Received: 6 March 2020 | Revised: 23 September 2020 | Accepted: 19 November 2020

DOI: 10.1111/anu.13205

ORIGINAL ARTICLE

Aquaculture Nutrition

WILEY

Dietary rutin promoted the growth, serum antioxidant response and flesh collagen, free amino acids contents of grass carp (*Ctenopharyngodon idella*)

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 31772825

Abstract

Rutin is a natural flavone derivative with many biological activities. To investigate the effects of dietary rutin on growth, serum antioxidant response and flesh quality of grass carp (*Ctenopharyngodon idella*), six diets were prepared with the rutin inclusion at 0 (control diet), 0.2, 0.4, 0.6, 0.8 and 1.0 g/kg, respectively. Grass carp with initial body weight of 18.5 ± 0.2 g were fed with the six diets for 60 days. The weight gain was increased from 348.5% (the control) to 364.4%, 366.5% ($p < .05$), and feed conversion ratio was decreased from 1.56 (the control) to 1.49, 1.48 ($p < .05$), respectively, by the supplementation of 0.8 and 1.0 g/kg rutin in diets. Serum SOD activity was increased by the addition of 0.2–1.0 g/kg rutin ($p < .05$), and serum MDA content was decreased by the addition of 0.4–1.0 g/kg rutin ($p < .05$). The inclusion of 1.0 g/kg rutin and 0.6–1.0 g/kg rutin significantly increased the collagen content and the total free amino acids in flesh ($p < .05$), respectively. The content of n-3 PUFA in flesh was also promoted by the inclusion of 0.2 and 0.6 g/kg rutin ($p < .05$). However, dietary rutin did not significantly affect the flesh texture and water-holding capacity. In conclusion, dietary rutin could improve the growth, antioxidant response ability and flesh quality of grass carp, and the recommended supplemental level of rutin was 0.8–1.0 g/kg.

KEYWORDS
Antioxidation, Flesh quality, Grass carp, Growth, Rutin

1 | INTRODUCTION

Food from aquaculture makes an important contribution to human nutrition and health. Maintaining the sustainable aquaculture production is a continuing challenge for society (Jennings et al., 2016). At present, the potential application of natural products and plant extracts is attracting more and more attentions in aquaculture (Reverter et al., 2014). Rutin is a natural flavone derivative widely existing in plants such as buckwheat, eucommia and tomatoes, which consists of disaccharide 6-o-l-rhamnosyl-d-glucose and aglycone (Figure 1). Rutin has been reported some biological activities, including antioxidation (Machawal & Kumar, 2014), anti-inflammatory (Yang et al., 2008), neuroprotective, anti-viral (Aron & Kennedy, 2008) and anti-carcinogenic effects (Javed et al., 2012). The supplementation of rutin in diets attenuated the expressions of IL-1b and IL-6 mRNA in colonic mucosa and ameliorated DSS (dextran sulphate sodium)-induced colitis by suppressing pro-inflammatory cytokines in mice (Kwon et al., 2005). Dietary rutin also decreased thiobarbituric acid reactive substances and lipid hydroperoxides and increased the non-enzymic antioxidants in rats (Kamalakkannan, & Prince, 2006).

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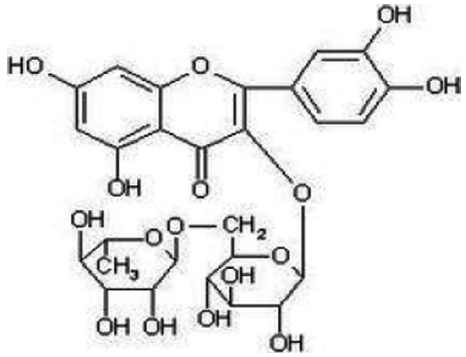

FIGURE 1 Chemical structure of rutin

In aquatic animals, intramuscular injection of rutin improved the immune ability and resistance against *Vibrio alginolyticus* infection in white shrimp (*Litopenaeus vannamei*) (Hsieh et al., 2008). Dietary rutin prevented the damage to the liver and reduced the oxidation in rainbow trout (*Oncorhynchus mykiss*) exposed to oxytetracycline (Nazeri et al., 2017). The protective effect and antioxidant effect of rutin were also reported in silver catfish (*Rhamdia quelen*) (Pês et al., 2016; Londero et al . 2020; Baldissera et al., 2020) and tilapia (*Oreochromis niloticus*) (Deng et al., 2019). In addition, the addition of rutin in tilapia diet improved the growth and reduced liver and myofibre damage induced by mycotoxin (T-2) (Deng et al., 2019).

TABLE 1 Ingredients and proximate composition of experimental diets (air dry basis, g/kg)

	Rutin Addition (g/kg)					
Ingredients[a]	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Fish meal	20.0	20.0	20.0	20.0	20.0	20.0
Soybean meal	180.0	180.0	180.0	180.0	180.0	180.0
Cottonseed meal	160.0	160.0	160.0	160.0	160.0	160.0
Rapeseed meal	180.0	180.0	180.0	180.0	180.0	180.0
Wheat bran	100.0	100.0	100.0	100.0	100.0	100.0
Rice bran	100.0	100.0	100.0	100.0	100.0	100.0
Wheat middling[b]	224.5	224.3	224.1	223.9	223.7	223.5
Soybean oil	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride (50%)	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix[b]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[c]	3.0	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate mphosphaphosphatephosphate	15.0	15.0	15.0	15.0	15.0	15.0
Rutin[d]	0.0	0.2	0.4	0.6	0.8	1.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Proximate composition						
Crude protein	325.0	324.8	325.3	325.6	325.1	324.3
Crude lipid	36.5	35.8	35.4	36.1	35.3	35.8
Ash	76.3	75.5	75.6	75.2	75.8	76.1
Moisture	85.3	85.5	85.5	85.2	85.4	85.0
Cross energy[e] MJ/kg	16.7	16.7	16.7	16.7	16.6	16.7

[a]The ingredients in formula were purchased from the Daqiao Feed Company (Shanghai, China), and the protein contents of the ingredients are as follow: fish meal (630 g/kg), soybean meal (442 g/kg), cottonseed meal (500 g/kg), rapeseed me al (377 g/kg), wheat middling (169 g/kg), rice bran (143 g/kg), wheat bran (147 g/kg).

[b]Vitamin premix (mg or IU/kg diet): vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; ascorbic acid, 1,000 mg; vitamin E, 150 IU; nicotinic acid, 100 mg; thiamine, 20 mg; riboflavin, 20 mg; pyridoxine HCl, 22 mg; cyanocobalamin, 0.15 mg; menadione, 12.17 mg; inositol, 500 mg; folic acid, 8 mg; biotin,0.6 mg.

[c]Mineral premix (mg/kg diet): magnesium, 180 mg; iron, 63 mg; zinc, 89 mg; manganese, 11.45 mg; copper, 3 mg; iodine, 1.5 mg; cobalt, 0.6 mg; selenium, 0.24 mg.

[d]Rutin was purchased from Shanghai Yuanye Bio-Tech Co., LTD (Shanghai, China) with a purity higher than 95%.

[e]Gross energy from the protein, the lipid and the carbohydrate were 23.6, 39.5 and 17.2 MJ/kg, respectively (Yang et al., 2018).

TABLE 2 Amino acid composition of the experimental diets (g/kg, dry matter)

	Rutin Addition (g/kg)					
Amino acid	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
EAA						
Val	10.3	10.1	10.3	10.3	10.5	10.6
Thr	11.1	11.5	11.8	11.2	10.9	11.2
Met	5.1	5.1	4.9	5.4	5.0	5.2
Ile	5.3	5.7	5.7	5.6	5.8	5.5
Leu	23.7	23.9	24.0	24.5	24.0	23.9
Phe	15.2	15.6	14.9	15.0	15.3	15.5
His	13.7	13.3	13.3	13.7	13.7	13.5
Lys	16.3	15.9	16.3	15.9	15.9	16.2
Arg	23.1	23.2	23.5	23.4	23.4	23.4
NEAA						
Asp	31.0	31.3	31.3	31.6	31.4	31.0
Ser	18.7	18.5	18.3	18.4	18.0	18.4
Glu	65.9	65.7	65.8	66.2	65.7	66.1
Gly	17.5	16.8	16.9	16.9	17.0	16.9
Ala	14.5	14.3	13.9	14.0	14.7	13.9
Cys	4.7	4.3	4.8	4.4	4.0	4.4
Tyr	8.7	8.9	8.8	8.5	8.8	8.6
Pro	15.0	15.2	14.9	15.0	15.3	15.4
TAA	299.8	299.3	299.4	300.0	299.4	299.7

Note: EAA = essential amino acids; NEAA = nonessential amino acids; TAA = total amino acids

Grass carp is the world's leading freshwater species in aquaculture, especially in China. To improve the growth, antioxidant response and flesh quality of grass carp, some plant extracts have been reported in the diet of this fish, including chlorogenic acid (Sun et al., 2017a), geniposide (Sun et al., 2017b), geniposidic acid (Sun et al., 2018). Rutin is one of the most common quercetin glycosides in nature (Gohlke et al., 2013), but there is no report in the effects of dietary rutin on the growth and flesh quality in grass carp. Therefore, the present study was conducted to investigate the effects of dietary rutin on growth performance, body proximate composition, free amino acid, fatty acid, texture characteristics, collagen, water-holding capacity and histology of flesh and antioxidant response of grass carp.

2 | MATERIALS AND METHODS

2.1 | Experimental diets and design

Six diets were formulated with the inclusion of rutin in basal diet at 0 (control diet), 0.2, 0.4, 0.6, 0.8 and 1.0 g/kg, respectively. Correspondingly, wheat middling was reduced to balance the formula composition. All ingredients were ground and passed through a 40 mesh sieve, then mixed with soybean oil and distilled water to form a homogeneous mixture. The mixture was granulated into sinking pellet with a diameter of 2 mm by using a single-screw extruder (SLP-45; Chinese Fishery Machinery and Instrument Research Institute, Shanghai, China). The pelleting temperature was 85–90°C, and the diets were air-dried and stored at 4°C until use.

Rutin was sourced from Shanghai Yuanye Bio-Tech Co., LTD (Shanghai, China) with a purity higher than 95%. The formulation and chemical composition of the experimental diets are shown in Table 1, and the amino acid composition is shown in Table 2. The protein and energy contents of experimental diets were 324.3–325.6 g/kg and 16.6–16.7 MJ/kg, respectively.

2.2 | Fish and feeding management

Grass carp were purchased from Jinshan Aquaculture Farm (Shanghai, China). After the transportation to the laboratory, the fish were fed with the control diet for one week to acclimate to the experimental environment. The fish with an initial body weight of 18.5 ± 0.2 g were randomly allocated into 18 cages (1.5 × 1.2 × 1.2 m, a water depth of 0.8 m) with 3 replicates (cages) per treatment and 20 fish per cage. Six cages from the six treatments were placed in one indoor cement pool without direct sunshine, and three pools (5.0 × 3.0 × 1.2 m) were used.

During the feeding period, the fish were fed by hand three times daily (08:00, 12:00, 16:00). The daily feeding rate was 3.0%-5.0% of body weight, and adjusted every three days according to the water

temperature and feeding behaviour. All cages were offered the same quantity of feed. About half of cultured water was exchanged with filtrated pond water, and the waste in pools was cleared by siphoning every 5 days. The water quality was monitored daily with pH meter (HI 98,128, Italy) and kits produced by Beijing Zhongda Ante Biochemical Technology Co., Ltd. (Beijing, China), and dissolved oxygen, temperature, pH, ammonia nitrogen and nitrite were > 5 mg/L, 25–30°C, 7.5–8.0, <0.2 mg/L and < 0.1 mg/L, respectively. The feeding trial was conducted at the Aquaculture Station of Shanghai Ocean University, and lasted for 60 days.

2.3 | Sample collection

After the end of the feeding trial, all grass carp were deprived of diets for 24 hr, and then were counted and bulk weighed per cage to calculate weight gain (WG), survival and feed conversion ratio (FCR) based on the initial body weight, initial fish number and feed intake. Three fish were randomly selected from each cage to measure body weight and body length, then the blood was drawn from caudal vein. After the centrifuging at 1,150 g for 10 min, the serum was collected and immediately stored at −80°C for the analysis of superoxide dismutase (SOD), catalase (CAT) activity and malondialdehyde (MDA) concentration. Then the three fish were dissected, and visceral, liver, and intestinal lipid were weighed to calculate condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat ratio (IFR). The dorsal muscle was sampled from the left side of the body and frozen at −20°C for the analysis of crude lipid, ash, crude protein, moisture, free amino acids, fatty acid and collagen. Three blocks of dorsal flesh (2–3 g) were sampled from the right side of the body for immediate measurement of water-holding capacity (WHC). The fourth block of flesh (2–3 g) from the same side was stored in fixative solution (saturated picric acid solution: methanol: acetic acid = 15:5:1) and then transferred to alcohol until use. The fifth block of dorsal flesh about 1 cm^3 was sampled for the immediate measurement of texture characteristics.

2.4 | Parameters and methods

2.4.1 | Growth performance and husbandry parameters

$$\text{Weight gain (WG, \%)} = 100 \times (\text{[final weight [g]} - \text{initital weight [g]]/initial weight [g]}).$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake [g]/weight gain [g]}.$$

$$\text{Survival (\%)} = 100 \times (\text{final number of fish/initial number of fish}).$$

$$\text{VSI (\%)} = 100 \times (\text{visceral weight [g]/body weight [g]}).$$

$$\text{HSI (\%)} = 100 \times (\text{liver weight [g]/body weight [g]}).$$

$$\text{IFR (\%)} = 100 \times (\text{intraperitoneal fat weight [g]/body weight [g]}).$$

$$\text{K (g/cm}^3\text{)} = 100 \times (\text{body weight [g]/body length [cm]}^3).$$

2.4.2 | Serum biochemical indices

The activity of SOD and CAT was determined by xanthine oxidase method and ammonium molybdate method, respectively. The content of MDA was measured by the thiobarbituric acid (TBA) method. All kits were sourced from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.4.3 | Proximate composition of flesh and diets

The proximate composition of flesh and diets was determined with the standard methods of AOAC (1995). The moisture and ash contents were measured by drying samples to constant weight at 105°C, and by combusting samples in a muffle furnace at 550°C for 6 hr. The crude protein content (N × 6.25) was determined using the Kjeldahl system method (2,300 Auto analyser; FOSS Tecator, AB, Hoganas, Sweden). The crude lipid content was determined after ether extraction using Soxtherm (SOX 416 Macro, Gerhardt, Germany).

2.4.4 | Amino acid composition of diets

The samples of diets (70 mg) were freeze-dried until constant weight, then hydrolysed with 6 mol/L hydrochloric acid at 110°C for 24 hr. The hydrolyzate (0.5 ml) was dried and diluted with 5 ml of diluent, then the hydrolyzate was injected into a sodium exchange column for amino acid analysis using a Symkam S-433D amino acid automatic analyzer (Seccam, Germany). Before the measurement of methionine (Met), the samples were hydrolysed with 2 ml performic acid at 55°C for 15 min.

2.4.5 | Free amino acid composition of flesh

Fresh flesh (40 mg) was mixed with 1.2 ml of extract (methanol: water = 4:1), then homogenized in ice-water bath with ultrasonic for 10 min and stored at −20°C for 2 hr. After centrifuging at 12,000 r/min for 30 min (4°C),the supernatant was collected for free amino acid analysis by using a Waters ACQUITY Ultra Performance LC/MS (Waters, USA).

2.4.6 | Fatty acid composition of flesh

The fatty acid was measured by the method of boron trifluoride methyl esterification. The extracted fat was dissolved in 1 ml of

TABLE 3 Growth performance of grass carp fed diets with various rutin levels for 60 days

Growth performance	Rutin Addition (g/kg)					
	0	0.2	0.4	0.6	0.8	1.0
IBW(g)	18.5 ± 0.2	18.5 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.4 ± 0.2	18.5 ± 0.1
FBW(g)	83.0 ± 1.7^{a}	82.7 ± 1.6^{a}	83.2 ± 1.8^{a}	84.6 ± 1.4^{ab}	85.9 ± 1.3^{b}	86.3 ± 1.1^{b}
WG(%)	348.5 ± 9.1^{a}	347.2 ± 8.6^{a}	349.5 ± 9.5^{a}	357.1 ± 7.5^{ab}	364.4 ± 6.9^{b}	366.5 ± 6.4^{b}
FCR	1.56 ± 0.04^{b}	1.56 ± 0.04^{b}	1.55 ± 0.04^{b}	1.52 ± 0.03^{ab}	1.49 ± 0.03^{a}	1.48 ± 0.02^{a}
S(%)	100	100	100	100	100	100
K(g/cm^3)	1.84 ± 0.08	1.87 ± 0.05	1.92 ± 0.04	1.82 ± 0.06	1.89 ± 0.15	1.91 ± 0.07
HSI(%)	8.10 ± 0.63	8.28 ± 0.74	8.16 ± 0.51	8.17 ± 0.37	8.17 ± 0.60	8.28 ± 0.59
VSI(%)	2.06 ± 0.17	2.10 ± 0.16	2.08 ± 0.06	2.08 ± 0.11	2.10 ± 0.14	2.07 ± 0.15
IFR(%)	1.46 ± 0.06	1.50 ± 0.11	1.43 ± 0.15	1.49 ± 0.17	1.51 ± 0.15	1.52 ± 0.15

Note: IBW, initial body weight (g); FBW, final body weight (g); WG, weight gain; FCR, feed conversion ratio; S, Survival; K, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index; IFR, intraperitoneal fat ratio.

Values are means ± *SD* of the triplicate samples. Values in the same row with different superscripts indicate significant differences according to Duncan's multiple-range tests ($p < .05$) ($n = 3$).

hexane, then 2 ml of 14% boron trifluoride methanol solution was added. After a water bathing of 100°C and 25 min (the first step of methyl esterification), benzene (2 ml) and methanol solution (2 ml) were added for another water bath (100°C, 25 min) (the second step of methyl esterification). Then the samples were mixed with distilled water and n-hexane. After centrifuging at 3,000 r/min for 10 min, the supernatant was collected for fatty acid analysis by using an Agilent Technologies 7890B GS System GC/MS (Agilent, USA).

2.4.7 | Texture characteristics of flesh

A block of fresh flesh about 1 cm^3 was used for texture profile analysis (TPA) using a Universal TA texture analyzer (Tengba, China), including hardness, elasticity, chewiness, adhesion, cohesiveness, recovery. A cylindrical probe was used with diameter and speed of 25 mm and 1 mm/s respectively. The deformation was 70% of the original thickness, and the contact induction force is 5 gf. Each sample was measured twice and averaged.

2.4.8 | Collagen content in flesh

Collagen is the main component of connective tissue and significantly influences the function and texture properties of flesh (Gordon & Hahn, 2010). The collagen content was calculated by multiplying the Hyp content by 8 with reference to AOAC method 990.26 (AOAC, 2000). The content of hydroxyproline (Hyp) in flesh was determined by alkaline hydrolysis method. The kit was obtained from Nanjing Biotechnology Institute (Nanjing, China). The principle is that the oxidation products produced by the reaction of hydroxyproline with an oxidizing agent react with dimethylaminobenzaldehyde to exhibit a purple-red colour, and the content is calculated based on the depth of the colour.

2.4.9 | Water-holding capacity of flesh

Steaming loss: a block of flesh (about 3 g,W1) was placed in gauze and steamed for 5 min. After cooling at room temperature and wiping off the surface water, the sample was weighed (W2).

Centrifugal loss: a block of flesh (about 2 g,W1) was centrifuged at 3,000 g for 10 min, then wiped off the surface water and weighed (W2).

Thawing loss: a block of flesh (about 3 g, W1) was placed at −20°C for 24 hr, then thawed at room temperature, wiped off the surface water and weighed (W2).

Cooking (centrifugal, thawing) loss (%) = 100*(W1-W2)/W1.

2.4.10 | Histological analyses of muscle

The muscle samples were dehydrated in a series of alcohol solutions and xylene, and then embedded in paraffin, sliced, and stained with haematoxylin–eosin. The morphological structure of muscle was observed using an imaging microscope (Nikon YS100, Japan). The number of muscle fibre per square millimetre (mm^2) and the muscle fibre diameter were measured.

2.5 | Statistical analysis

All the data were presented as the mean ± standard deviation (*SD*) and analysed using the Statistical Package for the Social Sciences (SPSS) 17.0. When the difference between the groups was detected

by one-way ANOVA, Duncan's multiple-range test was used to determine the significance among groups. The significance level for the differences among treatments was $p < .05$.

3 | RESULTS

3.1 | Growth performance

During the feeding period, no death was recorded. The fish fed diet supplemented with 0.8 and 1.0 g/kg rutin showed higher WG of 364.4%, 366.5% (+4.6%, +5.2%) and lower FCR of 1.49, 1.48 (−0.07, −0.08) than the control fish (348.5%, 1.56) ($p < .05$). There were no significant differences in CF, VSI, HSI and IFR among all the treatments (Table 3).

3.2 | Serum antioxidant response

The serum SOD activity was significantly increased by the addition of 0.2–1.0 g/kg rutin ($p < .05$), and the MDA content was significantly decreased by the addition of 0.4–1.0 g/kg rutin. The CAT activity showed no significant difference among all the groups (Table 4).

3.3 | Chemical composition of flesh

There were no significant differences in the proximate composition of flesh, including moisture, crude ash, crude lipid and crude protein contents among all the groups. The collagen content in flesh was significantly increased from 2.54 g/kg (the control) to 3.17 g/kg by the addition of 1.0 g/kg rutin ($p < .05$) (Table 5).

3.4 | Free amino acid composition in flesh

As shown in Table 6, the supplementation of 0.8, 1.0 g/kg rutin significantly increased the contents of total free amino acids (TFAA) (+113.1, +99.5 mg/kg) and delicious amino acids (DAA) (+28.3, +23.0 mg/kg) in flesh, and the content of TFAA was also increased by the addition of 0.6 g/kg rutin, when compared to the control group ($p < .05$).

3.5 | Fatty acid composition in flesh

As shown in Table 7, the content of n-3 PUFA was increased by the addition of 0.2 and 0.6 g/kg rutin ($p < .05$). There were no significant differences in the contents of SFA, MUFA, n-6PUFA and DHA/EPA ratio among all the groups.

3.6 | Texture characteristics, water-holding capacity and histology of flesh

No significant differences were observed in flesh hardness, springiness, chewiness, cohesiveness and resilience among all the groups, and the water-holding capacity also showed no significant difference among all the groups (Table 8).

In Table 8 and Figure 2, the muscle fibre density and muscle fibre diameter were 196.4–203.2 cell/mm^2, 79.2–80.5 μm, respectively, and there was no significant difference in muscle fibre density and diameter among all the groups.

4 | DISCUSSION

At present, the growth-promoting effect of rutin was only found in tilapia (Deng et al., 2019), where the supplementation of 0.3% rutin in diet significantly improved the weight gain of fish exposed to mycotoxin (T-2). However, relatively more studies have been conducted in quercetin, a chemical compound with similar structure to rutin. Rutin and quercetin are the most commonly consumed flavonoids in foods (Nakamura et al., 2000), and rutin is one of the most common quercetin glycosdes in nature (Gohlke et al., 2013). Rutin is degraded into quercetin by microorganisms and absorbed in the intestine (Gohlke et al., 2013). Then, quercetin is absorbed in the form of aglycone, and aglycones may be a favourable form to exert its pharmacological effects (Park et al., 2009). The supplementation of 0.2–1.6 g/kg quercetin in diets significantly increased weight gain and decreased FCR of tilapia (Zhai & Liu, 2013). The studies on olive flounder (*Paralichthys olivaceus*) (Shin et al., 2010), grass carp (Xu et al., 2019) and blunt snout bream (*Megalobrama amblycephala*) (Jia et al., 2019) also indicated that dietary quercetin improved the growth performance. In this study, the supplementation of 0.8 and 1.0 g/kg rutin

TABLE 4 Serum antioxidant response enzymes of grass carp fed diets with various rutin levels for 60 days

	Rutin Addition (g/kg)					
Serum enzymes	0	0.2	0.4	0.6	0.8	1.0
CAT (U/L)	1.65 ± 0.25	1.55 ± 0.09	1.56 ± 0.15	1.58 ± 0.11	1.70 ± 0.07	1.64 ± 0.13
SOD (U/ml)	224.99 ± 9.85^{a}	259.20 ± 6.01^{b}	268.99 ± 14.32^{b}	258.39 ± 16.73^{b}	265.01 ± 19.29^{b}	250.54 ± 9.04^{b}
MDA (nmol/ml)	16.33 ± 1.03^{c}	14.39 ± 1.39^{bc}	12.18 ± 1.22^{a}	12.59 ± 1.34^{ab}	13.05 ± 0.86^{ab}	11.91 ± 1.12^{a}

Note: CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde

Values are means ± *SD* of the triplicate samples. In the same row,values with different superscripts indicate significant differences according to Duncan's multiple-range tests ($p < .05$) ($n = 3$).

TABLE 5 Chemical composition of flesh of grass carp fed diets with various rutin levels for 60 days (g/kg, fresh tissue)

Chemical composition	Rutin Addition (g/kg)					
	0	0.2	0.4	0.6	0.8	1.0
Moisture	784.8 ± 5.9	780.9 ± 0.7	781.4 ± 0.3	784.4 ± 0.3	780.4 ± 4.9	779.1 ± 3.6
Crude ash	12.8 ± 0.4	12.6 ± 0.1	12.5 ± 0.1	12.9 ± 0.3	12.4 ± 0.4	12.8 ± 0.2
Crude lipid	10.8 ± 0.6	10.9 ± 0.7	10.7 ± 0.8	11.0 ± 0.7	11.3 ± 0.7	11.1 ± 0.1
Crude protein	192.5 ± 3.8	196.5 ± 1.5	196.5 ± 0.5	195.2 ± 2.4	196.7 ± 5.3	197.3 ± 2.0
Collagen	2.54 ± 2.22^{a}	2.67 ± 0.31^{ab}	2.79 ± 0.38^{ab}	2.83 ± 0.24^{ab}	2.88 ± 0.19^{ab}	3.17 ± 0.46^{b}

Note: Values are means ± *SD* of the triplicate samples. In the same row, values with different superscripts indicate significant differences according to Duncan's multiple-range tests ($p < .05$) ($n = 3$).

TABLE 6 Flesh free amino acid composition of grass carp fed diets with various rutin levels for 60 days (mg/kg, fresh tissue)

Free amino acid	Rutin Addition (g/kg)					
	0	0.2	0.4	0.6	0.8	1.0
Arg	48.4 ± 5.2^{a}	51.0 ± 0.1^{ab}	52.9 ± 3.9^{ab}	55.4 ± 4.2^{b}	56.5 ± 4.4^{b}	55.5 ± 0.9^{b}
His	168.1 ± 11.1	174.0 ± 10.4	178.0 ± 13.7	177.6 ± 8.5	173.5 ± 7.8	176.3 ± 5.3
Glu	22.5 ± 2.0	23.5 ± 2.3	24.5 ± 2.0	23.4 ± 1.2	22.8 ± 1.1	25.3 ± 2.8
Gln	222.5 ± 20.4	245.2 ± 8.0	243.6 ± 19.7	247.6 ± 17.4	251.8 ± 11.8	248.3 ± 8.2
Lys	217.6 ± 20.3^{a}	222.4 ± 14.1^{ab}	219.9 ± 17.1^{a}	246.1 ± 20.0^{b}	232.6 ± 13.6^{ab}	230.8 ± 3.4^{ab}
Asp	9.3 ± 1.5^{a}	13.8 ± 0.1^{b}	11.2 ± 1.0^{ab}	10.2 ± 1.6^{ab}	12.4 ± 3.2^{ab}	12.5 ± 0.5^{ab}
Asn	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Cys	1.9 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	2.1 ± 0.2	1.9 ± 0.2	1.8 ± 0.2
Thr	51.4 ± 2.0	50.8 ± 4.4	49.4 ± 3.0	51.5 ± 4.7	50.2 ± 4.3	49.7 ± 2.3
Val	3.0 ± 0.2	3.3 ± 0.3	3.2 ± 0.1	3.2 ± 0.2	3.4 ± 0.3	3.3 ± 0.1
Pro	77.8 ± 6.4^{a}	86.4 ± 10.2^{ab}	90.2 ± 8.2^{ab}	90.3 ± 8.4^{ab}	93.1 ± 4.7^{b}	89.8 ± 6.3^{ab}
Ser	51.4 ± 4.8	52.3 ± 5.4	53.2 ± 3.7	50.7 ± 4.8	53.5 ± 0.1	54.7 ± 1.4
Ala	285.5 ± 25.6^{ab}	281.5 ± 7.8^{ab}	279.7 ± 15.1^{a}	290.1 ± 11.4^{ab}	311.3 ± 15.9^{b}	304.7 ± 23.5^{b}
Try	22.8 ± 2.4	25.7 ± 2.0	27.9 ± 1.0	29.7 ± 2.7	29.8 ± 3.3	28.0 ± 1.3
Tyr	20.9 ± 0.7	20.0 ± 1.2	20.0 ± 2.8	18.6 ± 0.3	19.9 ± 1.7	19.0 ± 1.5
Phe	24.0 ± 1.7	23.7 ± 2.1	22.4 ± 0.8	22.9 ± 1.8	24.2 ± 0.7	23.8 ± 1.0
Met	2.7 ± 0.1^{a}	2.9 ± 0.2^{ab}	3.1 ± 0.3^{ab}	3.0 ± 0.1^{ab}	3.3 ± 0.1^{b}	3.0 ± 0.3^{ab}
Leu	3.9 ± 0.1^{a}	3.9 ± 0.1^{a}	4.2 ± 0.3^{ab}	4.5 ± 0.2^{b}	4.4 ± 0.3^{ab}	4.1 ± 0.3^{ab}
Ile	2.2 ± 0.2^{a}	4.1 ± 0.1^{b}	4.6 ± 0.3^{b}	5.2 ± 0.5^{b}	4.3 ± 0.4^{b}	4.5 ± 0.4^{b}
DAA	362.3 ± 9.2^{a}	362.4 ± 4.0^{a}	357.7 ± 10.4^{a}	365.2 ± 6.2^{a}	390.6 ± 12.1^{b}	385.3 ± 16.5^{b}
TFAA	1,236.2 ± 93.2^{a}	1,286.5 ± 20.3^{ab}	1,290.3 ± 67.3^{ab}	1,332.3 ± 119.8^{b}	1,349.3 ± 54.9^{b}	1,335.7 ± 30.5^{b}

Note: DAA (Asp, Gly, Glu, Ala, Phe, Tyr), delicious amino acids; TFAA: total free amino acids.

Values are means ± *SD* of the triplicate samples. In the same row, values with different superscripts indicate significant differences according to Duncan's multiple-range tests ($p < .05$) ($n = 3$).

also increased the weight gain and decreased FCR ($p < .05$) (Table 3). Due to the similarity in chemical structure, rutin may have a similar acting mechanism to quercetin. Guo et al. (2012) once reported that the supplementation of rutin promoted the release of growth hormone (GH), and up-regulated the gene expression of growth hormone receptors (GHR). GH can promote the growth by a direct action on the growth plates as well as the production of insulin-like growth factors (Dattani & Malhotra, 2019). In addition, some studies showed protein synthesis in muscle had positive relations with the growth, and isoflavones (a flavonoid) could promote the protein synthesis in the muscle of broiler (Kamboh & Zhu, 2013). Usually, the growth-promoting effect could be attributed to the increase in intestinal digestive enzyme activity, immunity status and antioxidant capacity in fish (Shin et al., 2010).

The majority of the biological activity of rutin, such as anti-inflammatory, anti-microbial, anti-tumour and anti-asthma, might be related to its powerful antioxidant capacity, especially as a free radical scavenger (Chua, 2013). Nazeri et al. (2017) found that

TABLE 7 Flesh fatty acid composition of grass carp fed diets with various rutin levels for 60 days (%, percentage of total fatty acids)

	Rutin Addition (g/kg)					
Fatty acid	0	0.2	0.4	0.6	0.8	1.0
C14:0	0.63 ± 0.03	0.65 ± 0.02	0.65 ± 0.06	0.6 ± 0.05	0.66 ± 0.03	0.62 ± 0.01
C16:0	16.81 ± 0.57	16.43 ± 0.33	16.77 ± 1.06	16.33 ± 0.16	16.74 ± 0.60	16.13 ± 0.28
C18:0	5.71 ± 0.30^{b}	5.31 ± 0.14^{ab}	5.59 ± 0.32^{ab}	5.23 ± 0.03^{a}	5.61 ± 0.23^{ab}	5.21 ± 0.05^{a}
C22:0	0.59 ± 0.03^{a}	0.59 ± 0.04^{ab}	0.64 ± 0.03^{ab}	0.67 ± 0.05^{b}	0.60 ± 0.01^{a}	0.56 ± 0.02^{a}
SFA	23.73 ± 0.84	22.98 ± 0.42	23.65 ± 1.45	22.82 ± 0.23	23.61 ± 0.83	22.52 ± 0.25
C16:1	2.27 ± 0.31^{a}	2.63 ± 0.11^{b}	2.62 ± 0.09^{b}	2.46 ± 0.21^{ab}	2.65 ± 0.16^{b}	2.44 ± 0.03^{ab}
C18:1	29.44 ± 0.96	29.87 ± 0.61	29.4 ± 2.04	29.31 ± 1.48	30.66 ± 1.14	29.59 ± 0.53
MUFA	31.71 ± 1.23	32.5 ± 0.58	32.02 ± 2.11	31.78 ± 1.33	33.31 ± 1.25	32.03 ± 0.56
C18:2	18.46 ± 0.51^{ab}	19.19 ± 0.20^{b}	18.69 ± 1.21^{ab}	17.67 ± 0.46^{a}	18.51 ± 0.79^{ab}	18.49 ± 0.62^{ab}
C20:2	0.87 ± 0.08^{a}	1.09 ± 0.10^{b}	1.05 ± 0.07^{b}	0.99 ± 0.02^{b}	1.10 ± 0.06^{b}	1.06 ± 0.04^{b}
C20:3	1.33 ± 0.13^{a}	1.42 ± 0.04^{b}	1.38 ± 0.07^{ab}	1.38 ± 0.05^{b}	1.31 ± 0.04^{a}	1.32 ± 0.03^{ab}
C20:4	5.36 ± 0.59^{ab}	5.22 ± 0.04^{ab}	5.18 ± 0.28^{ab}	5.44 ± 0.28^{b}	4.80 ± 0.25^{a}	5.01 ± 0.26^{ab}
n−6PUFA	26.02 ± 1.29	26.91 ± 0.05	26.29 ± 1.02	25.48 ± 0.77	25.72 ± 0.90	25.88 ± 0.32
C18:3	1.63 ± 0.20	1.82 ± 0.21	1.72 ± 0.22	1.80 ± 0.16	1.85 ± 0.23	1.62 ± 0.19
C20:5	0.48 ± 0.02^{ab}	0.54 ± 0.06^{b}	0.48 ± 0.05^{ab}	0.50 ± 0.04^{ab}	0.46 ± 0.04^{ab}	0.44 ± 0.05^{a}
C22:6	3.56 ± 0.17	3.96 ± 0.15	3.83 ± 0.29	3.93 ± 0.14	3.71 ± 0.10	3.80 ± 0.18
n−3PUFA	5.67 ± 0.35^{a}	6.32 ± 0.13^{b}	6.03 ± 0.56^{ab}	6.24 ± 0.29^{b}	6.02 ± 0.26^{ab}	5.86 ± 0.07^{a}
DHA/EPA	7.42 ± 0.64	7.46 ± 0.55	8.07 ± 0.44	7.88 ± 0.39	7.48 ± 0.57	8.02 ± 0.29

Note: SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

Values are means ± *SD* of the triplicate samples. In the same row, values with different superscripts indicate significant differences according to Duncan's multiple-range tests ($p < .05$) ($n = 3$).

TABLE 8 Flesh texture characteristics, water-holding capacity, histology of grass carp fed diets with various rutin levels for 60 days

	Rutin Addition (g/kg)					
Items	0	0.2	0.4	0.6	0.8	1.0
Texture characteristics						
Hardness (gf)	342.0 ± 34.7	338.3 ± 28.4	338.3 ± 32.9	350.0 ± 27.1	357.7 ± 25.6	352.9 ± 27.6
Springiness	0.55 ± 0.04	0.54 ± 0.06	0.53 ± 0.05	0.49 ± 0.04	0.51 ± 0.06	0.52 ± 0.03
Chewiness (gf)	122.2 ± 13.3	123.9 ± 12.3	120.5 ± 11.3	128.0 ± 2.6	127.5 ± 7.3	127.5 ± 10.2
Cohesiveness (gf)	0.59 ± 0.05	0.59 ± 0.03	0.60 ± 0.04	0.58 ± 0.03	0.59 ± 0.05	0.59 ± 0.04
Resilience	1.02 ± 0.09	0.98 ± 0.08	0.97 ± 0.07	0.99 ± 0.07	0.98 ± 0.04	1.03 ± 0.10
Water-holding capacity						
Centrifugal loss	18.6 ± 1.9	18.1 ± 1.6	18.6 ± 1.0	19.2 ± 1.8	19.2 ± 1.5	19.3 ± 2.0
Thawing loss	7.2 ± 1.0	7.3 ± 1.4	7.2 ± 1.1	7.4 ± 0.7	7.4 ± 1.2	7.1 ± 1.3
Steaming loss	27.9 ± 1.8	26.1 ± 1.0	25.4 ± 3.3	25.7 ± 1.0	26.8 ± 1.6	26.4 ± 3.1
Muscle histology						
Muscle fibre density(cell/mm^2)	201.5 ± 17.2	199.7 ± 7.4	202.7 ± 10.2	199.1 ± 5.4	196.4 ± 13.2	203.2 ± 9.9
Muscle fibre diameter (μm)	79.5 ± 5.2	79.9 ± 3.5	79.3 ± 7.3	80.0 ± 5.3	80.5 ± 2.1	79.2 ± 5.4

Note: Values are means ± *SD* of the triplicate samples. In the same row, values with different superscripts indicate significant differences according to Duncan's multiple-range tests ($p < .05$) ($n = 3$).

dietary rutin (0.5–2.0 g/kg) promoted the activity of SOD and CAT in the liver of rainbow trout fed diet containing oxytetracycline (OTC). Dietary rutin (0.15%) also increased SOD activity and diminished the oxidized glutathione (GSSG) content in muscle of silver catfish infected by *Aeromonas hydrophila* (Rosa et al., 2019), and enhanced the GST activity in liver of tilapia exposed to mycotoxin (T-2) (Deng et al., 2019). In addition, the supplementation of rutin in the diets (0.15% and 0.30%) of silver catfish significantly reduced the lipid peroxidation and increased the activity of SOD and GST in the brain and liver of silver catfish (Pês et al., 2016). In the present study, the serum SOD activity was significantly increased, and the serum MDA level was significantly decreased by the addition of 0.4–1.0 g/kg rutin, which were consistent with the previous observations. SOD can catalyse the dismutation of superoxide anion into hydrogen peroxide (H_2O_2), while CAT catalyses the conversion of H_2O_2 to water and molecular oxygen (Jose et al., 2018). The increased enzymes activity means an increased capacity to remove free radicals, thereby protecting the body from oxidative damage. Generally, flavonoids have a strong antioxidant capacity due to the presence of phenolic compounds, which can quench free radical and chelate metals (Hotta et al., 2002). In addition, rutin also shows the ability to decrease oxidative stress and augment antioxidant defence system by displaying a profound role in iNOS-Nrf2 signalling pathway (Singh et al., 2019) However, in the study of Bai (1992), channel catfish (*Ictalurus punctatus*) fed rutin-supplemented diets showed no significant difference in 2-thiobarbituric acid (TBA) level in fillets with the control group. The serum SOD activity of white shrimp exposed to *Vibrio alginolyticus* and injected with rutin extract did not show any changes (Hsieh et al., 2008). The different results might be related to the species, cultivation environment and diet composition.

As an effective antioxidant, rutin has been reported to decrease ROS and MDA levels, and increase SOD and CAT activity in rats (Alsaif, 2009). Rutin can also enhance the antioxidant defence mechanism of cells and protect the body from oxidative damage (Mahmoud, 2012). In this study, the serum MDA level was significantly decreased by the addition of 0.4–1.0 g/kg rutin. Nazeri et al. (2017) reported that rutin reduced protein carbonyl contents (an indicator reflecting protein oxidation) in the liver of rat. Rutin can inhibit the production of free radicals in three stages: the formation of superoxide ions, the production of cyclichydroxyl radicals in Fenton reaction and the formation of peroxy radicals (Afanasev et al., 1989).

Lipid composition is an important quality indicator of fish products considering that fish is the main source of long-chain polyunsaturated fatty acids (LC-PUFA) including docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) (Pratoomyot et al., 2011). Arslan et al. (2018) once reported an increased EPA ratio in rainbow trout fed diet containing grape seed oil rich in flavonoid. Dietary garlic extracts (rich in flavonoid) were also reported to increase EPA (C22:6n3) and DHA (C22:5n3) in the whole body of sterlet sturgeon (*Acipenser ruthenus*) (Lee et al., 2012). Asgari et al. (2020) found that dietary hydroalcoholic extract of honeybee pollen (rich in flavonoid) increased the n-3 long-chain polyunsaturated fatty acids level in the muscle of rainbow trout. The addition of barberry root and extract in diets significantly enhanced the proportion of C18:1n9, monounsaturated fatty acids (MUFAs), C18:3n3, C:226n3, n-3, and n-3/n-6 fatty acids (Ramezanzadeh et al., 2020). The current study revealed that n-3 PUFA content was increased by the addition of 0.2 and 0.6 g/kg rutin. Lipid peroxidation is a biological reaction initiated by reactive oxygen species (ROS), which extracts protons from fatty acids (FA), and the most likely reaction is on the polyunsaturated FA of the n-3 family (n-3 PUFA) (Kamal-Eldin & Yanishlieva, 2002). As a powerful antioxidant, rutin can prevent the peroxidation of lipid, which might be the reason for the increase of n-3 PUFA that are sensitive to oxidation in flesh (Ramezanzadeh et al., 2020). In addition, flavonoids might increase the intestinal absorption of EPA and DHA, and accelerate the synthesis of EPA and DHA from their precursor a-linolenic acid (Toufektsian, Salen, Laporte, Tonelli, & Lorgeril, 2011). However, the mechanism of rutin acting on fatty acid composition is unclear, and more research is needed in the future.

Free amino acids are generally used as quality indices in various fish and crustacean species and are responsible for flavour and taste (Li et al., 2017). For example, alanine and glycine have a sweet taste, and glutamic acid has an umami taste (Prakash et al., 2015). Mo et al. (2016) reported that the supplementation of Chinese herbal medicines (5 and 10 g/kg) in diets increased TFAA and DAA in flesh of hybrid snakehead (*Channa maculata*♀×*Channa argus*♂). Lee et al. (2012) reported garlic extracts (0.5%) significantly increased glutamic acid, alanine, valine, leucine and phenylalanine in muscle of sterlet sturgeon. In the current study, the supplementation of 0.8–1.0 g/kg rutin significantly increased the contents of TFAA and DAA in flesh. Similarly, our previous study also showed that dietary catechin (another flavonoid compound) increased the contents of TFAA and DAA in flesh of grass carp (Xu et al., 2020). These results indicated that flavonoids might play an important role in promoting flesh flavour and quality. In sterlet sturgeon, garlic extract was reported to promote the secretion of insulin, which could promote the FAA in blood transfer into muscle

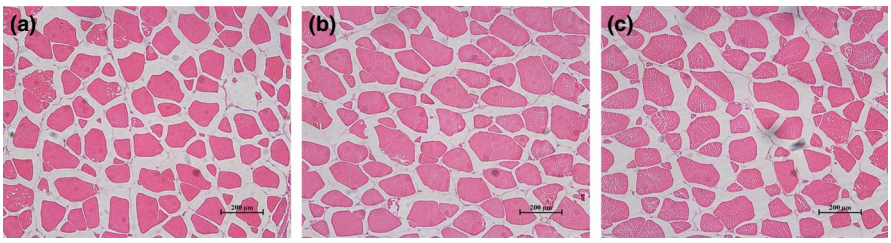

FIGURE 2 The muscle histology of grass carp fed diets containing various rutin levels (A, B, C represent the control, 0.4 and 1.0 g/kg rutin groups, respectively.)

(Lee et al., 2012). Rutin may have a similar action mechanism as garlic extract.

The main edible part and quality value of fish meat are the skeletal muscle tissue, which is composed of muscle fibres and intramuscular connective tissue (IMCT). IMCT is related to muscle stiffness, and it is mainly composed of collagen and elastin fibres (Purslow, 2005). Collagen is the main structural element that contributes to tissue stability and maintains its structural integrity, which plays an important role in the texture properties of flesh (Zhang et al., 2013). Collagen content, type and structure are important factors affecting fish muscle texture (Cheng et al., 2014). The increase of collagen enhanced the firmness and further improved the taste of flesh (Gordon & Hahn, 2010). The present result showed that the addition of 1.0 g/kg rutin significantly increased the collagen content in flesh. Rutin and its metabolites can inhibit glucose glycation of collagen I, thereby increasing the accumulation of collagen (Cervantes-Laurean et al., 2006). In rat cardiac fibroblasts, reactive oxygen species (ROS) was reported to reduce collagen synthesis by decreasing transcription of procollagen mRNA (Siwik et al., 2001). As an effective antioxidant, rutin can increase the SOD activity and the ability to scavenge free radicals, thus decreasing ROS levels and increasing collagen synthesis.

In addition, the flesh quality trait includes texture characteristics, water-holding capacity (WHC) and muscle histology. WHC and texture characteristics are important characteristics affecting the acceptability and mechanical processing of flesh (Lin et al., 2012). Texture characteristics, especially hardness, are related to the inherent structure and properties of meat (Rafael et al., 2004). Muscle fibre density is significantly correlated with textural characteristics and colour visualization (Valente et al., 2016). However, in this study, dietary rutin did not significantly affect the texture characteristics, WHC, histology and chemical composition in flesh of grass carp, which might be related to the small size of the fish and the short breeding period (60 days). In the future, adult fish should be used to investigate the dietary effect of rutin on flesh quality of grass carp with a longer feeding period.

5 | CONCLUSION

In the present study, dietary rutin improved the growth, antioxidant response and flesh quality of grass carp by increasing weight gain , serum SOD activity, collagen, fatty acids and free amino acids contents in flesh and decreasing serum MDA content. The recommended supplementation level of rutin in the diet of grass carp was 0.8–1.0 g/kg.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (31772825).

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this article.

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How to cite this article: Xu Z, Li XQ, Yang H, Poolsawat L, Wang P, Leng XJ. Dietary rutin promoted the growth, serum antioxidant response and flesh collagen, free amino acids contents of grass carp (*Ctenopharyngodon idella*). *Aquacult Nutr*. 2020;00:1–12. https://doi.org/10.1111/anu.13205