ChinaRxiv

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201711.00746

Effects of Dietary Cholesterol Content on Growth Performance, Tissue Biochemical Indices, and Liver Lipid Metabolism-Related Enzyme Activities in Orange-Spotted Grouper (Epinephelus coioides): Postprint

Authors: Zhang Wucai, Xiaohui Dong, Tan Beiping, Zhang Shuang, Chi Shuyan, Yang Qihui, Liu Hongyu, Chen Xiaoru

Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary cholesterol content on growth performance, tissue biochemical parameters, and lipid metabolism-related enzyme activities in orange-spotted grouper (Epinephelus coicoides). Six isonitrogenous and isoenergetic experimental diets were formulated by supplementing cholesterol at 0 (control), 5, 10, 15, 20, and 25 g/kg into a basal diet containing casein as the primary protein source, designated as C0, C0.5, C1, C1.5, C2, and C2.5, respectively, with measured dietary cholesterol contents of 2.6, 7.5, 12.7, 17.2, 22.2, and 27.2 g/kg. These diets were fed to 360 orange-spotted grouper with an average initial body weight of (84.30±0.25) g for 10 weeks. Each experimental diet was assigned to three replicates, with 20 fish per replicate. The results showed that with increasing dietary cholesterol content, weight gain rate (WGR), protein efficiency ratio (PER), and feed efficiency (FE) exhibited a trend of initially increasing and then decreasing. The C0.5 group showed the highest WGR, PER, and FE, which were significantly higher than those of the control group ($P<0.05$). Whole-body crude lipid content in all supplemented groups was significantly higher than that of the control group ($P<0.05$), while crude ash content was significantly lower ($P<0.05$). Liver total cholesterol (TC) and triglyceride (TG) contents increased with increasing dietary cholesterol content, whereas muscle TC and TG contents were not significantly affected by dietary cholesterol content ($P>0.05$). With increasing dietary cholesterol content, serum TC, TG, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents initially increased and then plateaued, while the serum HDL-C/LDL-C ratio exhibited a decreasing trend. Serum TG content in the C2.0 group was significantly higher

than that in the control group (P<0.05). Serum HDL-C content in the C2.5 group was significantly higher than that in the control group and the C0.5 and C1.5 groups (P<0.05). Serum LDL-C content in the C2.5 group was significantly higher than that in all other groups (P<0.05). The serum HDL-C/LDL-C ratio in the control group was significantly higher than that in all supplemented groups (P<0.05). With increasing dietary cholesterol content, hepatic malic enzyme (ME) activity in orange-spotted grouper exhibited an increasing trend, with the C1.5, C2.0, and C2.5 groups being significantly higher than the other groups (P<0.05). Hepatic carnitine palmitoyltransferase I (CPT I) activity initially decreased and then plateaued, with the control group being significantly higher than all other groups (P<0.05). Hepatic CYP7A1 activity initially increased and then plateaued, with the C2.5 group showing the highest activity, representing a 35.48% increase compared with the control group (P<0.05). In this experiment, broken-line model analysis using WGR and hepatic CYP7A1 activity as evaluation indices indicated that the dietary cholesterol requirement of orange-spotted grouper was 7.43 and 8.70 g/kg, respectively.

Full Text

Effects of Dietary Cholesterol Content on Growth Performance, Tissue Biochemical Indices and Liver Lipid Metabolism Related Enzyme Activities of Orange-Spotted Grouper (Epinephelus coioides)

ZHANG Wuchai[1], DONG Xiaohui[1]*, TAN Beiping[1], ZHANG Shuang[1], CHI Shuyan[1], YANG Qihui[1], LIU Hongyu[1], CHEN Xiaoru[2]

(1. Laboratory of Aquatic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China; 2. Tongwei Co., Ltd., Chengdu 610041, China)

Abstract: A feeding trial was conducted to evaluate the effects of dietary cholesterol content on growth performance, tissue biochemical indices, and liver lipid metabolism-related enzyme activities of orange-spotted grouper (Epinephelus coioides). Six isoenergetic and isonitrogenous experimental diets were formulated using casein as the primary protein source, with cholesterol supplementation levels of 0 (control group), 5, 10, 15, 20, and 25 g/kg, designated as C0, C0.5, C1, C1.5, C2, and C2.5, respectively. The measured cholesterol contents in these diets were 2.6, 7.5, 12.7, 17.2, 22.2, and 27.2 g/kg. A total of 360 orange-spotted grouper with an average initial body weight of (84.30±0.25) g were fed the experimental diets for 10 weeks. Each diet was randomly assigned to three replicates, with 20 fish per replicate. The results showed that weight gain rate (WGR), protein efficiency ratio (PER), and feed efficiency (FE) initially increased and then decreased with rising dietary cholesterol levels. The C0.5 group exhibited the highest WGR, PER, and FE, which were significantly higher than those of the control group (P<0.05). Whole-body crude lipid

content in all cholesterol-supplemented groups was significantly higher than in the control group ($P<0.05$), while crude ash content was significantly lower ($P<0.05$). Liver total cholesterol (TC) and triglyceride (TG) contents increased with dietary cholesterol elevation, whereas muscle TC and TG contents were not significantly affected ($P>0.05$). Serum TC, TG, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents initially increased and then plateaued with increasing dietary cholesterol, while serum HDL-C/LDL-C ratio showed a decreasing trend. The C2.0 group had significantly higher serum TG content than the control group ($P<0.05$). The C2.5 group showed significantly higher serum HDL-C content compared to the control group and the C0.5 and C1.5 groups ($P<0.05$), and significantly higher serum LDL-C content than all other groups ($P<0.05$). The control group had a significantly higher serum HDL-C/LDL-C ratio than all supplemented groups ($P<0.05$). Liver malic enzyme (ME) activity increased with dietary cholesterol content, with the C1.5, C2.0, and C2.5 groups showing significantly higher activity than other groups ($P<0.05$). Liver carnitine palmitoyltransferase I (CPT I) activity decreased initially and then stabilized, with the control group being significantly higher than other groups ($P<0.05$). Liver cholesterol 7 -hydroxylase (CYP7A1) activity increased initially and then plateaued, peaking in the C2.5 group with a 35.48% increase compared to the control group ($P<0.05$). Based on broken-line model analysis using WGR and liver CYP7A1 activity as evaluation indices, the dietary cholesterol requirement for orange-spotted grouper was determined to be 7.43 and 8.70 g/kg, respectively.

Keywords: orange-spotted grouper; cholesterol; growth performance; biochemical indices; lipid metabolism

Introduction

Fishmeal is a crucial ingredient in compound feeds, containing abundant cholesterol. In recent years, as fishery resources have become increasingly scarce, fishmeal has been largely replaced by other protein sources, resulting in progressively lower cholesterol content in some compound feeds. Fish fed low-fishmeal diets exhibit inferior growth performance compared to those fed high-fishmeal diets [1-4]. Cholesterol is an essential chemical component in fish, regulating cell membrane fluidity and serving as a critical precursor for adrenal hormones, sex hormones, vitamin D, and bile acids [5-7]. While cholesterol has been extensively studied in human health, research on cholesterol requirements in marine fish remains limited [6,8].

Researchers generally believe that supplemental cholesterol is unnecessary in fish diets [9]. Dietary cholesterol supplementation in animal protein-based diets has shown no significant effects on growth of Atlantic salmon [6] (Salmo salar L.), hybrid striped bass [7] (Morone chrysops×M. saxatilis), and cobia [10] (Rachycentron canadum). However, domestic scholars have reported that cholesterol supplementation in diets using defatted fish meal as the protein source promoted growth in turbot [11]. Other researchers found that adding

1% cholesterol to plant protein-based diets significantly enhanced fish growth and feed intake [12-15]. Rat studies have confirmed that cholesterol increases liver triglyceride accumulation and decreases fatty acid -oxidation [16]. International scholars also reported that dietary cholesterol elevated muscle crude lipid content and reduced fatty acid -oxidation in rainbow trout [17]. To date, research on cholesterol' s effects on lipid metabolism in fish remains scarce.

Orange-spotted grouper (Epinephelus coioides) is a tropical and subtropical demersal fish species that represents an important marine aquaculture variety in southern coastal China. However, most orange-spotted grouper culture still relies on trash fish feeding, with efficient and healthy compound feeds still under development. The role of cholesterol in orange-spotted grouper growth has not yet been reported. This study investigated the effects of dietary cholesterol content on growth performance, tissue biochemical indices, and liver lipid metabolism-related enzyme activities in orange-spotted grouper to provide experimental evidence for developing rational compound feeds.

1.1 Experimental Diets

Six isoenergetic and isonitrogenous experimental diets were formulated using casein (product of Gansu Hualing Industrial Group Co., Ltd.), Russian white fish meal, and gelatin (product of Rousselot Gelatin Co., Ltd.) as primary protein sources, with cholesterol supplementation levels of 0 (control), 5, 10, 15, 20, and 25 g/kg . The diets were designated as C0, C0.5, C1, C1.5, C2, and C2.5, with corresponding measured cholesterol contents of 2.6, 7.5, 12.7, 17.2, 22.2, and 27.2 g/kg. All ingredients were ground to pass through a 40-mesh sieve, mixed thoroughly in a V-type vertical mixer, and then blended with fish oil and corn oil in which cholesterol had been uniformly dissolved. The mixture was processed into sinking pellets (6.0 mm diameter) using an F-26 twin-screw extruder at room temperature, air-dried indoors to approximately 13% moisture content, sealed in plastic bags, and stored at -20 °C.

1.2 Experimental Fish and Feeding Management

Juvenile orange-spotted grouper were purchased from a fish farm in Dongjian Town, Donghai Island, Zhanjiang, Guangdong. The feeding trial was conducted in a recirculating aquaculture system. After acclimation in square cement tanks for two weeks while being fed the control diet, healthy fish with an average body weight of (84.30±0.25) g were selected and randomly distributed into six groups with three replicates each (20 fish per replicate). Each replicate was stocked in an indoor cylindrical fiberglass tank (1 m^3 volume, approximately 900 L water). During the trial, water temperature was maintained at 26-30 °C, salinity at 25-28, pH at 7.8-8.2, and dissolved oxygen concentration at 5 mg/L.

Fish were hand-fed to apparent satiation twice daily (08:00 and 17:00) with sand-filtered, settled seawater. Water exchange of 60-80% of total volume was performed each morning. The trial lasted for 10 weeks.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection At the end of the feeding trial, fish were fasted for 24 h before sampling. Fish in each tank were anesthetized with eugenol, weighed, and counted. Three fish per replicate were randomly selected and stored at -20 °C for whole-body proximate composition analysis. Another three fish per replicate were individually measured for body weight and length to calculate condition factor, then dissected to obtain viscera weight for viscerosomatic index. Abdominal fat and liver were separated and weighed to calculate intraperitoneal fat ratio and hepatosomatic index. Five fish per replicate were randomly selected for blood collection from the caudal vein. Blood samples were placed in centrifuge tubes, left at 4 °C for 6 h, then centrifuged at 4,000×g for 15 min at 4 °C. Serum was collected and stored at -80 °C for biochemical analysis. Finally, five fish per replicate were randomly selected for liver and muscle collection, stored at -80 °C for triglyceride (TG) and total cholesterol (TC) determination.

1.3.2 Analytical Methods Proximate composition of experimental diets and whole fish was determined according to AOAC (2006) [18]: moisture by oven-drying at 105 °C, crude protein by Kjeldahl method, crude lipid by Soxhlet extraction with petroleum ether, and ash by combustion at 550 °C in a muffle furnace. Dietary cholesterol content was determined according to Yun et al. [12]: 500 mg feed sample was extracted with chloroform:methanol (2:1, v/v) at 1:9 mass/volume ratio for 24 h, centrifuged at 3,500 r/min for 5 min, and 500 L of the supernatant was evaporated under nitrogen. The lipid residue was dissolved in 1 mL isopropanol containing 100 g/L Triton X-100, and TC was measured using a commercial kit from Nanjing Jiancheng Bioengineering Institute.

Serum TG, TC, HDL-C, LDL-C, alkaline phosphatase (AKP), and glutamic oxaloacetic transaminase (GOT) activities were measured using a Hitachi 7020 automatic biochemical analyzer. Liver and muscle TC and TG contents were processed according to Folch et al. [19] and Fletcher [20], respectively, and measured following Yun et al. [12].

Liver lipid metabolism-related enzyme activities were determined by homogenizing 1 g liver in 2 mL ice-cold phosphate buffer (pH 7.4) on ice, centrifuging at 3,000 r/min for 20 min at 4 °C, and storing the supernatant at -80 °C. Liver malic enzyme (ME) and carnitine palmitoyltransferase I (CPT I) activities were measured using kits from Nanjing Jiancheng Bioengineering Institute, while cholesterol 7 -hydroxylase (CYP7A1) activity was measured using an ELISA kit from Beijing Dongge Biology Co., Ltd., following manufacturer instructions.

1.4 Calculation Formulas

Weight gain rate (WGR, %) = 100 × (Wf - Wi) / Wi
Protein efficiency ratio (PER, %) = 100 × (Wf - Wi) / FP
Feed efficiency (FE) = (Wf - Wi) / FC

Survival rate (SR, %) = 100 × Nf / Ni
Feeding intake rate (FI, %) = 100 × FC / [(Wf + Wi) / 2] / D
Condition factor (CF, %) = 100 × W / L^3
Viscerosomatic index (VSI, %) = 100 × Wm / W
Intraperitoneal fat ratio (IPF, %) = 100 × Wz / W
Hepatosomatic index (HSI, %) = 100 × Wg / W

Where: Wi = average initial body weight (g); Wf = average final body weight (g); FP = average protein intake (g); FC = average feed intake (g); Ni = initial fish number; Nf = final fish number; D = feeding days; W = body weight (g); L = body length (cm); Wm = viscera weight (g); Wz = abdominal fat weight (g); Wg = liver weight (g).

1.5 Statistical Analysis

All data are expressed as mean ± standard deviation (SD) and analyzed by one-way ANOVA using SPSS 17.0 software. Duncan' s multiple range test was performed when significant differences were detected among groups. $P < 0.05$ was considered statistically significant.

Results

2.1 Effects of Dietary Cholesterol Content on Morphological Indices

As shown in Table 2 , viscerosomatic index and hepatosomatic index of orange-spotted grouper showed an upward trend with increasing dietary cholesterol content, but no significant differences were observed among groups ($P > 0.05$). Dietary cholesterol content had no significant effect on condition factor or intraperitoneal fat ratio ($P > 0.05$).

2.2 Effects of Dietary Cholesterol Content on Growth Performance

As presented in Table 3 , dietary cholesterol content had no significant effect on survival rate ($P > 0.05$), which ranged from 91.11% to 100.00% across all groups. Weight gain rate, protein efficiency ratio, and feed efficiency initially increased and then decreased with rising dietary cholesterol levels. The C0.5 group showed significantly higher weight gain rate than all other groups ($P < 0.05$). The C1.0 and C1.5 groups had significantly higher weight gain rate than the control group but lower than the C0.5 group ($P < 0.05$), with no significant differences from the remaining groups ($P > 0.05$). Protein efficiency ratio followed a similar pattern to weight gain rate, while the C2.5 group exhibited the lowest protein and feed efficiency. Feed intake showed a gradual upward trend with increasing dietary cholesterol content, though differences among groups were not significant ($P > 0.05$).

The relationship between dietary cholesterol content (x) and weight gain rate (y) is illustrated in Figure 1 [Figure 1: see original paper]. Broken-line model fitting yielded the equations: $y = 4.559x + 123.7$ ($R^2 = 1.000$) and $y = -0.822x$

+ 163.7 (R^2 = 0.920). Based on weight gain rate as the evaluation criterion, maximum weight gain rate was achieved at a dietary cholesterol content of 7.43 g/kg.

2.3 Effects of Dietary Cholesterol Content on Body Composition

As shown in Table 4 , no significant differences were observed in whole-body moisture or crude protein content among groups ($P > 0.05$). The control group had significantly lower whole-body crude lipid content than all supplemented groups ($P < 0.05$), while no significant differences existed among supplemented groups ($P > 0.05$). Whole-body ash content in the control group was significantly higher than in all supplemented groups ($P < 0.05$), with no significant differences among supplemented groups ($P > 0.05$).

2.4 Effects of Dietary Cholesterol Content on Serum, Liver, and Muscle Biochemical Indices

As presented in Table 5 , serum TG, TC, HDL-C, and LDL-C contents initially increased and then plateaued with rising dietary cholesterol levels. The C2.0 group had significantly higher serum TG content than the control group ($P < 0.05$), though not significantly different from other groups ($P > 0.05$). The C2.5 group showed significantly higher serum HDL-C content than the control, C0.5, and C1.5 groups ($P < 0.05$), but not significantly different from the C1.0 and C2.0 groups ($P > 0.05$). Serum LDL-C content in the C2.5 group was significantly higher than all other groups ($P < 0.05$), while the C1.0, C1.5, and C2.0 groups had significantly lower LDL-C than C2.5 but higher than the control group ($P < 0.05$). The control group exhibited significantly higher serum HDL-C/LDL-C ratio than all supplemented groups ($P < 0.05$), with the C0.5 group showing intermediate values significantly higher than C2.5 but lower than the control ($P < 0.05$). No significant differences were observed in serum AKP or GOT activities among groups ($P > 0.05$).

Liver TC content increased with dietary cholesterol elevation, with the C1.0, C1.5, C2.0, and C2.5 groups being significantly higher than the control group ($P < 0.05$). Liver TG content also increased with dietary cholesterol, with the C1.5 and C2.5 groups significantly higher than the control and C0.5 groups ($P < 0.05$). No significant differences were detected in muscle TG or TC contents among groups ($P > 0.05$).

2.5 Effects of Dietary Cholesterol Content on Liver Lipid Metabolism-Related Enzyme Activities

As shown in Table 6 , liver ME activity increased with dietary cholesterol content, with the C1.5, C2.0, and C2.5 groups being significantly higher than other groups ($P < 0.05$), representing an 18.81% increase in the C2.5 group compared to the control. Liver CPT I activity decreased initially and then stabilized, with the control group being significantly higher than all other groups ($P < 0.05$).

Liver CYP7A1 activity increased initially and then plateaued, with no significant differences among the C1.5, C2.0, and C2.5 groups ($P > 0.05$), peaking in the C2.5 group at 35.48% higher than the control group ($P < 0.05$).

The relationship between dietary cholesterol content (x) and liver CYP7A1 activity (y) is presented in Figure 2 [Figure 2: see original paper]. Broken-line model fitting produced the equations: $y = 21.571x + 352.71$ ($R^2 = 1.000$) and $y = 4.3741x + 502.24$ ($R^2 = 0.834$). Based on liver CYP7A1 activity as the evaluation criterion, the dietary cholesterol requirement for orange-spotted grouper was 8.70 g/kg.

Discussion

3.1 Effects of Dietary Cholesterol Content on Growth Performance

Research indicates that fish can synthesize cholesterol endogenously or utilize dietary cholesterol to meet physiological metabolic demands [21-22]. As fishery resources decline, fishmeal is increasingly replaced by plant protein sources to reduce costs. Plant protein sources in aquatic animal feeds affect feed digestibility [23], and extensive fishmeal replacement leads to reduced growth performance and feed utilization [24-26]. Some researchers found that cholesterol supplementation in animal protein-based diets had no significant effects on weight gain rate or feed utilization in Atlantic salmon [6], hybrid striped bass [7], and rainbow trout [17]. However, this study demonstrated significant effects of dietary cholesterol on weight gain rate, protein efficiency ratio, and feed efficiency in orange-spotted grouper fed casein-based diets, with these parameters initially increasing and then decreasing as dietary cholesterol content rose, reaching maximum weight gain rate at 7.43 g/kg cholesterol. This suggests that appropriate cholesterol supplementation in casein-based diets promotes growth in orange-spotted grouper, though the underlying mechanisms require further investigation. Similar results have been reported in turbot [12], channel catfish [13], Japanese flounder [14], and rainbow trout [27] fed high plant protein diets. However, when dietary cholesterol exceeded requirements, growth performance declined in orange-spotted grouper, with the lowest protein and feed efficiency observed at 27.2 g/kg cholesterol, possibly due to excessive metabolic burden. Interestingly, turbot fed defatted fish meal diets showed no growth inhibition from cholesterol oversupply [11], suggesting that differences among species, feeding habits, fish size, basal diet composition, and culture environment may influence these outcomes.

Cholesterol supplementation in plant protein-based diets has been shown to promote feed intake in turbot [12], Japanese flounder [14], and rainbow trout [15], with researchers speculating that cholesterol may act as a feeding stimulant when plant proteins predominate, though the specific mechanism remains unclear. In this study, feed intake tended to increase with dietary cholesterol content, and visual observations indicated faster feeding in cholesterol-supplemented groups compared to the control, though differences were not sta-

tistically significant, consistent with findings by Zhu et al. [11].

3.2 Effects of Dietary Cholesterol Content on Body Composition and Tissue Biochemical Indices

Dietary cholesterol supplementation has been shown to promote bile acid secretion and lipid deposition in turbot [12] and enhance intestinal lipase activity and apparent lipid digestibility in Japanese flounder [28]. In this study, dietary cholesterol significantly affected whole-body crude lipid content in orange-spotted grouper, consistent with results in turbot [11-12] and Japanese flounder [28]. The control group had significantly lower whole-body crude lipid than supplemented groups, indicating that appropriate dietary cholesterol enhanced lipid absorption and storage. However, dietary cholesterol had no significant effect on whole-body moisture or crude protein content, similar to findings in Atlantic salmon [6], turbot [11-12], and Japanese flounder [28]. The control group showed significantly higher whole-body ash content than supplemented groups, differing from results in turbot [11-12] and Japanese flounder [28], warranting further investigation.

Studies have demonstrated that cholesterol significantly increases TG and TC contents in rat liver [16]. Similarly, liver TG content in orange-spotted grouper increased with dietary cholesterol, indicating enhanced hepatic TG synthesis, consistent with reports in hybrid striped bass [7]. Liver TG content is an important indicator of lipid deposition, and elevated levels may induce fatty liver and disrupt hepatic lipid metabolism. As the primary site for cholesterol synthesis and the main source of cholesterol for extrahepatic tissues, liver TC content in orange-spotted grouper increased gradually with dietary cholesterol, matching results in turbot [11-12] and indicating promoted hepatic cholesterol deposition. Elevated liver TG and TC contents may cause hepatic lipid metabolism disorders, potentially contributing to the reduced growth performance observed in high-cholesterol groups.

Blood biochemical indices objectively reflect fish health status, and excessively high serum TG and TC contents may be detrimental [29-30]. In this study, serum TG, HDL-C, and LDL-C contents increased with dietary cholesterol, consistent with reports by Zhu et al. [11], Yun et al. [12], and Chen et al. [28], indicating positive correlations between serum lipid parameters and dietary cholesterol. As an energy source in fish, TG participates in energy release and storage, and increased serum TG suggests reduced hepatic lipid catabolism [31]. The HDL-C/LDL-C ratio reflects cholesterol transport status and atherosclerosis degree [12]. The decreasing serum HDL-C/LDL-C ratio with increasing dietary cholesterol in orange-spotted grouper aligns with findings in turbot [11-12], suggesting cholesterol accumulation in fish bodies [11].

3.3 Effects of Dietary Cholesterol Content on Liver Lipid Metabolism-Related Enzyme Activities

CYP7A1 is the key enzyme in hepatic cholesterol conversion to bile acids [32]. Bile acids, with their amphiphilic properties, effectively emulsify lipids, enhance digestive lipase activity, promote lipid absorption, and increase lipid deposition [33]. In this study, liver CYP7A1 activity initially increased and then stabilized with rising dietary cholesterol, consistent with results in turbot [11-12], indicating enhanced bile acid synthesis capacity. Using liver CYP7A1 activity as the evaluation criterion, the dietary cholesterol requirement for orange-spotted grouper was 8.70 g/kg, higher than the requirement based on weight gain rate (7.43 g/kg), suggesting that maintaining normal physiological function requires more cholesterol than supporting optimal growth.

Mammalian studies have confirmed that cholesterol affects fatty acid metabolism [34-35]. ME provides NADPH for hepatic fatty acid synthesis and is a key enzyme in this pathway. The increasing trend in liver ME activity with dietary cholesterol in orange-spotted grouper suggests enhanced hepatic fatty acid synthesis, which may contribute to elevated liver TG content. In contrast, rat studies showed that dietary cholesterol decreased liver ME activity [16], with the discrepancy likely attributable to different fatty acid metabolism mechanisms between mammals and fish [36-37].

The liver is the primary site for fatty acid -oxidation and plays a crucial role in regulating lipid storage [38]. CPT I is the key enzyme for fatty acid -oxidation in mitochondria, catalyzing the conversion of acyl-CoA to acylcarnitine for transport into the mitochondrial matrix [33]. Decreased CPT I activity may reduce fatty acid catabolism. Studies have shown that cholesterol reduces CPT activity and expression in rat liver [16] and decreases fatty acid -oxidation in rainbow trout [17]. In this study, all cholesterol-supplemented groups showed significantly lower liver CPT I activity than the control, indicating reduced hepatic fatty acid catabolism. Reduced fatty acid -oxidation is an important reason for conversion of fatty acids to TG and cholesterol esters [39], which may also contribute to increased liver TG content.

Conclusions

1. Based on broken-line regression analysis using weight gain rate and liver CYP7A1 activity as evaluation indices, the dietary cholesterol requirement for orange-spotted grouper was determined to be 7.43 and 8.70 g/kg, respectively.
2. Dietary cholesterol supplementation enhanced lipid deposition, increased liver TC and TG contents, and elevated ME activity, but decreased liver CPT I activity in orange-spotted grouper.

References

[1] WANG Shuwen, HUANG Xianzhi, LUO Li, et al. Effects of replacement of fish meal with silkworm pupae on growth performance, body composition and serum biochemical indices of GIFT tilapia [J]. Chinese Journal of Animal Nutrition, 2015, 27(9): 2774-2783.

[2] LIU Yunzheng, HE Gen, MAI Kangsen, et al. Effects of partial replacement of fish meal by a new composite animal and plant protein source on growth and flesh quality of juvenile turbot (Scophthalmus maximus) [J]. Journal of Ocean University of China, 2016, 46(1): 33-39.

[3] SHI Dongjie, LIANG Yongjun, SUN Yansheng, et al. Effects of replacement of fish meal with maggot meal on growth, muscle composition and liver non-specific immune indices of tilapia [J]. Fisheries Science & Technology Information, 2015, 42(6): 319-323.

[4] WANG Jia, XUE Min, WU Xiufeng, et al. Research progress on regulatory mechanisms of selective feeding in fish fed different protein source diets [J]. Chinese Journal of Animal Nutrition, 2014, 26(4): 833-842.

[5] SHEEN S S. Dietary cholesterol requirement of juvenile mud crab Scylla serrata [J]. Aquaculture, 2000, 189(3/4): 277-285.

[6] BJERKENG B, STOREBAKKEN T, WATHNE E. Cholesterol and short-chain fatty acids in diets for Atlantic salmon Salmo salar (L.): effects on growth, organ indices, macronutrient digestibility, and fatty acid composition [J]. Aquaculture Nutrition, 1999, 5(3): 181-191.

[7] SEALEY W M, CRAIG S R, GATLIN D M. Dietary cholesterol and lecithin have limited effects on growth and body composition of hybrid striped bass (Morone chrysops×M. saxatilis) [J]. Aquaculture Nutrition, 2001, 7(1): 25-31.

[8] ASCHOFF L. Observations concerning the relationship between cholesterol metabolism and vascular disease [J]. The British Medical Journal, 1932, 2(3755): 1131-1134.

[9] NRC. Nutrient requirement of fish [S]. Washington, D.C.: National Academy of Press, 1993: 114.

[10] LUO Yiwen, AI Qinghui, MAI KANGSEN, et al. Effects of dietary taurine and cholesterol on growth, body composition and blood indices of cobia (Rachycentron canadum) [J]. Journal of Ocean University of China, 2013, 43(8): 31-36.

[11] ZHU T F, AI Q H, MAI K S, et al. Feed intake, growth performance and cholesterol metabolism in juvenile turbot (Scophthalmus maximus L.) fed defatted fish meal diets with graded levels of cholesterol [J]. Aquaculture, 2014, 428-429: 290-296.

[12] YUN B, MAI K S, ZHANG W B, et al. Effects of dietary cholesterol on

growth performance, feed intake and cholesterol metabolism in juvenile turbot (Scophthalmus maximus L.) fed high plant protein diets [J]. Aquaculture, 2011, 319(1/2): 105-110.

[13] TWIBELL R G, WILSON R P. Preliminary evidence that cholesterol improves growth and feed intake soybean meal-based diets aquaria studies with juvenile channel catfish, Ictalurus punctatus [J]. Aquaculture, 2004, 236(1/2/3/4): 539-546.

[14] DENG J, MAI K, AI Q, et al. Interactive effects of dietary cholesterol and protein sources on growth performance and cholesterol metabolism of Japanese flounder (Paralichthys olivaceus) [J]. Aquaculture Nutrition, 2010, 16(4): 419-429.

[15] DENG J M, BI B L, KANG B, et al. Improving the growth performance and cholesterol metabolism of rainbow trout (Oncorhynchus mykiss) fed soyabean meal-based diets using dietary cholesterol supplementation [J]. British Journal of Nutrition, 2013, 110(1): 29-39.

[16] WANG Yuming, WANG Jingfeng, XUE Changhu. Mechanism of cholesterol affecting lipid metabolism in rats [J]. Acta Nutrimenta Sinica, 2007, 29(6): 530-534.

[17] NORAMBUENA F, LEWIS M, HAMID N K A, et al. Fish oil replacement in current aquaculture feed: is cholesterol a hidden treasure for fish nutrition? [J]. PLoS One, 2013, 8(12): e81705.

[18] AOAC. Official methods of analysis of AOAC international [S]. Gaithersburg, MD: AOAC International, 2006.

[19] FOLCH J, LEES M, SLOANE STANLEY G H. A simple method for the isolation and purification of total lipids from animal tissues [J]. Journal of Biological Chemistry, 1957, 226(1): 497-509.

[20] FLETCHER M J. A colorimetric method for estimating serum triglycerides [J]. Clinica Chimica Acta, 1968, 22(3): 393-397.

[21] LEAVER M J, VILLENEUVE L A N, OBACH A, et al. Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in Atlantic salmon (Salmo salar) [J]. BMC Genomics, 2008, 9(1): 299.

[22] PARISH E J, LI S, BELL A D. Chemistry of waxes and sterols [M]//AKOH C C, MIN D B. Food lipids: chemistry, nutrition, and biotechnology. 3rd ed. Boca Raton, FL: CRC Press, 2008: 99-123.

[23] YANG Qihui, TAN Beiping, ZHOU Xiaoqiu, et al. Study on replacement of fish meal with plant protein complex in diets for Litopenaeus vannamei [J]. Chinese Journal of Animal Nutrition, 2014, 26(6): 1486-1495.

[24] ZHANG Junzhi, LÜ Fu, HUAN Zhili, et al. Effects of replacement of fish meal with extruded soybean meal on growth performance, body composition,

intestinal digestive enzyme activities and serum biochemical indices of rice field eel (Monopterus albus) [J]. Chinese Journal of Animal Nutrition, 2015, 27(11): 3567-3576.

[25] SUN Hong, YE Youbiao, YAO Xiaohong, et al. Effects of partial replacement of fish meal with fermented cottonseed meal on growth performance, body composition and plasma biochemical indices of juvenile black seabream (Acanthopagrus schlegeli) [J]. Chinese Journal of Animal Nutrition, 2014, 26(5): 1238-1245.

[26] CHEN Wenyan, CHEN Yongjun, PENG Xianghe, et al. Study on replacement of fish meal with fermented mulberry leaf protein in low-fishmeal diets for tilapia [J]. Chinese Journal of Animal Nutrition, 2015, 27(12): 3968-3974.

[27] DENG J M, ZHANG X, LONG X W, et al. Effects of dietary cholesterol supplementation on growth and cholesterol metabolism of rainbow trout (Oncorhynchus mykiss) fed diets with cottonseed meal or rapeseed meal [J]. Fish Physiology and Biochemistry, 2014, 40(6): 1827-1838.

[28] CHEN Jinghua. Effects of microbial fermentation, exogenous enzymes and feeding stimulants on utilization of soybean meal protein by Japanese flounder (Paralichthys olivaceus) [D]. PhD thesis. Qingdao: Ocean University of China, 2006.

[29] FUNGWE T V, FOX J E, CAGEN L M, et al. Stimulation of fatty acid biosynthesis by dietary cholesterol cholesterol synthesis dietary fatty acid [J]. Journal of Lipid Research, 1994, 35(2): 311-318.

[30] BOND C E. Circulation, respiration, and the gas bladder [M]//BOND C E. Biology of fishes. London: W.B Saunders Company Press, 1979: 347-374.

[31] KIM K N, JOO E S, KIM K I, et al. Effect of chitosan oligosaccharides on cholesterol level and antioxidant enzyme activities in hypercholesterolemic rat [J]. Journal of the Korean Society of Food Science and Nutrition, 2005, 34(1): 36-41.

[32] HUBACEK J A, BOBKOVA D. Role of cholesterol 7 -hydroxylase (CYP7A1) in nutrigenetics and pharmacogenetics of cholesterol lowering [J]. Molecular Diagnosis & Therapy, 2006, 10(2): 93-100.

[33] ZOU Sixiang. Animal biochemistry [M]. 4th ed. Beijing: China Agriculture Press, 2005: 181-196.

[34] GARG M L, THOMSON A B, CLANDININ M T. Effect of dietary cholesterol and/or omega 3 fatty acids on lipid composition and delta 5-desaturase activity of rat liver microsomes [J]. The Journal of Nutrition, 1988, 118(6): 661-668.

[35] MURIANA F J G, RUIZ-GUTIERREZ V, VAZQUEZ C M. Influence of dietary cholesterol on polyunsaturated fatty acid composition, fluidity and

membrane-bound enzymes in liver microsomes of rats fed olive and fish oil [J]. Biochimie, 1992, 74(6): 551–556.

[36] VAGNER M, SANTIGOSA E. Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: a review [J]. Aquaculture, 2011, 315(1/2): 131–143.

[37] EMERY J A, HERMON K, HAMID N K A, et al. Δ-6 desaturase substrate competition: dietary linoleic acid (18:2n-6) has only trivial effects on -linolenic acid (18:3n-3) bioconversion in the teleost rainbow trout [J]. PLoS One, 2013, 8(2): e57463.

[38] LOFTUS T M, JAWORSKY D E, FREHYWOT G L, et al. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors [J]. Science, 2000, 288(5475): 2379–2381.

[39] FUKUDA N, ONTOKO J A. Interactions between fatty acid synthesis, oxidation, and esterification in the production of triglyceride-rich lipoproteins by the liver [J]. Journal of Lipid Research, 1984, 25(8): 831–842.

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

Source: ChinaXiv —Machine translation. Verify with original.