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Effects of dietary cholesterol on growth performance, feed intake and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets

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ABSTRACT

A 9-week growth trial was conducted to investigate the effects of dietary cholesterol supplementation on growth performance, feed intake and cholesterol metabolism of juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets. A fish meal diet (diet FM) with 58% FM was formulated, and this diet was used as control. The other four diets were formulated to contain 14.5% FM, 42.0% soybean meal (SBM), and 18.5% wheat gluten meal. The four diets were supplemented with 0.0%, 0.5%, 1.0% and 1.5% cholesterol, respectively, and were isonitrogenous and isolipidic to the diet FM. They were named as diet C-0.0%, C-0.5%, C-1.0% and C-1.5%, respectively. The final dietary cholesterol concentrations were 0.30%, 0.77%, 1.25%, and 1.78%, respectively. That in control diet was 0.63%. The results showed that weight gain rate (WGR) and feed efficiency rate in fish fed diet FM were significantly higher than those in fish fed other diets ($P<0.05$). Furthermore, compared with fish fed diet C-0.0%, fish fed diet C-1.0% significantly enhanced WGR, feed intake (FI) and cholesterol levels in plasma and liver. However, WGR and FI in fish fed diet C-1.5% were significantly lower than those in fish fed diet C-1.0% ($P<0.05$). Fish fed diet C-1.0% showed significantly higher whole-body lipid content than that of fish fed other diets ($P<0.05$). The total cholesterol (TC), free cholesterol (FC), cholesterol esters, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents in fish plasma, TC and cholesterol esters in fish liver, and TC in fish feces were significantly correlated with dietary cholesterol contents, and correlated coefficients were above 0.74. Fish fed diet C-0.0% showed the lowest fecal total bile acid and activity of cholesterol 7α hydroxylase among dietary treatments. These results suggested that 1.25% of dietary cholesterol is helpful for juvenile turbot fed high plant protein diets to get significantly better growth rate without negative effects.

1. Introduction

Cholesterol is an essential dietary nutrient for a variety of marine crustaceans (Hernandez et al., 2004; Holme et al., 2006; Teshima, 1997) as well as for abalone *Haliotis discus hannai* (Zhang et al., 2009). It is a vital component of cell membranes (Cheng and Hardy, 2004), and also serves as a precursor for many physiologically active compounds, including sex and molting hormones, adrenal corticoids, bile acids and vitamin D (Sheen, 2000). However, limited research has addressed the potential requirement for a dietary supply of cholesterol in fish primarily owing to the fact that vertebrates can synthesize cholesterol from sterol precursors (Deng et al., 2010; National Research Council, 1993; Sealey et al., 2001).

Turbot (*Scophthalmus maximus* L.) is widely farmed and an important commercial carnivorous fish in Europe and Asia because of its appreciated flesh and rapid growth. However, current diets used in turbot farming are based on high-quality fish meal (FM) as the main protein source (Bonaldo et al., 2011). High demands and limited supply have led to high price for FM. Therefore, replacing some of the FM with plant protein ingredients has become a focus (Gatlin et al., 2007; Tacon and Metian, 2008). However, growth reduction was observed in many carnivorous fish species fed plant protein sources, such as in rainbow trout (Kaushik et al., 1995), Atlantic salmon (Refstie et al., 1998), turbot (Regost et al., 1999), cobia (Chou et al., 2004) and cuneate drum (Wang et al., 2006). The reduced growth performance in fish fed plant protein sources is partially explained by the presence of anti-nutritional factors (ANFs) (Francis et al., 2001; Olli et al., 1995). However, differences of the nutritional contents between protein in plant and FM may result in growth reduction. For example, cholesterol is rich in FM but deficient in most plant-based ingredients (Cheng and Hardy, 2004). Furthermore, dietary addition of 1.0% cholesterol significantly improved the weight gain rate (WGR) and feed intake (FI) in channel catfish fed soybean meal (SBM)-based diet (Twibell and Wilson, 2004) and Japanese flounder fed SBM-based diet (Chen, 2006). However, dietary addition of 1.0% cholesterol had no effect on the WGR and FI in fish fed FM-based diet (Bjerkeng et al.,

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1999), casein-based diet (Sealey et al., 2001; Twibell and Wilson, 2004) and fish protein concentrate-based diet (Deng et al., 2010). It is supposed that the effect of dietary cholesterol on growth performance in fish depends on the types of dietary protein sources.

Plasma total cholesterol is significantly decreased in fish fed plant-based diets compared with those in fish fed FM-based diet. These studied species include rainbow trout (Kaushik et al., 1995), turbot (Regost et al., 1999), gilthead sea bream (Gomez-Requeni et al., 2004; Sitja-Bobadilla et al., 2005; Venou et al., 2006), Atlantic cod (Hansen et al., 2007) and parrot (Lim and Lee, 2009). Previous works suggest that a hypocholesterolemic effect was not attributable to the difference in the amino acid profiles between plant protein sources and animal protein sources (Madani et al., 1998, 2000). At present, it is still unknown which factors led to the hypocholesterolemic effect of fish fed plant-based diets compared with fish fed FM-based diet. The cholesterol metabolism is affected by *de novo* cholesterol synthesis in the liver (endogenous cholesterol) and dietary cholesterol contents (exogenous cholesterol) (Maita et al., 2006). Biosynthesis of cholesterol is controlled by a feedback mechanism, and the key enzyme is 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase (Maita et al., 2006). Maita et al. (2006) reported that relative expression of HMG-CoA reductase in liver of yellowtail fed the plant-based diets was significantly higher than that in liver of fish fed the FM-based diet. In addition, excretions of total bile acid (TBA) are major factors involved in the homeostasis of cholesterol (Matsumoto et al., 2005). Cholesterol 7α hydroxylase (CYP7A1) is a rate-limiting enzyme in hepatic TBA synthesis (Matsumoto et al., 2005).

Previous studies have focused on growth performance and FI of fish fed dietary cholesterol (Bjerkeng et al., 1999; Chen, 2006; Deng et al., 2010; Sealey et al., 2001; Twibell and Wilson, 2004). At present, however, it is unknown how dietary cholesterol contents affected cholesterol metabolism of fish fed high plant protein diets. The objective of the present study was, therefore, to investigate the effects of dietary cholesterol supplementation on growth performance, FI and cholesterol metabolism of juvenile turbot fed high plant protein diets.

Table 1
Formulation (%), proximate composition (%) and energy content (MJ kg^{-1}) of the experimental diets.

Ingredients	FM	C-0.0%	C-0.5%	C-1.0%	C-1.5%
Fish meal	58.00	14.50	14.50	14.50	14.50
Soybean meal	0.00	42.00	42.00	42.00	42.00
Wheat gluten meal	0.00	18.50	18.50	18.50	18.50
Wheat flour	31.80	9.55	9.05	8.55	8.05
Fish oil	2.00	6.00	6.00	6.00	6.00
Soybean oil	4.00	3.00	3.00	3.00	3.00
Soybean lecithin	1.00	1.00	1.00	1.00	1.00
Sodium alginate	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_3)_2$	0.00	0.50	0.50	0.50	0.50
Vitamin premix[a]	0.85	0.85	0.85	0.85	0.85
Mineral premix[b]	0.50	0.50	0.50	0.50	0.50
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Lys-H_2SO_4	0.00	0.75	0.75	0.75	0.75
DL-methionine	0.00	0.60	0.60	0.60	0.60
L-threonine	0.00	0.40	0.40	0.40	0.40
L-arginine	0.50	0.30	0.30	0.30	0.30
L-valine	0.30	0.50	0.50	0.50	0.50
Cholesterol	0.00	0.00	0.50	1.00	1.50
Analyzed nutrients compositions (dry matter basis)					
Cholesterol	0.63	0.30	0.77	1.25	1.78
Dry matter	93.63	93.46	93.87	93.86	93.75
Crude protein	49.85	50.44	50.12	50.34	49.86
Crude lipid	13.69	13.64	14.15	14.60	15.00
Gross energy	20.89	21.62	21.64	22.92	22.45
Ash	9.96	7.01	7.39	7.03	6.95

FM, diet fish meal; C-0.0%, high plant protein diet without cholesterol supplementation; C-0.5%, addition of 0.5% cholesterol based on high plant protein; C-1.0%, addition of 1.0% cholesterol based on high plant protein; C-1.5%, addition of 1.5% cholesterol based on high plant protein;

[a] Vitamin premix supplied the diet with (mg kg^{-1} diet) the following compounds: retinyl acetate, 32; vitamin D_3, 5; DL-α-tocopherol acetate, 240; vitamin K_3, 10; thiamin, 25; riboflavin (80%), 45; pyridoxine hydrochloride, 20; vitaminB_{12} (1%), 10; L-ascorbyl-2-monophosphate-Na (35%), 2000; calcium Pantothenate, 60; nicotinic acid, 200; inositol, 800; biotin (2%), 60; folic acid, 20; choline chloride (50%), 2500; cellulose, 2473.

[b] Mineral premix consisted of (mg kg^{-1} diet) the following ingredients: $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $CuSO_4 \cdot 5H_2O$, 10; $MnSO_4 \cdot H_2O$, 45; KI, 60; $CoCl_2 \cdot 6H_2O$ (1%), 50; Na_2SeO_3 (1%), 20; $MgSO_4 \cdot 7H_2O$, 1200; calcium propionate, 1000; zoelite, 2485.

2. Materials and methods

2.1. Feed ingredients and diet formulation

Cholesterol (>99% pure), was obtained from Tianqi Chemicals (Anhui, China) Co., Ltd. FM, SBM and wheat gluten meal were used as the primary protein sources. Fish oil and soybean oil were used as the lipid sources. Wheat flour was used as the carbohydrate sources. Lysine–H_2SO_4, DL-methionine, L-threonine, L-arginine and L-valine (Crystalline amino acids) were supplemented to meet the essential amino acid (EAA) requirements of juvenile turbot based on the whole body amino acid profile (Kaushik, 1998). A fish meal diet (diet FM) was formulated with 58% FM, and this diet was used as control. The other four diets were formulated to contain 14.5% FM, 42.0% SBM, and 18.5% wheat gluten meal. The four diets were supplemented with 0.0%, 0.5%, 1.0% and 1.5% cholesterol at isonitrogenous and isolipidic as diet FM, respectively (Table 1). They were named as diet C-0.0%, C-0.5%, C-1.0% and C-1.5%, respectively. The final dietary cholesterol concentrations were 0.30%, 0.77%, 1.25%, and 1.78%, respectively, and that of the control diet (diet FM) was 0.63%.

Ingredients were ground into fine powder through a 246-μm mesh. Cholesterol was blended into menhaden fish oil. Then all the ingredients were thoroughly mixed with the menhaden fish oil, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China) and dried for about 12 h in a ventilated oven at 45 °C, and kept in freezer at −20 °C.

2.2. Fish, experimental conditions and samples collection

Juvenile turbot were obtained from Haiyang fish farm (Haiyang, Shandong, China). Fish were acclimated to the system and fed with the diet FM for 2 weeks before the trials. Juvenile turbot (initial body weight: 5.84 ± 0.02 g) were randomly distributed into 15 tanks with flat bottom (filled with 300 L seawater). Seawater, continuously pumped from the coast adjacent to the experiment station, passed through sand filters into each tank at approximately 1.5 L/min. Three replicates tanks were randomly assigned to each diet group and 40 fish were bulk weighed and stocked in each tank. During the 9-week feeding period, fish were fed the experimental diets to apparent satiation twice daily at 07:00 and 18:00 respectively. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70 °C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying and reweighing.

Before the experiment, 20 fish from the same population were randomly selected for determination of initial whole-body proximate composition. At the end of the experiment, 4 fish were randomly sampled in each tank and stored frozen −20 °C for whole body composition analysis. All experimental fish were anesthetized with eugenol (1:10,000) (Shanghai Reagent Corporation, Shanghai, China) before sampling. Blood samples were taken from the caudal vein using heparinized syringes to obtain plasma samples after centrifugation (4000 g for 10 min) at 4 °C and immediately stored at −20 °C until analysis. Liver samples were frozen in liquid nitrogen, and then stored at −80 °C for subsequent determination of lipid contents