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Effects of Dietary Mulberry Leaf Flavonoids on Growth Performance, Body Composition, Antioxidant Indices, and Nitrite Stress Resistance in GIFT Tilapia (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary mulberry leaf flavonoids on growth performance, body composition, serum and liver antioxidant indices, and anti-nitrite stress capability in juvenile GIFT tilapia (Oreochromis niloticus). A total of 840 juvenile tilapia with an initial body weight of (1.51$±$0.02) g were randomly divided into 6 groups, with 4 replicates per group and 35 fish per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, 300, 500 and 1 000 mg/kg mulberry leaf flavonoids, respectively. The feeding trial lasted 56 days. At the end of the feeding trial, a 72-h stress test was conducted using sodium nitrite in each group. The results showed that: 1) There were no significant differences in weight gain, feed conversion ratio, or protein efficiency ratio of GIFT tilapia among all groups (P>0.05). No significant differences were observed in moisture, crude protein, crude lipid, or crude ash content of the fish body among all groups (P>0.05). 2) Serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and total antioxidant capacity (T-AOC) in the experimental groups were all higher than those in the control group, among which serum SOD activity in the 100, 300, 500 and 1 000 mg/kg mulberry leaf flavonoid groups, serum GSH-Px activity in the 50, 500 and 1 000 mg/kg mulberry leaf flavonoid groups, and serum T-AOC in the 100 and 500 mg/kg mulberry leaf flavonoid groups were significantly higher than those in the control group (P<0.05). Serum malondialdehyde (MDA) content in the experimental groups was significantly lower than that in the control group (P<0.05). Liver catalase (CAT) and SOD activities and T-AOC in the 100, 300 and 500 mg/kg mulberry leaf flavonoid groups were significantly higher than those in the control group (P<0.05); liver GSH-Px activity was highest in the 500 mg/kg

mulberry leaf flavonoid group, which was significantly higher than that in the 1 000 mg/kg mulberry leaf flavonoid group (P<0.05). 3) The cumulative mortality at 48 and 72 h of nitrite-nitrogen stress in the 50, 100 and 500 mg/kg mulberry leaf flavonoid groups was significantly lower than that in the control group (P<0.05). These results indicate that dietary mulberry leaf flavonoids had no significant effect on the growth performance of GIFT tilapia, but improved serum and liver antioxidant indices and anti-nitrite stress capability. Under the conditions of this experiment, through regression equation analysis and using serum SOD activity and T-AOC as evaluation indices, the optimal supplementation level of mulberry leaf flavonoids in juvenile GIFT tilapia feed was determined to be 100 mg/kg; using liver SOD activity and T-AOC as evaluation indices, the optimal supplementation level was determined to be 371.00–441.75 mg/kg.

Full Text

Effects of Dietary Mulberry Leaf Flavonoids on Growth Performance, Body Composition, Antioxidant Indices, and Resistance to Nitrite Stress in GIFT Tilapia

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Abstract: This experiment was conducted to investigate the effects of dietary mulberry leaf flavonoids (MLF) on growth performance, body composition, serum and liver antioxidant indices, and resistance to nitrite stress in juvenile genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). A total of 840 GIFT with an initial body weight of (1.51$±$0.02) g were randomly divided into 6 groups with 4 replicates per group and 35 fish per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, 300, 500, and 1,000 mg/kg MLF, respectively. The feeding trial lasted for 56 days. Following the feeding trial, a 72-hour nitrite stress test was conducted using sodium nitrite in each group. The results showed that: 1) No significant differences were observed in weight gain, feed conversion ratio, or protein efficiency ratio among all groups (P>0.05). Similarly, no significant differences were found in whole-body moisture, crude protein, crude lipid, or ash contents among all groups (P>0.05). 2) Serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, and total antioxidant capacity (T-AOC) in the experimental groups were higher than those in the control group. Specifically, serum SOD activities in the 100, 300, 500, and 1,000

mg/kg MLF groups, serum GSH-Px activities in the 50, 500, and 1,000 mg/kg MLF groups, and serum T-AOC in the 100 and 500 mg/kg MLF groups were significantly higher than those in the control group ($P<0.05$). Compared with the control group, serum malondialdehyde (MDA) contents in all experimental groups were significantly decreased ($P<0.05$). Liver catalase (CAT), SOD activities, and T-AOC in the 100, 300, and 500 mg/kg MLF groups were significantly higher than those in the control group ($P<0.05$). The highest liver GSH-Px activity was observed in the 500 mg/kg MLF group, which was significantly higher than that in the 1,000 mg/kg MLF group ($P<0.05$). 3) After 48 and 72 hours of nitrite stress, the cumulative mortality rates in the 50, 100, and 500 mg/kg MLF groups were significantly lower than those in the control group ($P<0.05$). In conclusion, dietary MLF supplementation did not significantly affect growth performance but improved serum and liver antioxidant indices and enhanced resistance to nitrite stress in GIFT. Under the experimental conditions, regression analysis indicated that the optimal dietary MLF supplementation level for juvenile GIFT was 100 mg/kg based on serum SOD activity and T-AOC, and 371.00–441.75 mg/kg based on liver SOD activity and T-AOC.

Keywords: mulberry leaf flavonoids; *Oreochromis niloticus*; growth performance; antioxidant; nitrite

Introduction

Mulberry leaves are widely cultivated in China and contain various natural bioactive substances. Flavonoids are one of the main functional components in mulberry leaves, accounting for 1–3% of dry weight [1], and exhibit extensive physiological functions including antitumor, blood pressure reduction, hypoglycemic, antioxidant, and anti-aging activities [2]. Several studies have demonstrated that mulberry leaf flavonoids can significantly scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, indicating strong antioxidant capacity [3-4]. Pan et al. [5] found that mulberry leaf flavonoids possess strong scavenging activity against three oxygen-centered radicals: hydroxyl radicals (·OH), singlet oxygen ($^1O_2$), and superoxide anion radicals ($O_2^{-}\cdot$). Mulberry leaf flavonoids can increase superoxide dismutase (SOD) activity in rats [6] and enhance the antioxidant capacity of human hepatoma cells (HepG2 cells) induced by oxidative stress from 2,2' -azobis(2-amidinopropane) dihydrochloride (AAPH) and copper ions (Cu^{2+}) [4]. However, no studies have been reported on the application of mulberry leaf flavonoids in aquatic animals.

Tilapia is an important aquaculture species worldwide. GIFT (Genetically Improved Farmed Tilapia) is a genetically enhanced strain of Nile tilapia (*Oreochromis niloticus*) characterized by rapid growth, high fillet yield, and stable genetic traits [7]. With increasing intensification of aquaculture, various oxidative stress factors have emerged, severely affecting tilapia growth and disease resistance. Therefore, utilizing natural plant bioactive substances to enhance

fish antioxidant capacity is crucial for healthy tilapia farming. This study investigated the effects of dietary MLF supplementation at different levels on growth performance, body composition, serum and liver antioxidant indices, and resistance to nitrite stress in GIFT, aiming to provide a basis for the rational application of MLF in tilapia feed.

1.1 Experimental Diets

Mulberry leaf flavonoids (purity 77.9%) were provided by the Sericulture and Farm Product Processing Research Institute of Guangdong Academy of Agricultural Sciences. A basal diet was formulated using fish meal, soybean meal, and rapeseed meal as protein sources, high-gluten flour as carbohydrate source, and soybean oil and lecithin as lipid sources. The composition and nutrient levels of the basal diet are shown in Table 1 .

The control group was fed the basal diet, while experimental groups were fed the basal diet supplemented with 50, 100, 300, 500, and 1,000 mg/kg MLF, respectively. Feed ingredients were ground and passed through a 60-mesh sieve. Micro-ingredients were added using the progressive expansion method. MLF was first dissolved in water and then mixed into each group' s feed. After mixing in a mixer, appropriate water was added and thoroughly blended in a blender. The mixture was processed into 1.5 mm pellets using an SLX-80 twin-screw extruder and G-500 pelletizer (both manufactured by the Science and Technology Industry General Factory of South China University of Technology). The pellets were dried at 55°C, naturally cooled, sealed in bags, and stored at -20°C until use.

1.2 Experimental Fish and Culture Management

GIFT were obtained from Guangdong Tilapia Breeding Farm. After two weeks of acclimation with the basal diet, the feeding trial was conducted in an indoor recirculating aquaculture system at the Aquaculture Research Laboratory of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. The system consisted of 24 fiberglass tanks (350 L capacity each, 90 cm diameter × 80 cm height, with 300 L water volume) and two filter tanks filled with coral stones and activated carbon, with a water inflow rate of 1.5 L/min.

At the start of the experiment, 840 healthy and active GIFT with an initial body weight of (1.51$±$0.02) g were randomly divided into 6 groups with 4 replicates per group and 35 fish per replicate. Fish were hand-fed to satiation twice daily at 08:30 and 18:00. The water source was sand-filtered and disinfected tap water. Water temperature was maintained at 25–31°C, with continuous aeration ensuring dissolved oxygen >7.0 mg/L, pH 7.5–8.0, ammonia nitrogen $ $0.10 mg/L, and nitrite $ $0.01 mg/L. The feeding trial lasted for 56 days.

1.3 Sample Collection

At the end of the feeding trial, fish were fasted for 24 hours before final body weight measurement and survival rate calculation. From each replicate, 5 fish were randomly selected for body length and weight measurement to calculate condition factor. Another 3 fish per replicate were randomly collected for whole-body composition analysis. Blood samples were collected from the caudal vein of 10 fish per replicate, allowed to clot at 4°C for 2 hours, then centrifuged at 3,000 r/min for 10 minutes. The serum was collected and stored at -20°C for antioxidant index analysis. Additionally, 5 fish per replicate were randomly selected for viscera and liver separation and weighing to calculate viscerosomatic index and hepatosomatic index. Liver samples were stored at -80°C for further analysis.

Liver supernatant preparation: A known weight of liver sample was homogenized with 9 volumes of 0.80% pre-cooled physiological saline in an ice-water bath. The homogenate was centrifuged at 3,000 r/min for 10 minutes, and the supernatant was collected and stored at -20°C for liver antioxidant index determination.

1.4.1 Calculation of Growth Performance and Morphological Indices

- Survival rate (SR, %) = 100 × (final fish number)/(initial fish number)
- Weight gain (WG, %) = 100 × [final average body weight (g) − initial average body weight (g)]/initial average body weight (g)
- Feed conversion ratio (FCR) = total feed intake (g)/[final body weight (g) − initial body weight (g)]
- Protein efficiency rate (PER, %) = 100 × [final body weight (g) − initial body weight (g)]/[dry matter intake (g) × dietary crude protein content (%)]
- Condition factor (CF, g/cm^3) = 100 × body weight (g)/body length (cm^3)
- Viscerosomatic index (VSI, %) = 100 × viscera weight (g)/body weight (g)
- Hepatosomatic index (HSI, %) = 100 × liver weight (g)/body weight (g)

1.4.2 Proximate Composition Analysis of Feed and Whole Fish

Moisture content was determined by oven drying at 105°C to constant weight (GB/T 6435-1986). Crude protein content was measured using the Kjeldahl method with a semi-automatic Kjeldahl analyzer (GB/T 6432-1994). Crude lipid content was determined by ether extraction (GB/T 6433-1994). Ash content was measured by incineration at 550°C to constant weight (GB/T 6438-1992).

1.4.3 Antioxidant Index Determination

Serum and liver supernatant antioxidant indices were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute, following the man-

ufacturer' s instructions. SOD activity was determined by the hydroxylamine method, GSH-Px activity by colorimetry, catalase (CAT) activity by visible light spectrophotometry, T-AOC by colorimetry, and MDA content by the thiobarbituric acid (TBA) method.

1.5 Nitrite Stress Test

After the feeding trial, a nitrite stress test was conducted using sodium nitrite. Each group had 4 replicates with 10 fish per replicate. During the stress test, water recirculation was stopped, and sodium nitrite solution was added to the water (100 L volume) to achieve a nitrite-nitrogen concentration of 16 mg/L. Fish behavior and mortality were observed, and the number of dead fish was recorded to calculate cumulative mortality rates (CMR) at 24, 48, and 72 hours:

Cumulative mortality rate (%) = 100 × (number of dead fish after stress)/(number of fish subjected to stress)

1.6 Data Processing

Statistical analysis was performed using SPSS 20.0 software. One-way ANOVA and Duncan' s multiple range test were used to analyze significant differences among treatment means. Results are expressed as mean ± standard deviation, with $P<0.05$ indicating statistical significance.

Results

2.1 Effects of MLF on Growth Performance and Feed Utilization

As shown in Table 2 , no significant differences were observed in weight gain among all groups ($P>0.05$), although the 500 mg/kg MLF group showed a 6.9% increase compared with the control group. Feed conversion ratios in all experimental groups were lower than that of the control group, but the differences were not significant ($P>0.05$). No significant differences were found in protein efficiency ratio among all groups ($P>0.05$). Similarly, no significant differences were detected in condition factor or viscerosomatic index among all groups ($P>0.05$), while the hepatosomatic index in the 1,000 mg/kg MLF group was significantly lower than that in the control group ($P<0.05$).

2.2 Effects of MLF on Whole-Body Composition

Table 3 shows that no significant differences were observed in whole-body crude protein, crude lipid, ash, or moisture contents among all groups ($P>0.05$). However, crude protein contents in the 500 and 1,000 mg/kg MLF groups increased by 5.6% and 7.6%, respectively, compared with the control group.

2.3 Effects of MLF on Serum and Liver Antioxidant Indices

As shown in Table 4 , no significant differences were observed in serum CAT activity among all groups ($P>0.05$). Serum GSH-Px activities in all experimental groups were higher than that in the control group, with the 1,000 mg/kg MLF group showing significantly higher activity than all other groups ($P<0.05$), and the 50 and 500 mg/kg MLF groups showing significantly higher activity than the control group ($P<0.05$). Serum SOD activities in the 100, 300, 500, and 1,000 mg/kg MLF groups were significantly higher than that in the control group ($P<0.05$), with the highest value observed in the 300 mg/kg MLF group. Serum T-AOC in all experimental groups was higher than that in the control group, with significant differences observed in the 100 and 500 mg/kg MLF groups ($P<0.05$). Serum MDA content showed a decreasing trend with increasing MLF supplementation levels, and all experimental groups had significantly lower MDA contents compared with the control group ($P<0.00$).

Based on broken-line model regression analysis of the relationship between MLF supplementation level (Y) and serum SOD activity and T-AOC, the optimal dietary MLF level for juvenile GIFT was determined to be 100.00 mg/kg when evaluated by serum SOD activity (X), yielding the equation $Y = 41.31 - 0.043(100.00 - X)$ ($R^2 = 0.7352$). When evaluated by serum T-AOC (X), regression analysis yielded equations $Y = 0.0117X + 8.3550$ ($R^2 = 0.7352$) and $Y = -0.0008X + 9.4314$ ($R^2 = 0.9894$), with the broken-line method indicating an optimal MLF level of 98.75 mg/kg.

As shown in Table 5 , liver CAT activities in the 100, 300, 500, and 1,000 mg/kg MLF groups were significantly higher than that in the control group ($P<0.05$), with the highest value observed in the 500 mg/kg MLF group. Liver GSH-Px activity was highest in the 500 mg/kg MLF group, which was significantly higher than that in the 1,000 mg/kg MLF group ($P<0.05$) but not significantly different from other groups ($P>0.05$). Liver SOD activity and T-AOC showed an increasing trend followed by a decreasing trend with increasing MLF supplementation levels, with significantly higher values observed in the 100, 300, and 500 mg/kg MLF groups compared with the control group ($P<0.05$). No significant differences were observed in liver MDA content among all groups ($P>0.05$).

Parabolic model regression analysis of the relationship between MLF supplementation level (Y) and liver SOD activity and T-AOC indicated that the optimal dietary MLF level was 371.00 mg/kg when evaluated by liver SOD activity (X), based on the equation $Y = -0.0003X^2 + 0.2226X + 168.4234$ ($R^2 = 0.9535$). When evaluated by liver T-AOC (X), the optimal level was 441.75 mg/kg, based on the equation $Y = -0.000002X^2 + 0.001767X + 0.366188$ ($R^2 = 0.9526$).

2.4 Effects of MLF on Resistance to Nitrite Stress

The cumulative mortality rates of GIFT during nitrite stress at 24, 48, and 72 hours are presented in Table 6 . At 24 hours, no significant differences were observed among groups ($P>0.05$), with the control group showing the highest

mortality rate of 22.7% and experimental groups ranging from 7.5% to 17.7%. At 48 hours, the control group mortality rate of 41.3% was significantly higher than those of the 50, 100, and 500 mg/kg MLF groups ($P<0.05$), which all showed mortality rates around 20%, similar to the control group value at 24 hours. At 72 hours, all experimental groups showed lower mortality rates than the control group, with the 50, 100, and 500 mg/kg MLF groups being significantly lower ($P<0.05$).

Discussion

3.1 Effects of MLF on Growth Performance and Feed Utilization

Currently, mulberry leaf resources are primarily used in aquaculture as a feed ingredient to replace fish meal. Some studies have found that replacing partial fish meal with mulberry leaf meal does not reduce digestibility and can even improve specific growth rate in fish, suggesting that mulberry leaves contain growth-promoting active components [8-9]. Flavonoids are abundant active components in mulberry leaves. Qu et al. [10] reported that mulberry leaf flavonoids significantly increased body length index and weight gain in weaned calves. Some natural flavonoids also affect animal growth. For instance, dihydromyricetin, a quercetin analog, significantly improved weight gain rate and specific growth rate in tilapia when added at 2,400 mg/kg [11], and flavonoids from *Sophora* plants significantly increased tilapia weight gain rate at 200 mg/kg [12]. Shin et al. [13] found that dietary quercetin at 0.5% significantly improved weight gain rate in olive flounder (*Paralichthys olivaceus*). In this study, dietary MLF supplementation did not significantly affect weight gain or feed conversion ratio in GIFT, indicating that certain MLF levels do not influence growth performance. These results differ from previous reports, possibly due to inconsistent survival rates affecting growth performance in this experiment or because MLF may not possess growth-promoting effects in GIFT. Currently, no consensus exists regarding the effects of flavonoids on animal growth. Studies on sheep [14] and Chongren partridge chickens [15] found that dietary alfalfa flavonoids did not significantly affect growth performance. Zhao et al. [16] reported that seabuckthorn leaf flavonoids did not significantly affect average daily gain or feed-to-gain ratio in broilers.

Soybean isoflavones significantly promoted growth in tilapia [17] and common carp (*Cyprinus carpio*) [18] by stimulating pituitary growth hormone (GH) production and release [17]. However, no isoflavone phytoestrogens have been identified in mulberry leaf flavonoids, suggesting different mechanisms of action between mulberry leaf flavonoids and soybean isoflavones. Nutrient metabolism is an important factor affecting animal growth. Mulberry leaf flavonoids can improve dietary metabolizable energy and nitrogen biological value in pre-weaned calves and increase total energy metabolic rate and nitrogen utilization in post-weaned calves [19], which may be one reason for improved tilapia growth. However, limited research is available, and the mechanisms by which mulberry leaf flavonoids promote tilapia growth require further investigation.

In this study, dietary MLF supplementation did not significantly affect condition factor or viscerosomatic index in GIFT, while the 1,000 mg/kg MLF group showed significantly lower hepatosomatic index compared with the control group. These findings differ from the observation that *Sophora* flavonoids significantly increased tilapia condition factor [12], possibly due to differences in flavonoid sources and animal growth stages. Other studies have shown that dietary soybean isoflavones did not significantly affect hepatosomatic index, condition factor, or viscerosomatic index in turbot (*Scophthalmus maximus*) [20-21], and mango leaf flavonoids did not significantly affect condition factor, hepatopancreatic index, or viscerosomatic index in common carp [22].

3.2 Effects of MLF on Whole-Body Composition

This study found that dietary MLF supplementation did not significantly affect whole-body crude lipid, crude protein, ash, or moisture contents in GIFT, although crude protein content was somewhat increased. The effects of flavonoids on animal body composition are inconsistent. Soybean isoflavones significantly reduced crude lipid and crude protein contents in juvenile turbot [20-21], while dietary daidzein did not significantly affect body composition in American eel (*Anguilla rostrata*) [23]. Quercetin reduced fat accumulation in hyperlipidemic rats [24], and seabuckthorn leaf flavonoids increased muscle fat content in broilers [16]. These discrepancies may be attributed to differences in animal species, growth stages, flavonoid sources, and supplementation levels.

3.3 Effects of MLF on Serum and Liver Antioxidant Capacity

Animals produce numerous free radicals through cellular activities and various stressors. Excessive oxygen free radicals can damage cell membrane integrity, causing oxidative stress. When oxidative stress exceeds the protective capacity of the antioxidant defense system, tissue and cellular oxidative damage occurs [25-26]. Reactive oxygen species (ROS) act as intracellular messengers in cell signaling, gene expression, and cell proliferation regulation, but excessive ROS can also cause oxidative stress and lipid peroxidation in tissues and cells [27-28]. SOD, widely present in various tissues, catalyzes the conversion of oxygen free radicals to hydrogen peroxide (H_2O_2), which is then decomposed to H_2O by CAT and GSH-Px, thereby eliminating free radicals. The sulfhydryl groups in GSH-Px can also bind with ROS to inhibit lipid peroxide formation [29]. MDA, a lipid peroxidation product that can cause abnormal biological membrane structure and function, serves as both an indicator of antioxidant status and an indirect measure of cellular damage.

In this study, serum SOD activities in the 100, 300, 500, and 1,000 mg/kg MLF groups and liver SOD activities and T-AOC in the 100, 300, and 500 mg/kg MLF groups were higher than those in the control group, indicating that MLF can enhance the elimination of excess ROS in tilapia. This was further confirmed by reduced MDA contents in corresponding treatment groups. Regression analysis indicated that the optimal dietary MLF level for juvenile GIFT was 100 mg/kg

based on serum SOD activity and T-AOC, and 371.00–441.75 mg/kg based on liver SOD activity and T-AOC. Previous studies reported that oral administration of mulberry leaf flavonoids increased liver SOD activity and reduced serum MDA content in alloxan-induced diabetic mice [6,30]. In vitro cell experiments showed that quercetin, a flavonoid from mulberry leaf extract, enhanced antioxidant capacity in HepG2 cells under oxidative stress induced by AAPH and Cu^{2+} [4]. Three flavonoids isolated from mulberry leaf methanol extract (quercetin, kaempferol, and rutin) showed time- and dose-dependent inhibition of AAPH-induced hemolysis in human erythrocytes, with rutin significantly reducing glutathione depletion [31]. These findings suggest that mulberry leaf flavonoids not only scavenge free radicals through their own reductive hydroxyl groups but also regulate antioxidant enzyme activities. Mechanistic studies on flavonoids have shown that quercetin can translocate the transcription factor DAF-16 to the nucleus, thereby regulating oxidative stress and aging in *Caenorhabditis elegans* through signaling pathways [32]. It can be speculated that mulberry leaf flavonoids may modulate antioxidant capacity in GIFT by affecting related signaling pathways, though further research is needed.

3.4 Effects of MLF on Resistance to Nitrite Stress in GIFT

Nitrite is a common stressor that causes oxidative stress in fish, primarily affecting normal respiration and metabolism and severely impacting physiological indices and tissue organs [33]. No previous studies have reported the effects of mulberry leaf flavonoids on fish resistance to nitrite stress. In this study, after 24, 48, and 72 hours of nitrite stress, mortality rates in all MLF-supplemented groups were lower than that in the control group, with significant reductions observed in the 50, 100, and 500 mg/kg MLF groups at the latter two time points. Further analysis revealed that when cumulative mortality reached approximately 20%, this occurred at 24 hours in the control group but was delayed until 48 hours in the experimental groups. These results demonstrate that MLF can enhance the resistance of juvenile GIFT to nitrite stress, not only reducing mortality within a certain period after high-concentration nitrite exposure but also delaying the onset of mortality.

The mechanism by which MLF reduces nitrite stress-induced mortality in tilapia may involve several pathways. Nitrite can cause excessive ROS accumulation in blood cells [34], while mulberry leaf flavonoids can scavenge ROS [4]. MLF may utilize its reductive hydroxyl groups to eliminate free radicals and modulate antioxidant enzyme activities to enhance free radical scavenging capacity.

Conclusions

1. Dietary MLF supplementation did not significantly affect growth performance in GIFT but improved serum and liver antioxidant indices and enhanced resistance to nitrite stress.
2. Under the experimental conditions, regression analysis indicated that the

optimal dietary MLF supplementation level for juvenile GIFT was 100 mg/kg based on serum SOD activity and T-AOC, and 371.00–441.75 mg/kg based on liver SOD activity and T-AOC.

References

[1] LIAO Sentai, XIAO Gengsheng. Research on Active Substances in Mulberry Trees [M]. Beijing: China Agricultural Science and Technology Press, 2012: 13.

[2] ZHU Dan, YUAN Fang, MENG Kun, et al. Research progress on flavonoids [J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2007, 22(6): 387–389.

[3] KIM S Y, GAO J J, LEE W C, et al. Antioxidative flavonoids from the leaves of *Morus alba* [J]. Archives of Pharmacal Research, 1999, 22(1): 81–85.

[4] KIM G N, JANG H D. Flavonol content in the water extract of the mulberry (*Morus alba* L.) leaf and their antioxidant capacities [J]. Journal of Food Science, 2011, 76(6): C869–C873.

[5] PAN Jianyong, WANG Zhiping, DANG Jiangbo, et al. Study on in vitro antioxidant activity and main functional components of summer mulberry leaves [J]. Journal of Nuclear Agricultural Sciences, 2011, 25(4): 754–759.

[6] LI Xiangrong, FANG Xiao, YU Lingying. Antioxidant and protein glycation inhibitory effects of mulberry leaf flavonoids [J]. Journal of Zhejiang University: Agriculture and Life Sciences, 2005, 31(2): 203–206.

[7] DEY M M, GUPTA M V. Socioeconomics of disseminating genetically improved Nile tilapia in Asia: an introduction [J]. Aquaculture Economics & Management, 2000, 4(4): 5–11.

[8] MONDAL K, KAVIRAJ A, MUKHOPADHYAY P K. Effects of partial replacement of fishmeal in the diet by mulberry leaf meal on growth performance and digestive enzyme activities of Indian minor carp *Labeo bata* [J]. International Journal of Aquatic Science, 2012, 3(1): 72–83.

[9] KAVIRAJ A, MONDAL K, MUKHOPADHYAY P K, et al. Impact of fermented mulberry leaf and fish offal in diet formulation of Indian major carp (*Labeo rohita*) [J]. Proceedings of the Zoological Society, 2013, 66(1): 64–73.

[10] QU Peibin. Effects of Mulberry Leaf Flavonoids and *Candida tropicalis* on Growth Performance, Slaughter Performance, Meat Quality and Serum Indices of Calves [D]. Master' s Thesis. Handan: Hebei University of Engineering, 2015.

[11] CAI Yinghua, SHU Xugang, ZHANG Lei. Effects of dihydromyricetin on growth, immunity and antioxidant function of tilapia [J]. Guangdong Feed, 2010, 19(12): 19–21.

[12] LIU Shulan. Effects of Different Levels of *Sophora* Flavonoids on Growth and Hepatopancreatic Antioxidant Capacity of Tilapia [D]. Master' s Thesis.

Xiamen: Jimei University, 2012.

[13] SHIN H S, YOO J H, MIN T S, et al. Effect of quercetin on the activity and mRNA expression of antioxidant enzymes and physiological responses in olive flounder (*Paralichthys olivaceus*) exposed to cadmium [J]. Asian-Australasian Journal of Animal Sciences, 2010, 23(6): 742-749.

[14] WANG Mengzhu, LIU Yanfeng, WANG Wenqi, et al. Effects of alfalfa flavonoids on growth performance and serum indices of sheep [J]. China Animal Husbandry & Veterinary Medicine, 2015, 42(9): 2345-2351.

[15] OUYANG Kehui, XIONG Xiaowen, WANG Wenjun, et al. Effects of alfalfa flavonoids on growth performance and muscle chemical composition of Chongren partridge chickens [J]. Acta Prataculturae Sinica, 2013, 22(4): 340-345.

[16] ZHAO Wei, CHEN Xin, LIU Hongnan, et al. Effects of seabuckthorn leaf flavonoids on growth performance and carcass quality of broilers [J]. Chinese Journal of Animal Nutrition, 2012, 24(1): 117-123.

[17] YU Zugong, XIA Dequan, WU Tingting. Effects of daidzein on growth, related hormone levels and blood biochemical indices of *Oreochromis aureus* [J]. Chinese Journal of Veterinary Science, 2006, 26(2): 183-185.

[18] XU Zenghong. Application of daidzein in aquaculture [J]. Freshwater Fisheries, 2005, 35(6): 41-43.

[19] YANG Chuntao, DIAO Qiyu, QU Peibin, et al. Effects of *Candida tropicalis* and mulberry leaf flavonoids on nutrient metabolism and rumen fermentation in calves [J]. Chinese Journal of Animal Nutrition, 2016, 28(1): 224-234.

[20] GUO Haiyan, ZHANG Yanjiao, MAI Kangsen, et al. Effects of genistein on growth, digestive enzyme activities and intestinal histology of turbot (*Scophthalmus maximus*) [J]. Journal of Ocean University of China: Natural Science Edition, 2014, 40(9): 30-36.

[21] HU Haibin, LIU Jintao, LI Yanxian, et al. Effects of dietary daidzein on growth, digestive enzyme activities, antioxidant capacity and intestinal structure of turbot (*Scophthalmus maximus*) [J]. Journal of Fisheries of China, 2014, 38(9): 1503-1513.

[22] LÜ Xiaowen, HAO Qian, CHEN Yeyuan, et al. Dietary mango leaf flavonoid extract promotes fish growth [J]. Transactions of the Chinese Society of Agricultural Engineering, 2013, 29(18): 277-283.

[23] YE Jidan, CHEN Xuehao. Effects of daidzein on growth and several metabolic enzyme activities in liver and intestine of American eel (*Anguilla rostrata*) [J]. Journal of Jimei University: Natural Science Edition, 2008, 13(1): 1-6.

[24] ZHOU Yusheng, YANG Xuefeng. Therapeutic effect of quercetin on alcoholic fatty liver in rats [J]. Journal of China Prescription Drug, 2005(10): 80-81.

[25] RITOLA O, LYYTIKÄINEN T, PYLKKÖ P, et al. Glutathione-dependent defence system and monooxygenase enzyme activities in Arctic charr *Salvelinus alpinus* (L.) exposed to ozone [J]. Aquaculture, 2000, 185(3/4): 219-233.

[26] BHOR V M, RAGHURAM N, SIVAKAMI S. Oxidative damage and altered antioxidant enzyme activities in the small intestine of streptozotocin-induced diabetic rats [J]. The International Journal of Biochemistry & Cell Biology, 2004, 36(1): 89-97.

[27] SOHN J H, HAN K L, LEE S H, et al. Protective effects of panduratin A against oxidative damage of tert-butylhydroperoxide in human HepG2 cells [J]. Biological & Pharmaceutical Bulletin, 2005, 28(12): 1083-1086.

[28] LI Jianxi, YANG Zhiqiang, WANG Xuezhi. Biological role of reactive oxygen species in animal body [J]. Progress in Veterinary Medicine, 2006, 27(10).

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