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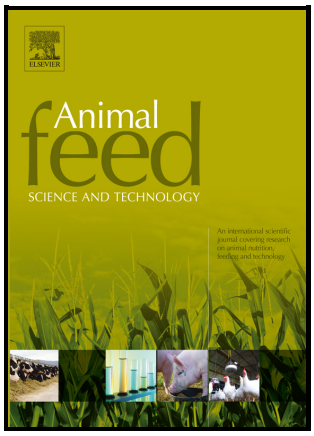

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Dietary supplementation of kaempferol improved the growth, lipid metabolism and flesh quality of juvenile grass carp (*Ctenopharyngodon idellus*) based on metabolomics

Zhen Xu[a,b,c], Hang Yang[a,b,c], Xiaoqin Li[a,b,c], Xiaoying Xu[a,b,c], Hongxin Tan[a,b,c*], Xiangjun Leng[a,b,c*]

[a] National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, China

[b] Centre for Research on Environmental Ecology and Fish Nutrition (CREEFN) of the Ministry of Agriculture, Shanghai Ocean University, Shanghai 201306, China

[c] Shanghai Collaborative Innovation for Aquatic Animal Genetics and Breeding, Shanghai Ocean University, Shanghai 201306, China

***Corresponding author:** Xiangjun Leng, E-mail: xjleng@shou.edu.cn. Hongxin Tan is the co-corresponding author (E-mail: hxtan@shou.edu.cn).

The first author is Zhen Xu, the co-first author is Hang Yang. These authors contributed equally to this work.

Address: Hucheng Ring Road 999, Lingang New City, Shanghai 201306, China.

ABSTRACT

Kaempferol is a common flavonoid with various biological and pharmacological functions. To investigate the effects of dietary kaempferol on growth, lipid metabolism and flesh quality of juvenile grass carp (*Ctenopharyngodon idell*a), five diets with kaempferol inclusion at 0 (control diet), 0.2, 0.4, 0.6 and 0.8 g/kg were fed to fish (17.0 ± 0.2 g) for 60 days. The results indicated that dietary kaempferol level linearly affected weight gain (WG) and feed conversion ratio (FCR), and the supplementation of 0.6 g/kg, 0.8 g/kg kaempferol increased WG by 4.9%, 5.6% and decreased FCR by 0.08, 0.08, respectively. Flavour amino acids content and activities of superoxide dismutase, catalase and glutathione peroxidase in flesh were linearly increased, and total free amino acids content, hardness, chewiness and adhesiveness of flesh were linearly and quadratically increased with the increasing kaempferol level. Moreover, the increasing dietary kaempferol linearly decreased intraperitoneal fat ratio, malondialdehyde content in flesh and triacylglycerol and cholesterol in serum and liver. In metabolomics profiling of flesh, 102 and 133 named differential metabolites were identified in 0.4 g/kg and 0.8 g/kg kaempferol groups, and these metabolites were mainly involved in the second-grade pathways of "lipid metabolism" and "amino acid metabolism". In gene expression, the mRNA levels of mammalian target of rapamycin (*mTOR*), catalase (*CAT*), peroxisome proliferators-activated receptor α (*PPARα*) in flesh as well as *PPARα* in liver were up-regulated, and *FAS* gene expression in liver was down-regulated by dietary supplementation of 0.4 and 0.8 g/kg kaempferol. In summary, dietary kaempferol improved the growth, lipid metabolism and flesh quality of juvenile grass carp, which might be associated with the insulin-like growth factor 1/mammalian target of rapamycin (*IGF-1/mTOR*), *PPAR* and nuclear factor-E2 related factor 2 (*Nrf2*) pathways. The recommended supplementation level of kaempferol in diet was 0.8 g/kg for juvenile grass carp.

Key words: Grass carp; Kaempferol; Growth; Flesh quality; Metabolomics

1 Introduction

Kaempferol (3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-benzopyran-4-one, Figure 1) is a common flavonoid found in tea, grape fruit, broccoli, apples, etc. (Chen and Chen, 2013; Park et al., 2006), which has been proved various biological and pharmacological functions as antioxidant, anti-inflammatory, anticancer and antimicrobial (Rajendran et al., 2014; Crespo et al., 2008). In the previous study, kaempferol significantly reduced the ROS-induced hemolysis of human erythrocyte by reducing malondialdehyde (MDA) content and increasing the activies of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Liao et al. 2016). Dietary kaempferol supplementation of 10 mg/kg body weight daily enhanced the activities of SOD, CAT, glutathione, GPx, membrane-bound enzymes and mitochondrial enzymes in the liver of rats exposed to aluminium sulphate (Usharani and Anuradha, 2018). In broiler chicken, the addition of 0.3% and 0.6% kaempferol in diets improved carcase characteristics by reducing abdominal fat percentage, subcutaneous fat thickness and the content of MDA in muscle (Xiao et al., 2014). Muhammad et al. (2019) reported that oral kaempferol (1-2 mg/kg BW per day) significantly improved the weight gain of chicken challenged with *Eimeria tenella.* However, the supplemental effects of kaempferol has not been reported in aquatic feeds.

Kaempferol, quercetin and rutin belong to flavonoids with similar chemical structures, thus they may have similar biological functions. Some studies have shown that rutin improved the antioxidant capacity (Pês et al., 2016) and anti-inflammatory responses (Zheng et al., 2017) of fishes. Dietary supplementation of 1.5 g/kg rutin was reported to protect the liver of silver catfish (*Rhamdia quelen*) from the oxytetracycline-induced oxidative stress and

apoptosis (Londero et al., 2020). Baldissera et al. (2020) found that dietary rutin (3 g/kg) increased the creatine kinase activity and polyunsaturated fatty acids content in flesh of silver catfish exposed to trichlorfon. Moreover, the addition of 8 g/kg quercetin in diet promoted weight gain, nitric oxide content, the gen expression of sirtuin 1 (*SIRT1*), Cu/Zn-superoxide dismutase (*Cu/Zn-SOD*), catalase (*CAT*), glutathione peroxidase (*GPx*), and lowering glucose, triacylglycerol (TG) and cholesterol (CHO) contents in plasma of blunt snout bream (*Megalobrama amblycephal*a) (Jia et al., 2019).

Metabolomics is a quantitative analysis of all small molecule metabolites in organism as cells, tissues or biological fluids using a high-throughput approach (Zhao et al., 2018). Recently, metabolomics based on nuclear magnetic resonance (NMR) has been used to estimate the effects of environments (Jiang et al., 2019) and feed composition (Wagner et al., 2019) on tissues metabolism in aquaculture. Flesh metabolomics profiling can reveal the differences in metabolites and indicate the possible changes in flesh quality affected by the environment and diet.

Aquaculture has developed rapidly in recent years, and the flesh quality improvement of aquatic animals is an increasing challenge for aquaculture at present and in the future (Jennings et al., 2016). In the past years, grass carp was selected and a series of studies were carried out to investigate the effects of dietary flavonoids on the growth and flesh quality, including rutin and quercetin, as this fish has the highest aquaculture production in China and in the world. The results showed that dietary supplementation of 800 mg/kg rutin and 400 mg/kg quercetin promoted the growth and flesh quality of grass carp by increasing the contents of flesh collagen and amino acids (Xu et al., 2021; Xu et al., 2019). As kaempferol

shares the similar chemical structure to quercetin and rutin, it was speculated that kaempferol might have the same function of promoting growth and flesh quality of fish. Therefore, kaempferol was supplemented in diets to explore the effects on the growth, lipid metabolism and flesh quality of juvenile grass carp, and the acting mechanism of kaempferol was also investigate by using metabonomics methods and real-time fluorescent quantitative PCR technology (RT-qPCR).

2 Materials and methods

2.1 Experimental diets and design

Five diets were designed with the kaempferol inclusion of 0 (control diet), 0.2, 0.4, 0.6 and 0.8 g/kg in basal diet. Kaempferol was purchased from Nanjing Yuanzhi Bio-Tech Co., LTD (Nanjing, China) with purity higher than 95%. All ingredients were grounded and passed through a 40 mesh sieve, and then made sinking pellets with diameter of 2 mm by a single-screw extruder (TR-85; Tianruo Machinery Co., Ltd., Hebei). The pelleting temperature was 85-90 °C, and the diets were air-dried and stored at 4 °C until use. The feed formulation and chemical composition are listed in Table 1.

2.2 Fish and feeding management

Grass carp were obtained from Jinshan Aquaculture Farm (Shanghai, China), and then transported to Binhai Station of Shanghai Ocean University for two weeks to adapt to the breeding environment. A total of 300 grass carp with initial body weight of 17.0 ± 0.2 g were randomly assigned into 15 cages (1.4 × 1.0 × 1.0 m) with 20 fish per cage. During the feeding period, the fish were fed manually three times daily (07:30, 11:30, 16:30) for 8 weeks. The daily feeding rate was adjusted according to the water temperature and feeding status. About

a half of cultured water was renewed by filtrated pond water, and the waste in bottom of pools was siphoned twice every 5 days. The water dissolved oxygen and temperature were 5.0 ± 0.5 mg/L and 28.0 ± 3.0 ℃, and the range of pH, ammonia nitrogen and nitrite were 7.8 ± 0.3, 0.1 ± 0.01 mg/L and 0.05 ± 0.01 mg/L, respectively.

2.3 Sample collection

At the termination of the feeding trial, all grass carp were deprived of diets for 24 h, then anaesthetized before sampling and measured body weight of each cage to calculate weight gain (WG) and feed conversion ratio (FCR). Three fish were randomly sampled from each cage to measure morphometric parameters, then blood were drawn from the caudal vein, centrifuged at 4 ℃ for 10 min (1200 g) and stored at -80 ℃ for measuring serum biochemical indices. After the dissection, the visceral, liver, intraperitoneal lipid were weighed to measured condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat ratio (IFR). The liver samples were collected and stored at -80 ℃ for measuring triacylglycerol (TG) and cholesterol(CHO). The dorsal flesh were sampled for analyzing texture characteristics, histology, proximate composition, antioxidant indices, free amino acid, fatty acid, collagen, RT-qPCR and metabolomics.

2.4 Parameters and methods

2.4.1 Growth performance and husbandry parameters

WG (%) = 100 × (final weight - initial weight) / initial weight

FCR = feed intake / weight gain

Survival (%) = 100 × (final number of fish / initial number of fish)

VSI (%) = 100 × (visceral weight / body weight)

HSI (%) = 100 × (liver weight / body weight)

IFR (%) = 100 × (intraperitoneal fat weight / body weight)

K (g/cm^3) = 100 × (body weight / body length^3)

2.4.2 Serum and liver biochemical indices

The liver sample was mixed with 4 times of the pre-cooled normal saline for homogenizing, then centrifuged for 15 min at 4℃ (2 000 g/min). The supernatants of the liver, together with the serum, were used to detect the contents of triglyceride (TG) and cholesterol (CHO) using the kits sourced from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.4.3 Flesh antioxidant indices

The flesh were added with 4 times of the pre-cooled normal saline, then homogenized and centrifuged for 15 min at 4℃ (2 000 g/min). The supernatants were collected for measuring of the activities of SOD, CAT and GPx and the contents of MDA, protein carbonyl (PC) and lactic acid (LD) using the kit provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.4.4 Proximate composition of flesh and diets

The proximate composition of flesh and diets were analyzed with the standard methods of AOAC (1995). The moisture and ash contents were measured by drying samples to constant weight at 105 ℃, and by combusting samples in a muffle furnace at 550 ℃ for 7 h, respectively. The crude protein content (N × 6.25) was estimated using the Kjeldahl system method (2300 Auto analyser; FOSS Tecator, Sweden). The crude lipid content was determined using the chloroform-methanol method.

2.4.5 Collagen content in flesh

The collagen content in flesh was calculated using the hydroxyproline (Hyp) kit with alkaline hydrolysis method provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The collagen content was calculated by multiplying the Hyp content by 8 according to AOAC method 990.26 (AOAC 2005).

2.4.6 Free amino acid composition of flesh

Free amino acid in flesh was analyzed referring to the method of Xu et al. (2021). Fresh flesh (0.5 g) was mixed with 15 mL of 5% trichloroacetic acid, then homogenized with ultrasonic in ice-water bath for 20 minutes and stored at -20 °C for 3 hours. After centrifuging at 14800 g/min for 20 min (4 °C), the supernatant was sampled and adjusted pH to 2.0 for determining free amino acid with Waters ACQUITY Ultra Performance LC/MS (Waters, USA).

2.4.7 Fatty acid composition of flesh

Fatty acid composition in flesh was measured with the method of boron trifluoride methyl esterification according to Xu et al. (2021). The extracted fat was dissolved in 2 mL of 14% boron trifluoride methanol solution. After 25 min of water bathing at 100 °C, benzene (2 mL) and methanol solution (2 mL) were added for another water bathing (100 °C, 25 min). Then, the samples were mixed with distilled water and n-hexane. After centrifuging at 1250 g/min for 10 min, the supernatant was used to analyze the fatty acid composition with Agilent Technologies 7890B GS Syetem GC/MS (Agilent, USA).

2.4.8 Texture characteristics of flesh

A block of fresh dorsal flesh about 1 cm^3 was used to perform the texture profile analysis (TPA) with a Universal TA texture analyzer (Tengba, China). Test conditions were as follows: cylindrical probe with diameter and speed of 25 mm and 1 mm/s, 70% deformation of the original thickness, and contact induction force of 5 gram force (gf).

2.4.9 Metabolomic analysis.

According to the growth results, the control group, 0.4 g/kg (Kae1) and 0.8 g/kg (Kae2) kaempferol supplemented groups were selected for muscle metabolomics analysis. Flesh sample (50 mg) were mixed with 400 μL solution of methanol and water (4:1), added 0.02 mg/mL L-2-chlorophenylalanin as internal standard, and then used LC-MS/MS for analysis. Chromatographic separation of the metabolites was executed with ExionLCTMAD system (AB Sciex, USA). After UPLC-TOF/MS analyses, peak detection and alignment of raw data were determined using the Progenesis QI 2.3 (Non-linear Dynamics, Waters, USA) (Wang et al. 2019).

2.4.10 RNA extraction and RT-qPCR

Based on the growth and the physiological and biochemical indices of serum and liver, the control group, 0.4 g/kg and 0.8 g/kg kaempferol supplemented groups were selected for analysis of related gene expression. The total RNA in flesh was extracted using RNAiso Plus kit (Takara, Dalian, China). The quality and OD 260/280 value of total RNA were tested by agarose gel electrophoresis and protein and nucleic acid analyzer (Agilent 2100, USA), respectively. The reverse transcription reactions and RT-qPCR were executed by the PrimeScript RT Reagent kit with gDNA Eraser and SYBR Premix Ex Taq kit (Takara, Dalian,

China), respectively. The primers were designed according to the GenBank (Table 2), and the reactions followed the standard protocols. The $2^{-\Delta\Delta Ct}$ method based on the cycle threshold (Ct) value was used to measure the relative expression of the target gene. *18S* rRNA was used as the internal reference gene.

2.5 Statistical analysis

The data were presented as the mean±standard deviation (SD), and analyzed with the Statistical Package for the Social Sciences (SPSS) 25.0. Tukey's test was used to analyze the significance level among treatments, and denoted by $P < 0.05$. Orthogonal polynomial contrasts for trend analysis was performed to determine whether the significant effect was linear and/or quadratic..

3 Results

3.1 Growth performance

During the feeding period, no death was recorded. Significant linear effects of dietary kaempferol levels were found on FBW, WG, FCR and IFR ($P < 0.05$). The fish fed diets supplemented with 0.6 and 0.8 g/kg kaempferol showed higher WG (+ 4.9%, + 5.6%) and lower FCR (- 0.08, - 0.08) than the control fish ($P < 0.05$). IFR was significantly decreased by dietary addition of 0.8 g/kg kaempferol ($P < 0.05$). There were no significant linear and quadratic effects of dietary kaempferol on CF, VSI and HSI ($P > 0.05$) (Table 3).

3.2 Serum and liver biochemical indices

As shown in Table 4, dietary kaempferol linearly and negatively affected the TG and CHO contents in serum and liver. The group of 0.8 g/kg kaempferol showed significantly lower TG and CHO contents in serum and liver than the control ($P < 0.05$)

3.3 Flesh antioxidant response

The activities of SOD, CAT, GPx and MDA in flesh were linearly affected by dietary kaempferol levels ($P < 0.05$). The CAT and GPx activities in 0.6, 0.8 g/kg kaempferol groups were significantly higher, while the MDA content in 0.8 g/kg kaempferol group was lower than those in the control ($P < 0.05$). Moreover, no significant differences were observed in LD and PC contents among all the treatments ($P > 0.05$) (Table 5).

3.4 Chemical composition of flesh

The supplementation of kaempferol in diets did not significantly affect the contents of moisture, crude ash, crude lipid, crude protein and collagen in flesh ($P > 0.05$) (Table 6).

3.5 Free amino acid composition in flesh

As shown in Table 7, proline (Pro) and threonine (Thr) were the most abundant free amino acids in all the groups. aspartic acid (Asp), serine (Ser), valine (Val), arginine (Arg) and total free amino acids (TFFA) contents in flesh showed positively linear and quadratic relationship with dietary kaempferol levels ($P < 0.05$), and Thr and flavour amino acids (FAA) were linearly and positively correlated with dietary kaempferol ($P < 0.05$). Moreover, Pro content were quadratically affected by dietary kaempferol levels ($P < 0.05$).

3.6 Fatty acid composition in flesh

The contents of saturated fatty acids (SFA), monounsaturated fatty acid (MUFA), n-6 polyunsaturated fatty acids (n-6PUFA), n-3 polyunsaturated fatty acids (n-3PUFA) and eicosapentaenoic acid/docosahexaenoic acid (DHA/EPA) ratio in flesh exhibited no significant differences among all the groups ($P > 0.05$), except a significant positive linear trend was found between C20:1 content and kaempferol levels ($P < 0.05$) (Table 8).

3.7 Texture characteristics of flesh

As shown in Table 9, both linear and quadratic effects of dietary kaempferol levels were observed on flesh hardness, chewiness and adhesiveness ($P < 0.05$), while the resilience only showed positively quadratic relationship with dietary kaempferol ($P < 0.05$). There were no significant differences in springiness and cohesiveness among all the groups ($P > 0.05$).

3.8 Metabolite profiles

Metabolic profiling results showed that a total of 858 and 1049 different metabolites, including 102 and 133 named metabolites were identified between the Kae1 group and control group, and between the Kae2 group and control group, respectively (Figure 2A and 2B).

In the Kae1 *VS* control group, the identified metabolites were assigned to the KEGG database, and 41 differential metabolites were classified into 18 second-grade pathways. The top priority was “lipid metabolism”, followed by “cancers: overview”, “digestive system”, “metabolism of cofactors and vitamins” and “amino acid metabolism” (Figure 3 A). In the Kae2 *VS* control group, 39 differential metabolites were classified into 19 second-grade pathways, and the top priority was “lipid metabolism”, followed by “nucleotide metabolism”, “metabolism of cofactors and vitamins”, “cancers: overview” and “amino acid metabolism” (Figure 3 B). The analysis of metabolic pathway enrichment showed that the pathways as “linoleic acid metabolism”, “beta-Alanine metabolism”, “PPAR signaling pathway” were significantly changed in the Kae1 group, and “purine metabolism”, “taste transduction” and “lysosome” pathways were significantly changed in the Kae2 group, when compared to the control respectively (Figure 3C and 3D).

3.9 The expression of related genes of growth, lipid metabolism and anti-oxidation

In growth-related genes, a significant ($P < 0.05$) positive linear trend was found between the increasing kaempferol level and *mTOR* mRNA expression in flesh. The *AKT* gene expression was linearly and quadratically affected by dietary kaempferol levels ($P < 0.05$), and it was significantly promoted by the addition of 0.8 g/kg kaempferol ($P < 0.05$). There were no significant difference in flesh *IGF-1* and *PI3K* genes expression among the three groups (Figure 4 A).

In terms of anti-oxidation genes, there was a significant linear relationship between *CuZn-SOD*, *CAT*, *GPx, CAT* and *Nrf2* mRNA levels and dietary kaempferol levels. The supplementation of 0.8 g/kg kaempferol in diet significantly up-regulated *CuZn-SOD*, *CAT*, *GPx* and *Nrf2* mRNA levels in flesh, while 0.4 g/kg kaempferol supplementation only up-regulated *CAT* mRNA expression ($P < 0.05$) (Figure 4 A).

In lipid metabolism, the *CPT1* and *PPARα* mRNA levels in flesh and liver were linearly and positively correlated with dietary kaempferol levels ($P < 0.05$). Dietary kaempferol produced negatively linear and quadratic effects on *FAS* gene expression in liver ($P < 0.05$) and negatively linear effects on *FAS* gene expression in flesh ($P < 0.05$). There were no significantly linear and quadratic effects of dietary kaempferol on the expression of *ACC* and *PPARγ* genes in flesh and liver (Figure 4 B).

4 Discussion

4.1 Growth performance

At present, no report about the inclusion of kaempferol was found in aquaculture animals. However, some studies have been conducted in the other two flavonoids with similar

chemical structures to kaempferol, quercetin and rutin. Jia et al. (2019) reported that dietary quercetin (800 mg/kg) significantly improved the growth performance of blunt snout bream (*Megalobrama amblycephala*). The supplementation of 2 g/kg quercetin and 3g/kg rutin significantly increased the weight gain of tilapia exposed to mycotoxin (T-2) (Deng et al., 2019). In this study, dietary keampferol (0.6-0.8 g/kg) also significantly increased the weight gain and decreased FCR of grass carp, which were similar to the previous studies on quercetin and rutin (Xu et al., 2021; Xu et al., 2019). Phosphatidylinositol 3-kinase (*PI3K*) is a key molecule in the signal pathway *IRS-PI3K-AKT*, which can phosphorylate many downstream *proteins* including *AKT* and *mTOR*. *AKT* plays an important role in cell growth and apoptosis (Holz and Blenis, 2005). After the activation, *AKT* phosphorylate some downstream proteins including a variety of enzymes, kinases and transcription factors, thereby regulating cell functions. *mTOR* is a part of the signal pathway that senses nutrients, and it participates in the transcription, translation, protein degradation and synthesis. Its reduced expression level cause protein synthesis inhibition, transcription changes and autophagy, etc. (Wu et al., 2019). When *mTOR* is phosphorylated, it regulate the expression of genes involved in intermediate metabolism, protein accumulation and cell growth (Skiba et al., 2008). The present results showed that dietary kaempferol (0.8 g/kg) significantly up-regulated *mTOR* and *AKT* mRNA levels in flesh, which might be the reason that kaempferol promoted the growth of grass carp.

In this study, TFFA content in flesh showed positively linear and quadratic relationship with dietary kaempferol levels. Moreover, the amino acids metabolites in flesh were also significantly changed by the addition of 0.4 and 0.8 g/kg kaempferol when compared with the

control group, including the upregulation of 5'-methylthioadenosine, spermine and P-salicylic acid in the 0.4 g/kg kaempferol group, and the upregulation of acetyl-CoA, indole and spermidine in the 0.8 g/kg kaempferol group. *IGFs* affect amino acids (AA) metabolism, and there is a correlation between the *IGFs* system and AA signals (Codina et al., 2008; Montserrat et al., 2012). *mTOR* is the main regulator of cell growth and factor linking *IGFs* and AA, and the activated *mTOR* modulate the synthesis of protein (Kim et al., 2009). The amino acid transporters deliver AA into cells, and the increase of AA contents in cells regulate protein translation by activating the target of *mTOR* signaling pathway (Dai et al., 2013; Jewell et al., 2013). Dietary kaempferol might stimulate AA transport and protein translation by regulating the expression of related genes such as *mTOR* and *AKT*, thereby promoting the accumulation of AA metabolites and muscle growth. Meanwhile, free amino acids are also important indices reflecting the flavor and taste of flesh. Alanine and glycine have a sweet taste, while glutamic acid has an umami taste (Prakash et al., 2015). In this study, the increased FAA content by dietary kaempferol indicated that kaempferol had positive effects on the improvement of flesh quality for grass carp.

4.2 Lipid metabolism

Lipid is an important energy source for the growth of fish, but high content of intraperitoneal fat reduced the flesh production (Xiao et al., 2017). The results of the current study revealed that intraperitoneal fat ratio and the contents of TG and CHO in liver and serum were significantly decreased by the addition of 0.8 g/kg kaempferol in diet. Xiao et al (2014) also reported that the diets containing 3 and 6 g/kg kaempferol significantly reduced the abdominal fat percentage, the subcutaneous fat thickness, plasma and hepatic total

cholesterol in broiler chicken. The liver is the most important organ of lipid metabolismin in organisms. The synthesis and catabolism of triacylglycerol are factors regulating lipid accumulation in specific tissues. Gómez-Zorita et al (2017) found that the supplementation of 25 μM kaempferol in culture medium reduced the TG content in human adipocytes. Similarly, the inclusion of 20μM kaempferol also significantly decreased the TG levels in HepG2, THP-1, and Caco2 cells (Hoang et al., 2019). Peroxisome proliferators-activated receptor γ (*PPARγ*) regulate the transcription of some fat synthesis target genes such as Acetyl CoA carboxylase (*ACC*), Fatty acid synthase (*FAS*) and Stearoyl-CoA desaturase 1 (Lee and Hossner, 2002). *FAS* is a key enzyme of lipid de novo synthesis and affect the efficiency of fatty acid production. Peroxisome proliferators-activated receptor α (*PPARα*), Carnitine palmitoyltransferase (*CPT-1*) and *ACC* are the main rate-limiting enzymes involved in lipid catabolism. *PPARα* can increase the mRNA expression of fatty acid oxidation-related genes, thereby inhibiting the lipid accumulation in liver and reducing the serum lipid level. Both *CPT1* and *ACC* are rate-limiting enzymes in catalyzing β-oxidation of fatty acids (Yan et al., 2017). In the present study, dietary kaempferol down-regulated *FAS* mRNA level, and up-regulated the expression of *CPT1* and *PPARα* gens, which revealed that kaempferol might regulate the expression of *FAS* and *CPT1* genes by activating the *PPAR* signaling pathway, thereby reducing lipid accumulation in fish. Hoang et al. (2019) also suggested that kaempferol reduced TG mainly by activating *PPARα*, *PPARδ* and regulating the expression of *PPAR* target genes related to fatty acid oxidation and synthesis.

In flesh metabolomics, seven lipid metabolites, including lysoPC(16:0), lysoPC (22:4(7Z,10Z,13Z,16Z)), 2-acetyl-1-alkyl-sn-glycero-3-phosphocholine, were found to be up-regulated in the 0.4 and 0.8 g/kg kaempferol groups, indicating that the effect of kaempferol on lipid metabolism in muscle is similar to that in liver and serum. However, dietary kaempferol did not significantly affect the crude lipid content and fatty acid composition of flesh, which may be due to the low lipid content in flesh. The addition of kaempferol only changed the expression levels of some genes and small molecular metabolites, but it was not enough to produce significant differences in macroscopic substances, which needs a further study in the future.

4.3 Flesh antioxidant response

Oxidative stress is caused by the destruction of the antioxidant system in the body, thereby increasing the reactive oxygen species production including superoxide anion free radicals, hydroxyl free radicals and lipid peroxides (Dias et al., 2013). Lipid peroxidation products might destroy the structural of cell membranes, thereby causing oxidative damage (Chen and Yan, 2005). The lipid and protein in fish flesh are easily oxidized during the processing and storage (Richards and Hultin, 2002). Due to the polyphenol structure, flavonoids have been found to have strong antioxidant capacity, which was closely related to the biological and pharmacological effects of flavonoids (Leung et al., 2007). Nirmala and Ramanathan (2011) reported that the supplementation of 200 mg/kg kaempferol in culture medium reduced the thiobarbituric acid level and increased the activities of CAT, SOD and GPx in the liver of rats induced by 1,2-dimethylhydrazine. Moreover, as antioxidant, kaempferol prevented the production of MDA, and increased SOD and GSH activities in

vascular smooth muscle cells induced by hydrogen peroxide (Yang et al., 2016). In the current study, the supplementation of 0.6-0.8 g/kg kaempferol significantly increased the activities of SOD, CAT and GPx in flesh, and the MDA content was also significantly decreased by dietary supplementation of 0.8 g/kg kaempforel. The activities of antioxidant enzymes are related to their original mRNA levels, which could reflect the de novo synthesis of enzymes (Jiang et al., 2015). The present results indicated a significant linear relationship between *CuZn-SOD*, *CAT*, *GPx, CAT* mRNA levels and dietary kaempferol levels, which was consistent with the results of enzyme activities.

Nuclear factor E2-related factor 2 (*Nrf2*) is a regulator factor for controlling the body's antioxidant stress. After being stimulated by ROS, *Nrf2* will transfer to the nucleus and combine with the antioxidant reactant (*ARE*), thus starting a series of downstream antioxidant genes expression (Montserrat et al., 2011). In this study, *Nrf2* mRNA level was significantly up-regulated by the supplementation of 0.8 g/kg kaempferol, indicating that kaempferol might enhance the expression of related antioxidant enzymes by activating the *Nrf2* pathway. A similar report was also found in quercetin, where quercetin increased the expression of *HO-1* by activating the *Nrf2* pathway, thus protecting the liver from the oxidative stress induced by chronic drinking (Nussler et al., 2010).

4.4 Flesh texture characteristics and chemical composition

Texture characteristics as hardness, chewiness adhesiveness and resilience is the most important quality indicators, estimating the acceptability and mechanical processing of flesh (Lin et al., 2012). Hardness indicates the maximum force required to compress the flesh (Viji et al., 2015). Chewing is the sensation of continuous chewing, and it is a important factor

affecting the crispness of flesh (Xu et al., 2020). Our results showed that dietary kaempforel significantly increased the hardness, adhesiveness, chewiness and resilience of flesh. The main factors affecting flesh texture characteristics include lipid and collagen contents as well as fiber density and diameter (Pearce et al., 2011). Yu et al. (2019) reported that hardness in fish flesh was positively correlated with collagen content, and negatively correlated with flesh fiber diameter. However, in present study, no significant differences were observed in the contents of crude lipid and collagen. Maybe the texture characteristics were not only associated with the crude lipd and collagen contents, but some other factors may have more effects on flesh texture. Moral et al. (2002) suggested that the type and function of protein significantly affect the flesh texture, and the hardness is associated with myofibrillar protein and sarcoplasmic protein.

5 Conclusion

In the present study, dietary kaempferol improved the growth, promoted lipid metabolism by decreasing intraperitoneal fat ratio, TG and CHO contents in serum and liver, and enhanced flesh quality by increasing flesh free amino acids contents and the activies of SOD, CAT, GPx and decreasing MDA content in flesh of juvenile grass carp. These improvements might be associated with *IGF-1/mTOR*, *PPAR* and *Nrf2* pathways. The recommended supplementation level of kaempferol in the diet of juvenile grass carp was 0.8 g/kg.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgments

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Figures and Tables Caption

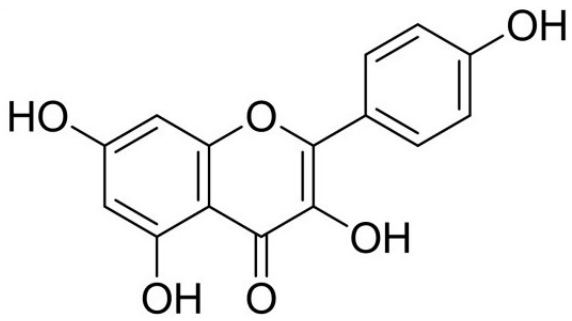


Figure 1 The molecular structure of kaempferol.

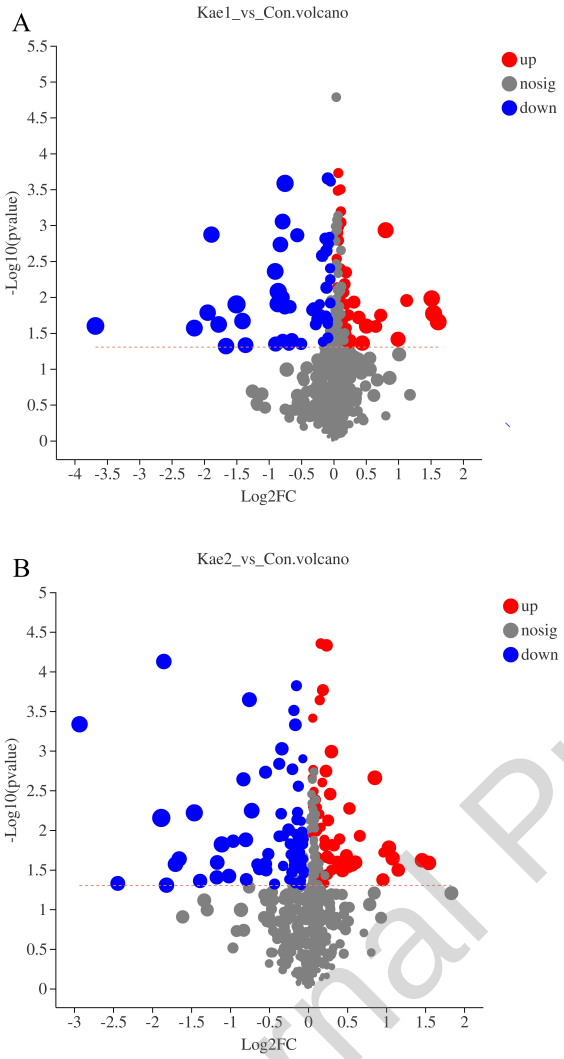
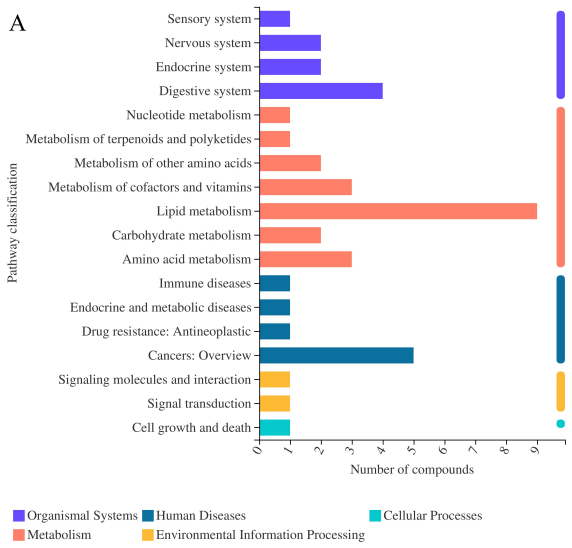
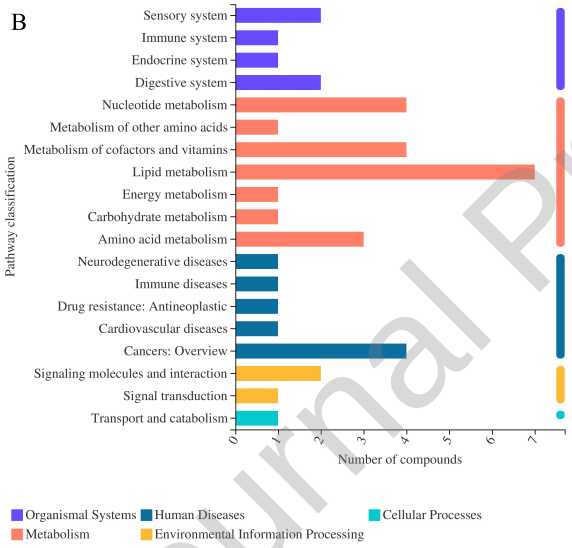
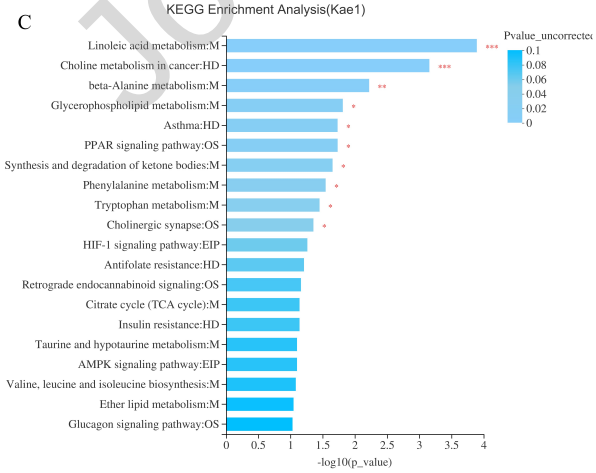


Figure 2 Volcano plot of differently expressed metabolites between the Kae1 and control group (A) as well as between the Kae2 and control group (B).

Note: the horizontal line is twice of the difference threshold, and the vertical line is *p*-value. Red and blue dots represent the significantly up-regulated and down-regulated metabolites, respectively.







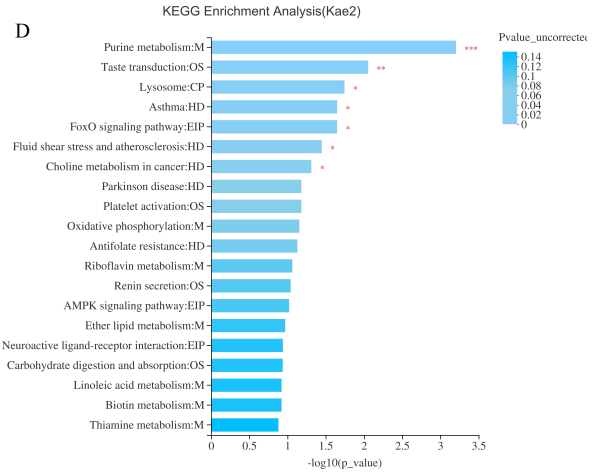


Figure 3 A and B, KEGG pathway classification: metabolites detected and annotated between the Kae1 and control group, and between the Kae2 and control group; C and D, metabolic pathway enrichment study of differently presented metabolites between the Kae1 and control group, and between the Kae2 and control group.

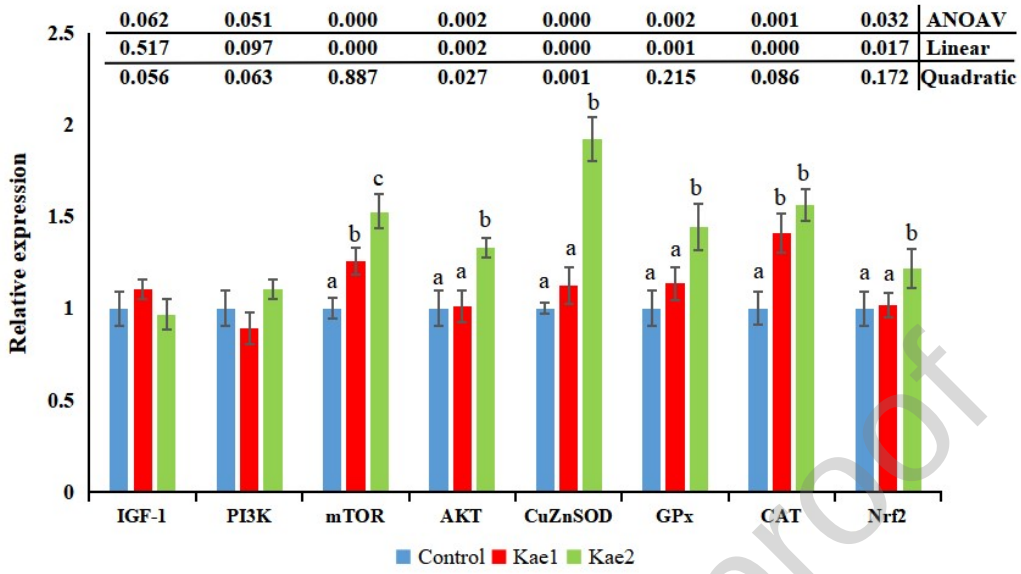


Figure 4 A Expression of growth-related and anti-oxidation-related genes in flesh of grass carp

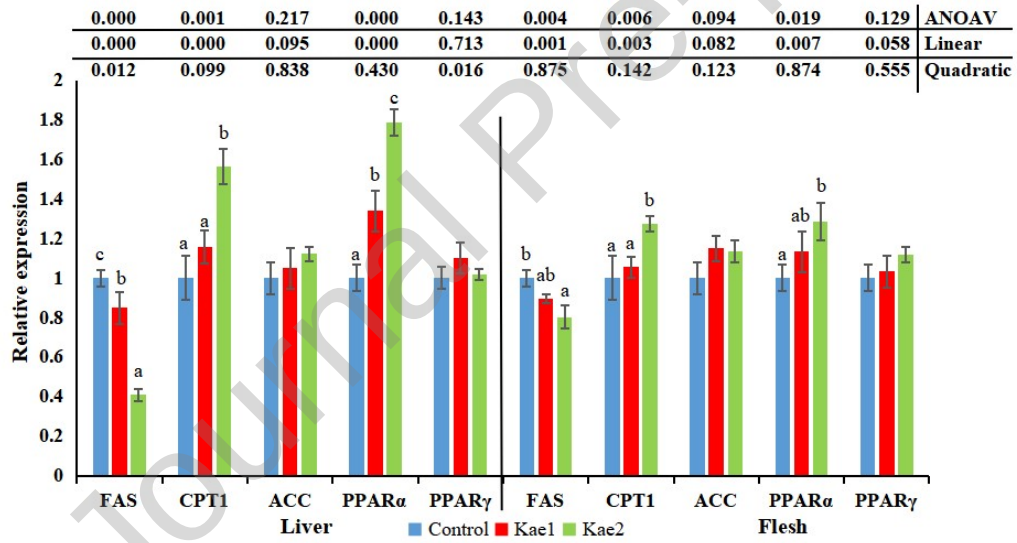


Figure 4 B Expression of lipid metabolism-related genes in flesh and liver of grass carp

Table 1 Ingredients and proximate composition of experimental diets (dry weight basis, g/kg).

Ingredients[a]	Kaempferol addition (g/kg)				
	0	0.2	0.4	0.6	0.8
Fish meal	20	20	20	20	20
Soybean meal	180	180	180	180	180
Cottonseed meal	160	160	160	160	160
Rapeseed meal	180	180	180	180	180
Wheat bran	100	100	100	100	100
Rice bran	100	100	100	100	100
Wheat middling	224.5	224.3	224.1	223.9	223.7
Soybean oil	10	10	10	10	10
Choline chloride (50%)	5	5	5	5	5
Vitamin premix[b]	2.5	2.5	2.5	2.5	2.5
Mineral premix[c]	3	3	3	3	3
Monocalcium phosphate	15	15	15	15	15
Kaempferol	0	0.2	0.4	0.6	0.8
Total	1000	1000	1000	1000	1000
Proximate composition					
Crude protein	304.8	304.5	305.5	305.8	305.1
Crude lipid	35.5	35.9	36.3	34.9	35.5
Crude ash	74.9	75.3	74.6	74.5	74.9
Moisture	95.5	94.7	94.2	95.1	95.4

[a]The protein contents of fish meal, soybean meal, cottonseed meal, rapeseed meal, wheat middling, rice bran, wheat bran were 635 g/kg, 475 g/kg, 455 g/kg, 382 g/kg, 150 g/kg, 135 g/kg, 130 g/kg.

[b]Vitamin premix (mg/kg diet): VA, 20 mg; VD3, 10 mg; VE, 300 mg; VK3, 12.17 mg; VB1, 20 mg; VB2, 20 mg; VB3, 100 mg; VB6, 22 mg; VB12, 0.15 mg; VC, 1,000 mg; VB7, 0.6 mg; VB9, 8 mg; inositol, 500 mg.

[c]Mineral premix (mg/kg diet): KIO_3, 2.5 mg; $CoCl_2 \cdot 6H_2O$, 2.4 mg; $CuSO4 \cdot 5H_2O$, 12 mg; $FeSO4 \cdot H_2O$, 200 mg; $ZnSO4 \cdot H_2O$, 250 mg; $MnSO4 \cdot H_2O$, 35 mg; Na_2SeO_3, 0.86; $MgSO_4 \cdot 7H_2O$, 900 mg.

Table 2 The primers for real-time PCR

Primer name	Sequence from 5′ to 3′	GenBank accession no
18S rRNA	F:GGAATGAGCGTATCCTAAACCC R:CTCCCGAGATCCAACTACAAGC	EU047719.1
IGF-1	F:CTTTAAGTGTACCATGCGCTGT R:ATTTGCCGGTTTTCACGGGT	EU051323.1
PI3K	F:CAGTCTACGCACACCAGAGG R:CTCGGTAGCAGCCTTACTGG	KY763989.1
AKT	F:AGAGGATGGTATGGAGCCGT R:GCAGAGGCAGATATCGGGAC	KY763985.1
mTOR	F:GGTACTGCAGAGAACTGATG R:GAAAGTATGAGGCTGGAAGG	JX854449.1
PPARα	F:TCAGGATACCACTATGGAGTTCAC R:TACAGCGGCGTTCACACTTG	FJ595500.1
CPT-1	F:GGCATTGACCGCCATCTCTT R:TGTCTCGCCAGGTCAAACAG	JF728839.1
PPARγ	F:CGCTCATCTCCTACGGTCAG R:ATGTCGCTGTCGTCCAACTC	GU997136.1
ACC	F:GTGGGCACACAGTGTAATCGTAGG R:CAGTCTTAAAAGCAGAGTCAGGGA	HM142590.1
FAS	F:CCTCAGCTTACAGCAGAATC R:CTCTTCAGCAAGGGAGTTTAG	MK111644.1
CuZnSOD	F:CGCACTTCAACCCTTACA R:ACTTTCCTCATTGCCTCC	KP143135.1
GPx	F:TGCAACCAGTTCGGACATCA R:CATCAGGGACACAGCGTCAT	EU828796.2
CAT	F:GCGGAGAACTGGAAGTGGAA R:GCCGATGTGTGTCTGGGTAA	MG821473.1
Nrf2	F:GGAGAAGAGCGAACGTAGCA R:GGAACGAGAAAAACGGTGCC	KX243419.1

Note: *IGF-1*: Insulin-like growth factor 1; *PPARα*: Peroxisome proliferators-activated receptor α; *PI3K*: Phosphatidylinositol 3-kinase; *AKT*: Protein kinase B; *CAT*: Catalase; *CPT-1*: Carnitine palmitoyltransferase; *Nrf2*: Nuclear factor-E2 related factor 2; *PPARγ*: Peroxisome proliferators-activated receptor γ; *CuZnSOD*: Cu/Zn-superoxide dismutase; *mTOR*: Mammalian target of rapamycin; *ACC*: Acetyl CoA carboxylase; *GPx*: Glutathione peroxidase; *FAS*: Fatty acid synthase.

Table 3 Growth performance of grass carp fed kaempferol supplemented diets.

Growth performance	Kaempferol addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
IBW(g)	17.0±0.2	17.0±0.1	17.1±0.1	17.1±0.2	16.9±0.1	0.943	0.67	0.933
FBW(g)	82.2±1.1^{a}	82.9±1.8^{abc}	82.5±2.0^{ab}	85.4±1.3^{bc}	85.8±1.5^{c}	0.022	0.003	0.352
WG(%)	383.2±6.9^{a}	387.5±10.5^{abc}	385.2±11.9^{ab}	402.2±7.5^{bc}	404.5±9.0^{c}	0.037	0.005	0.394
FCR	1.53±0.03^{c}	1.51±0.04^{abc}	1.52±0.05^{bc}	1.45±0.03^{ab}	1.45±0.03^{a}	0.022	0.003	0.357
S(%)	100	100	100	100	100	-	-	-
K(g/cm^{3})	1.92±0.05	1.93±0.10	1.92±0.10	1.92±0.09	1.94±0.10	0.992	0.778	0.956
HSI(%)	9.69±0.91	9.76±0.85	9.74±0.69	9.90±0.44	9.90±1.00	0.977	0.543	0.946
VSI(%)	2.23±0.27	2.26±0.22	2.24±0.14	2.26±0.12	2.27±0.18	0.998	0.838	0.808
IFR(%)	2.28±0.14^{a}	2.22±0.22^{a}	2.28±0.27^{a}	2.24±0.26^{a}	1.96±0.12^{b}	0.048	0.033	0.084

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

Values in the same row with different superscripts indicate significant differences according to Tukey's tests (P<0.05). Pr>F: significant probability associated with the F-statistic, the same below.

Table 4 Serum and liver biochemical indices of grass carp fed kaempferol supplemented diets.

Iterms	Kaempferol addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
Serum								
TG (mmol/L)	4.61±0.49^{b}	4.50±0.46^{b}	4.50±0.57^{b}	4.61±0.29^{b}	3.58±0.48^{a}	0.028	0.045	0.121
CHO (mmol/L)	9.33±0.33^{b}	9.64±0.28^{b}	9.56±0.42^{b}	8.98±0.67^{ab}	7.12±0.73^{a}	0.009	0.004	0.057
Liver								
TG (μmol/g)	11.86±0.64^{b}	11.92±0.92^{b}	11.87±0.84^{b}	11.47±0.70^{ab}	10.94±0.45^{a}	0.029	0.048	0.241
CHO (μmol/g)	17.84±0.71^{b}	17.34±0.92^{b}	17.17±0.79^{b}	17.35±0.54^{b}	16.18±0.92^{a}	0.023	0.004	0.449

TG, Triacylglycerol; CHO, Cholesterol.

Table 5 Flesh antioxidant response of grass carp fed kaempferol supplemented diets.

SOD, superoxide dismutase; MDA, malondialdehyde; PC, Protein carbonyl; CAT, catalase; LD, lactic acid; GPx, glutathione peroxidase.

Serum enzymes	Kaempforel addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
SOD U/mg	16.61±0.67^{a}	18.29±0.17^{bc}	17.65±0.73^{b}	18.99±0.33^{c}	18.90±0.2^{c}	0.001	0.000	0.166
CAT U/mg	5.05±0.17^{a}	5.14±0.17^{ab}	5.32±0.06^{abc}	5.59±0.28^{bc}	5.55±0.31^{c}	0.041	0.004	0.724
GPx U/mg	30.48±1.82^{a}	32.26±1.26^{ab}	32.69±1.41^{ab}	33.60±1.89^{b}	33.44±1.21^{b}	0.046	0.022	0.313
MDA nmol/g	222.09±9.08^{b}	218.22±7.74^{b}	215.12±9.52^{b}	215.50±12.32^{b}	203.49±4.19^{a}	0.025	0.035	0.524
PC μmol/g	5.52±0.16	5.50±0.20	5.56±0.08	5.62±0.15	5.59±0.24	0.921	0.438	0.973
LD μmol/g	0.45±0.04	0.45±0.05	0.44±0.04	0.45±0.02	0.47±0.02	0.941	0.616	0.518

Table 6 Flesh chemical composition of grass carp fed kaempferol supplemented diets (g/kg, fresh weight).

Chemical composition	Kaempforel addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
Moisture	781.6±1.5	774.1±1.2	776.9±0.8	781.6±12.7	778.9±2.8	0.513	0.840	0.390
Crude ash	11.8±0.1	12.2±0.1	12.1±0.1	11.6±0.3	11.6±0.2	0.120	0.115	0.064
Crude protein	189.1±2.0	189.8±1.8	188.1±0.1	183.4±10.2	189.6±2.6	0.492	0.542	0.456
Crude lipid	11.5±0.4	11.5±1.6	11.3±1.3	11.4±0.9	11.3±1.1	1.000	0.867	0.989
Collagen	2.44±0.25	2.48±0.36	2.54±0.24	2.49±0.35	2.53±0.17	0.948	0.51	0.743

Table 7 Flesh free amino acid composition of grass carp fed kaempferol supplemented diets (mg/kg, fresh tissue).

Free amino acid	Kaempferol addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
Asp	5.6±0.7^{a}	10.8±0.5^{bc}	10.8±1.2^{bc}	11.4±1.6^{c}	8.6±1.1^{b}	0.002	0.025	0.000
Thr	452.0±6.4^{a}	485.3±37.6^{ab}	502.6±19.6^{ab}	497.8±68.5^{ab}	547.7±23.0^{b}	0.017	0.014	0.894
Ser	55.8±4.7^{ab}	69.6±4.0^{b}	53.0±11.7^{a}	69.1±3.3^{b}	95.4±10.8^{c}	0.000	0.000	0.006
Glu	96.7±9.3^{bc}	75.1±10.2^{a}	104.7±12.0^{c}	86.5±3.2^{bc}	82.3±10.9^{ab}	0.025	0.345	0.542
Gly	293.8±36.4	323.7±37.1	355.8±36.1	364.4±48.0	376.0±19.3	0.264	0.037	0.566
Ala	273.6±35.6	261.5±30.8	288.1±28.8	280.1±36.8	265.2±29.7	0.960	0.985	0.712
Cys	38.1±3.3^{b}	27.1±2.0^{a}	34.9±4.1^{ab}	38.8±4.3^{b}	34.9±3.5^{ab}	0.026	0.593	0.381
Val	25.2±2.9^{a}	71.9±8.2^{bc}	66.9±10.7^{b}	87.2±8.1^{c}	74.8±9.0^{bc}	0.000	0.000	0.000
Met	12.5±0.6	11.8±1.9	12.5±1.3	14.8±2.0	14.6±2.1	0.231	0.055	0.508
Ile	18.1±1.4^{b}	21.3±1.3^{bc}	14.4±1.8^{a}	22.5±2.7^{c}	19.1±1.9^{bc}	0.003	0.365	0.697
Leu	34.9±2.7^{ab}	37.4±3.9^{b}	26.4±2.5^{a}	45.3±4.1^{b}	39.8±5.0^{b}	0.026	0.122	0.288
Tyr	20.0±2.6	34.9±2.4	30.8±3.8	41.7±4.7	35.6±2.4	0.141	0.099	0.571
Phe	61.1±8.2	62.1±6.7	58.5±6.7	55.3±4.1	58.7±6.1	0.828	0.417	0.417
Lys	258.0±37.2	287.4±29.3	338.1±40.7	307.7±32.1	263.5±27.7	0.463	0.773	0.098
His	387.9±33.5	393.3±19.6	389.3±6.4	378.0±23.7	393.3±8.5	0.885	0.906	0.781
Arg	254.0±34.7^{a}	302.1±22.8^{ab}	518.0±23.6^{d}	411.1±11.3^{c}	362.1±10.8^{bc}	0.004	0.019	0.004
Pro	498.2±52.5^{a}	648.2±54.2b	599.7±49.2^{a}	514.3±34.4^{a}	528.0±56.1^{a}	0.030	0.466	0.024
TFAA	2795.6±131.9^{a}	3123.6±231.0^{bc}	3404.5±108.9^{c}	3225.7±320.9^{b}	3199.5±29.5^{bc}	0.035	0.028	0.019
FAA	760.9±69.9^{a}	768.1±79.7^{a}	848.7±62.5^{b}	839.2±47.0^{b}	826.3±71.7^{b}	0.32	0.032	0.204

FAA (Asp, Gly, Glu, Ala, Phe, Tyr), flavour amino acids; TFAA: total free amino acids.

Table 8 Flesh fatty acid composition of grass carp fed kaempferol supplemented diets (%, percentage of total fatty acids).

Fatty acid	Kaempferol addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
C14:0	0.96±0.08	0.91±0.05	0.97±0.08	0.99±0.11	0.99±0.08	0.779	0.430	0.749
C16:0	14.10±0.45	14.30±0.15	14.00±0.20	14.24±0.36	13.95±0.82	0.857	0.666	0.662
C18:0	4.48±0.15	4.36±0.20	4.33±0.27	4.47±0.34	4.42±0.40	0.954	0.990	0.631
SFA	19.54±0.39	19.56±0.11	19.30±0.35	19.69±0.56	19.36±0.94	0.896	0.824	0.960
C16:1	5.66±0.13	5.53±0.46	5.48±0.15	5.58±0.17	5.67±0.08	0.829	0.872	0.274
C18:1	34.68±0.61	35.17±0.22	34.52±0.38	34.23±0.88	34.54±0.85	0.525	0.328	0.985
C20:1	1.82±0.01^{a}	1.79±0.05^{a}	1.90±0.04^{b}	1.84±0.06^{ab}	1.88±0.08^{ab}	0.008	0.009	0.922
MUFA	42.16±0.53	42.49±0.63	41.89±0.47	41.65±0.82	42.10±0.78	0.620	0.439	0.684
C18:2n6	16.90±0.68	16.89±0.54	17.19±0.21	17.59±0.35	17.12±0.64	0.491	0.258	0.481
C20:2n6	2.02±0.09	2.01±0.12	2.02±0.21	1.98±0.06	1.98±0.15	0.988	0.653	0.922
C20:3n6	2.91±0.10	2.87±0.04	2.90±0.04	2.93±0.04	2.90±0.02	0.498	0.806	0.851
C20:4n6	6.01±0.17	6.04±0.04	6.03±0.20	5.98±0.15	6.05±0.29	0.991	0.992	0.917
n-6PUFA	27.85±0.69	27.81±0.70	28.14±0.29	28.24±0.41	28.04±0.62	0.861	0.450	0.665
C18:3n3	2.05±0.13^{b}	1.90±0.10^{a}	2.02±0.15^{b}	2.06±0.14^{b}	2.05±0.14^{b}	0.561	0.546	0.534
C20:5n3	0.11±0.01	0.11±0.01	0.11±0.01	0.10±0.01	0.11±0.01	0.737	0.692	0.325
C22:5n3	3.89±0.17	3.81±0.09	4.02±0.15	3.86±0.11	3.95±0.13	0.416	0.511	0.893
C22:6n3	4.39±0.06	4.31±0.08	4.51±0.12	4.39±0.15	4.40±0.15	0.431	0.709	0.589
n-3PUFA	10.45±0.25	10.13±0.21	10.67±0.13	10.43±0.03	10.50±0.40	0.174	0.369	0.975
DHA/EPA	39.24±2.56	38.43±2.46	40.63±0.99	39.03±1.74	38.87±0.21	0.656	0.963	0.538

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 9 Flesh texture characteristics of grass carp fed kaempferol supplemented diets.

Texture characteristics	Kaempferol addition (g/kg)					Pr > F		
	0	0.2	0.4	0.6	0.8	ANOAV	Linear	Quadratic
Hardness (gf)	226.4±35.33^{a}	200.67±45.0^{a}	417.0±53.28^{c}	388.0±53.25^{c}	321.5±21.25^{b}	0.000	0.000	0.001
Adhesiveness (gf-mm)	1.25±0.16^{c}	0.67±0.06^{b}	0.26±0.08^{a}	0.35±0.08^{a}	0.81±0.07^{b}	0.000	0.000	0.000
Springiness	0.54±0.04	0.53±0.02	0.59±0.02	0.58±0.04	0.57±0.02	0.200	0.220	0.122
Chewiness (gf)	77.3±13.6^{a}	64.3±8.0^{a}	135.3±9.4^{b}	124.1±20.3^{b}	110.9±17.6^{b}	0.001	0.003	0.028
Cohesiveness (gf)	0.62±0.05	0.58±0.07	0.59±0.03	0.59±0.03	0.61±0.04	0.483	0.355	0.679
Resilience	0.97±0.09^{a}	0.94±0.04^{a}	1.10±0.08^{b}	1.07±0.11^{b}	1.08±0.05^{b}	0.015	0.182	0.029

Author statement

Zhen Xu and Hang Yang completed the experiment and prepared the manuscript. Xiao-Qin Li and Xiao-ying Xu carried out the growth experiment and analysed the samples. Xiang-Jun Leng and Hong-Xin Tan designed the experiment and revised the manuscript. The final manuscript has been approved by all authors prior to submission.

Declaration of interests

☑ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

1. A major concern is that the flesh quality of aquatic has been declining in recent years with poor taste and loose texture. We explored the effects of kaempferol on flesh quality grass carp (*Ctenopharyngodon idellus*).
2. The supplementation of kaempferol in diet effectively improved the growth, lipid metabolism and flesh quality of grass carp (*Ctenopharyngodon idellus*).
3. Metabonomics methods and real-time fluorescent quantitative PCR technology (RT-qPCR) were first used to explore the acting mechanism of kaempferol.