



Original Research Article

Dietary xanthophyll improved growth, antioxidant, pigmentation and meat quality in the southern catfish (*Silurus soldatovi meridionalis* Chen)

Chaoqun He ^a, Haoyu Geng ^a, Yawen Qin ^a, Peng Yang ^a, Wenqiang Wang ^a, Kangsen Mai ^a, Fei Song ^{a, b, *}

^a Institute of Modern Aquaculture Science and Engineering (IMASE), Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitoring, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou, 510631, China

^b Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai, 519000, China

ARTICLE INFO

Article history:

Received 3 August 2022

Received in revised form

14 December 2022

Accepted 21 December 2022

Available online 27 December 2022

Keywords:

Xanthophyll

Growth performance

Antioxidant capacity

Fish quality

Southern catfish (*Silurus soldatovi meridionalis* Chen)

ABSTRACT

Xanthophyll has multiple physiological functions to improve the quality of farmed animals. The present study aimed to investigate the effects of xanthophyll on the growth performance, antioxidation, immunity, pigmentation and meat quality of southern catfish (*Silurus soldatovi meridionalis* Chen). Juvenile southern catfish (18.35 ± 0.04 g) were randomly allocated into 24 cages (30 juveniles per cage), and fed diets with different dietary xanthophyll levels (at 14, 42, 80, 108, 126 and 152 mg/kg, dry matter of diet) twice daily for 8 weeks. Results indicated that the diet with 80 mg/kg xanthophyll induced a higher specific growth rate (SGR), feed efficiency ratio and protein productive value. Moreover, the 80 mg/kg xanthophyll diet also increased complement 3, immunoglobulin M (IgM) and lysozyme content more than the other groups. The mRNA expression level of inflammation-related genes and antioxidant enzyme activities further confirmed the effects of 80 mg/kg dietary xanthophyll on improving immune response. The present study also found that the 126 mg/kg xanthophyll diet significantly enhanced the content of total carotenoids and xanthophyll, hydroxyproline, collagen and amino acid in muscle. The diet with 126 mg/kg xanthophylls also induced lower drip loss, thawing loss, centrifugal loss, cooking loss and higher muscle adhesiveness, cohesiveness, springiness, gumminess and chewiness than the other treatments. In conclusion, quadratic regression model analysis based on SGR and IgM revealed that the optimum xanthophyll level in the diet was 86.78 and 84.63 mg/kg, respectively. Moreover, broken line regression analysis based on xanthophyll content in dorsal skin and chewiness in muscle demonstrated that the optimal xanthophyll level was between 89.73 and 108.66 mg/kg in the diet of southern catfish (*S. soldatovi meridionalis* Chen).

© 2023 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Xanthophyll is a natural carotenoid synthesized only by plants (Do et al., 2022). Previous studies have demonstrated that

xanthophyll is an effective functional compound with many biological properties that benefit the health and meat quality of animals (Bédécarrats and Leeson, 2006; Rajput et al., 2013; Xu et al., 2014; Yuan et al., 2021; Yang et al., 2022). Nowadays, it has been widely used in diverse fields, especially in the feed industry (Breithaupt, 2007). Lutein and zeaxanthin are two active components of xanthophyll which have showed good performance in enhancing health status and body color (Yi et al., 2014; Herath et al., 2016; Wang et al., 2020; Yeşilayer et al., 2020). Therefore, revealing the effects of xanthophyll on improving immune response and pigmentation in skin will provide a theoretical basis for improving its application in the feed industry.

* Corresponding author.

E-mail address: songfei@m.scnu.edu.cn (F. Song).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



Production and Hosting by Elsevier on behalf of KeAi

Xanthophyll is an active compound that participates in multiple physiological functions in humans to invertebrates (Thomas and Johnson, 2018). Previous studies have reported that lutein, the active ingredient of xanthophyll, has great effects on stimulating immune and antioxidant systems, as well as reducing feed consumption. Wang et al. (2020) showed that dietary xanthophyll supplementation significantly enhanced the stress response by regulating the activities and mRNA expression of the key enzymes and regulators involved in antioxidant capacity in Chinese soft-shelled turtle (*Pelodiscus sinensis*). Moreover, zeaxanthin is an excellent pigment to improve skin color. Some researchers conducted experiments in animals to elucidate the effects of zeaxanthin on improving the yellowness and carotenoid content both in abdominal and dorsal skin (Sun et al., 2012; Wang et al., 2012; Yi et al., 2014; Yeşilayer et al., 2020). Turkey meat quality could also be improved by dietary protein-xanthophyll supplementation (Dolatowski et al., 2008). On the whole, although some related studies have been carried out in aquatic animals, the effects of xanthophyll on the regulation of growth, metabolism and health in fish species remains to be elucidated.

Southern catfish (*Silurus soldatovi meridionalis* Chen) is a commonly cultured species of catfish due to its high nutritional value, rapid growth, strong reproduction and disease resistance abilities (Wei et al., 2014). It is mainly distributed in the southern region of the Yangtze River in China (Li and Xie, 2019). However, intensive farming brings a series of problems. An increasing rate of disease outbreaks and decline in the quality of products has severely restricted the development of this industry (Zhang et al., 2018; Fu et al., 2019a and b). Therefore, developing new additives to solve these problems has become a key factor to overcome these challenges in the aquaculture industry. In the present study, the effects of xanthophyll addition in the diet as a new additive regulating the growth, health and quality of fish were evaluated, providing a solution to solve the problems in the aquaculture industry.

2. Materials and methods

2.1. Ethics statement

Procedures used in this study strictly complied with the regulations of the University Animal Care and Use Committee of South China Normal University (approval reference number 1002019-02-0016).

2.2. Experimental diets

The basal diet was formulated using fish meal, poultry by-product meal and cottonseed meal as the main protein sources, and soybean oil and soybean phospholipid oil as the main sources of lipids (Table 1). Meanwhile, methionine, lysine and threonine were added to balance the amino acid requirements. Different levels (0.0%, 0.3%, 0.6%, 0.9%, 1.2% and 1.5%, dry matter of diet) of xanthophyll (lutein 2%, zeaxanthin 35%; Guangzhou Lidar Biotechnology Co., Ltd., Guangzhou, China) were added to the basal diet to formulate 6 experimental diets. The tested contents of xanthophyll in the 6 diets were 14, 42, 80, 108, 126 and 152 mg/kg, respectively. Raw materials were thoroughly mixed and sieved through 320 μm mesh screens. After the addition of water and oil, the diets were homogenized using a blender. After that, the mixture was wet-extruded into particles using a granulator (F-26, South China University of Technology, China). All diets were dried at 40 °C for 24 h, then stored at -20 °C until use.

2.3. Experiment and sample collection

The culture system was an outdoor pool (length 20.0 m \times width 3.0 m \times depth 2.0 m) with a water inlet and outlet system, a microporous oxygenation system which contained a 1.5-kW Roots blower and 36 microporous oxygenation facilities, in which 24 breeding cages (length 1.0 m \times width 1.0 m \times depth 1.0 m) were suspended inside. Water temperature, dissolved oxygen content, pH and the ammonia nitrogen content were detected by a water quality testing meter (U fish, 959, Guangzhou, China). After 2 weeks adaptation to the experimental conditions, 720 juvenile southern catfish (IBW = 18.4 \pm 0.02 g) with good vibrancy were selected and randomly divided into 24 cages at the stocking density of 30 fish per cage. The 24 cages were randomly divided into 6 groups, each fed a different experimental diet. Fish were fed twice daily for 8 weeks (07:30 and 17:30) to apparent satiety for 25 to 35 min. For the whole experimental period, the water temperature was controlled at 25–30 °C, the dissolved oxygen content was \geq 5.0 mg/L, the pH was 7.5–8.0, and the ammonia nitrogen content was maintained at <0.08 mg/L.

2.4. Sample collection

Before sampling, all fish were weighed and counted after 24 h of starvation. Six fish were randomly selected from each cage, then photographed, and body weight was recorded individually after anesthesia with 100 mg/L MS-222 (Jinjiang Aquatic Supplies Co., Ltd., China). Blood samples were collected by using the caudal venipuncture method, and placed into anticoagulation tubes (sodium heparin) for plasma and blood cell preparation. Visceral mass and liver were dissected and weighed, while liver, midgut, dorsal kidney, dorsal muscle, dorsal skin and abdominal skin were dissected and placed in RNA-free enzyme tubes and frozen in liquid nitrogen immediately. Additionally, 2 fish randomly selected from each cage was dissected to obtain the dorsal muscle of the same position and size (3 cm \times 3 cm \times 0.5 cm). The whole fish were also collected to analyze the nutrient composition.

2.5. Proximate composition of whole fish and muscle

Proximate composition of whole body and muscle were measured using standard methods (AOAC, 2005). The moisture content was detected by continuously drying the samples at 105 °C to a constant weight. Crude protein content was estimated using the Dumas nitrogen determination apparatus (Dumatherm DT N PRO, Gerhart Analytical Systems, Germany). The Soxtec 2055 extraction (Soxtec 8000, FOSS Co., Ltd., Denmark) by using petroleum ether (boiling point 30–60 °C for 3 h) was used for crude lipid determination. Ash content was analyzed by combusting samples in a muffle furnace (FO610C, Yamato Scientific Co., Ltd., Japan) at 550 °C for 8 h until samples reached a constant weight.

2.6. Biochemical analysis of plasma

Physiological and biochemical parameters of plasma indices were measured according to the method of Wang et al. (2020) and Yang et al. (2020). An automated biochemical analyzer (CHEMIX 800, Sysmex Corporation, Japan) was used to determine albumin (ALB), globulin (GLOB), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), glutamate aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) contents.

Table 1
Ingredients and proximate composition of experimental diets (% dry weight).

Item	Diets					
	X14	X42	X80	X108	X126	X152
Ingredients						
Fish meal ¹	25.0	25.0	25.0	25.0	25.0	25.0
Poultry by-product meal ²	24.0	24.0	24.0	24.0	24.0	24.0
Cottonseed meal ³	10.0	10.0	10.0	10.0	10.0	10.0
Corn gluten meal ⁴	8.50	8.50	8.50	8.50	8.50	8.50
Soybean meal ⁵	2.00	2.00	2.00	2.00	2.00	2.00
Wheat meal ⁶	17.30	17.3	17.3	17.3	17.3	17.3
Soybean oil ⁷	4.20	4.20	4.20	4.20	4.20	4.20
Soybean lecithin oil ⁸	3.50	3.50	3.50	3.50	3.50	3.50
Monocalcium phosphate ⁹	1.50	1.50	1.50	1.50	1.50	1.50
Choline chloride ¹⁰	0.40	0.40	0.40	0.40	0.40	0.40
Mineral premix ¹¹	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix ¹²	0.50	0.50	0.50	0.50	0.50	0.50
Lysine ¹³	0.25	0.25	0.25	0.25	0.25	0.25
Methionine ¹⁴	0.15	0.15	0.15	0.15	0.15	0.15
Threonine ¹⁵	0.15	0.15	0.15	0.15	0.15	0.15
Xanthophyll ¹⁶	0.00	0.30	0.60	0.90	1.20	1.50
Ethoxyquin ¹⁷	0.01	0.01	0.01	0.01	0.01	0.01
Propionic acid ¹⁸	0.07	0.07	0.07	0.07	0.07	0.07
Microcrystalline cellulose ¹⁹	1.97	1.67	1.37	1.07	0.77	0.47
Total	100	100	100	100	100	100
Proximate composition						
Dry matter	89.7	88.1	89.6	88.7	89.1	89.4
Crude protein	47.3	47.1	46.9	47.1	46.8	46.9
Crude lipid	14.1	14.0	14.1	13.9	13.9	14.0
Xanthophyll, mg/kg	14.0	42.0	80.0	108	126	152

Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively.

¹ Fish meal (crude protein: 66.8%, crude lipid: 7.80%) obtained from TASA Fish Product Co., Ltd., Peru.

² Poultry by-product meal (crude protein: 65.8%, crude lipid: 12.4%) obtained from American Proteins Inc., USA.

³ Cottonseed meal (crude protein: 60.7%, crude lipid: 7.20%) obtained from Xinjiang Jinlan Plant Protein Co., Ltd., Shihezi, Xinjiang, China.

⁴ Corn gluten meal (crude protein: 60.5%, crude lipid: 0.93%) obtained from Heilongjiang Golden Elephant Biochemical Co., Ltd., Harbin, Heilongjiang, China.

⁵ Soybean meal (crude protein: 43.2%, crude lipid: 0.00%) obtained from China National Textile Group Co., Ltd., Beijing, China.

⁶ Wheat meal obtained from Yihai Jiali Golden Dragon Fish Grain, Oil and Food Co., Ltd., Shanghai, China.

⁷ Soybean oil obtained from Cargill Cereals and Oils (Yangjiang) Co., Ltd., Yangjiang, Guangdong, China.

⁸ Soybean lecithin oil obtained from Tianbao Animal Nutrition Technology Co., Ltd., Chuxiong, Yunnan, China.

⁹ Monocalcium phosphate obtained from Dahai Grain and Oil Industry (Fangchenggang) Co., Ltd., Fangchenggang, Guangxi, China.

¹⁰ Choline chloride obtained from Guangdong Haida Group Co., Ltd., Guangzhou, Guangdong, China.

¹¹ Mineral premix (Guangdong Haida Group Co., Ltd., Guangzhou, Guangdong, China) provides the following (mg/kg, dry matter diet): Cu (CuSO₄), 2.00; Zn (ZnSO₄), 34.40; Mn (MnSO₄), 6.20; Fe (FeSO₄), 21.10; Ca (Ca (IO₃)₂), 1.63; Se (Na₂SeO₃), 0.18; Co (COCl₂), 0.24; Mg (MgSO₄·H₂O), 52.70.

¹² Vitamin premix (Guangdong Haida Group Co., Ltd., Guangzhou, Guangdong, China) provides the following (mg/kg, dry matter diet): Vitamin A, 16,000 IU; vitamin D₃, 8,000 IU; vitamin K₃, 14.72; vitamin B₁, 17.80; vitamin B₂, 48; vitamin B₆, 29.52; vitamin B₁₂, 0.24; vitamin E, 160; vitamin C, 800; niacinamide, 79.20; calcium pantothenate, 73.60; folic acid, 6.40; biotin, 0.64; inositol, 320; L-carnitine, 100.

¹³ Lysine obtained from Guangzhou Haida Feed Co., Ltd., Guangzhou, Guangdong, China.

¹⁴ Methionine obtained from Guangzhou Haida Feed Co., Ltd., Guangzhou, Guangdong, China.

¹⁵ Threonine obtained from Guangzhou Haida Feed Co., Ltd., Guangzhou, Guangdong, China.

¹⁶ Xanthophyll (Dry matter of product, mg/g) obtained from Guangzhou Lidar Biotechnology Co., Ltd., Guangzhou, Guangdong, China. Lutein, 20; Zeaxanthin, 350.

¹⁷ Ethoxyquin obtained from Yixing Tianshi Feed Co., Ltd., Wuxi, Jiangsu, China.

¹⁸ Propionic acid obtained from Shandong Tongtai Weirun Food Technology Co., Ltd., Zaozhuang, Shandong, China.

¹⁹ Microcrystalline cellulose obtained from Shandong Liujia Pharmaceutical Accessories Co., Ltd., Jining, Shandong, China.

2.7. ROS and apoptosis rate of blood cells

Reactive oxygen species (ROS) content and apoptosis rate of blood cells were determined using a reactive oxygen species assay kit (S003SS; Beyotime, Nantong, China) and annexin V-FITC apoptosis detection kit (CLO62L; Beyotime, Nantong, China). After passing through a 70- μ m cell sieve, blood cells were processed according to the manufacturer's instructions and analyzed by flow cytometry (LSR Fortessa X-20, BD, Biosciences, US). The average fluorescence intensity was used to indicate the content of ROS in blood cells, and the proportion of cells in apoptotic periods was used to indicate the apoptosis rate.

2.8. Immunoproteins and antioxidant enzymes in plasma

Plasma immunoglobulin M (IgM), complement 3 (C3), complement 4 (C4) contents and lysozyme (LZM) activity were determined using the immunoprotein assay kit (H109-1-1, H186-1-1, H186-2-1, A150-1-1; Jiancheng Bioengineering Institute, Nanjing, China) by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The contents of IgM, C3 and C4 were calculated by the standard curve method and LZM activity was determined using the turbidimetric method. The activities of total antioxidant capacity (T-AOC) and superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and content of malondialdehyde (MDA) in plasma were determined by using the kits (A105-1-1, A001-1-2, A006-1-1, A007-2-1, A003-1-2; Jiancheng Bioengineering Institute, Nanjing, China). T-AOC and SOD, GSH, CAT and MDA of plasma were determined by the ferric ion reducing antioxidant power (FRAP) method, hydroxylamine method, spectrophotometric method and thiobarbituric acid (TBA) method, respectively.

2.9. RT-PCR for immune factor genes

According to the method of Qin et al. (2021) and W Wang et al. (2021), total mRNA was extracted by using RNA isolator total extraction reagent (R401-01; Vazyme, Nanjing, China). RNA was dissolved in diethyl pyrocarbonate (DEPC) treated water, and then was tested for purity and integrity by using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Thermo, US) and 1.2% agarose gel electrophoresis. One microgram RNA was sucked and reverse transcribed into cDNA by using the Prime Script RT kit (R223-01; Vazyme, Nanjing, China). A 20- μ L q-PCR system was formulated with a cDNA template, quantitative primers (Table 2), ddH₂O and the fluorescent quantitative enzyme SYBR qPCR Master Mix (R311-02; Vazyme, Nanjing, China). Then, RT-PCR was performed using a real-time PCR machine (CFX96, BIO-RAD, USA) to determine the target gene expression levels. Melting curve analysis was executed to confirm that only a single product was present in the reaction. Relative expression levels of target genes were calculated using the 2^{- $\Delta\Delta$ Ct} method.

2.10. Total concentration of carotenoids and xanthophyll in tissues

The total carotenoids in skin and muscle were determined according to the method of Yanar et al. (2004). A 200 mg sample and 100 mg of homogenized anhydrous sodium sulfate were added to 1.5 mL of acetone, and stored at 4 °C for 3 days. Then, 200 μ L supernatant was separated and measured at 480 nm by using an enzyme marker (M200 PRO, TECAN, Switzerland). The xanthophyll in diets, skin and muscle was analyzed according to the method of Quackenbush and Miller (1972). A 200 mg sample was mixed with 1.5 mL of extraction solution (hexane:acetone:anhydrous ethanol:toluene = 10:7:6:7) in a brown stopper sample bottle for

1 min with full rotation. Then 200 μ L 40% potassium hydroxide-methanol solution was added with rotation and shaking for 1 min. After being heated up in a water bath at 55.5 °C for 20 min, the sample was then cooled down in the dark for 1 h. After adding 1.5 mL of hexane and shaking for 1 min, the volume was kept at 5 mL by using 10% sodium sulfate solution and shaken vigorously for 1 min. After keeping in the dark for 1 h, 0.5 mL of supernatant was separated and diluted with hexane. The absorbance value was measured at 474 nm and the total xanthophyll content was calculated according using a standard curve.

2.11. Muscle pH and water-holding capacity (WHC)

Muscle pH was determined according to Wei et al. (2016). Fresh muscle samples were placed in plastic sealed pockets and put in the 4 °C refrigerator for 45 min, then the meat infusion was prepared by using ultrapure water. WHC was determined referring to the methods of Wei et al. (2016), Brinker and Reiter (2011) and Su et al. (2022). Fresh muscle samples were weighed and hung in bags, placed in a refrigerator at 4 °C for 24 h, then dried and weighed to calculate the drip loss. Fresh muscle samples were weighed and wrapped in filter paper and centrifuged (500 \times g, 10 min at 10 °C) in a centrifuge tube (with filter paper at the bottom of the centrifuge tube) to calculate the centrifugal loss. After that, fresh muscle samples were weighed and cooked at 70 °C for 20 min to calculate the cooking loss.

2.12. Muscle texture

Muscle texture was tested using a texture analyzer (TA. XT Plus, Stable Micro Systems, English) at room temperature and a flat-bottomed column probe P/36, simulating human teeth chewing food, and by performing 2 compression texture profile analysis tests on fresh samples. The test conditions were as follows: pre-test rate 5 mm/s, test rate 1 mm/s, post-test rate 5 mm/s, compression level 60%, dwell interval 5 s, weighted probe type auto-5 g, and data collection rate 200 times/s.

2.13. Muscle hydroxyproline (Hyp) and collagen content

Hyp was determined using a test kit (A030-2-1; Jiancheng Bioengineering Institute, Nanjing, China). Samples were processed according to the instructions using the alkaline hydrolysis method. Collagen determination was performed according to Crouse et al. (1986). Hyp content in collagen was 13.4%, and the conversion of collagen content was performed.

2.14. Muscle amino acid composition

Fresh muscle samples were fully dried by a freeze dryer (GAMMA 1-16/2-16LSC, Christ, Germany), and 2 g of freeze-dried

muscle samples were hydrolyzed with 6 mol/L hydrochloric acid. After digestion, vacuum drying and dilution, samples were ground through a 0.22- μ m filter membrane and measured by amino acid analyzer (L-8900, Hitachi, Japan).

2.15. Heat stress test

After the 8-week experiment, 8 fish from each cage were selected to do the heat stress test. They were put into a recirculating water system with temperature control equipment. The water temperature rose from 28 to 34 °C in 2 days. The volume of each tank was about 500 L. The water source was urban tap water, which was used after full aeration. The flow rate of the water inlet of each tank was about 363 L/h. After the water temperature reached 34 °C, heat stress test began. During this experiment, the experimental fish were fed until apparent satiety. All fish were put into high water temperature tanks (34 \pm 1 °C) for 1 week. The number of fish was recorded every day to calculate mortality. A blood sample was obtained after a 1-week challenge for physiological and biochemical analysis.

2.16. Statistical analysis

Data were analyzed by SPSS 26.0 (IBM SPSS Statistics, Version 26.0, Armonk, NY, USA). Results were presented as mean \pm standard error of mean (SEM), and significant differences among treatment groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple range test, with the level of significance set at $P < 0.05$. Meanwhile, representative data were used to simulate the best curve of the effects of different xanthophyll levels on the growth, immunity, body color and meat quality of southern catfish.

3. Results

3.1. Growth performance and body proximate composition

The growth performance and body proximate composition after feeding with different diets is presented in Table 3. The final body weight (FBW), weight gain rate (WGR), special growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER) and protein productive value (PPV) significantly improved with increased dietary xanthophyll level up to 80 mg/kg diet, and then decreased. Whereas the hepatosomatic index (HSI), viscerasomatic index (VSI) and condition factor presented a decreased pattern after xanthophyll was gradually added, and the 152 mg/kg group had the lowest HSI and VSI out of all groups ($P < 0.05$). Moreover, xanthophyll supplementation had no effects on changing the feed intake (FI) ($P > 0.05$). The crude protein content also increased with dietary xanthophyll and the highest was in the group fed with 126 mg/kg diet ($P < 0.05$). Meanwhile, the crude lipid content showed a

Table 2

The sequences of the primers used in the RT-PCR.

Gene	Forward sequence (5'–3')	Reverse sequence (5'–3')	Reference
β -actin	GGCATCACACCTTCTACAACGA	ACGCTCTGTCAGGATCTTCA	Dong et al. (2013)
<i>IL-1β</i>	GAACATCCAGTCCACCGAGT	GGAAGATTCTCCTCCGTACCA	Cloned by degenerate PCR strategy
<i>C4</i>	ATCTGGCGTATGTCTCGCA	ATGGTTTGTCTGACAGCC	Cloned by degenerate PCR strategy
<i>C3</i>	TGTGCCAAAACAGTCGGAAGTC	GCTGAACCTGTCTGAGCGTGT	Fu et al. (2019a)
<i>C9</i>	GGGCATCGGGGAAGGTCAG	GAAATCAAAGACCCAAAGAAGACG	Fu et al. (2019b)
<i>Hsc70</i>	CAAGATCAGTGACGAGGACAAG	GGTTACAGACTTCTCCAGTTCC	(Refaey and Li, 2018)
<i>Hsp70</i>	CTTGATGTTACCCCTCTGTCTCT	TCAGAGTAGTGGTGAAAGTCTG	(Refaey and Li, 2018)
<i>Hsp90</i>	ATCTGAAGGAGGATCAGACAGAG	CGCTCCTCTTACAAAGAGTG	(Refaey and Li, 2018)
<i>IκB-α</i>	TGAACGAGGAGCCGAGGATTA	GCCGTCTGTCTGTTAGTTC	Cloned by degenerate PCR strategy

IL-1 β = interleukin 1 β ; *C4* = complement 4; *C3* = complement 3; *C9* = complement 9; *Hsc70* = heat shock cognate 70; *Hsp70* = heat shock protein 70; *Hsp90* = heat shock protein 90; *I κ B- α* = nuclear factor κ B inhibitory protein α .

Table 3
Growth performance and proximate composition of whole fish of southern catfish fed different xanthophyll levels for 8 weeks.

Item	Diets						P-value
	X14	X42	X80	X108	X126	X152	
Growth performance							
IBW, g	18.4 ± 0.02	18.4 ± 0.01	18.4 ± 0.01	18.4 ± 0.01	18.4 ± 0.01	18.4 ± 0.01	0.980
FBW, g	149 ± 4.49 ^a	160 ± 2.12 ^{ab}	181 ± 5.33 ^b	162 ± 1.58 ^{ab}	167 ± 7.60 ^{ab}	152 ± 3.37 ^a	0.005
WGR, %	712 ± 24.9 ^a	774 ± 10.8 ^{ab}	884 ± 28.6 ^b	781 ± 8.07 ^{ab}	810 ± 41.6 ^{ab}	730 ± 17.9 ^a	0.005
SGR, %/d	4.19 ± 0.06 ^a	4.34 ± 0.02 ^{ab}	4.57 ± 0.06 ^b	4.36 ± 0.02 ^{ab}	4.41 ± 0.09 ^{ab}	4.31 ± 0.05 ^{ab}	0.011
FER	1.06 ± 0.04 ^a	1.22 ± 0.01 ^{bc}	1.28 ± 0.06 ^c	1.23 ± 0.01 ^{bc}	1.13 ± 0.02 ^{ab}	1.12 ± 0.01 ^{ab}	0.002
FI, g/d	2.49 ± 0.16	2.35 ± 0.05	2.54 ± 0.12	2.34 ± 0.01	2.66 ± 0.08	2.38 ± 0.10	0.193
PER	1.06 ± 0.04 ^a	1.18 ± 0.02 ^{abc}	1.28 ± 0.06 ^c	1.23 ± 0.01 ^{bc}	1.17 ± 0.03 ^{abc}	1.13 ± 0.01 ^{ab}	0.006
PPV, %	35.2 ± 1.08 ^a	38.9 ± 0.92 ^{abc}	42.3 ± 1.64 ^c	40.5 ± 0.19 ^{abc}	38.1 ± 0.56 ^{abc}	36.5 ± 0.18 ^{ab}	0.038
HSI, %	1.99 ± 0.03 ^e	1.90 ± 0.06 ^{de}	1.79 ± 0.02 ^{cd}	1.79 ± 0.03 ^{bc}	1.59 ± 0.06 ^{ab}	1.50 ± 0.04 ^a	0.000
VSI, %	7.69 ± 0.19 ^b	7.44 ± 0.14 ^b	7.29 ± 0.07 ^{ab}	7.22 ± 0.10 ^{ab}	7.19 ± 0.04 ^{ab}	6.80 ± 0.18 ^a	0.012
CF, g/cm ³	0.75 ± 0.01 ^b	0.66 ± 0.00 ^a	0.69 ± 0.01 ^a	0.67 ± 0.00 ^a	0.68 ± 0.01 ^a	0.69 ± 0.00 ^a	0.000
Moisture, %	74.2 ± 0.24	73.8 ± 0.31	74.0 ± 0.18	74.5 ± 0.41	74.4 ± 0.06	74.7 ± 0.32	0.261
Proximate composition of whole fish							
Crude protein, %	15.4 ± 0.06 ^a	15.5 ± 0.04 ^{ab}	15.6 ± 0.07 ^{ab}	15.7 ± 0.07 ^{abc}	15.9 ± 0.05 ^c	15.8 ± 0.01 ^{bc}	0.001
Crude lipid, %	7.47 ± 0.17 ^b	7.04 ± 0.33 ^{ab}	6.97 ± 0.14 ^{ab}	6.88 ± 0.05 ^{ab}	6.62 ± 0.03 ^a	6.65 ± 0.03 ^a	0.034
Ash, %	2.68 ± 0.01	2.70 ± 0.01	2.72 ± 0.00	2.74 ± 0.04	2.76 ± 0.02	2.75 ± 0.02	0.113

IBW = initial body weight; FBW = final body weight; WGR = weight gain rate; SGR = special growth rate; FER = feed efficiency ratio; FI = feed intake; PER = protein efficiency ratio; PPV = protein productive value; HSI = hepatosomatic index; VSI = viscerasomatic index; CF = condition factor.

Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively.

Data are presented as the mean ± SEM ($n = 4$), values in the same row with different superscripts are significantly different ($P < 0.05$).

For the following equations, W_0 and W_t represented the body weight (g) of initial fish and final fish, respectively, while t represented the rearing days of this experiment.

Weight gain rate (WGR, %) = $100 \times (W_t - W_0)/W_0$.

Specific growth rate (SGR, %/day) = $100 \times (\ln W_t - \ln W_0)/t$.

Feed intake (FI, g/d) = dry feed intake/ t .

Feed efficiency ratio (FER) = $(W_t - W_0)/(\text{dry feed intake})$.

Protein efficiency ratio (PER) = $(W_t - W_0)/(\text{dry feed intake} \times \text{protein percent in dry diet})$.

Protein productive value (PPV, %) = $100 \times (W_t \times \text{final carcass protein content} - W_0 \times \text{initial carcass protein content})/\text{protein intake}$.

Hepatosomatic index (HSI, %) = $100 \times (\text{liver wet weight}/\text{whole wet weight})$.

Viscerasomatic index (VSI, %) = $100 \times (\text{viscera wet weight}/\text{body wet weight})$.

Condition factor (CF, g/cm³) = $100 \times (\text{final body weight}/\text{body length}^3)$.

decreased trend after fish were gradually supplemented with xanthophyll, resulting in the 126 mg/kg group having lowest crude lipid content compared with the other groups ($P < 0.05$). No significant differences were observed in moisture and ash contents among all groups ($P > 0.05$). The quadratic regression model based on SGR showed that the optimum xanthophyll supplementation level was 86.78 mg/kg (Fig. 1).

3.2. Plasma biochemistry

The plasma biochemical indices of the catfish fed the test diets are presented in Table 4. The fish fed 80 mg/kg diet had higher activities of ALB and lower ALP in plasma than the fish fed 14 mg/kg

diet ($P < 0.05$). However, a higher levels xanthophyll supplementation increased the AST and ALT concentration in plasma ($P < 0.05$). The highest activities of AST and ALT were found in the 126 and 152 mg/kg groups ($P < 0.05$).

3.3. ROS production and apoptosis rate in blood cells

The ROS production and apoptosis rate of blood cells in each group are presented in Fig. 2. The concentration of ROS production in blood cells was significantly enhanced as the xanthophyll level increased up to 80 mg/kg diet, and then decreased. Conversely, the apoptosis rate of blood cells showed the opposite pattern with the lowest in fish fed with xanthophyll at 80 mg/kg diet.

3.4. Antioxidative parameters

The antioxidant parameters in the plasma are presented in Fig. 3. The concentration of T-AOC was decreased with increased levels of xanthophyll, and lowest in the 152 mg/kg xanthophyll group. While the MDA level significantly decreased as the xanthophyll level increased up to 80 mg/kg diet, and then increased ($P < 0.05$). On the contrary, the SOD and CAT levels showed the opposite pattern being the highest in fish fed xanthophyll at a level of 80 mg/kg diet. Moreover, the GSH level gradually increased with higher levels of xanthophyll, and was highest in the 152 mg/kg xanthophyll supplementation group ($P < 0.05$).

3.5. Mortality and plasma biochemical changes after heat stress test

The mortality and plasma biochemical changes after the heat stress test are shown in Table 5. Reared in $34 \pm 1^\circ\text{C}$ for 1 week, the mortality rate was significantly reduced as the xanthophyll level

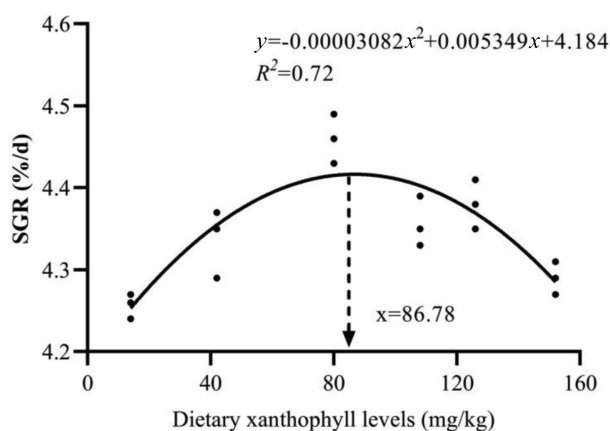


Fig. 1. Quadratic regression model analysis based on special growth rate (SGR) providing the optimal xanthophyll levels of southern catfish.

Table 4
Plasma physiological and biochemical indices of southern catfish after feeding different xanthophyll levels for 8 weeks.

Item	Diets						P-value
	X14	X42	X80	X108	X126	X152	
ALB, g/L	10.1 ± 0.03 ^a	10.9 ± 0.03 ^c	11.2 ± 0.03 ^d	10.9 ± 0.00 ^c	10.5 ± 0.03 ^b	10.5 ± 0.00 ^b	0.000
AST, U/L	68.3 ± 0.33 ^b	74.7 ± 0.33 ^c	74.3 ± 0.33 ^c	68.3 ± 0.88 ^b	77.7 ± 0.33 ^d	61.3 ± 0.33 ^a	0.000
ALT, U/L	10.7 ± 0.88 ^a	15.7 ± 0.33 ^b	15.0 ± 0.00 ^b	15.0 ± 0.00 ^b	14.0 ± 0.00 ^b	18.0 ± 0.00 ^c	0.000
ALP, U/L	4.33 ± 0.33 ^b	5.67 ± 0.33 ^{cd}	2.67 ± 0.33 ^a	6.00 ± 0.00 ^d	5.00 ± 0.00 ^{bcd}	4.67 ± 0.33 ^{bc}	0.000

ALB = albumin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase. Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Data are presented as the mean ± SEM (n = 4), values in the same row with different superscripts are significantly different (P < 0.05).

increased up to 108 mg/kg diet, and then elevated. For the biochemical changes, the activities of ABL, GLOB, AST, HDL and LDL in plasma were markedly increased as the xanthophyll level increased up to 80 mg/kg diet, and then decreased. The activities of LDH and ALT also increased as the xanthophyll level increased up to 42 and 126 mg/kg diet, respectively, and then decreased.

3.6. Plasma complement, immunoglobulin contents and lysozyme activity

As shown in Fig. 4, the content of C3, C4, IgM and LZM activity in plasma were significantly increased as the xanthophyll level increased up to 80 mg/kg diet, and then decreased. Furthermore,

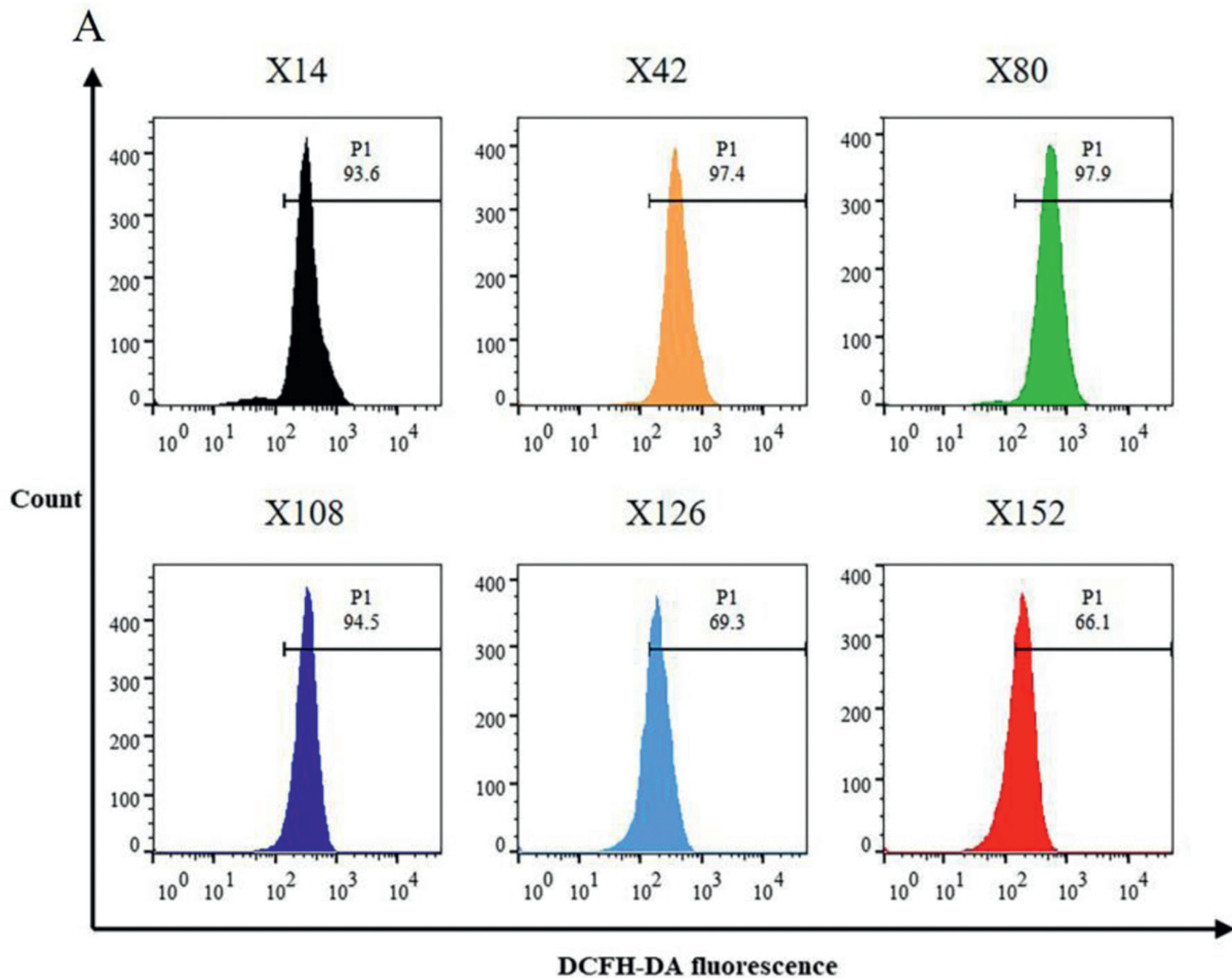


Fig. 2. Effects of dietary xanthophyll supplementation levels on (A, C) the content of ROS and (B, D) apoptosis rate in blood cells. Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Values in the same bars with different superscripts are significantly different (P < 0.05). ROS = reactive oxygen species; DCFH-DA = dichlorodihydrofluorescein diacetate; PI = propidium iodide; FITC = fluorescein isothiocyanate.

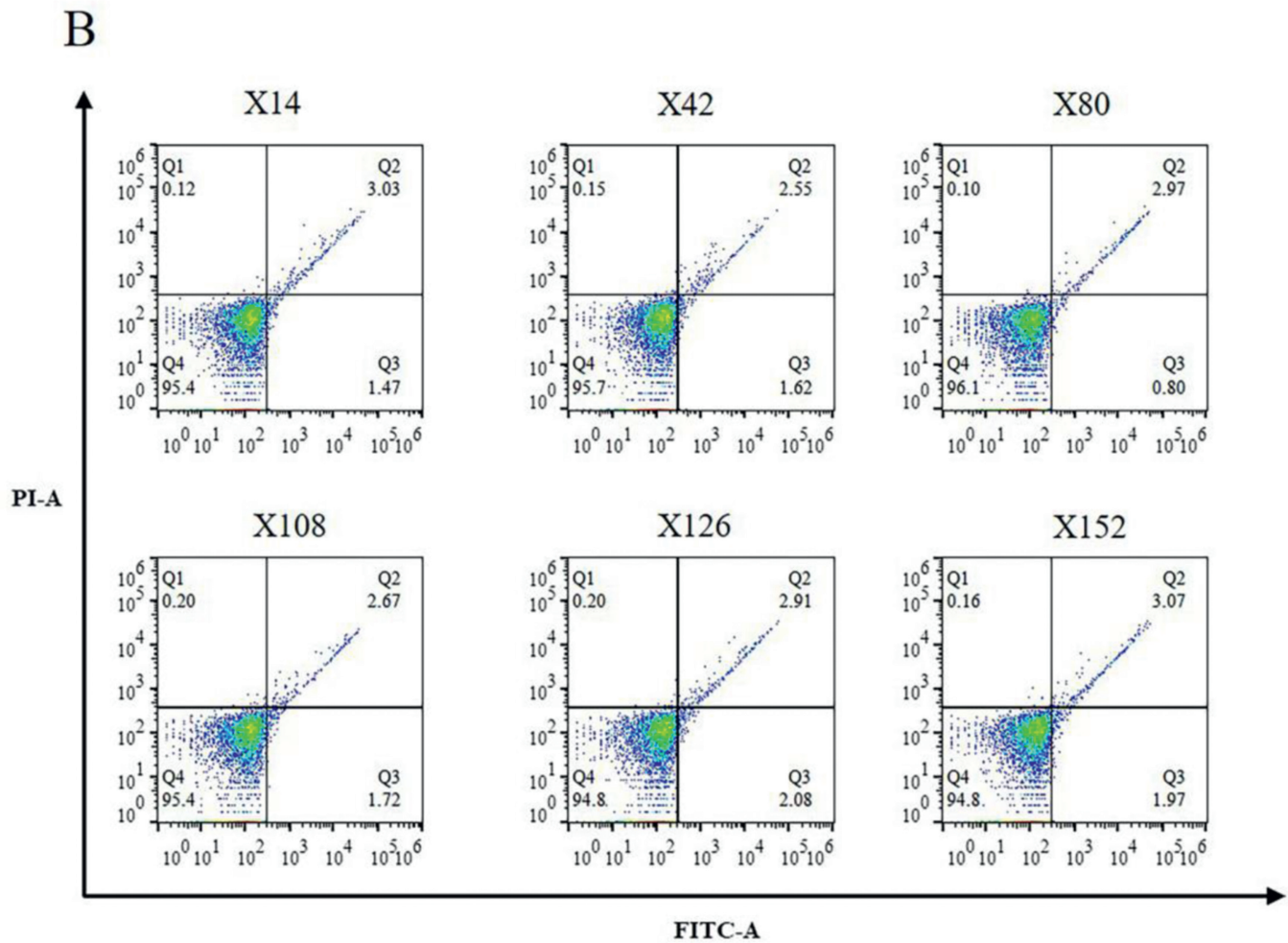


Fig. 2. Continued

the activity of C3, C4, IgM and LZM showed a quadratic response to dietary xanthophyll levels. The optimum dietary xanthophyll dose for southern catfish juveniles was estimated to be 84.63 mg/kg diet by quadratic regression analysis based on the content of IgM in plasma.

3.7. Inflammation-related gene expression

The mRNA expression of inflammation-related genes in the kidney and intestine are shown in Fig. 5. In the intestine, the gene expression levels of interleukin 1 β (*IL-1 β*), C4, C3, complement 9 (C9), heat shock cognate 70 (*Hsc70*), heat shock protein 70 (*Hsp70*), heat shock protein 90 (*Hsp90*) and nuclear factor κ B inhibitory protein α (*I κ B- α*) were significantly up-regulated as the xanthophyll level increased up to 80 mg/kg diet, and then down-regulated. In kidney, the gene expression of C3, C4, C9, *Hsc70*, *Hsp70*, *Hsp90* and *I κ B- α* were significantly up-regulated as the xanthophyll level increased up to 80 mg/kg diet, and then down-regulated. Meanwhile *IL-1 β* expression level was up-regulated with the increased levels of xanthophyll, and the highest expression level was presented in 152 mg/kg xanthophyll group.

3.8. Carotenoid concentration in tissues

As shown in Fig. 6, total carotenoid and xanthophyll contents in dorsal skin, abdominal skin and muscle progressively increased

with higher levels of xanthophyll, and the highest expression level was presented in the 152 mg/kg xanthophyll group. Moreover, the content of total carotenoid and xanthophyll were higher in dorsal skin than in abdominal skin and muscle ($P < 0.05$). The content of total carotenoid and xanthophyll showed a broken line response to dietary xanthophyll level. The optimum dietary xanthophyll for juvenile southern catfish was estimated to be 89.73 mg/kg diet by broken line regression analysis based on the content of xanthophyll in dorsal skin.

3.9. The nutrient content in muscle

The effect of different proportions of xanthophyll supplementation on regulating muscle nutrient content is presented in Tables 6 and 7. The Hyp, collagen and crude protein contents significantly increased as the xanthophyll level increased up to 126 mg/kg diet, and then decreased. On the contrary, the crude lipid content gradually decreased with increasing xanthophyll supplementation levels, and the lowest level was presented in the 152 mg/kg xanthophyll group ($P < 0.05$). As presented in Table 7, compared with other diets, the 152 mg/kg xanthophyll group had significant effect on elevating the phenylalanine (Phe), methionine (Met), tyrosine (Tyr) and cysteine (Cys) content in muscle. Moreover, the 80 mg/kg group also had the effects on enhancing the content of lysine (Lys) and histidine (His) in muscle ($P < 0.05$). On the other hand, xanthophyll supplementation decreased the

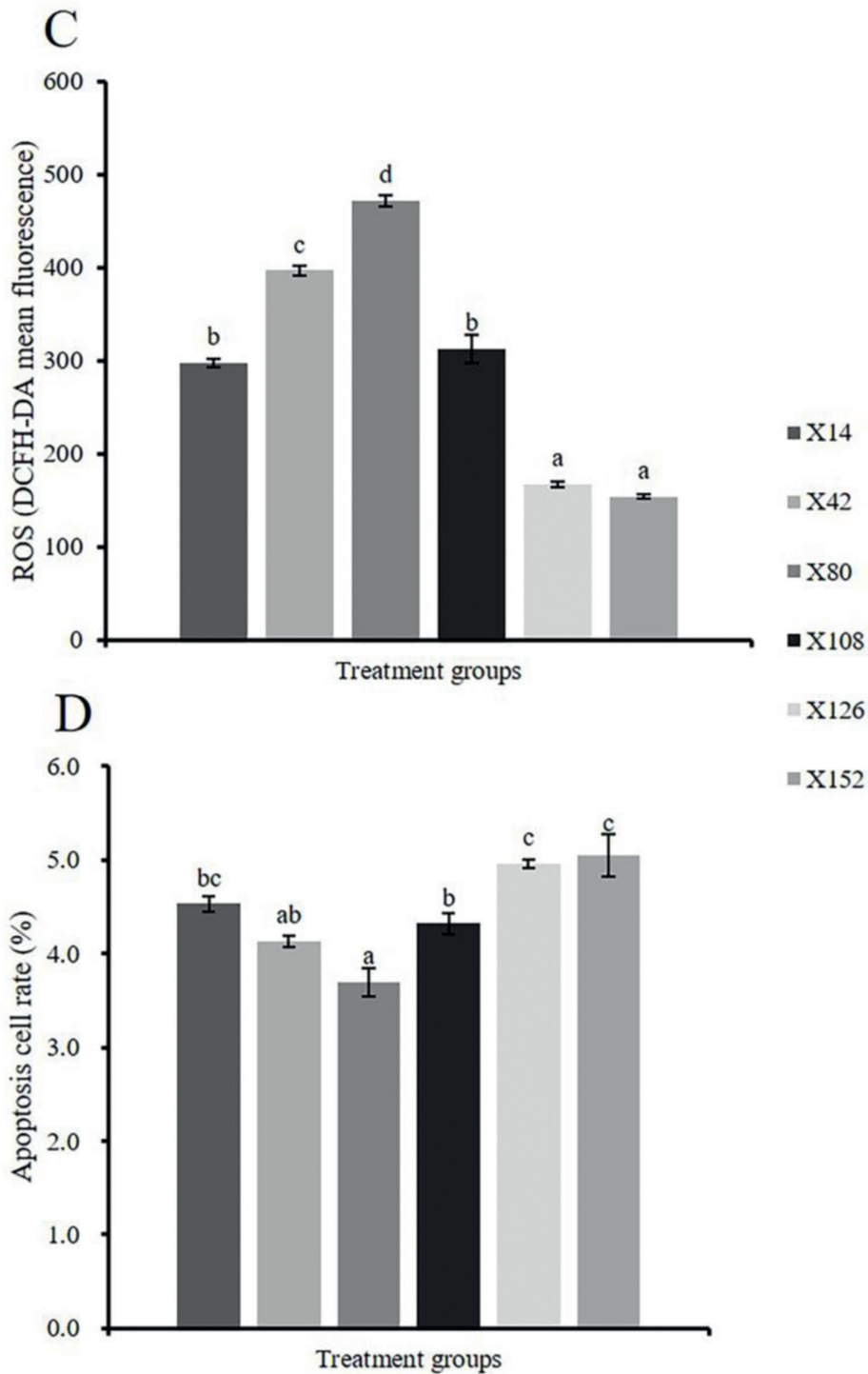


Fig. 2. Continued

glycine (Gly) content of muscle ($P < 0.05$). No significant difference was found in the content of other amino acids ($P > 0.05$).

3.10. Muscle pH, water-holding capacity (WHC) and texture

The pH, WHC and texture in muscle across different groups are presented in supplementary Table S1. The pH in muscle increased

continuously with increasing xanthophyll supplementation levels, and the highest level was presented in the 152 mg/kg xanthophyll group. While for drip loss, thawing loss, centrifugal loss and cooking loss, all the indices significantly decreased as the xanthophyll level increased up to 126 mg/kg diet, and then increased. Apart from that, the muscle hardness gradually increased with increasing xanthophyll supplementation levels, and the highest

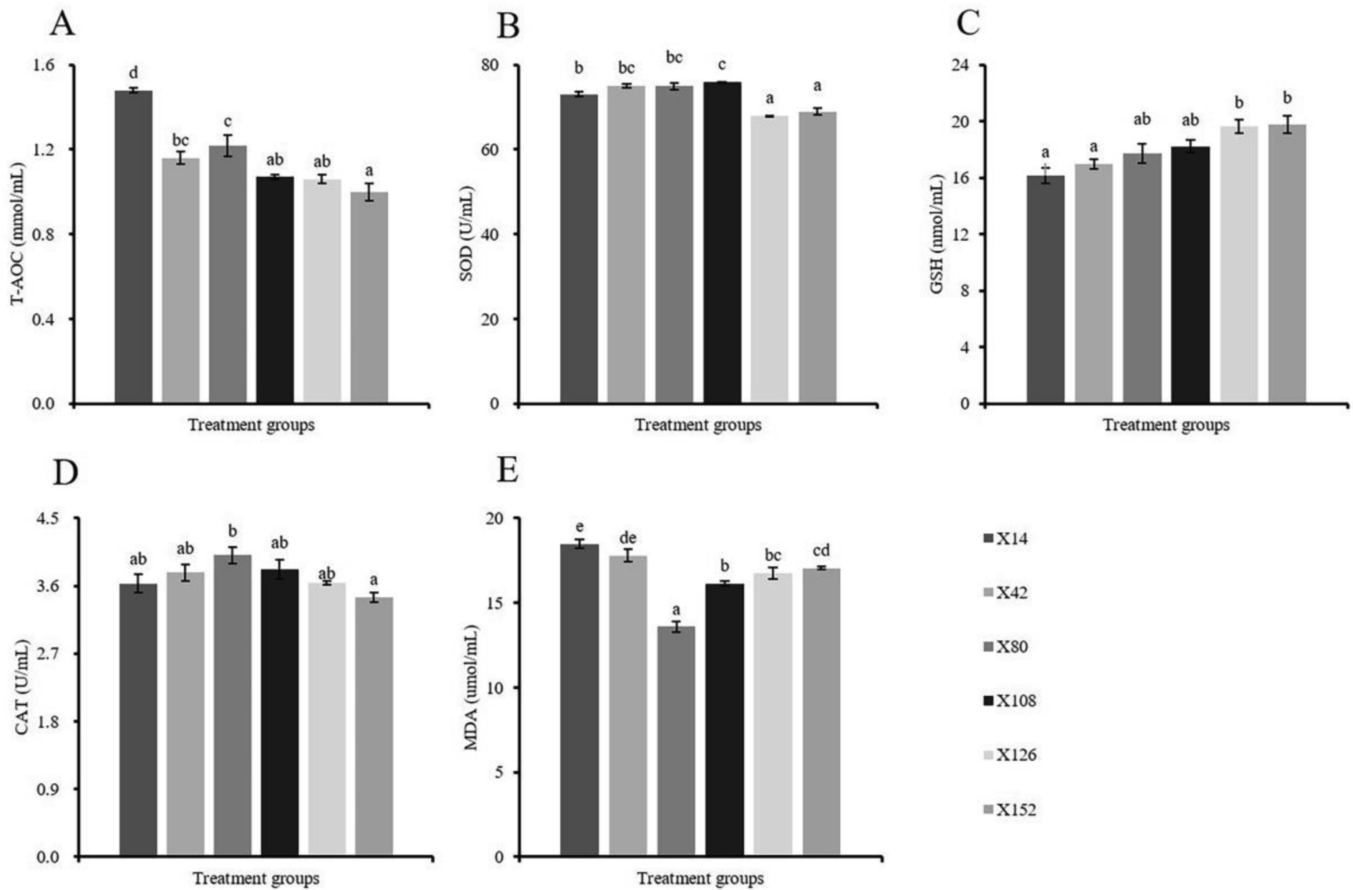


Fig. 3. Effects of dietary xanthophyll levels on plasma antioxidative parameters in southern catfish. (A) T-AOC = total antioxidant capacity; (B) SOD = superoxide dismutase; (C) GSH = glutathione; (D) CAT = catalase; (E) MDA = malondialdehyde. Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Values in the same bars with different superscripts are significantly different ($P < 0.05$).

level was presented in the 152 mg/kg xanthophyll group. Different from hardness, the muscle adhesiveness, cohesiveness, springiness, gumminess and chewiness significantly increased as the xanthophyll level increased up to 126 mg/kg diet, and then decreased. Broken-line regression analysis based on the chewiness of muscle analysis revealed that the optimum xanthophyll supplementation level was 108.66 mg/kg (supplementary Fig. S1).

4. Discussion

Using feed additives to improve fish growth, meat quality and maintain health is a vital way to achieve the healthy sustainable development of aquaculture. Xanthophyll as a carotenoid pigment which has multiple physiological functions including promoting phenotype response, organoleptic properties, antioxidant capacity

Table 5

Mortality and plasma physiological and biochemical indices of southern catfish under high temperature conditions ($34 \pm 1^\circ\text{C}$) after feeding different xanthophyll levels for 8 weeks.

Item	Diets						P-value
	X14	X42	X80	X108	X126	X152	
Mortality ¹ , %	58.3 ± 4.81 ^b	36.1 ± 2.78 ^{ab}	33.3 ± 4.81 ^{ab}	25.0 ± 0.00 ^a	38.9 ± 1.11 ^{ab}	41.7 ± 4.81 ^{ab}	0.028
ALB, g/L	8.50 ± 0.00 ^a	9.50 ± 0.00 ^c	9.83 ± 0.03 ^d	9.27 ± 0.33 ^b	9.07 ± 0.09 ^b	9.63 ± 0.03 ^c	0.000
GLOB, g/L	24.5 ± 0.06 ^a	26.0 ± 0.00 ^c	26.8 ± 0.03 ^d	25.20 ± 0.10 ^b	25.3 ± 0.23 ^b	26.3 ± 0.07 ^{cd}	0.000
AST, U/L	118 ± 0.58 ^a	163 ± 0.33 ^d	183 ± 0.33 ^f	159 ± 0.33 ^c	167 ± 1.20 ^e	147 ± 0.33 ^b	0.000
ALT, U/L	17.0 ± 0.00 ^a	26.0 ± 0.00 ^c	22.0 ± 0.00 ^b	25.7 ± 0.33 ^c	29.0 ± 0.00 ^d	18.3 ± 0.33 ^a	0.000
LDH, U/L	646 ± 1.15 ^c	797 ± 1.00 ^e	512 ± 0.88 ^b	714 ± 2.03 ^d	737 ± 4.51 ^d	394 ± 0.88 ^a	0.000
HDL, mmol/L	1.71 ± 0.01 ^a	1.90 ± 0.00 ^c	2.01 ± 0.01 ^d	1.82 ± 0.00 ^b	1.84 ± 0.01 ^b	1.88 ± 0.00 ^c	0.000
LDL, mmol/L	0.39 ± 0.00 ^a	0.53 ± 0.00 ^c	0.77 ± 0.10 ^d	0.49 ± 0.03 ^b	0.53 ± 0.03 ^c	0.47 ± 0.03 ^b	0.000

ALB = albumin; GLOB = globulin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; HDL = high density lipoprotein; LDL = low density lipoprotein.

Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively.

Data are presented as the mean ± SEM ($n = 4$), values in the same row with different superscripts are significantly different ($P < 0.05$).

¹ Mortality (%) = $100 \times [1 - (\text{final number of fish}/\text{initial number of fish})]$.

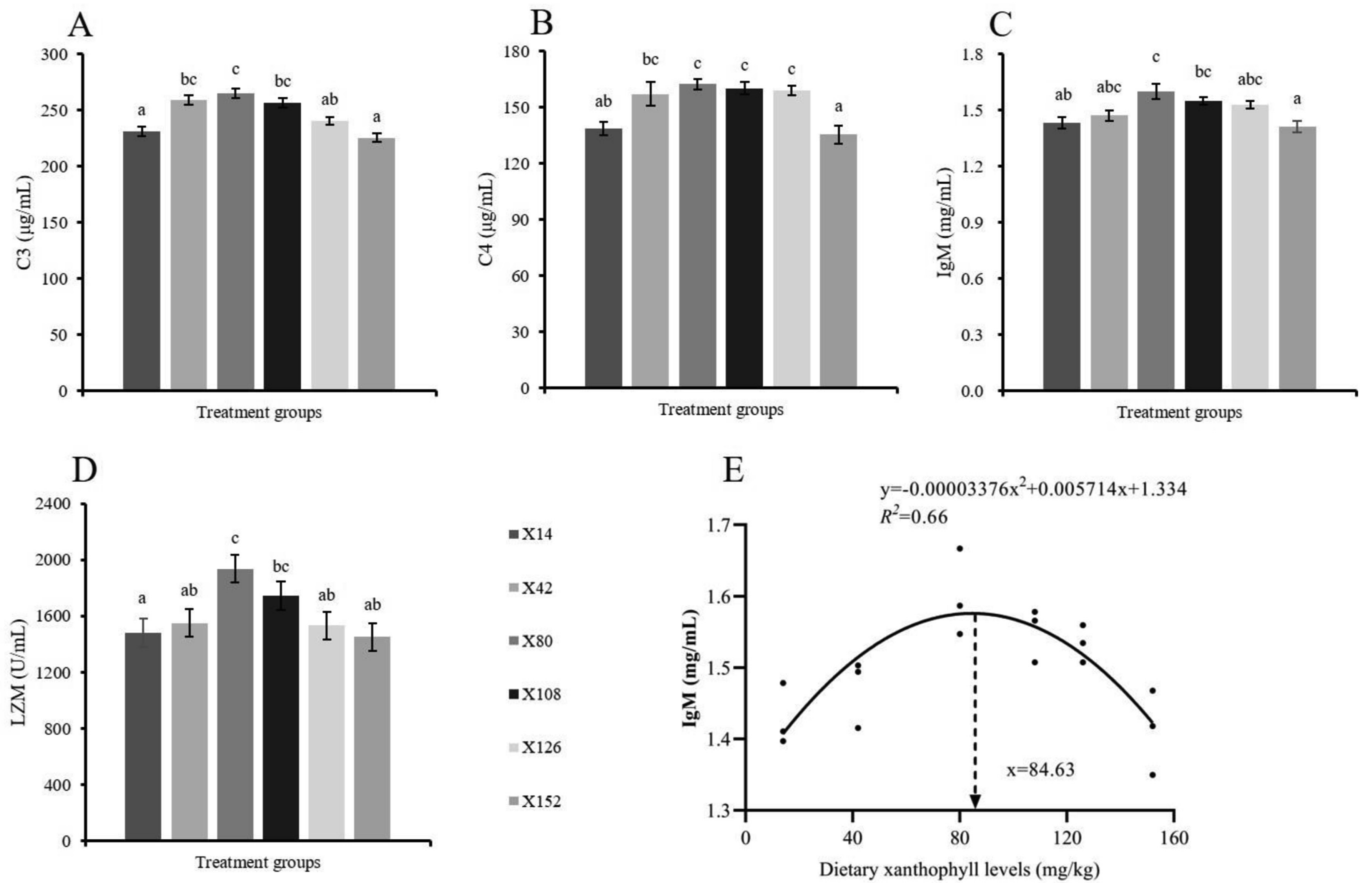


Fig. 4. Plasma complement, immunoglobulin contents and lysozyme activity after fish were fed diets with different xanthophyll levels. (A) C3 = complement 3; (B) C4 = complement 4; (C) IgM = immunoglobulin M; (D) LZM = lysozyme; (E) Quadratic regression model analysis based on the content of IgM. Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Values in the same bars with different superscripts are significantly different ($P < 0.05$).

and meat quality in farmed animals (Hu et al., 2012; Rajput et al., 2014; Mansour et al., 2020; P Wang et al., 2021). The results of a previous study had confirmed the effects of dietary xanthophyll supplementation on growth performance, antioxidant capacity and meat quality in aquatic animals (Wang et al., 2017). However, the optimal supplemental levels of xanthophyll in different aquatic animals were not determined. Therefore, in the present study, the optimal supplemental level of xanthophyll in Southern Catfish (*S. soldatovi meridionalis* Chen) was evaluated based on organoleptic properties, antioxidant capacity and meat quality.

Xanthophyll significantly improved the WGR and SGR of southern catfish. A quadratic regression model based on SGR revealed that the optimal dietary xanthophyll level was 86.78 mg/kg. Moreover, FER, PER and PPV were also increased after incremental xanthophyll additions, with the 80 mg/kg xanthophyll addition group reaching peak values. All the results were consistent with previous studies on oriental river prawn (*Macrobrachium nipponense*) fed a lutein supplemented diet (Etefaghdoost and Haghghi, 2021), Lake Kurumoi rainbow fish, *Melanotaenia parva* (Allen) fed with astaxanthin, canthaxanthin or lutein supplemented diets (Meiliszka et al., 2017) and Chinese soft-shelled turtle (*P. sinensis*) fed with 80 mg/kg xanthophyll supplementation (Wang et al., 2020). The growth-promoting role of xanthophyll might be related to the capability of carotenoids to inhibit nicotinamide adenine dinucleotide phosphoric acid (NADPH) reductase, which saves energy consumption, allowing for more energy to be used for

growth in aquatic animals (Ohno et al., 2011). However, higher xanthophyll supplementation decreased the WGR and SGR compared with optimal xanthophyll addition. Furthermore, xanthophyll had no effect on feeding attraction, since FI did not change significantly when fish were fed with the different diets. The growth performance results demonstrated that xanthophyll supplementation could elevate feed efficiency and further promote body nutrient deposition. Data on HSI and VSI showed that xanthophyll supplementation significantly decreased both indices. Moreover, body lipid composition also showed a downward trend when fish were fed a diet with graded xanthophyll supplementation. This suggests that xanthophyll supplementation could improve lipid metabolism, resulting in lower condition factor and FBW and enhanced physical indicators. Therefore, lipid metabolism should be investigated in further study.

Beyond growth performance, dietary xanthophyll also had great effect on regulating antioxidant capacity and immune response. Dietary xanthophyll levels significantly altered the content of plasma ROS, apoptosis, AST, ALT and SOD in the current study, which elucidated the regulatory effects of xanthophyll on antioxidant capacity. A heat stress test further confirmed this effect. Xanthophyll supplementation decreased mortality when fish were reared at 34 ± 1 °C. A level of 80 mg/kg xanthophyll in the diet significantly elevated the concentration of plasma physiological and biochemical indices. Similarly, a study on immune response also showed that moderate xanthophyll supplementation markedly

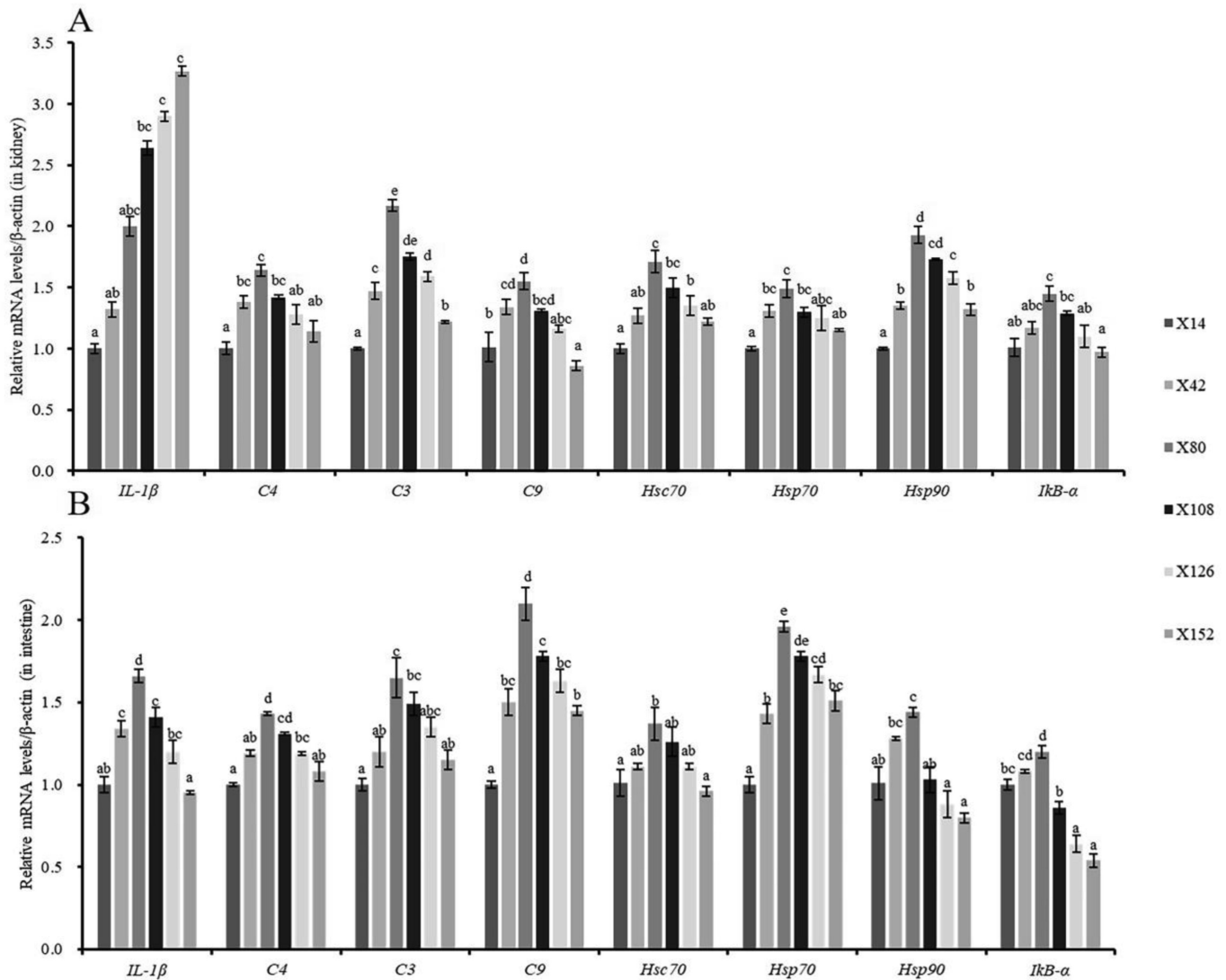


Fig. 5. The effects of dietary xanthophyll levels on the expression of regulators related to immune response in the (A) kidney and (B) intestine, respectively. Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Values in the same bars with different superscripts are significantly different ($P < 0.05$). *IL-1 β* = interleukin 1 β ; *C4* = complement 4; *C3* = complement 3; *C9* = complement 9; *Hsc70* = heat shock cognate 70; *Hsp70* = heat shock protein 70; *Hsp90* = heat shock protein 90; *I κ B- α* = nuclear factor κ B inhibitory protein α .

increased the concentration of plasma immunoproteins and mRNA expression levels of key regulators involved in the immune response. In the feeding trial of Luo et al. (2020), five immune and antioxidant stress related proteins (leucocyte cell-derived chemotaxin 2 (LECT2), small ubiquitin-like modifier (SUMO), HSP70, glutathione S-transferase (GST) and charged multivesicular body protein 4C (CHMP4C)) were up-regulated after xanthophyll supplementation. This effect was also observed in previous studies by Alonso-Alvarez et al. (2008) and Li et al. (2014). All the results demonstrated that moderate xanthophyll addition could stimulate antioxidant and immune response, while excessive xanthophyll supplementation might inhibit endogenous antioxidant and immune homeostasis. Resistance to environmental stress was more dependent on exogenous xanthophyll to provide higher antioxidant capacity.

Carotenoids are often used as a pigment to improve fish color. Generally, skin coloration is an important quality parameter to reflect the freshness and health of fish species and is considered

one of the most important price criteria for southern catfish. In the present study, total carotenoids and xanthophyll in the dorsal skin, abdominal skin and muscle were examined after fish were fed diets with different xanthophyll levels. Compared with the 14 mg/kg diet, dietary xanthophyll supplementation significantly increased the concentration of total carotenoids and xanthophyll in dorsal skin, abdominal skin and muscle in a dose-dependent manner. Carotenoids deposited in specific tissues and organs such as muscle, gonad (Svensson et al., 2005) and some specific zones of the skin. A previous study on discus fish (*Symphysodon aequifasciatus*) found that carotenoids accumulated more in dorsal fin than in skin and muscle (Song et al., 2017). A study in yellow catfish (*Pelteobagrus fulvidraco*) by Liu et al. (2022) also showed that carotenoids were more likely to be deposited in the skin than in the liver. Consistent with these studies, we found that the concentration of total carotenoids and xanthophyll were higher in dorsal skin than abdominal skin and muscle. Combined with the above results, broken-line regression analysis based on the xanthophyll in dorsal

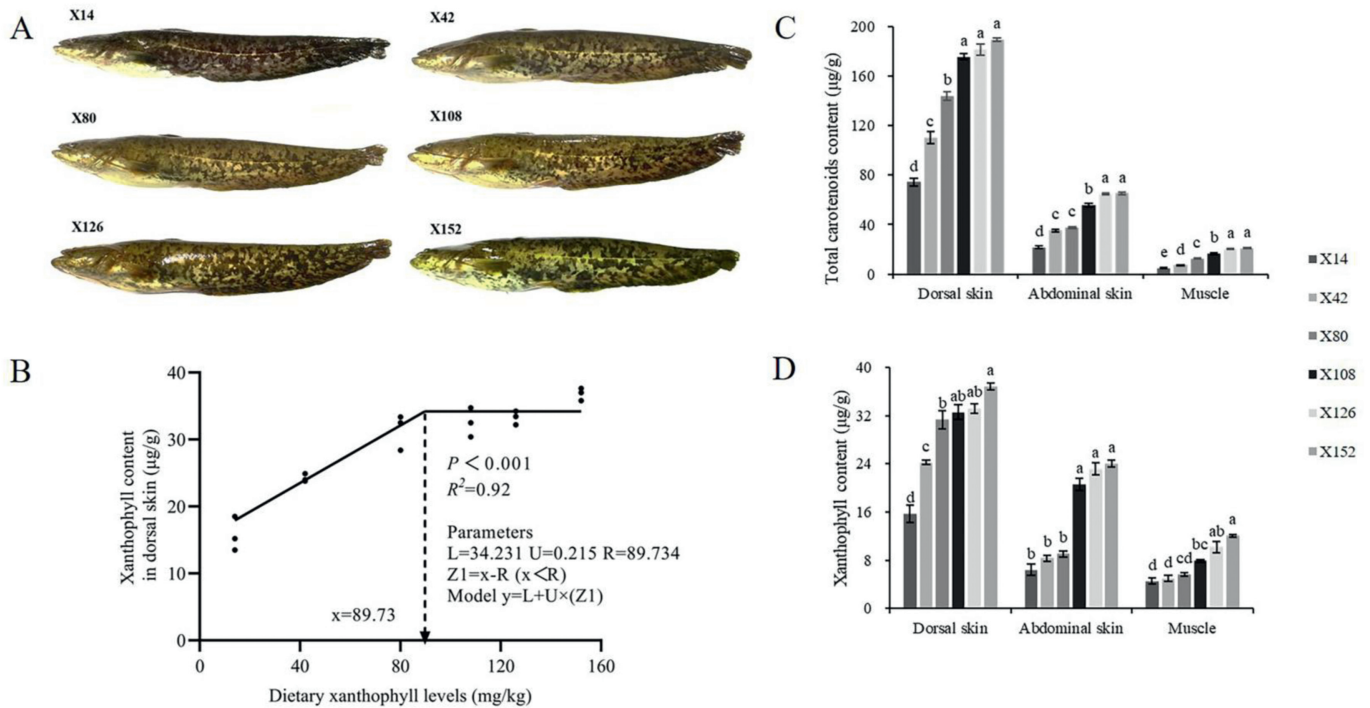


Fig. 6. The changes of total carotenoid and xanthophyll contents in dorsal skin, abdominal skin and muscle. (A) The skin color of southern catfish. (B) Broken-line regression model based on xanthophyll contents of dorsal skin. (C, D) The effects of different xanthophyll levels on the contents of total carotenoids and xanthophyll in skin and muscle of southern catfish, respectively. Parameters are defined as L = plateau, U = slope and R = breaking point. Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Values in the same bars with different superscripts are significantly different ($P < 0.05$).

skin suggested that the optimal dietary xanthophyll level in southern catfish was 108.66 mg/kg. The underlying mechanisms of how dietary xanthophyll level regulates skin coloration should be investigated in further study.

Studies on promoting meat quality with xanthophyll supplementation have not been explored clearly. Wang et al. (2020) also reported that in Chinese soft-shelled turtle, 80 mg/kg xanthophyll diet had marked effects on increasing the total, essential, and delicious amino acids of muscle. Meanwhile, in turkeys and pigs, Dolatowski (2008) obtained good quality meat and products after applying 1.5% protein-xanthophyll concentrate from alfalfa. In this study, increases in muscle pH, WHC and texture were found when southern catfish were fed with the 126 mg/kg diet. However, no significant effect was detected in total amino acid content of muscle after different levels of xanthophyll supplementation. Notably, xanthophyll supplementation decreased Gly content of muscle, while the content of Hyp and collagen were elevated after

xanthophyll was added to the diet. A previous study on piglets demonstrated that Gly could synthesize Hyp in different tissues (Hu et al., 2021; Li and Wu, 2018). Based on these studies, we deduced that dietary xanthophyll could stimulate Gly to synthesize Hyp and increase collagen content. Beyond that, our data on anti-oxidant capacity also showed that xanthophyll supplementation worked significantly in regulating oxidative stress. Inhibition of collagen production has been reported previously in fibroblasts after exposure to oxidative stress environments (Siwik et al., 2001). The effect of ROS in the reduction of collagen synthesis was due to interference with extracellular matrix remodelling and alterations in collagen biosynthesis by fibroblasts damaged by ROS (Archile-Contreras and Purslow, 2011). Decreasing collagen solubility in muscle possibly affected meat hardness. The present study indicated that the 126 mg/kg xanthophyll diet could decrease the concentration of ROS in plasma, and increase the content of collagen of muscle.

Table 6

Hydroxyproline (Hyp) and collagen contents and proximate composition of muscle in southern catfish fed different xanthophyll levels for 8 weeks.

Item	Diets						P-value
	X14	X42	X80	X108	X126	X152	
Hyp, mg/g	0.61 ± 0.01 ^a	0.73 ± 0.00 ^b	0.82 ± 0.02 ^c	0.83 ± 0.00 ^c	1.02 ± 0.01 ^e	0.94 ± 0.00 ^d	0.000
Collagen, mg/g	8.58 ± 0.14 ^a	10.3 ± 0.03 ^b	11.6 ± 0.22 ^c	11.8 ± 0.03 ^c	14.4 ± 0.15 ^e	13.2 ± 0.18 ^d	0.000
Proximate composition, %							
Moisture	78.9 ± 0.16	78.6 ± 0.25	78.7 ± 0.14	79.1 ± 0.11	78.8 ± 0.09	78.7 ± 0.25	0.568
Crude protein	18.4 ± 0.04 ^a	18.5 ± 0.05 ^{ab}	18.5 ± 0.04 ^{ab}	18.6 ± 0.09 ^{ab}	18.7 ± 0.04 ^b	18.6 ± 0.03 ^{ab}	0.047
Crude lipid	1.43 ± 0.08 ^b	1.36 ± 0.04 ^{ab}	1.34 ± 0.05 ^{ab}	1.26 ± 0.01 ^{ab}	1.22 ± 0.05 ^{ab}	1.18 ± 0.06 ^a	0.037
Ash	1.24 ± 0.01	1.28 ± 0.01	1.27 ± 0.01	1.23 ± 0.02	1.25 ± 0.01	1.26 ± 0.01	0.065

Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively. Data are presented as the mean ± SEM (n = 4), values in the same row with different superscripts are significantly different ($P < 0.05$).

Table 7
Amino acid composition of muscle in southern catfish fed different xanthophyll levels for 8 weeks (% dry weight).

Item	Diets						P-value
	X14	X42	X80	X108	X126	X152	
Thr	4.30 ± 0.08	4.14 ± 0.04	4.37 ± 0.15	4.04 ± 0.09	3.70 ± 0.25	3.91 ± 0.18	0.050
Val	4.29 ± 0.09	4.21 ± 0.03	4.49 ± 0.15	4.25 ± 0.08	4.13 ± 0.10	4.20 ± 0.12	0.267
Ile	4.43 ± 0.11	4.17 ± 0.05	4.46 ± 0.14	4.05 ± 0.15	3.64 ± 0.35	4.27 ± 0.22	0.090
Leu	7.50 ± 0.14	7.08 ± 0.01	7.55 ± 0.30	6.98 ± 0.09	6.93 ± 0.09	7.00 ± 0.21	0.074
Lys	8.97 ± 0.16 ^b	8.43 ± 0.02 ^{ab}	8.94 ± 0.33 ^b	8.64 ± 0.11 ^{ab}	8.02 ± 0.14 ^a	8.41 ± 0.19 ^{ab}	0.025
Phe	3.70 ± 0.09 ^{ab}	3.63 ± 0.04 ^{ab}	3.79 ± 0.09 ^b	3.62 ± 0.06 ^{ab}	3.45 ± 0.05 ^a	3.74 ± 0.02 ^b	0.024
Met	1.51 ± 0.18 ^{ab}	1.08 ± 0.11 ^a	0.97 ± 0.04 ^a	1.72 ± 0.27 ^{ab}	1.95 ± 0.36 ^b	2.29 ± 0.22 ^c	0.009
His	2.34 ± 0.04 ^{bc}	2.26 ± 0.01 ^{abc}	2.39 ± 0.03 ^c	2.13 ± 0.07 ^{ab}	2.09 ± 0.04 ^a	2.31 ± 0.05 ^{bc}	0.003
Arg	5.37 ± 0.05	5.35 ± 0.12	5.33 ± 0.27	5.25 ± 0.09	4.96 ± 0.27	5.39 ± 0.11	0.217
Cys	0.63 ± 0.03 ^{ab}	0.58 ± 0.01 ^a	0.54 ± 0.03 ^a	0.67 ± 0.03 ^{ab}	0.71 ± 0.03 ^b	0.73 ± 0.02 ^b	0.002
Tyr	2.82 ± 0.07 ^{ab}	2.63 ± 0.05 ^{ab}	2.51 ± 0.05 ^a	2.80 ± 0.03 ^{ab}	2.81 ± 0.07 ^{ab}	2.95 ± 0.15 ^b	0.022
Asp	9.04 ± 0.13	8.88 ± 0.02	9.41 ± 0.34	9.09 ± 0.13	8.68 ± 0.12	8.97 ± 0.38	0.387
Gly	4.74 ± 0.21 ^b	4.11 ± 0.07 ^a	4.11 ± 0.14 ^a	4.08 ± 0.15 ^a	4.21 ± 0.15 ^a	4.46 ± 0.12 ^{ab}	0.036
Ala	5.43 ± 0.13	5.23 ± 0.10	5.51 ± 0.26	5.17 ± 0.12	4.73 ± 0.32	5.35 ± 0.12	0.098
Glu	13.6 ± 0.28	13.1 ± 0.10	13.8 ± 0.51	13.1 ± 0.26	12.9 ± 0.21	13.4 ± 0.44	0.086
Ser	3.77 ± 0.07	3.51 ± 0.02	3.80 ± 0.16	3.67 ± 0.03	3.29 ± 0.20	3.51 ± 0.12	0.067
Pro	2.94 ± 0.09	2.87 ± 0.13	2.82 ± 0.16	2.73 ± 0.05	2.63 ± 0.13	2.93 ± 0.01	0.388
TAA	83.5 ± 1.17	82.2 ± 1.30	83.2 ± 1.35	82.0 ± 1.37	80.8 ± 1.46	83.3 ± 2.36	0.863
EAA ¹	42.4 ± 1.23	40.9 ± 0.82	41.5 ± 0.74	40.9 ± 0.63	40.2 ± 0.73	41.7 ± 1.21	0.672
NEAA ²	38.8 ± 0.12	37.9 ± 0.54	39.0 ± 0.85	37.6 ± 0.80	37.2 ± 0.72	38.1 ± 1.08	0.620
DAA ³	38.2 ± 0.58	36.8 ± 0.49	37.3 ± 0.54	36.6 ± 0.76	36.1 ± 0.70	37.0 ± 1.04	0.493

Thr = threonine; Val = valine; Ile = isoleucine; Leu = leucine; Lys = lysine; Phe = phenylalanine; Met = methionine; His = histidine; Arg, arginine; Cys = cysteine; Tyr = tyrosine; Asp = asparagine; Gly = glycine; Ala = alanine; Glu = glutamate; Ser = serine; Pro = proline; TAA = total amino acids; EAA = essential amino acid; NEAA = nonessential amino acids; DAA = delicious amino acids.

Diets X14, X42, X80, X108, X126 and X152 contained 14.0, 42.0, 80.0, 108, 126 and 152 xanthophyll (mg/kg, dry matter of diet), respectively.

Data are presented as the mean ± SEM (n = 4), values in the same row with different superscripts are significantly different (P < 0.05).

¹ EAA = Thr + Val + Ile + Leu + Lys + Phe + Met + His + Arg.

² NEAA = Asp + Gly + Ala + Glu + Ser + Pro.

³ DAA = Arg + Asp + Gly + Ala + Glu.

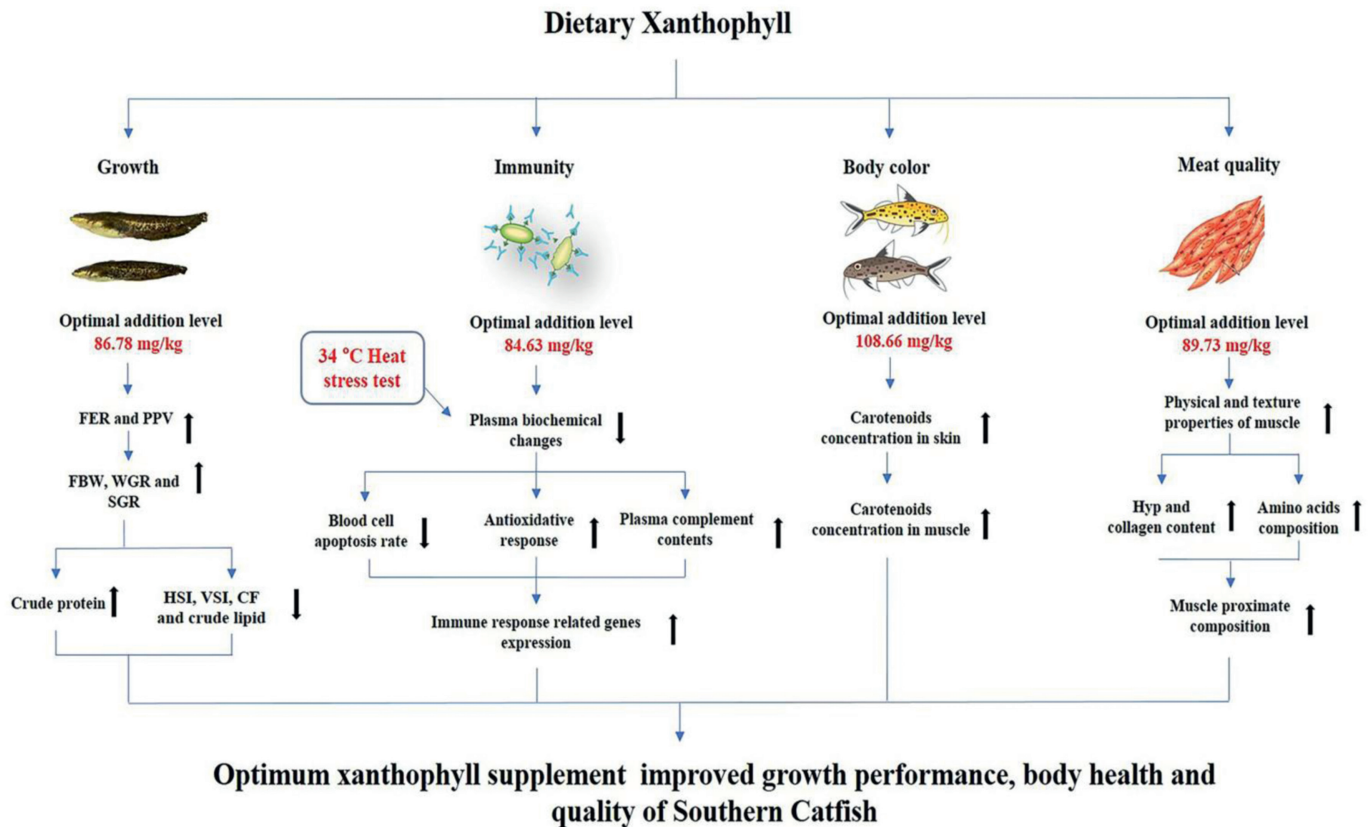


Fig. 7. The effect of optimum xanthophyll supplementation on the relationships between the main test indicators. FER = feed efficiency ratio; PPV = protein productive value; FBW = final body weight; WGR = weight gain rate; SGR = special growth rate; HSI = hepatosomatic index; VSI = viscerasomatic index; CF = condition factor.

5. Conclusion

In conclusion, current study indicated that an optimal level of dietary xanthophyll supplementation could enhance growth, antioxidant capacity, immune response, skin pigmentation and meat quality through regulating phenotypic response to gene expression (Fig. 7). A quadratic regression model based on SGR and IgM found that the optimum xanthophyll supplementation level was 86.78 and 84.63 mg/kg diet, respectively. Moreover, broken line regression analysis based on xanthophyll content in dorsal skin and chewiness revealed that the optimal xanthophyll level was between 89.73 and 108.66 mg/kg in diet of southern catfish (*S. soldatovi meridionalis* Chen). These results may have implications for using xanthophyll to eliminate oxidative stress and improve fish quality in aquaculture industry.

Author contributions

Chaoqun He and Haoyu Geng conducted the study. Yawen Qin, Peng Yang and Wenqiang Wang analyzed the data. Fei Song and Kangsen Mai designed the study and revised the paper. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgement

This work was supported by the Foundation and Applied Basic Research Fund Project of Guangdong Province (2019A151110606), Innovation Group Project of Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai).

Appendix Supplementary data

Appendix Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2022.12.005>.

References

- Alonso-Alvarez C, Pérez-Rodríguez L, Mateo R, Chastel O, Vinuela J. The oxidation handicap hypothesis and the carotenoid allocation trade-off. *J Exp Biol* 2008;21(6):1789–97.
- AOAC. Official methods of analysis. 18th ed. Arlington, VA, USA: Association of Official Analytical Chemists; 2005.
- Archile-Contreras AC, Purslow PP. Oxidative stress may affect meat quality by interfering with collagen turnover by muscle fibroblasts. *Food Res Int* 2011;44(2):582–8.
- Bédécarrats GY, Leeson S. Dietary lutein influences immune response in laying hens. *J Appl Poultry Res* 2006;15(2):183–9.
- Breithaupt DE. Modern application of xanthophylls in animal feeding—a review. *Trends Food Sci Technol* 2007;18(10):501–6.
- Brinker A, Reiter R. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: effects on feed utilization and fish quality. *Aquaculture* 2011;310(3–4):350–60.
- Crouse JD, Calkins CR, Seideman SC. The effects of rate of change in body weight on tissue development and meat quality of youthful bulls. *J Anim Sci* 1986;63(6):1824–9.
- Do CVT, Nguyen NTT, Pham MHT, Pham TYT, Le TG, Tran TD. Central composite design for simultaneously optimizing biomass and lutein production by a mixotrophic *Chlorella sorokiniana* TH01. *Biochem Eng J* 2022;177:108231.
- Dolatowski ZJ. Meat quality and their products from pigs and turkeys fed diet supplementation with protein-xanthophylls (PX) concentrate of alfalfa. In: 3rd International Conference “Feed and food additives” Alfalfa in human and animal nutrition. Monographic by ER Grela Edition; 2008. p. 21–8. Dzierżówka-Lublin, Poland.
- Dong R, Yang S, Jiao J, Wang T, Shi H, Zhou L, et al. Characterization of Stra8 in Southern catfish (*Silurus meridionalis*): evidence for its role in meiotic initiation. *BMC Mol Cell Biol* 2013;14(1):1–9.
- Ettefaghdoost M, Haghighi H. Impact of different dietary lutein levels on growth performance, biochemical and immune-physiological parameters of oriental river prawn (*Macrobrachium nipponense*). *Fish Shellfish Immunol* 2021;115:86–94.
- Fu YW, Zhu CK, Zhang QZ, Hou TL. Molecular characterization, expression analysis, and ontogeny of complement component C9 in southern catfish (*Silurus meridionalis*). *Fish Shellfish Immunol* 2019a;86:449–58.
- Fu YW, Zhu CK, Zhang QZ. Molecular characterization and expression analysis of complement component C3 in southern catfish (*Silurus meridionalis*) and a whole mount in situ hybridization study on its ontogeny. *Fish Shellfish Immunol* 2019b;84:865–75.
- Herath SS, Haga Y, Satoh S. Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*. *Aquaculture* 2016;464:205–12.
- Hu CH, Wang DG, Pan HY, Zheng WB, Zuo AY, Liu JX. Effects of broccoli stem and leaf meal on broiler performance, skin pigmentation, antioxidant function, and meat quality. *Poultry Sci* 2012;91(9):2229–34.
- Hu S, He W, Wu G. Hydroxyproline in animal metabolism, nutrition, and cell signaling. *Amino Acids* 2021:1–16.
- Li J, Xie X. Effects of waterborne cadmium exposure on growth performance, specific dynamic action, and energy budget of southern catfish *Silurus meridionalis*. *Aquaculture* 2019;511:634194.
- Li M, Wu W, Zhou P, Xie F, Zhou Q, Mai K. Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. *Aquaculture* 2014;434:227–32.
- Li P, Wu G. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino Acids* 2018;50(1):29–38.
- Liu C, Liu H, Zhu X, Han D, Jin J, Yang Y, et al. The effects of dietary arthrospira platensis on oxidative stress response and pigmentation in yellow catfish *Pelteobagrus fulvidraco*. *Antioxidants* 2022;11(6):1100.
- Luo K, Li J, Chen J, Pan Y, Zhang Y, Zhou H, et al. Proteomics analysis of skin coloration of large yellow croaker *Larimichthys crocea* fed different dietary carotenoids. *Aquacult Nutr* 2020;26(6):1981–93.
- Mansour AT, El-Feky MM, El-Beltagi HS, Sallam AE. Synergism of dietary co-supplementation with lutein and bile salts improved the growth performance, carotenoid content, antioxidant capacity, lipid metabolism, and lipase activity of the marbled spinefoot rabbitfish, *Siganus rivulatus*. *Animals* 2020;10(9):1643.
- Meilisa N, Jusadi D, Zairin JM, Artika IM, Priyo-Utomo NB, Kadarini T, et al. Digestibility, growth and pigmentation of astaxanthin, canthaxanthin or lutein diets in Lake Kurumoi rainbowfish, *Melanotaenia parva* (Allen) cultured species. *Aquacult Res* 2017;48(11):5517–25.
- Ohno M, Darwish WS, Ikenaka Y, Miki W, Ishizuka M. Astaxanthin can alter CYP1A-dependent activities via two different mechanisms: induction of protein expression and inhibition of NADPH P450 reductase dependent electron transfer. *Food Chem Toxicol* 2011;49(6):1285–91.
- Qin Y, He C, Wang W, Yang P, Wang J, et al. Changes in growth performance, nutrient metabolism, antioxidant defense and immune response after fishmeal was replaced by low-gossypol cottonseed meal in golden pompano (*Trachinotus ovatus*). *Front Mar Sci* 2021:1687.
- Quackenbush FW, Miller SL. Composition and analysis of the carotenoids in marigold petals. *J Am Chem Soc* 1972;55(3):617–21.
- Rajput N, Ali S, Naeem M, Khan MA, Wang T. The effect of dietary supplementation with the natural carotenoid curcumin and lutein on pigmentation, oxidative stability and quality of meat from broiler chickens affected by a coccidiosis challenge. *Br Poultry Sci* 2014;55(4):501–9.
- Rajput N, Naeem M, Al S, Zhang JF, Zhang L, Wang T. The effect of dietary supplementation with the natural carotenoid curcumin and lutein on broiler pigmentation and immunity. *Poultry Sci* 2013;92(5):1177–85.
- Refaey MM, Li D. Transport stress changes blood biochemistry, antioxidant defense system, and hepatic HSPs mRNA expressions of channel catfish *Ictalurus punctatus*. *Front Physiol* 2018;9:1628.
- Siwik DA, Pagano PJ, Colucci WS. Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am J Physiol Cell Physiol* 2001;280(1):C53–60.
- Song X, Li X, Chen Z, Wang L, Huan D, Leng X. Effect of dietary xanthophyll on the growth and body color of discus fish (*Symphysodon aequifasciata*). *J Fish China* 2017;41(4):566–72.
- Sun X, Chang Y, Ye Y, Ma Z, Liang Y, Li T, et al. The effect of dietary pigments on the coloration of Japanese ornamental carp (koi, *Cyprinus carpio* L.). *Aquaculture* 2012;342:62–8.
- Su Y, Sun X, Zhao S, Hu M, Li D, Qi S, et al. Dietary alfalfa powder supplementation improves growth and development, body health, and meat quality of Tibetan sheep. *Food Chem* 2022;369:133709.
- Svensson PA, Forsgren E, Amundsen T, Sköld HN. Chromatic interaction between egg pigmentation and skin chromatophores in the nuptial coloration of female two-spotted gobies. *J Exp Biol* 2005;208(23):4391–7.
- Thomas SE, Johnson EJ. Xanthophylls. *Adv Nutr* 2018;9(2):160–2.

- Wang L, Xue M, Wang J, Wu X, Zheng Y, Cao C. Effect of natural xanthophylls on growth performance and body pigmentation of yellow catfish (*Pelteobagrus fulvidraco*). *J Fish China* 2012;36(7):1102–10.
- Wang P, Li X, Wang X, Peng N, Luo Z. Effects of dietary xanthophyll supplementation on growth performance, body color, carotenoids, and blood chemistry indices of Chinese soft-shelled turtle *Pelodiscus sinensis*. *N Am J Aquacult* 2020;82(4):394–404.
- Wang P, Zhou X, Xiong G, Zeng D, Ge L, Luo Z, et al. Effect of dietary canthaxanthin and xanthophyll on growth, antioxidant capacity, body colour, and BCO2, CAT and SOD2 gene expression in Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Aquacult Nutr* 2021;27(6):2365–77.
- Wang S, Zhang L, Li J, Cong J, Gao F, Zhou G. Effects of dietary marigold extract supplementation on growth performance, pigmentation, antioxidant capacity and meat quality in broiler chickens. *Asian-Australas J Anim Sci* 2017;30(1):71.
- Wang W, Xu Y, Chi S, Yang P, Mai K, et al. Dietary lysine regulates body growth performance via the nutrient-sensing signaling pathways in largemouth bass (*Micropterus salmoides*). *Front Mar Sci* 2020;7:595682.
- Wang W, Yang P, He C, Chi S, Li S, et al. Effects of dietary methionine on growth performance and metabolism through modulating nutrient-related pathways in largemouth bass (*Micropterus salmoides*). *Aquac Rep* 2021;20:100642.
- Wei J, Qi W, Zhou Y, Zhang X, Dong R, Zhou L, et al. Establishment and characterization of an ovarian cell line from Southern catfish (*Silurus meridionalis*). *Fish Physiol Biochem* 2014;40(5):1383–91.
- Wei Z, Ma J, Pan X, Mu H, Li J, Shentu J, et al. Dietary hydroxyproline improves the growth and muscle quality of large yellow croaker *Larimichthys croceus*. *Aquaculture* 2016;464:497–504.
- Xu CZ, Wang HF, Yang JY, Wang JH, Duan ZY, Wang C, et al. Effects of feeding lutein on production performance, antioxidative status, and milk quality of high-yielding dairy cows. *J Dairy Sci* 2014;97(11):7144–50.
- Yanar Y, Çelik M, Yanar M. Seasonal changes in total carotenoid contents of wild marine shrimps (*Penaeus semisulcatus* and *Metapenaeus monoceros*) inhabiting the eastern Mediterranean. *Food Chem* 2004;88(2):267–9.
- Yang P, Wang W, Chi S, Mai K, Song F, Wang L. Effects of dietary lysine on regulating GH-IGF system, intermediate metabolism and immune response in largemouth bass (*Micropterus salmoides*). *Aquac Rep* 2020;17:100323.
- Yang X, Liu J, Wan P, Guo D, Chen DW. Use of egg yolk to imitate meat aroma. *Food Chem* 2022;371:131112.
- Yeşilayer N, Mutlu G, Yildirim A. Effect of nettle (*Urtica* spp.), marigold (*Tagetes erecta*), alfalfa (*Medicago sativa*) extracts and synthetic xanthophyll (zeaxanthin) carotenoid supplementations into diets on skin pigmentation and growth parameters of electric yellow cichlid (*Labidochromis caeruleus*). *Aquaculture* 2020;520:734964.
- Yi X, Xu W, Zhou H, Zhang Y, Luo Y, Zhang W. Effects of dietary astaxanthin and xanthophylls on the growth and skin pigmentation of large yellow croaker *Larimichthys croceus*. *Aquaculture* 2014;433:377–83.
- Yuan H, Liu Y, Luo R, Qi Z, Qi H, Du Y, et al. Effects of lutein-phospholipids emulsion on oxidatively stressed egg yolk protein. *Lebensm Wiss Technol* 2021;152:112187.
- Zhang Z, Xu W, Tang R, Li L, Refaey MM, Li D. Thermally processed diet greatly affects profiles of amino acids rather than fatty acids in the muscle of carnivorous *Silurus meridionalis*. *Food Chem* 2018;256:244–51.