen content was caused by the decreased glycogen synthesis (*gys*) and increased glycogenolysis (*pygl*) in hepatopancreas and decreased glucose absorption (*sglt1* and *glut2*) in foregut. It is indicated that the absorption of glucose and glycogenesis were affected by tea polysaccharides via regulating the related-genes expression. Intestinal barrier function is an important aspect for intestinal health. The study of cyclophosphamide (Cy)-induced BALB/c mice showed that the colonic TLR4/MyD88/NF-kB p65 and JAK2/STAT3 pathway was activated by pectic heteropolysaccharides and the expression of *claudin1*, *claudin5* and *occludin1* were significantly increased (50). In addition, the expression levels of *claudin1* and *claudin5* were significantly promoted in the colonic tissues of mice in the polysaccharides from the tea flower (TFPS) treatment group (51). Those results indicated that the tea polysaccharides play beneficial role in intestinal health by regulating intestinal barrier related genes expression. The regulatory mechanism underlying the impact of tea polysaccharides on intestinal barrier related genes expression of fish should be investigated in future studies.
Conclusion

In conclusion, our present results suggested that tea polysaccharides promoted immunity, antioxidative capacity and intestinal barrier function and reduced lipogenesis and glucose transporter of common carp. The results of this study will provide a theoretical foundation of tea polysaccharides application in aquaculture.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The animal study was reviewed and approved by Animal Care and Use Ethics Committee of the Henan Normal University.

Author contributions

GY, XL, JH: conceived and performed the experiments, formal analysis, writing, and original draft. XM, GY: Reviewing and editing. WH, KL, XC, YZ: Supervision, reviewing, and editing. CL, YS, XZ: analyzed the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors WH and KL were employed by Henan JinBaiHe Biotechnology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Barbosa V, Maulvault AL, Anacleto P, Santos M, Mai M, Oliveira H, et al. Effects of steaming on health–valuable nutrients from fortified farmed fish: Gilthead seabream (Sparus aurata) and common carp (Cyprinus carpio) as case studies. Food Chem Toxicol an Int J published by Br Ind Biol Res Assoc (2021) 152.112218. doi: 10.1016/j.fct.2021.112218
2. Tian J, Wu F, Yu JJ, Lu X, Jiang M, Liu W, et al. The effects of high-macronutrient (protein, fat and carbohydrate) diets on growth performance and muscular metabolic responses in grass carp. Aquacult Nutr (2020) 26:2135–46. doi: 10.1111/anj.15152
3. Wu CL, Ye JY, Gao JZ, Chen L, Lu ZB. The effects of dietary carbohydrate on the growth, antioxidant capacities, innate immune responses and pathogen resistance of juvenile black carp mylopharyngodon piceus. Fish Shellfish Immunol (2016) 49:132–42. doi: 10.1016/j.fsi.2015.12.030
4. Lin SM, Shi CM, Ma MM, Chen YJ, Luo L. Effect of high dietary starch levels on growth, hepatic glucose metabolism, oxidative status and immune response of juvenile largemouth bass, micropterus salmoides. Fish Shellfish Immunol (2018) 78:121–6. doi: 10.1016/j.fsi.2018.04.046
5. Zhou CP, Ge XP, Lin HZ, Niu J. Effect of dietary carbohydrate on non-specific immune response, hepatic antioxidative abilities and disease resistance of juvenile golden pompano (Trachinotus ovatus). Fish Shellfish Immunol (2014) 41:183–90. doi: 10.1016/j.fsi.2014.08.024
6. Zhao H, Luo YE, Wu ZX, Zhou Y, Guo DY, Wang H, et al. Hepatic lipid metabolism and oxidative stress responses of grass carp (Ctenopharyngodon idella) fed diets of two different lipid levels against aeromonas hydrophila infection. Aquaculture (2019) 509:149–58. doi: 10.1016/j.aquaculture.2019.05.029
7. Meng XL, Wu SK, Hu WP, Zhu ZX, Yang GK, Zhang YM, et al. Clostridium butyricum improves immune responses and remodels the intestinal microbiota of common carp (Cyprinus carpio L.). Aquaculture (2021) 530:735753. doi: 10.1016/j.aquaculture.2020.735753
8. Church NA, McKillip JL. Antibiotic resistance crisis: challenges and imperatives. Biologia (2021) 76:1535–50. doi: 10.1017/s13756-021-00697-x
9. Choi WM, Mo WY, Wu SC, Mak NK, Bian ZX, Nie XP, et al. Effects of traditional Chinese medicines (TCM) on the immune response of grass carp (Ctenopharyngodon idella). Aquacult Int (2014) 22:361–77. doi: 10.1007/s10499-013-9644-7
10. Zhao F. A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture (2020) 526:735422. doi: 10.1016/j.aquaculture.2020.735422
11. Pu HY, Li XY, Du QB, Cui H, Xu YP. Research progress in the application of Chinese herbal medicines in aquaculture: A review. Engineering-Proc (2017) 3:791–7. doi: 10.1016/j.eng.2017.03.017
12. Beliveau R, Gingras D. Green tea: prevention and treatment of cancer by nutraceuticals. Lancet (2004) 364:1021–2. doi: 10.1016/s0140-6736(04)17076-1
13. Koo MW, Cho CH. Pharmacological effects of green tea on the gastrointestinal system. Eur J Pharmacol (2004) 500:177–85. doi: 10.1016/j.ejphar.2004.07.023
14. Nie SP, Xie MY. A review on the isolation and structure of tea polysaccharides and their bioactivities. Food Hydrocoll (2011) 25:144–9. doi: 10.1016/j.foodhyd.2010.04.010
Beer, K., and A. K. Wachs. 2020. In vitro biocompatibility of polysaccharide conjugates prepared from fresh tea leaves and their improving impaired glucose tolerance. Int J Food Sci Tech (2018) 53:2335–31. doi: 10.1111/ijf.13825

24. Chen XQ, Fang YP, Nishikawa K, We H, Sun CC, Li JR, et al. Physicochemical characteristics of polysaccharide conjugates prepared from fresh tea leaves and their improving impaired glucose tolerance. Carbohydr Polymers (2014) 112:77–84. doi: 10.1016/j.carbpol.2014.05.030

25. Xie X, Franek R, Pienicka M, Chen F, Kaipar V. Optimization of in vitro culture conditions of common carp germ cells for purpose of surrogate production. Front Vet Sci (2022) 9:1036495. doi: 10.3389/fvets.2022.1036495

26. Sotnikov A, Rodina M, Stechkina T, Benevente CF, Gela D, Boryshpolets S, et al. Characterization of polysaccharides and polyphenols fractions of yellow tea of Ctenopharyngodon idellus: Molecular identifications, structural features, and antioxidant activity in gastric cancer mice. J Food Sci Tech (2019) 61:103470. doi: 10.1016/j.jfst.2019.06.024

27. Yuan C, Li Z, Peng F, Xiao F, Ren D, Xue H, et al. Combination of selenium-enriched green tea polysaccharides and huo-jin polysaccharides synergistically enhances antioxidant and immune activity in mice. J Sci Food Agric (2015) 95:3211–7. doi: 10.1002/jsfa.7287

28. Yang JJ, Chen B, Gu Y. Pharmacological evaluation of tea polysaccharides with antioxidant activity in gastric cancer mouse. Carbohydr Polymers (2012) 90:943–7. doi: 10.1016/j.carbpol.2012.06.024

29. Liu Q, Nie SP, Sheng MY, Hu IL, Yu Q, Gong DM, et al. Tea polysaccharide inhibits colitis-associated colorectal cancer via interleukin-6/STAT3 pathway. J Agric Food Chem (2016) 64:4894–93. doi: 10.1021/acs.jafc.5b04616

30. Yang G, Zhao W, Qin C, Yang L, Meng X, Lu R, et al. Igfbp3 in grass carp (Ctenopharyngodon idellus) modulate gut health and ameliorate cyclophosphamide-induced immunosuppression. J Funct Food (2019) 61:103470. doi: 10.1016/j.jff.2019.10.003

31. Fan S, Zhang Y, Hu N, Sun Q, Ding X, Li G, et al. Hypoglycemic effect of soluble polysaccharide and catechins from green tea on inhibiting intestinal transport of glucose. J Sci Food Agric (2020) 100:3979–86. doi: 10.1002/jsfa.10442

32. Chen D, Chen GJ, Ding Y, Wang Y, Chen CX, et al. Polysaccharides from the flowers of tea (Camellia sinensis L.) modulate gut health and ameliorate cyclophosphamide-induced immunosuppression. J Funct Food (2019) 61:103470. doi: 10.1016/j.jff.2019.10.003

33. Chen HX, Zhang M, Xie BJ. Components and antioxidant activity of polysaccharide conjugate from green tea. Food Chem (2005) 90:17–21. doi: 10.1016/j.foodchem.2004.03.001

34. Li SQ, Chen HX, Wang J, Wang XM, Hu B, Lu FN. Involvement of the PI3K/Akt signal pathway in the hypoglycemic effects of tea polysaccharides on diabetic mice. Int J Biol Macromol (2015) 81:967–74. doi: 10.1016/j.ijbiomac.2015.09.037

35. Chen XQ, Lin Z, Ye Y, Zhang R, Yin JF, Jiang YW, et al. Suppression of diabetes in non-obese diabetic (NOD) mice by oral administration of water-soluble and alkali-soluble polysaccharide conjugates prepared from green tea. Carbohydr Polymers (2010) 82:28–33. doi: 10.1016/j.carbpol.2010.04.017

36. Xu AN, Lai WY, Chen P, Awasu MK, Chen XQ, Wang YF, et al. A comprehensive review on polysaccharide conjugates derived from tea leaves: Composition, structure, function and application. Trends Food Sci Tech (2021) 114:83–99. doi: 10.1016/j.tifs.2021.05.020

37. Xu Y, Zhang M, Wu T, Dai S, Xu J, Zhou Z. The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Food Funct (2015) 6:297–304. doi: 10.1039/c4fo00970c

38. Sun Y, Yang L, Xu L, Wang D, Zhao Y. Protective effects of keemun black tea polysaccharides on acute carbon tetrachloride-induced oxidative hepatotoxicity in mice. Food Chem Toxicol on an Int J Biol Ind Biol Res Assoc (2013) 58:184–92. doi: 10.1016/j.fct.2013.04.034

39. Cao H. Polysaccharides from Chinese tea: recent advances on bioactivity and function. Int J Biol Macromol (2013) 62:76–9. doi: 10.1016/j.ijbiomac.2012.08.033

40. Miao Y, Wei BY, Teng JW, Xia N, Zhao MM, Huang L, et al. Polysaccharides from Chinese lipao dark tea and their protective effect against hyperlipidemia. Int J Food Sci Tech (2018) 53:599–607. doi: 10.1111/ijf.13633

41. Chen GJ, Yuan QX, Saeeduddin M, Ou SY, Zeng XX, Ye H. Recent advances in tea polysaccharides: Extraction, purification, physicochemical characteristics and bioactivities. Carbohydr Polymers (2016) 153:663–78. doi: 10.1016/j.carbpol.2016.08.022

42. Liu Q, Nie SP, Sheng MY, Hu IL, Yu Q, Gong DM, et al. Tea polysaccharides inhibit colitis-associated colorectal cancer via interleukin-6/STAT3 pathway. J Agric Food Chem (2016) 64:4894–93. doi: 10.1021/acs.jafc.5b04616

43. Yang JJ, Chen B, Gu Y. Pharmacological evaluation of tea polysaccharides with antioxidant activity in gastric cancer mouse. Carbohydr Polymers (2012) 90:943–7. doi: 10.1016/j.carbpol.2012.06.024

44. Yuan C, Li Z, Peng F, Xiao F, Ren D, Xue H, et al. Combination of selenium-enriched green tea polysaccharides and huo-jin polysaccharides synergistically enhances antioxidant and immune activity in mice. J Sci Food Agric (2015) 95:3211–7. doi: 10.1002/jsfa.7287

45. Zhai X, Ren D, Luo Y, Hu Y, Xue H. Chemical characteristics of an ilex kuding tea polysaccharide and its protective effects against high fructose-induced liver injury and vascular endothelial dysfunction in mice. Food Funct (2017) 8:2536–47. doi: 10.1039/c7fo00490g

46. Tian C, Ye X, Zhang R, Long J, Ren W, Ding S, et al. Green tea polysaccharides reduced fat deposits in high-fat-fed rats via erk1/2-FPR2amma-gamma-adiponectin pathway. PloS One (2013) 8:e53796. doi: 10.1371/journal.pone.0053796

47. Chen N, Bezzina R, Hinch E, Lewandowski PA, Cameron-Smith D, Mathai ML, et al. Green tea, black tea, and epigallocatechin modify body composition, glucose tolerance, and body fat deposition in mice. J Sci Food Agric (2016) 96:1016–25. doi: 10.1002/jsfa.7941

48. Fan S, Zhang Y, Hu N, Sun Q, Ding X, Li G, et al. Extract of kuding tea polysaccharide and its protective effects against high fructose-induced liver injury and vascular endothelial dysfunction in mice. Food Funct (2017) 8:2536–47. doi: 10.1039/c7fo00490g

49. Tian C, Ye X, Zhang R, Long J, Ren W, Ding S, et al. Green tea polysaccharides reduced fat deposits in high-fat-fed rats via erk1/2-FPR2amma-gamma-adiponectin pathway. PloS One (2013) 8:e53796. doi: 10.1371/journal.pone.0053796

50. Chen D, Ding Y, Ye H, Sun Y, Zeng XX. Effect of long-term consumption of tea (Camellia sinensis L.) on glucose and insulin resistance in alloxan-induced diabetic mice. J Food Sci (2012) 77:3846–52. doi: 10.1111/j.1538-0264.2012.02950.x

51. Chen D, Ding Y, Wang Y, Peng YJ, Chen CX, et al. Polysaccharides from the flowers of tea (Camellia sinensis L.) modulate gut health and ameliorate cyclophosphamide-induced immunosuppression. J Funct Food (2019) 61:103470. doi: 10.1016/j.jff.2019.10.003

52. Chen D, Ding Y, Wang Y, Peng YJ, Chen CX, et al. Polysaccharides from the flowers of tea (Camellia sinensis L.) modulate gut health and ameliorate cyclophosphamide-induced immunosuppression. J Funct Food (2019) 61:103470. doi: 10.1016/j.jff.2019.10.003
Glossary

TG  triglyceride
MDA  malondialdehyde
SOD  superoxide dismutase
GPX  glutathione peroxidase
ACC1  acetyl-CoA carboxylase 1
FAS  fatty acid synthase
SREBP1c  sterol regulatory element binding protein 1c
LPL  lipoprotein lipase
PPARY  peroxisome proliferator activated receptor γ
PYGL  glycogen phosphorylase
CAT  catalase
MnSOD  manganese superoxide dismutase
HO-1  heme oxygenase-1
GR  glutathione reductase
OCC  occludin
GLUT2  glucose transporter 2
GYS  glycogen synthase
SGLT1  sodium/glucose cotransporter 1
HMTP  Huangshan Maofeng
GIP  glucose-dependent insulinotropic polypeptide
PTPS  puerh tea polysaccharides
GTPS  green tea polysaccharides
BTPS  black tea polysaccharides
KBTP  Keemun black tea polysaccharides
CLTPS  Chinese Liupao tea polysaccharides
CAC  colitis-associated cancer
AOM/DSS  Azoxymethane/Dextran sulfate sodium
NC  native control
GTE  green tea extract
WSP  water soluble polysaccharides derived from green tea
TFPS  polysaccharides from tea flower
IKTP  Ilex Kuding tea polysaccharides
TPs  tea polysaccharides.