

Effects of Tea Polyphenols on Growth, Digestive Function, Immune Performance and Disease Resistance of Nile Tilapia (*Oreochromis niloticus*) Postprint

Authors: Liang Gaoyang, Li Xiaoqin, Yang Hang, POOLSAWAT Lumpan, Gao Bowei, Leng Xiangjun

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of tea polyphenols on growth, digestive function, immune performance, and disease resistance of tilapia (*Oreochromis niloticus* × *O. aureus*). Six experimental diets were formulated by supplementing 0 (control group), 100, 200, 400, 600, and 800 mg/kg tea polyphenols into the basal diet, which were fed to tilapia with initial body weight of (6.09±\$0.07) g for 9 weeks, with 3 replicates per group and 25 fish per replicate.

The results showed that: compared with the control group, the weight gain rate and feed conversion ratio of tea polyphenol-supplemented groups showed no significant changes ($P>0.05$). However, the 100 and 200 mg/kg tea polyphenol groups showed an increasing trend in weight gain rate, while the 400-800 mg/kg tea polyphenol groups showed a decreasing trend, with the 200 mg/kg tea polyphenol group achieving the highest weight gain rate.

Dietary tea polyphenols had no significant effect on whole-body moisture, crude protein, crude ash content, and apparent digestibility of crude protein in tilapia ($P>0.05$). However, compared with the control group, high supplementation levels (600, 800 mg/kg) of tea polyphenols significantly reduced the apparent digestibility of dry matter, intestinal amylase and protease activities, and whole-body crude lipid content ($P<0.05$).

Compared with the control group, dietary supplementation with 200, 400, 600, and 800 mg/kg tea polyphenols significantly increased serum superoxide dismutase (SOD) and lysozyme (LZM) activities ($P<0.05$), while serum malondialdehyde (MDA) content showed a trend of first decreasing and then increasing with increasing tea polyphenol supplementation. The 200 mg/kg tea polyphenol

group had the highest serum SOD activity and the lowest serum MDA content, while the 400 mg/kg tea polyphenol group had the highest serum LZM activity.

After challenge with *Aeromonas hydrophila*, the cumulative mortality at 48 and 96 h in the 200, 400, 600, and 800 mg/kg tea polyphenol groups was significantly lower than that in the control group ($P < 0.05$), with the 400 mg/kg tea polyphenol group showing the lowest cumulative mortality.

In summary, tea polyphenols can improve the immune performance of tilapia and reduce mortality after *Aeromonas hydrophila* challenge, but have no significant effect on growth performance. The recommended dietary supplementation level of tea polyphenols for tilapia is 200–400 mg/kg.

Full Text

Effects of Tea Polyphenols on Growth, Digestive Function, Immune Performance and Disease Resistance of Hybrid Tilapia (*Oreochromis niloticus* × *O. aureus*)

LIANG Gaoyang¹²³, LI Xiaoqin¹²³, YANG Hang¹²³, POOLSAWAT Lumpan¹²³, GAO Bowei¹²³, LENG Xiangjun^{123*}

¹National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, China

²Centre for Research on Environmental Ecology and Fish Nutrition of the Ministry of Agriculture, Shanghai Ocean University, Shanghai 201306, China

³Shanghai Collaborative Innovation for Aquatic Animal Genetics and Breeding, Shanghai Ocean University, Shanghai 201306, China

Abstract

This study investigated the effects of tea polyphenols (TP) on growth, digestive function, immune performance, and disease resistance in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Six experimental diets were formulated by supplementing a basal diet with 0 (control), 100, 200, 400, 600, or 800 mg/kg TP. Each diet was fed to triplicate groups of 25 fish with an initial body weight of (6.09 ± 0.07) g for 9 weeks. The results showed that compared with the control group, TP supplementation did not significantly affect weight gain rate or feed conversion ratio ($P > 0.05$). However, 100 and 200 mg/kg TP tended to improve weight gain rate, while 400–800 mg/kg TP tended to reduce it, with the highest weight gain rate observed in the 200 mg/kg group. Whole-body moisture, crude protein, and ash contents, as well as apparent crude protein digestibility, were not significantly affected by dietary TP ($P > 0.05$). In contrast, high TP levels (600 and 800 mg/kg) significantly reduced dry matter apparent digestibility, intestinal amylase and protease activities, and whole-body crude lipid content ($P < 0.05$). Supplementation with 200, 400, 600, and 800 mg/kg TP significantly increased serum superoxide dismutase (SOD) and lysozyme

(LZM) activities ($P < 0.05$), while serum malondialdehyde (MDA) content first decreased and then increased with rising TP levels. The 200 mg/kg group exhibited the highest serum SOD activity and lowest MDA content, whereas the 400 mg/kg group showed the highest LZM activity. Following challenge with *Aeromonas hydrophila*, cumulative mortality at 48 and 96 h was significantly lower in the 200, 400, 600, and 800 mg/kg groups compared with the control ($P < 0.05$), with the 400 mg/kg group showing the lowest mortality. In conclusion, TP can enhance immune performance and reduce mortality after *A. hydrophila* challenge in hybrid tilapia, though it does not significantly affect growth performance. The recommended dietary TP supplementation level for hybrid tilapia is 200–400 mg/kg.

Keywords: tea polyphenols; hybrid tilapia; growth; digestibility; immune performance; disease resistance

Tea polyphenols are polyhydroxy phenolic compounds extracted from tea leaves and tea by-products, accounting for approximately 30% of tea dry weight. Their main components include catechins, flavonoids, phenolic acids, anthocyanins, and their derivatives [1]. Tea polyphenols exhibit antioxidant, immune-enhancing, disease-preventing, and lipid metabolism-regulating effects [2-5], and have been widely applied in food preservation, clinical medicine, and animal husbandry. Recent research has begun to focus on their applications in aquaculture. Xu et al. [6] reported that dietary supplementation with 25–100 mg/kg TP significantly improved liver immune enzyme activities in rainbow trout (*Oncorhynchus mykiss*). Hwang et al. [7] found that feeding juvenile black rockfish (*Sebastes schlegeli*) diets containing 1%–5% green tea extract significantly improved weight gain rate and stress resistance. Long et al. [8-9] demonstrated that 50 and 100 mg/kg TP supplementation significantly increased weight gain rate and liver antioxidant capacity in juvenile Wuchang bream (*Megalobrama amblycephala*), while reducing mortality after *A. hydrophila* infection.

The long-term use of antibiotics has led to serious problems with pathogen resistance and drug residues, posing significant threats to aquaculture development [10]. Consequently, identifying safe and effective antibiotic alternatives has become a research priority. Tea polyphenols possess a broad antimicrobial spectrum and can inhibit the growth of various pathogens [11-12], while also enhancing fish immune performance [13-14], making them a potential antibiotic substitute. This study used hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) as the experimental model to investigate the effects of different dietary TP levels on growth, digestive function, immune performance, and resistance to *A. hydrophila* infection, providing a theoretical basis for TP application in aquaculture.

1.1 Experimental Diets

A basal diet was formulated using fish meal, soybean meal, cottonseed meal, and rapeseed meal as primary protein sources, and soybean oil as the main lipid source (containing 32.06% crude protein and 5.62% crude lipid). Six experimental diets were prepared by supplementing the basal diet with 0 (control), 100, 200, 400, 600, or 800 mg/kg TP [14]. Yttrium oxide (Y_2O_3) was added at 0.05% to all diets to determine nutrient apparent digestibility. Major feed ingredients were ground to pass through a 40-mesh sieve, mixed progressively, and processed into 2 mm diameter hard pellet sinking diets using a single-screw extruder (SLP-45, Fishery Machinery Research Institute, Chinese Academy of Fishery Sciences) at a processing temperature of (85 ± 5) °C. The pellets were dried at 55 °C and stored at 4 °C. The composition and nutrient levels of the basal diet are presented in Table 1. Tea polyphenols (98% purity) were provided by Shandong Fengtai Biological Technology Co., Ltd.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

| Items | Content |
|---------------------------------|---------|
| Ingredients¹⁾ | |
| Fish meal | |
| Soybean meal | |
| Cottonseed meal | |
| Rapeseed meal | |
| Wheat middling | |
| Rice bran | |
| Soybean oil | |
| $Ca(H_2PO_4)_2$ | |
| Vitamin premix ²⁾ | |
| Mineral premix ³⁾ | |
| Choline chloride (50%) | |
| Y_2O_3 | |
| Total | |
| Nutrient levels | |
| Dry matter (DM) | |
| Crude protein (CP) | |
| Ether extract (EE) | |
| Crude ash (Ash) | |

¹⁾ Feed ingredients were purchased from Shanghai Nonghao Feed Co., Ltd. The crude protein content of fish meal (Peru), soybean meal, rapeseed meal, and cottonseed meal was 65.18%, 44.65%, 36.64%, and 44.77%, respectively.

²⁾ The vitamin premix provided the following per kg of diet: VA 6,000 IU, VD₃ 1,000 IU, VE 60 IU, VK 5 mg, VB₁ 15 mg, VB₂ 15 mg, VB₃ 30 mg, VB₅ 35

mg, VB₆ 20 mg, biotin 2 mg, folic acid 3 mg, VB₁₂ 0.03 mg.

³) The mineral premix provided the following per kg of diet: Ca(IO₃)₂ 0.04 g, CoCl₂ · 6H₂O 0.01 g, FeSO₄ · H₂O 0.446 g, ZnSO₄ · H₂O 0.232 g, MnSO₄ · H₂O 0.063 g, NaSeO₃ · 5H₂O 0.01 g, MgSO₄ · 7H₂O 0.645 g.

1.2 Experimental Fish and Rearing Management

The feeding trial was conducted at the Binhai Special Aquaculture Farm of Shanghai Ocean University for 9 weeks. Hybrid tilapia were purchased from Shanghai Xinchang Tilapia Breeding Farm and acclimated to the control diet for one week prior to the experiment. A total of 450 fish with an average initial body weight of (6.09 ± 0.07) g were randomly distributed into 18 net cages (1.5 m × 1.0 m × 1.2 m) at a density of 25 fish per cage (00, 12 : 00, and 17 : 00). During the trial, water was exchanged twice weekly (one – third of the volume each time). Water temperature was maintained at $(28 \pm 2)^\circ\text{C}$, pH at 7.5 ± 0.5 , dissolved oxygen concentration >6.0 mg/L, and ammonia nitrogen concentration <0.2 mg/L.

1.3 Sampling and Analysis

1.3.1 Growth Indices At the end of the 9-week feeding trial, fish were fasted for 24 h before final counting and weighing to calculate growth indices. Three fish were randomly selected from each cage for body length measurement and dissection to obtain viscera and liver weights for morphological index calculation. The following formulas were used:

- Weight gain rate (WGR, %) = $100 \times (\text{final mean weight} - \text{initial mean weight}) / \text{initial mean weight}$
- Feed conversion ratio (FCR) = $\text{total feed intake} / (\text{final mean weight} - \text{initial mean weight})$
- Survival rate (SR, %) = $100 \times \text{final fish number} / \text{initial fish number}$
- Feed intake (FI, g) = $\text{total feed intake} / \text{fish number}$
- Hepatosomatic index (HSI, %) = $100 \times \text{liver weight} / \text{body weight}$
- Viscerosomatic index (VSI, %) = $100 \times \text{viscera weight} / \text{body weight}$
- Condition factor (CF, g/cm³) = $100 \times \text{body weight} / \text{body length}^3$

1.3.2 Proximate Composition Analysis After weighing, three fish per cage were randomly selected for whole-body proximate composition analysis. Moisture content was determined by oven drying at 105 °C, crude protein by the Kjeldahl method using an automatic Kjeldahl analyzer (Kjeltec-2300, FOSS, Sweden), crude lipid by Soxhlet extraction using a fat analyzer (SOX-416, Gerhardt, Germany), and crude ash by muffle furnace incineration at 550 °C using a programmable box furnace (SXL-1008, Shanghai Jinghong Experimental Equipment Co., Ltd.).

1.3.3 Serum Immune Indices Three additional fish per cage were randomly selected for blood collection from the caudal vein. Blood samples were centrifuged at 3,000 r/min for 10 min at 4 °C, and serum was harvested and

stored at -80 °C until analysis. Serum superoxide dismutase (SOD), alkaline phosphatase (AKP), lysozyme (LZM) activities, and malondialdehyde (MDA) content were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer' s instructions. SOD and AKP activities were determined using a microplate reader (Synergy 2, BioTek, USA), while MDA content and LZM activity were measured using a spectrophotometer (Model 722, Shanghai Precision Scientific Instrument Co., Ltd.).

1.3.4 Intestinal Digestive Enzyme Activity The anterior intestine (from stomach to the first intestinal bend) was dissected from the three fish sampled in Section 1.3.3, rinsed with physiological saline to remove digesta, and stored at -80 °C. For enzyme analysis, intestinal samples were thawed at 4 °C, homogenized with 9 volumes of ice-cold physiological saline, and centrifuged at 3,000 r/min for 10 min at 4 °C. The supernatant was used for enzyme activity assays.

- Amylase activity was measured using a commercial kit (Nanjing Jiancheng Bioengineering Institute), defined as the amount of enzyme that hydrolyzes 10 mg starch in 30 min at 37 °C per mg protein.
- Protease activity was determined by the Folin-phenol method using 2% casein as substrate, defined as the amount of enzyme that produces 1 g tyrosine per minute at pH 7.2 and 37 °C per g tissue protein.
- Intestinal protein content was measured by the Coomassie brilliant blue method.

1.3.5 Nutrient Apparent Digestibility Feces were continuously collected for 10 days starting from day 30 of the feeding trial to determine nutrient apparent digestibility. Two hours after each feeding, feces were collected by siphoning. Intact and continuous fecal samples were oven-dried at 60 °C and stored at -20 °C until analysis. Yttrium oxide content in diets and feces was determined by inductively coupled plasma optical emission spectrometry (Optima 8000DV, PerkinElmer, USA). Crude protein content in feces was analyzed as described in Section 1.3.2.

- Dry matter apparent digestibility (%) = $100 \times (1 - b/B)$
- Crude protein apparent digestibility (%) = $100 \times [1 - (A/a \times b/B)]$

Where: a and A represent crude protein content in diet and feces, respectively; b and B represent Y_2O_3 content in diet and feces, respectively.

1.3.6 Aeromonas hydrophila Challenge Test At the end of the feeding trial, 12 fish from each cage were challenged with *Aeromonas hydrophila*. The bacterial strain was obtained from the National Aquatic Animal Pathogen Bank (Lü Liqun Laboratory) at Shanghai Ocean University. The bacteria were cultured on nutrient agar plates at 28 °C for 24 h, then a single colony was inoculated into sterile nutrient broth and incubated at 28 °C for 30 h. The culture was centrifuged at 3,500 r/min for 5 min, the supernatant was removed, and the pellet was resuspended in sterile physiological saline to a concentration of

1.1×10^8 CFU/mL (determined by preliminary experiments). Each fish received an intraperitoneal injection of 0.2 mL bacterial suspension. Mortality was recorded at 1, 24, 48, and 96 h post-challenge, and cumulative mortality was calculated as:

- Cumulative mortality (CM, %) = $100 \times \text{cumulative deaths} / \text{total initial fish number}$

1.4 Statistical Analysis All data (except survival rate) are expressed as mean \pm standard deviation (SD) and were analyzed by one-way ANOVA using SPSS 22.0 software. When significant differences were detected, Duncan's multiple range test was applied. Statistical significance was set at $P < 0.05$.

2.1 Effects of TP on Growth and Physical Indices

The effects of dietary TP on growth and physical indices are presented in Table 2. After the 9-week feeding trial, survival rate was 100% in all groups, with no significant differences in feed intake ($P > 0.05$). Compared with the control group, TP supplementation did not significantly affect weight gain rate or feed conversion ratio ($P > 0.05$). However, 100 and 200 mg/kg TP tended to improve weight gain rate, while 200, 400, and 800 mg/kg TP tended to reduce it. Notably, the 800 mg/kg group showed significantly lower weight gain rate than the 200 mg/kg group ($P < 0.05$). Quadratic regression analysis of weight gain rate (y) against TP supplemental level (x) yielded the equation $y = -0.0003x^2 + 0.1224x + 1500$ ($R^2 = 0.5084$), with the maximum weight gain rate predicted at 204 mg/kg TP. Regarding morphological indices, the hepatosomatic index was significantly lower in the 600 and 800 mg/kg groups compared with the control ($P < 0.05$), while condition factor and viscerosomatic index showed no significant differences among groups ($P > 0.05$).

Table 2 Effects of TP on growth and physical indices of hybrid tilapia

| Items | TP supplemental level (mg/kg) | 0 | 100 | 200 | 400 | 600 | 800 |
|------------------------------|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Initial body weight (IBW, g) | | 6.11 ± 0.09 | 6.07 ± 0.05 | 6.12 ± 0.04 | 6.11 ± 0.11 | 6.07 ± 0.06 | 6.12 ± 0.07 |

In the same row, values with no letter or the same letter superscripts indicate no significant difference ($P > 0.05$), while different letters indicate significant difference ($P < 0.05$). The same applies below.

2.2 Effects of TP on Body Composition

The effects of TP on whole-body composition are shown in Table 3 . Compared with the control group, dietary supplementation with 600 and 800 mg/kg TP significantly reduced whole-body crude lipid content ($P<0.05$). No significant differences were observed among groups in whole-body moisture, crude protein, or ash contents ($P>0.05$).

Table 3 Effects of TP on body composition of hybrid tilapia (wet weight basis)

| Items | TP supplemental level (mg/kg) | | | | | | |
|--------------|-------------------------------|------------------|------------------|------------------|------------------|------------------|--------------------------------|
| | 0 | 100 | 200 | 400 | 600 | 800 | |
| Moisture (%) | 72.06 \pm 1.46 | 71.83 \pm 1.12 | 72.57 \pm 0.85 | 71.78 \pm 0.83 | 72.27 \pm 1.32 | 72.27 \pm 0.99 | Crudeprotein(CP, \pm 0.74 16 |

2.3 Effects of TP on Nutrient Apparent Digestibility and Intestinal Digestive Enzyme Activity

The effects of TP on nutrient apparent digestibility and intestinal digestive enzyme activities are presented in Table 4 . Dry matter apparent digestibility increased initially and then decreased with rising TP levels, reaching a maximum in the 200 mg/kg group that was significantly higher than all other groups ($P<0.05$). Crude protein apparent digestibility was not significantly affected by TP supplementation ($P>0.05$). Compared with the control, high TP levels (600 and 800 mg/kg) significantly reduced intestinal protease and amylase activities ($P<0.05$).

Table 4 Effects of TP on nutrient apparent digestibility and intestinal digestive enzyme activities of hybrid tilapia

| Items | TP supplemental level (mg/kg) | | | | | | |
|--|--|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| | 0 | 100 | 200 | 400 | 600 | 800 | |
| Nutrient apparent digestibility (%) | | | | | | | |
| Dry matter (DM) | 67.24 \pm 0.29b | 68.12 \pm 0.42c | 69.79 \pm 0.62d | 68.33 \pm 0.58c | 66.30 \pm 0.28a | 66.43 \pm 0.18a | Crudeprotein(CP) 8 |
| | *Intestinaldigestiveenzymeactivities(U/mgprot)* | | | | | | |
| | * Protease 1383.7 \pm 79.1b 1462.6 \pm 78.4b 1456.1 \pm 61.4b 1369.8 \pm 59.8b 1177.8 \pm 47.7a 1124.6 \pm 41.0a | | | | | | |

2.4 Effects of TP on Serum Immune Indices

The effects of TP on serum immune indices are shown in Table 5 . Compared with the control, dietary supplementation with 200, 400, 600, and 800 mg/kg TP

significantly increased serum SOD and LZM activities ($P < 0.05$). Quadratic regression analysis indicated that maximal SOD and LZM activities were achieved at TP levels of 438.89 and 412.35 mg/kg, respectively (Figure 1 [Figure 1: see original paper]). Serum MDA content decreased initially and then increased with rising TP levels, reaching its minimum in the 200 mg/kg group. No significant differences were observed in serum AKP activity among groups ($P > 0.05$).

Table 5 Effects of TP on serum immunological indexes of hybrid tilapia

| Items | TP supplemental level (mg/kg) | 0 | 100 | 200 | 400 | 600 | 800 |
|----------------------------------|-------------------------------|-------------------|--------------------|-------------------|-------------------|-------------------|-------------------|
| Superoxide dismutase (SOD, U/mL) | | 88.09 \pm 3.80a | 42.24 \pm 1.16ab | 56.71 \pm 5.08c | 56.30 \pm 4.46c | 47.22 \pm 3.84b | 44.42 \pm 2.28b |
| Lysozyme (LZM, U/mL) | | 1.12 \pm 0.05a | 1.12 \pm 0.05a | 1.12 \pm 0.05a | 1.12 \pm 0.05a | 1.12 \pm 0.05a | 1.12 \pm 0.05a |

Figure 1 Relationships between TP supplemental level and serum SOD or LZM activities of hybrid tilapia

2.5 Effects of TP on Cumulative Mortality After *A. hydrophila* Challenge

As shown in Figure 2 [Figure 2: see original paper], no mortality was observed in any group at 1 h post-challenge (100% survival). At 24 h, cumulative mortality was significantly lower in the 100, 200, 400, and 600 mg/kg groups compared with the control ($P < 0.05$). At 48 and 96 h, the 200, 400, 600, and 800 mg/kg groups all exhibited significantly lower cumulative mortality than the control ($P < 0.05$), with the 400 mg/kg group showing the lowest mortality.

Figure 2 Effects of TP on cumulative mortality of hybrid tilapia challenged with *Aeromonas hydrophila*

Data points at the same time point with different letters indicate significant difference ($P < 0.05$).

3.1 Effects of TP on Growth and Digestive Function

Research on tea polyphenols in aquatic animals remains limited. Studies have shown that dietary supplementation with 50–100 mg/kg TP significantly improved weight gain rate and specific growth rate in Wuchang bream, upregulating the expression of growth-related gene MaGHR2 [8]. In rainbow trout, 25–100 mg/kg TP numerically increased weight gain rate, though the difference was not statistically significant [6]. In Nile tilapia (*Oreochromis niloticus*), 166 mg/kg TP significantly improved weight gain rate, whereas 333 mg/kg showed no beneficial effects [14]. Similarly, 50 mg/kg TP significantly increased specific

growth rate in black carp (*Mylopharyngodon piceus*), while 500 mg/kg significantly reduced it [15]. In the present study, dietary TP at 100–800 mg/kg did not significantly affect growth performance in hybrid tilapia, but 100 and 200 mg/kg tended to improve weight gain rate, while 400, 600, and 800 mg/kg tended to reduce it, with the 800 mg/kg group showing significantly lower weight gain rate than the 200 mg/kg group. These findings suggest that high TP levels may inhibit fish growth. Zheng et al. [16] reported that green tea residue supplementation exceeding 3.6% reduced weight gain rate and increased feed conversion ratio in tilapia. Welker et al. [17] also found that rainbow trout fed diets containing 4% green tea showed significantly reduced weight gain rate and increased feed conversion ratio, likely due to high TP content. As phenolic compounds extracted from tea, tea polyphenols are generally considered anti-nutritional factors that can reduce nutrient utilization and animal growth performance [18–19].

In this study, 100–400 mg/kg TP significantly increased dry matter apparent digestibility in hybrid tilapia, whereas high levels (600 and 800 mg/kg) significantly reduced dry matter digestibility and intestinal amylase and protease activities. In black carp, 50 mg/kg TP significantly increased intestinal protease and lipase activities, while 500 mg/kg significantly reduced them [15]. In mice, dietary supplementation with 0.4% TP significantly reduced crude protein apparent digestibility [20], and oral administration of 0.4% TP significantly decreased intestinal digestibility of moisture, glucose, cholesterol, amino acids, and minerals [21]. Tea polyphenols can alleviate intestinal oxidative stress and promote the growth of beneficial intestinal bacteria [22], which may enhance digestive function. However, excessive TP, like other polyphenols, may bind to proteins, carbohydrates, and minerals, thereby reducing their digestibility [23–25]. The mechanisms underlying TP effects on fish digestive function require further investigation.

3.2 Effects of TP on Body Composition

Tea polyphenols can reduce body fat content in high-fat diet-fed mice by regulating lipid metabolism-related hormones such as insulin-like growth factor I, leptin, and adiponectin, as well as associated signaling pathway genes [26–28]. In broiler chickens, oral administration of 80 and 160 mg/kg TP significantly reduced subcutaneous fat thickness, intermuscular fat width, and abdominal fat percentage [29]. Dietary supplementation with 6.4% green tea residue significantly reduced whole-body crude lipid content in tilapia without affecting moisture or crude protein content [16]. Similar results were observed in the present study, where high TP levels (600 and 800 mg/kg) significantly reduced whole-body crude lipid content without affecting moisture or crude protein content. Comparable findings have been reported in rainbow trout, where low TP levels (25–500 mg/kg) did not affect whole-body crude lipid content, but high levels (1,000 mg/kg) significantly reduced it [6]. In Wuchang bream, low TP levels (25 and 50 mg/kg) increased muscle crude protein content without signif-

icantly affecting muscle crude lipid content [8]. These results indicate that TP effects on lipid deposition in fish are dose-dependent, with high levels generally inhibiting fat accumulation.

3.3 Effects of TP on Immune Performance and Disease Resistance

Previous studies have demonstrated that tea polyphenols can enhance fish immune performance. For instance, 25-100 mg/kg TP supplementation significantly increased liver SOD, AKP, and acid phosphatase (ACP) activities in rainbow trout [6]. In Wuchang bream, 50 mg/kg TP significantly elevated serum SOD activity under ammonia stress and improved stress resistance [30]. In black carp, 100 mg/kg TP significantly increased serum SOD activity and reduced MDA content [15]. In the present study, 200-800 mg/kg TP significantly increased serum SOD and LZM activities, decreased MDA content, and enhanced resistance to *A. hydrophila* infection in hybrid tilapia. The reduced mortality following *A. hydrophila* challenge may be attributed to both enhanced immune performance and the antibacterial properties of TP, as in vitro studies have shown that TP can inhibit *A. hydrophila* growth [31]. Similar results have been reported with green tea or green tea extract supplementation. Dietary inclusion of 1%-5% green tea extract enhanced plasma LZM activity, shortened stress recovery time after 2-phenoxyethanol immersion, and reduced mortality after air exposure in black rockfish [7]. Channel catfish (*Ictalurus punctatus*) fed diets containing 1%, 2%, or 4% green tea showed significantly reduced mortality after *A. hydrophila* challenge [32]. Nile tilapia fed diets with 0.125-2.000 g/kg green tea also exhibited significantly reduced mortality following *A. hydrophila* challenge in a dose-dependent manner [33].

Conclusion

Dietary supplementation with 400 mg/kg tea polyphenols enhanced immune performance and reduced cumulative mortality after *Aeromonas hydrophila* challenge in hybrid tilapia. The optimal dietary TP supplementation level for hybrid tilapia is recommended to be 200-400 mg/kg.

References

- [1] KHAN N, MUKHTAR H. Tea polyphenols health promotion[J]. Life Sciences, 2007, 81(7): 519-533.
- [2] FREI B, HIGDON J V. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies[J]. The Journal of Nutrition, 2003, 133(10): 3275S-3284S.
- [3] BOSE M, LAMBERT J D, et al. The major green polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice[J]. The Journal of Nutrition, 2008, 138(9): 1677-1683.
- [4] RIEGSECKER S, WICZYNSKI D, KAPLAN M J, et al. Potential benefits of green tea polyphenol EGCG in the prevention and treatment of vascular

- inflammation in rheumatoid arthritis[J]. *Life Sciences*, 2013, 93(8): 307-312.
- [5] PENG A, YE T, RAKHEJA D, et al. The green tea polyphenol (–)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis[J]. *Kidney International*, 2011, 80(6): 601-611.
- [6] XU Qiyu, LI Chan, XU Hong, et al. Effects of tea polyphenols on growth performance, biochemical indices, and non-specific immune indices of rainbow trout[J]. *Chinese Journal of Animal Nutrition*, 2008, 20(5): 547-553.
- [7] HWANG J H, LEE S W, RHA S J, et al. Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, *Sebastes schlegeli*[J]. *Aquaculture International*, 2013, 21(3): 525-538.
- [8] LONG Meng, HOU Jie, SU Yujing, et al. Effects of dietary selenium yeast and tea polyphenols on growth, expression of growth axis genes, nutritional quality, and disease resistance of juvenile Wuchang bream (*Megalobrama amblycephala*)[J]. *Journal of Fisheries of China*, 2015, 39(1): 97-107.
- [9] LONG Meng, HOU Jie, SU Yujing, et al. Effects of dietary selenium yeast and tea polyphenols on hepatic antioxidant enzyme activities and gene expression in juvenile Wuchang bream (*Megalobrama amblycephala*)[J]. *Journal of Fishery Sciences of China*, 2015, 22(2): 259-268.
- [10] CHEN Changfu, WANG Yutang. Current status, problems, and countermeasures of antibiotic use in aquaculture (Part 1)[J]. *China Fisheries*, 2015(4): 65-68.
- [11] DONG Jinfu, LI Yaoqing, HONG Shaomei. Study on minimum inhibitory concentration of tea polyphenols (TPP) against 8 pathogenic bacteria[J]. *Food Science*, 1995, 16(1): 6-12.
- [12] WU Linyi. Metabolism of EGCG in grass carp and its antibacterial efficacy against pathogenic bacteria[D]. Master's thesis. Hefei: Anhui Agricultural University, 2015.
- [13] SHEIKHZADEH N, NOFOUZI K, DELAZAR A, et al. Immunomodulatory effects of decaffeinated green tea (*Camellia sinensis*) on the immune system of rainbow trout (*Oncorhynchus mykiss*)[J]. *Fish & Shellfish Immunology*, 2011, 31(6): 1268-1269.
- [14] LIU Zhenxing, KE Hao, HAO Le, et al. Effects of tea polyphenols on growth performance, antioxidant function, and non-specific immune indices of tilapia[J]. *Guangdong Agricultural Sciences*, 2012, 39(23): 113-115.
- [15] LI Jinlong. Effects of tea polyphenols on growth, immunity, and lipid metabolism of juvenile black carp (*Mylopharyngodon piceus*)[D]. Master's thesis. Changsha: Hunan Agricultural University, 2013.
- [16] ZHENG Q M, HAN C Y, ZHONG Y M, et al. Effects of dietary supplementation with green tea waste on growth, digestive enzyme, and lipid metabolism of juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*[J]. *Fish Physiology and Biochemistry*, 2017, 43(2): 361-371.
- [17] WELKER T L, WAN X C, ZHOU Y B, et al. Effect of dietary green tea supplementation on growth, fat content, and muscle fatty acid profile of rainbow trout (*Oncorhynchus mykiss*)[J]. *Aquaculture International*, 2016, 25(3): 1073-1094.

- [18] MAITRA S, RAY A K. Inhibition of digestive enzymes in rohu, *Labeo rohita* (Hamilton), fingerlings by tannin: an in vitro study[J]. Aquaculture Research, 2003, 34(1): 93-95.
- [19] KUO K L, WENG M S, CHIANG C T, et al. Comparative studies on the hypolipidemic and growth suppressive effects of oolong, black, pu-erh, and green tea leaves in rats[J]. Journal of Agricultural and Food Chemistry, 2005, 53(2): 480-489.
- [20] OHNISHI R, IGA K, KIRIYAMA S. Green tea polyphenols reduce protein digestibility and suppress cecal fermentation in rats[J]. Nippon Eiyo Shokuryo Gakkaishi, 2005, 58(4): 199-208.
- [21] FREJNAGEL S, WROBLEWSKA M. Comparative effect of green tea, chokeberry and honeysuckle polyphenols on nutrients and mineral absorption and digestibility in rats[J]. Annals of Nutrition and Metabolism, 2010, 56(3): 163-169.
- [22] WANG Xiaohong, WU Shugeng, WANG Xiaocui, et al. Biological functions of tea polyphenols and their application in poultry production[J]. Chinese Journal of Animal Nutrition, 2016, 28(6): 1641-1648.
- [23] FRAZIER R A, DEAVILLE E R, GREEN R J, et al. Interactions of tea tannins and condensed tannins with proteins[J]. Journal of Pharmaceutical and Biomedical Analysis, 2010, 51(2): 490-495.
- [24] LACASSAGNE L, FRANCESCH M, CARRÉ B, et al. Utilization of tannin-containing and tannin-free faba beans (*Vicia faba*) by young chicks: effects of pelleting feeds on energy, protein and starch digestibility[J]. Animal Feed Science and Technology, 1988, 20(1): 59-68.
- [25] ŞENGÜL İ A, ÖZACAR M. Competitive biosorption of Pb^{2+} , Cu^{2+} and Zn^{2+} ions from aqueous solutions by valonia tannin resin[J]. Journal of Hazardous Materials, 2009, 166(2/3): 1488-1494.
- [26] SHEN C L, CAO J J, DAGDA R Y, et al. Green tea polyphenols benefit body composition and improve bone quality in long-term high-fat diet-induced obese rats[J]. Nutrition Research, 2012, 32(6): 448-457.
- [27] WU T, GUO Y, LIU R, et al. Black tea polyphenols and polysaccharides improve body composition, increase fecal fatty acid, and regulate fat metabolism in high-fat diet-induced obese rats[J]. Food & Function, 2016, 7(5): 2469-2478.
- [28] TIAN C, YE X L, ZHANG R, et al. Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPAR γ -adiponectin pathway[J]. PLoS One, 2013, 8(1): e53796.
- [29] HUANG Jinbao, WAN Bei, GE Gaofei. Effects of tea polyphenols on blood lipid levels, fat distribution, and tissue fatty acid composition in broiler chickens[J]. Science and Technology of Food Industry, 2017, 38(15): 290-295.
- [30] LONG Meng, LIN W, HOU J, et al. Dietary supplementation with selenium yeast and tea polyphenols improve growth performance and nitrite tolerance of Wuchang bream (*Megalobrama amblycephala*)[J]. Fish & Shellfish Immunology, 2017, 68: 74-83.
- [31] LU Chunxia, WANG Hongxin, LÜ Wenping, et al. Antibacterial effects of compound plant extracts against *Aeromonas hydrophila*[J]. Journal of Food

Science and Biotechnology, 2011, 30(2): 178-184.

[32] ZHANG Y B P, ZHOU Y B, SANG B Y, et al. Effect of dietary Chinese tea on growth performance, disease resistance and muscle fatty acid profile of channel catfish (*Ictalurus punctatus*)[J]. Aquaculture International, 2015, 23(2): 683-698.

[33] ABDEL-TAWWAB M, AHMAD M H, SEDEN M E A, et al. Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection[J]. Journal of the World Aquaculture Society, 2010, 41(Suppl. 2): 203-213.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv –Machine translation. Verify with original.