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Synergistic effects of dietary cholesterol and taurine on growth performance and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets

饲料胆固醇和牛磺酸对大菱鲆幼鱼生长表现和胆固醇代谢的协同作用（高植物蛋白饲料中）

Biao Yun, Qinghui Ai *, Kangse

The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao, 266003, PR China

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ABSTRACT

本研究是调查胆固醇，牛磺酸和二者在饲料中组合对于大菱鲆生长和胆固醇代谢的影响，在饲喂高植物蛋白饲料基础之上。基础饲料（对照组）是14.5%鱼粉和50%粗蛋白。
其他三组等氮和等脂的实验饲料，由基础饲料中添加1%胆固醇，1%牛磺酸和1%胆固醇和1%牛磺酸的组合。
结果表明WGR在这三组中显著高于对照组。
TC组有在所有处理中有最高的生长表现。
血浆总胆固醇，游离胆固醇和低密度脂蛋白胆固醇水平在TC组明显比C组低。
C组的CYP7A1活性显著比对照组高，并且CYP7A1的活性在T组中是最高的。
兼并引物扩增的HMG-CoAr的PCR产物是331bp。HMG-CoAr的mRNA水平在TC组显著比C组低。
这些结果表明1%胆固醇和1%牛磺酸的组合有助于大菱鲆幼鱼在高植物蛋白的饲料中获得更好的生长而没有负面效果。

of cholesterol, taurine and combination of dietary cholesterol
ol metabolism in juvenile turbot (*Scophthalmus maximus* L.)
with 14.5% fish meal and 50% crude protein was formulated.
erimental diets were prepared with the supplementation of
f 1.0% cholesterol and 1.0% taurine to the basal diet, which
. The results showed that the weight gain rate in fish fed C-
than that in fish fed the control diet. Especially, fish fed TC
ata among dietary treatments. The plasma total cholesterol,
terol levels were significantly lower in fish fed TC diet com-
ish fed C-1.0% diet showed significantly higher activity of
trol diet ($P<0.05$), and activity of CYP7A1 in fish fed T-1.0%
e PCR product of 3-hydroxy-3-methylglutaryl-Coenzyme A
te primers was 331 bp. The HMG-CoAr mRNA levels were
hat in fish fed C-1.0% diet ($P<0.05$). These results suggested
urine is helpful for juvenile turbot fed high plant protein diets
ive effects.

兼并引物

1. Introd

牛磺酸是氨基酸衍生物，首次从牛的胆汁中分离出来（Park and Lee，1998）。在哺乳动物和鱼中，已知牛磺酸在膜稳定、抗氧化、解毒（Wright et al.,1986）、渗透调节（Thurston et al.,1980）,与胆汁酸结合（Goto et al.,1996）起到重要的生理作用。但是，虽然牛磺酸能由鱼部分合成，合成的牛磺酸不能满足大部分鱼类的需求（Gaylord et al., 2006; Kim et al., 2005b; Yokoyama et al., 1997, 2001). Yokoyama et al. (1997）证明虹鳟能由半胱氨酸合成牛磺酸。
L-半胱氨酸脱羧酶是肝牛磺酸合成的限速酶（Kim et al.,2005b），而且这种酶的活性在不同种类和不同大小的鱼中也是不同的（Yokoyama et al.,2001）。
当移走鱼粉时，常常补充EAAs，脂肪和矿物质。但是很多必须营养经常被忽视。例如， 多数植物原料缺乏牛磺酸（Yamamoto et al.,1998）。植物饲料中牛磺酸补充提高了牙鲆的WGR和FER（Kim et al.,2005a;Park et al.,2002）虹鳟（Gaylord et al.,2006）,军曹鱼（Lunger et al.,2007）和黄尾鱼（Takagi et al.,2008）。因此，在饲喂植物饲料时可能需要牛磺酸的补充。

Tauri … derivative and was … and Lee, 1998). In … n to play an impo… bilization, antioxida… egulation (Thursto… oto et al., 1996). H… y by fish, synthesiz… h species (Gaylord… 7, 2001). Yokoyan… d synthe- size taur… ylase is a rate-limi… , 2005b), and activ… ecies and sizes (Yo… erals are often supplemented when fish meal (FM) is removed. However numerous essential nutrients are often overlooked. For example, most plant-based ingredients are deficient in taurine (Yamamoto et al., 1998). Dietary taurine supplementation improved weight gain rate (WGR) and feed efficiency rate in Japanese flounder (Kim et al., 2005a; Park et al., 2002), rainbow trout (Gaylord et al., 2006), cobia (Lunger et al., 2007) and yellowtail (Takagi et al., 2008) fed plant-based diets. Therefore, taurine supplementation may be required when fish are fed plant-based diets.

Cholesterol is an essential dietary nutrient for a variety of marine crus… ima, 199… ntial requ… arily owi… ste- rol … 993; Seal… vels are … g et al., 2… nifi- cant… fish (Twibell and Wilson, 2004) fed soybean meal (SBM)-based diet and Japanese flounder fed diet including 41% SBM (Chen, 2006).

胆固醇是许多海洋甲壳动物的必须饲料营养（Hernandez et al., 2004; Holme et al., 2006; Teshima,1997）。但是由于脊椎动物能由固醇前体合成胆固醇，有限的调查是关于饲料胆固醇的潜在需求（Deng et al., 2010; National Research Council, 1993;Sealey et al., 2001）。鱼粉富含胆固醇，但在大多数植物饲料中水平低（Cheng and Hardy, 2004; Deng et al., 2010 ）。
另外，饲料胆固醇补充显著提高了叉尾鮰的FI和WGR（Twibell and Wilson，2004）在SBM饲料中，和牙鲆在含41%SBM饲料中（Chen，2006）。

* Corresponding author. Tel./fax: +86 532 82031943.
E-mail addresses: qhai@ouc.edu.cn, aiqinghui@163.com (Q. Ai).

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Dietary taurine supplementation has been shown to affect plasma cholesterol level in rats (Murakami et al., 1996; Yokogoshi et al., 1999), mice (Chen et al., 2005; Murakami et al., 1999), hamsters (Murakami

饲料牛磺酸调价已经被证明影响大鼠血浆胆固醇水平（ Murakami et al., 1996; Y okogoshi et al., 1999），小鼠 (Chen et al., 2005; Murakami e t al., 1999)，仓鼠 (Murakami et al ., 2002)和人(Mizushima et a l., 1996)。CYP7A1是由胆固醇合成肝胆汁酸的限速酶(Mu rakami et al., 1996, 1999; Murakami et al., 2002; Yokogoshi et al.,1999)。牛磺酸的降血浆胆固醇效应是由提高高胆固醇大鼠的CYP7A1活性来完成的（Gandhi et al., 1992; Venkatesan et al., 1993）。但是，牛磺酸补充对补充胆固醇饲喂的兔子的血清或组织胆固醇水平没有影响(Petty et al., 1990)。因此，结果随着动物种类而变化。而且，血浆胆固醇水平由肝中的内源性胆固醇合成影响(Maita et al., 2006)。胆固醇的生物合成由负反馈机制调控，关键酶是HMG-CoAr(Maita et al., 2006)。到目前为止，仍不清楚在高植物蛋白饲料中，饲料牛磺酸含量是如何影响鱼的胆固醇合成的。

ductase (HMG-CoAr) (Maita et al., 2006). To date, it is unclear how dietary taurine contents affect cholesterol synthesis of fish fed high plant protein diets.

Turbot is a carnivorous species widely cultured in Europe and Asia because of its high quality flesh and rapid growth. A previous study

大菱鲆是在欧洲和亚洲广泛养殖的肉食性种类，由于高品质肉和快速的生长。之前的研究表明GR与大菱鲆幼鱼的牛磺酸水平关联密切（Conceiçao et al., 1997）。但是，胆固醇，牛磺酸及它们的组合对大菱鲆幼鱼的生长表现的影响并没有报道。也不清楚饲料牛磺酸含量是如何影响高植物蛋白饲喂鱼的胆固醇代谢的。因此，本实验的目的是调查在高植物蛋白饲料中，胆固醇或牛磺酸和二者组合对大菱鲆幼鱼生长表现和胆固醇代谢的影响。

diets. Therefore, the objectives of this study were to investigate effects of either cholesterol or taurine and their combination on growth performance and cholesterol metabolism in juvenile turbot fed high plant protein diets.

2. Materials and methods

2.1. Feed ingredients and diet formulation

Taurine (>99.8% purity) and cholesterol (>99% purity) were obtained from Changshu Yudong chemical plant and Tianqi Chemicals (

购买牛磺酸（纯度高于99.8%）和胆固醇（纯度高于99%）。
FM，SBM和WGM作为主要蛋白源，鱼油和豆油作为脂肪源，小麦粉作为糖原。赖氨酸，蛋氨酸，苏氨酸，精氨酸和缬氨酸（晶体氨基酸）补充进去来满足EAA的需要，该需要是根据大菱鲆幼鱼总体氨基酸组分来的（Kaushik，1998）。
基础饲料含14.5%FM和50%粗蛋白。
其他三组等氮等脂饲料在基础饲料上添加1%胆固醇，1%牛磺酸，和二者组合。相应的饲料牛磺酸水平是0.12%，1.14%和1.13%

and the combination of 1.0% cholesterol and 1.0% taurine to the basal diet, which were named as C-1.0%, T-1.0% and TC, respectively. The corresponding levels of dietary cholesterol were 1.25%, 0.40%, and 1.12%, respectively. The corresponding levels of dietary taurine were 0.12%, 1.14%, and 1.13%, respectively.

Ingredients were ground into fine powder through a 246-μm mesh. Cholesterol was blended into menhaden fish oil and taurine was mi

原料通过246-μm筛。胆固醇与鲱鱼油混合，牛磺酸与水混合。然后再将所有原料完全与鲱鱼油混合，然后加水产生硬面团。硬面团再用饲料粉碎机制粒，在45°C通风炉中干燥12小时，在-20°C下保存。

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 Yellow Sea Fisheries Co., Ltd. (Haiyang, Shandong, China). Fish were acclimated to the system and

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

	Control	C-1.0%	T-1.0%	TC
Fish meal[a]	14.50	14.50	14.50	14.50
Soybean meal (dehulled)	42.00	42.00	42.00	42.00
Wheat gluten meal[b]	18.50	18.50	18.50	18.50
Wheat flour	9.55	8.55	8.55	7.55
Fish oil	6.00	6.00	6.00	6.00
Soybean oil	3.00	3.00	3.00	3.00
Soybean lecithin (98%)	1.00	1.00	1.00	1.00
Sodium alginate	1.00	1.00	1.00	1.00
$Ca(H_2PO_3)_2$	0.50	0.50	0.50	0.50
Vitamin premix[c]	0.85	0.85	0.85	0.85
Mineral premix[d]	0.50	0.50	0.50	0.50
Amino acid premix[e]	2.55	2.55	2.55	2.55
Ethoxyquin	0.05	0.05	0.05	0.05
Cholesterol		1.00		1.00
Taurine			1.00	1.00
Analyzed nutrients compositions (dry matter basis)				
Cholesterol	0.30	1.25	0.40	1.12
Taurine	0.11	0.12	1.14	1.13
Dry matter	93.46	93.87	93.00	94.08
Crude protein	50.44	50.12	51.40	50.40
Crude lipid	13.64	14.61	13.50	14.41
Gross energy (MJ/kg)	21.62	22.92	22.09	22.33
Ash	7.01	7.39	6.88	6.81

Control, a basal diet; C-1.0%, addition of 1.0% cholesterol to the basal diet; T-1.0%, addition of 1.0% taurine to the basal diet; TC, addition of both 1.0% cholesterol and taurine to the basal diet.

[a] Fish meal: steam dried fish meal, (COPENCA Group, Lima, Peru), with crude protein: 74.6%, crude lipid: 9.2%.

[b] Wheat gluten meal: wheat flour was further processed including crude protein: 79.7%, crude lipid: 2.0%.

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

[d] Mineral premix consisted of (mg kg^{-1} diet) the following: $FeSO_4 \cdot H_2O$, 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.

[e] Aminoacid premix (g/100 g diet): L-arginine 0.30, lysine-H_2SO_4 0.75, DL-methinine 0.6, L-threonine 0.4, valine 0.5.

fed with the control diet for 2 weeks before the trials. Juvenile turbot (initial body weight: 5.84 ± 0.02 g) were randomly distributed into 12 tanks with flat bottom (filled with 300 L seawater). Seawater,

海阳购买大菱鲆幼鱼。实验之前用基础饲料暂养两周，使鱼适应系统。
大菱鲆幼鱼（初始体重5.84±0.02g）随机分配到12个平底桶中（添加300L海水）。海水从海边抽到实验地点,通过沙滤进入每个桶中，流速1.5L/min。三个重复的桶随机分配到每个饲料组，40尾鱼称重并储存在每个桶中。饲喂期间（九周），饱食投喂每天两次，在7:00和18:00。一小时后收集残饵，70°C干燥至恒重然后称重。通过一定方法估计残饵溶失。

out 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

实验前，从同一批鱼中随机选择20尾鱼来确定鱼体初始体组分。实验结束，取样相似重量的4尾鱼，储存在-20°C来分析体组成，每桶的另外5尾鱼取样来测量形态参数。记录单体重量，体长，肝重和内脏重来计算其状态因子，肝指数和内脏指数。所有实验用鱼用丁香酚麻醉（1:10，000）。
每桶的六尾鱼血样使用注射器抽取尾静脉来获取血浆样品，然后离心（4000g10min）在4°C，迅速储存在-80°C直至分析。肝脏样品用液氮冷冻并储存在-80°C以便后续检测脂肪含量，CYP7A1活性，和用于RNA分离。粪便的收集方法与Deng et al.(2010)描述相同。

centrifugation (4000 g for 10 min) at 4 °C and immediately stored at −80 °C until analysis. Liver samples were frozen in liquid nitrogen and stored at −80 °C for subsequent determination of lipid content, activity of CYP7A1, and RNA isolation. The feces collection method was the same as described in Deng et al. (2010).

Durin… s controlled at 19–22 °C … nitrogen was lower th… and dissolved oxygen … pplied to each tank 24 h daily.

在饲养期间，水温控制在19-22°C，pH7.5-8.0，盐度30-33‰。氨态氮低于0.4mg/L;亚硝酸盐低于0.1mg/L,溶解氧高于6.0mg/L。每天向每桶24小时充气。

2.3. Chemical analyses

2.3.1. Body composition and energy assays

Dr… analyzed for ing… tter was analyz… . Crude protei… ation of Officia… ltiplying nitrog… on using Soxhle… furnace at 550… utomatic Bomb Calorimeter (Parr, Moline, IL, USA). Duplicate analyses were conducted for each sample.

分析原料，实验饲料和鱼样品的干重，粗蛋白，粗脂肪，灰分和能量。
在105°C，通过干燥样品至恒重来分析分析干重。
用凯氏定氮来检测粗蛋白（Association of Official Analytical Chemist, AOAC, 1995 ）再乘以6.25。
用索氏抽提法乙醚抽提来测量粗脂肪。
用马弗炉在550°C燃烧16小时来检测灰分。
用Parr 1281自动弹式量热器来检测总能（Parr，Moline，IL，USA）。每个样品使用同样分析。

2.3.2. Cholesterol, taurine and total bile acid assays

The concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in plasma were determined according to the method described by Deng et al. (2010). The concentration of free cholesterol (FC) in plasma was measured by peroxidase–anti-peroxidase (PAP) complex method using commercial kits (Shanghai Mingdian Bioengineering Co. … at a w… ces wit… on- ten… ned usi… was ma… ter of … am, and … hol con… and live… ing the … diet wa… LC) according to the method of Sakai and Nagasawa (1992). Assay of fecal total bile acid (TBA) was performed according to the method described by Madani et al. (1998). Duplicate analyses were conducted for each sample.

血浆中总胆固醇，高密度脂蛋白，和低密度脂蛋白的检测是根据Deng et al（2010）描述的方法来检测。
血浆中游离胆固醇通过使用试剂盒过氧化物-抗-过氧化物（PAP）复合方法来检测，根据制造商的方案在波长500nm下检测。
在从500mg肝脏，饲料和粪便中用氯仿：甲醇（2:1，v/v）提出脂肪之后（Folch et al., 1957），肝，饲料和粪便中的总胆固醇含量，和肝脏中的游离胆固醇含量就用与血浆检测相同的试剂盒来检测。溶解脂肪体积和氯仿：甲醇（2:1，v/v）共同制成10ml。1ml该提取物作为样品，在纯氮气中干燥，获得的残余物与1ml含100g氚核 X-100/L的异丙醇混合（Reagent Grade）。
在血浆和肝脏中，胆固醇酯的量通过从总胆固醇中减去游离胆固醇来计算。
饲料牛磺酸含量根据Sakai and Nagasawa (1992)的方法通过高表现液相色谱分析法（HPLC）来检测。总胆汁酸的分析根据Madani et al. (1998)的方法来完成。每个样品重复分析。

2.3.3. Mi… ts and activity of CYP7A1 assays

微粒体准备，总蛋白质含量，和CYP7A1活性分析

Approximately 500 mg liver tissue were minced in Tris–HCl buffer (0.1 … MSF, pH 7… ation (Hit… rude mic… fuga- tion, … final volu… 0MX Prep… 4 °C (Ras… were susp… PO_4, 0.1 … later prot… icro- som… 976) using bovine serum albumin (Sigma A-2153) as a standard. The activity of CYP7A1 was measured using a commercial fish CYP7A1 ELISA kit (purchased from R&D, Minneapolis, USA). Duplicate analyses were conducted for each sample.

在Tris-HCL缓冲液中（含0.5MKCL，1mMEDTA和0.1mMPMSF，pH7.4）研磨大约500mg肝组织，以1：4（重量/体积）的比例。
10300g，4°C下的20min离心后，再使用超离心准备上清液（粗物质）。因为超离心，上清液被溶解于相同的Tris-HCL缓冲液至大约6ml体积，在105000g 4°C离心60min（Rasmussen et al., 2011 ）。然后微粒小球悬浮在含50mM Tris – HCl, 10 mM KH2PO4,0.1 mM EDTA, 20% 甘油(pH 7.4)的缓冲液中，并储存在-80°C，以便后面的蛋白质和酶分析实验。
根据Bradford（1976）的方法来分析蛋白质在微粒体中的含量，使用牛血清蛋白作为标准。CYP7A1的活性使用商品鱼CYP7A1 ELISA试剂盒来检测。每个杨平重复分析。

2.3.4. RNA extraction, cDNA synthesis and partial sequence cloning of HMG-CoAr gene

Total RNA was extracted from turbot liver using Trizol Reagent (Invitrogen, USA). The quantity and quality of isolated RNA was determined by spectrophotometry with an Nano Drop® ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA), and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. Three microgram (3 µg) of total RNA was subjected to reverse transcription by PrimerScript® RT Enzyme using Oligo-dT primer (Takara, Japan) in 1… enerate prim… -3′) and HM… esigned base… sapiens (NP… s scrofa (NP… 81280), chic… s labrax (AA… ebrafish Dan… (http:// bioi… ymerase chai… nducted on a… amplify HM… s: initial den… cles of den… , primer exte… The PCR frag… gel for leng… Japan). After transforming into the competent cells of Escherichia coli DH5α, the recombinants were identified through blue–white color selection in ampicillin-containing LB plates and confirmed by PCR. Three positive clones in each PCR fragment were sequenced in both directions and these resulting sequences were verified and subjected to cluster analysis in NCBI.

用Trizol Reagent来从大菱鲆肝脏中提取总RNA。用分光光度法来检测提取的RNA数量和质量，用Nano Drop® ND-1000分光光度计，在1.2%变性琼脂糖凝胶电泳来检测完整性。
根据Reagent的说明，3μg总RNA通过 PrimerScript®反转录酶使用Oligo-dT引物在10μL中体系中 来反转录。
HMG-CoAr的一对兼并引物是F（5＇-GCACATCTACTTCCARTTYCA RA A-3＇)和R（5＇-CGGACATGCAGCCGAAR CARCACAT-3＇），是根据人，家鼠，猪，非洲爪蛙，鸡，欧洲鲈，大西洋鲑，斑马鱼的HMG-CoAr氨基酸序列，通过使用CODEHOP软件来设计的。用PCR来获取HMG-CoAr片段来扩增HMG-CoAr 的cDNA片段。PCR过程如下：开始变性步骤是在94°C2min，然后是循环35次在94°C变性30s，在55°C引物退火30s，在72°C引物延伸1min，最后在72°C延伸5min。PCR片段在1. 2%琼脂糖凝胶中由于长度不同而电泳并且克隆成pMD-18T菌体。在导入大肠杆菌DH5α感受态细胞之后，再通过蓝白色选择在含氨苄西林的LB平板上识别重组体，并用PCR来确认。在每个PCR片段中的3个阳性克隆来在两个方向测序，这些结果序列已被证实，并在NCBI中集群分析。

2.3.5. Real-time PCR analysis of HMG-CoAr mRNA expression

Total RNA from individual liver samples, using 3 individuals per tank, was extracted from turbot liver using Trizol Reagent (Invitrogen, U… th… Re… D… qu… D… W… ra… sa… th… by… m… ti… nu… P… ep… SY… pr… pr… (5… le… ac… CATCTTCTCCCTGTT-3′) was used for internal control. The real-time PCR amplification began with 30 s at 95 °C, followed by 35 cycles of 5 s at 95 °C, 25 s at 56.2 °C, and 30 s at 72 °C. No template controls were run

来自个体肝样品的总RNA，使用每个桶3个个体，用Trizol Reagent来从大菱鲆肝中提取，在1.2%变性琼脂糖凝胶电泳来测试完整性。根据厂家的说明，20mg的总RNA用重组DNA酶I（无RNA酶）处理，来移除可能存在的DNA污染物。总RNA 的数量和质量用Nano Drop® ND-1000分光光度计来评估。所有样品在260/280nm的吸收比率范围是2.00到2.40，表明RNA样品的纯度令人满意。根据reagent的说明，纯化的RNA通过PrimerScript®反转录酶使用Oligo-dT引物和Random 6 mers在20μl体积中来进行反转录。PCR引物用Primer Premier 5.00根据克隆的大菱鲆HMG-CoAr基因核苷酸序列来设计。实时定量PCR分析通过在一个最终25μl体积包含 2 × SYBR® PremixExTaq™，每个引物0.5μl，1μlcDNA混合物中使用PCR仪来进行。HMG-CoAr基因特异性引物HMG-CoAr F和HMG-CoAr R应用于评估大菱鲆中HMG-CoArmRNA水平。参考β-肌动蛋白基因被用作内部控制。实时定量PCR扩增开始在95°C持续30s，然后循环，在95°C5s，在56.2°C25s，在72°C30s。每个PCR分析没有模板控制运行。四次的稀释用来评估每个分析的PCR效率，定量为6个含量。引物扩增效率对每一对引物最优化，结果是对HMG-CoAr0.9847，对β-actin0.9772。ΔCT斜率(HMG-CoArCT—β-act in CT)绝对值是0.0187，表明对目的基因的相对量ΔΔCT计算可以使用。HMG-CoAr表达水平通过2-ΔΔCT法来计算，这个值代表相对于校准值n倍的差异(Livak and Schmittgen, 2001)。

for each PCR assay. A four-fold serial dilution was used to assess PCR efficiencies for each assay, quantifying six concentrations. The primer amplification efficiency was optimized for each pair of primers, resulting in: 0.9847 for HMG-CoAr and 0.9772 for β-actin. The absolute ΔC_T value (HMG-CoArC_T − β-actin C_T) of the slope is 0.0187, which indicated the $\Delta\Delta C_T$ calculation for the relative quantification of target genes might be used. The expression level of HMG-CoAr was calculated by $2^{-\Delta\Delta CT}$ method, and the value stood for n-fold difference relative to the calibrator (Livak and Schmittgen, 2001).

2.4. Calculations and statistical methods

Growth parameters were calculated as follows:

Weight gain rate, WGR (%) = 100 × [(final body weight − initial body weight) / initial body weight].
Feed intake, FI (%/d) = 100 × total amount of the feed consumed (g) / [(initial body weight + final body weight) / 2] / days.

WGR
FI
FCR
PPV
EPV
SR
CF
HS
VSI
SPSS11.5软件用来数据评估。所有数据用单因素方差分析(ANOVA)然后T检验。当P<0.05时为差异显著。

Software SPSS, 11.5 was used for all statistical evaluations. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when $P<0.05$.

3. Results

3.1. Survival rate, growth performance and somatic indexes

Survival rate was higher than 96% in all treatments (Table 2), and no significant difference was found among dietary treatments ($P>0.05$). FI of fish fed T-1.0% diet was significantly lower than that of

存活率高于96%，处理间无显著差异。
T组FI显著低于其他组，C组FI显著高于其他组。
FBW和WGR在补充了胆固醇，牛磺酸或二者后显著增加，TC组的生长最好。
FCR在T组合TC组显著低于对照组。PPV,EPV,CF,VSI在各处理中无显著差异。

fish fed the control diet ($P<0.05$). No significant differences were detected among dietary treatments with respect to PPV, EPV, CF, or VSI ($P>0.05$).

3.2. Body composition

The whole-body lipid content in fish fed C-1.0% diet was significant

C组的总体脂肪含量显著高于对照组，而T组显著低于对照组。这几组的鱼体灰分含量显著低于对照组。在几个饲料处理之间的总体蛋白含量没有显著差异。

differences in whole-body protein content of fish among dietary treatments ($P>0.05$).

3.3. Plasma, liver and feces cholesterol

Fish fed C-1.0% diet showed significantly higher plasma TC, FC, cholesterol esters, HDL-C and LDL-C levels compared to the control diet, ($P<0.05$, Table 4). The plasma TC, FC and LDL-C levels were

Table 2
Growth performance and survival of turbot fed the experimental diets (n = 3).

	Control	C-1.0%	T-1.0%	TC	Pooled S.E	*F* value	*P* value
IBW[1]	5.83	5.84	5.84	5.84	0.0048	0.083	0.967
FBW[2]	24.3^{a}	26.7^{bc}	26.1^{b}	27.7^{c}	0.4096	12.853	0.002
WGR[3]	316.9^{a}	357.1^{bc}	346.4^{b}	374.2^{c}	7.0227	10.763	0.004
FI[4]	1.89^{b}	1.96^{c}	1.83^{a}	1.91^{b}	0.0151	17.948	0.001
FCR[5]	0.97^{b}	0.97^{b}	0.93^{a}	0.93^{a}	0.0064	8.133	0.008
PPV[6]	31.4	31.2	31.0	32.7	0.3126	1.824	0.221
EPV[7]	26.1	26.0	25.6	26.9	0.3019	1.629	0.247
SR[8]	99.2	99.2	96.7	96.7	0.8613	0.632	0.615
CF[9]	3.21	3.29	3.28	3.32	0.0381	0.285	0.835
HSI[10]	1.39^{ab}	1.76^{b}	1.09^{a}	1.50^{ab}	0.1072	2.207	0.165
VSI[11]	5.62	5.67	5.51	5.50	0.1035	0.124	0.943

Values in the same row with no common superscripts are significantly different ($P<0.05$).
[1] IBW, initial body weight.
[2] FBW, final body weight.
[3] Weight gain rate, WGR (%) = 100 × [(FBW − IBW) / IBW].
[4] Feed intake, FI (%/d) = 100 × total amount of the feed consumed (g) / [(IBW + FBW) / 2] / day.
[5] Feed conversion rate, FCR = total amount of the feed consumed (g) / weight gained (g).
[6] Protein productive value, PPV (%) = 100 × (whole-body protein gain / protein consumption).
[7] Energy productive value, EPV (%) = 100 × (whole-body energy gain / energy consumption).
[8] Survival rate (SR) (%) = 100 × (final fish number / initial fish number).
[9] Condition factor, CF = 100 × fish weight / (body length)^3.
[10] Hepatosomatic index, HSI (%) = 100 × (liver weigh / body weight).
[11] Viserosomatic index, VSI (%) = 100 × (visceral weight / body weight).

C组比对照组表现出更高的血浆TC,FC,胆固醇酯，HDL-C,LDL-C水平。血浆TC,FC,LDL-C水平在TC组中显著比C组低。
肝TC和胆固醇酯水平在C组和TC组中显著比对照组高。肝FC水平在各组中没有显著差异。
C组和TC组粪便TC显著高于对照组，在各饲料处理中粪便中TBA没有显著差异。

($P>0.05$).

3.4. Activity of CYP7A1

The fish fed T-1.0% diet had the highest activity of CYP7A1 ($P<0.$

T组的CYP7A1活性最高，C组CYP7A1活性显著高于对照组。在大菱鲆肝脏中的CYP7A1活性在对照组和TC组中没有显著差异。

ference between the control and TC diet ($P>0.05$).

3.5. Cloning of partial cDNA sequence of the HMG-CoAr and expression of HMG-CoAr in liver

The PCR product amplified by the degenerate primers was 331 bp, and its nucleotide sequences was significantly homologous to yellowtail *Seriola quinqueradiata* (GenBank accession number: AB218826)

Table 3
Proximate composition in whole body of turbot fed the experimental diets (% diet on wet basis; n = 3).

	Control	C-1.0%	T-1.0%	TC	Pooled S.E	*F* value	*P* value
Moisture	76.5	76.2	76.9	76.4	0.1209	2.548	0.129
Crude protein	15.1	15.1	15.0	15.2	0.0756	0.374	0.774
Crude lipid	4.9^{b}	5.4^{c}	4.6^{a}	4.9^{b}	0.1146	3.163	0.086
Ash	3.5^{b}	3.2^{a}	3.3^{a}	3.2^{a}	0.0391	6.435	0.016

Values in the same row with no common superscripts are significantly different ($P<0.05$).

Table 4
Lipid profiles in plasma, liver and feces of turbot fed the experimental diet (n = 3).

	Control	C-1.0%	T-1.0%	TC	Pooled S.E	*F* value	*P* value
Plasma (mmol/L)							
Total cholesterol	3.27[a]	7.13[c]	2.66[a]	5.63[b]	0.5594	67.025	0.000
Free cholesterol	2.03[a]	3.59[c]	1.51[a]	2.89[b]	0.2566	18.958	0.001
Cholesterol esters	1.23[a]	3.54[b]	1.15[a]	2.74[b]	0.3287	18.506	0.001
HDL-C[1]	2.09[ab]	4.46[c]	1.85[a]	3.42[bc]	0.3699	7.664	0.010
LDL-C[2]	1.43[a]	2.88[b]	1.42[a]	2.10[a]	0.2080	8.484	0.007
HDL-C/LDL-C	1.46	1.52	1.31	1.66	0.1847	0.376	0.798
Liver (g/kg wet liver)							
Total cholesterol	2.97[a]	4.03[b]	3.44[ab]	4.07[b]	0.1799	3.720	0.061
Free cholesterol	1.22	1.38	1.22	1.41	0.0388	2.340	0.150
Cholesterol esters	1.75[a]	2.65[b]	2.22[ab]	2.66[b]	0.1501	3.510	0.069
Faeces (g/kg dry matter)							
Total cholesterol	5.0[a]	19.8[b]	5.1[a]	19.9[b]	2.2487	134.14	0.000
Bile acid	0.72	0.82	0.73	0.85	0.0299	1.240	0.357

Values in the same row with no common superscripts are significantly different ($P<0.05$).
[1] HDL-C, high-density lipoprotein cholesterol.
[2] LDL-C, low-density lipoprotein cholesterol.

用兼并引物扩增的PCR产物是331bp，并且其核苷酸序列与黄尾鱼，欧洲鲈和大西洋鲑的同源性很高，HMG-CoAr mRNA水平在TC组显著低于C组。

(identities cession number: *S. salar* (GenBank 80%). HMG-CoA TC diet compared

4. Discussion

FM contains approximately 500–700 mg taurine per 100 g dry matter, while plant proteins, such as SBM, contain only trace amounts of taurine (Yamamoto et al., 1998). Therefore, replacement of FM with plant protein source results in lower dietary levels of taurine. It has been reported that growth and feed efficiency of red sea bream (Takagi et al., 2006), cobia (Lunger et al., 2007), rainbow

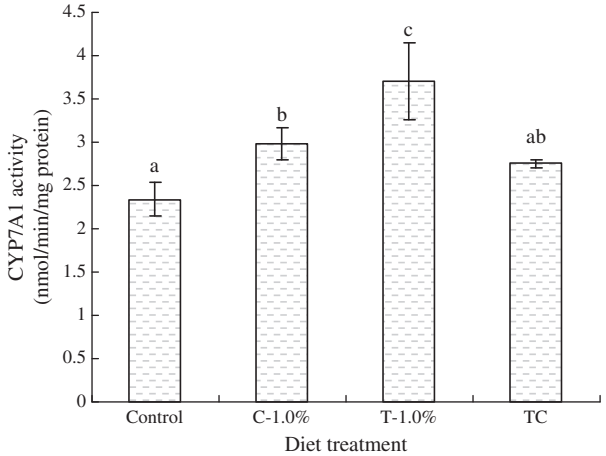

Fig. 1. Activity of cholesterol 7α hydroxylase (CYP7A1) in liver of turbot *Scophthalmus maximus* L. fed the experimental diets. Data of the four experimental diets are presented as mean ± S.E. (n = 3). Different letters above the bars denote significant differences between diet groups at the $P<0.05$ level.

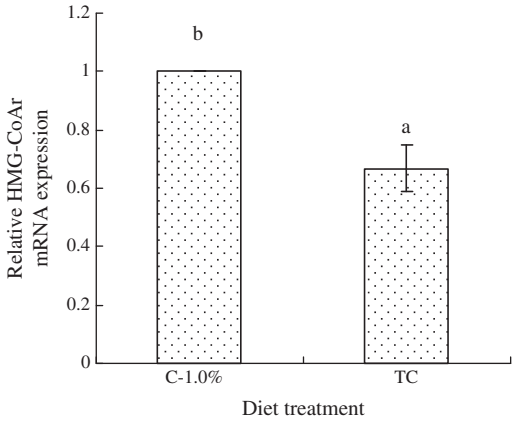

Fig. 2. Relative 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoAr) mRNA levels in liver of turbot *Scophthalmus maximus* L. after being fed the experimental diets including C-1.0%, and TC diets. HMG-CoAr mRNA levels were evaluated by real-time quantitative PCR and expressed relative to β-actin levels. Data of the two experimental diets are presented as mean ± S.E. (n = 3). Different letters above the bars denote significant differences between diet groups at the $P<0.05$ level.

trout (Gaylord et al., 2006, 2007) and yellowtail (Takagi et al., 2008) fed low levels of FM in diets based on high plant protein sources are improved by dietary taurine supplementation. In the present study, fish fed high plant protein diets with 1.14% taurine (T-1.0%) had significantly higher WGR compared to fish fed diet with 0.11% taurine (control). Thus, juvenile turbot appears to have a requirement for taurine when fish was fed high plant protein including SBM and WGM. This result was supported by the findings of Conceinçao et al. (1997) who reported that growth rates were positively correlated w a- ti t fo er so as a), ra), ar t st at in n- pr d co e bi n- br l., 19 h bi d no y re x (C of di n, is sufficient to maintain growth.

鱼粉每100g干物质中包含大约500-700mg牛磺酸，但是植物蛋白，如SBM，只含有很少的牛磺酸(Yamamoto et al., 1998)。因此，用植物蛋白源替代鱼粉会导致饲料牛磺酸水平下降。已有报道，真鲷(Takagi et al., 2006)，军曹鱼(Lunger et al., 2007)，虹鳟(Gaylord et al., 2006, 2007）和黄尾鱼(Takagi et al., 2008)饲喂低鱼粉高植物蛋白饲料的生长和饲料效率由于饲料牛磺酸的补充而提高。

在本研究中，高植物蛋白饲料含1.14%牛磺酸WGR显著高于饲料含0.11%牛磺酸（对照组）。由此，大菱鲆幼鱼似乎在当饲喂高植物蛋白包括SBM和WGM时对牛磺酸有需求。这个结果由Conceinçao et al.(1997)的发现所支持，他报道在大菱鲆幼鱼中GR与牛磺酸水平很相关。牛磺酸是氨基酸衍生物并且拥有刺激鱼类摄食的特点，例如低分子量，氮含量，水溶性和酸性（Carr，1982）。牛磺酸在欧洲鲈(Martinez et al., 2004)，虹鳟(Gaylord et al., 2006），黄尾鱼(Takagi et al., 2008)，牙鲆（Kim et al.,2005b）中用作主要的诱食剂。但是，在本实验中，T组的FI显著低于对照组。这就可能是牛磺酸的促进生长作用不是由于饲料摄入的增多。生长的促进可以归于一些与辅助作用相关的生物现象，如膜建立，抗氧化，解毒作用(Wright et al.,1986)，渗透调节(Thurston et al., 1980)，与胆汁酸结合(Goto et al., 1996)。但是，当与饲喂鱼粉饲料时，如之前报道的在虹鳟 (Gaylord et al., 2006)和牙鲷(Chatzifotis et al., 2008)中一样，牛磺酸补充并没增加生长。似乎表明饲料牛磺酸的水平，与一些内源性产物结合，对于维持生长是充足的。

Similarly, cholesterol is rich in FM, but only low levels are present in most plant sources (Cheng and Hardy, 2004; Deng et al., 2010). It h nd W al., 2 es w er, g ed b 0) fe le- m ed w dy, c ur-

同样的，胆固醇在鱼粉中也很丰富，在植物源中只存在少量(Cheng and Hardy, 2004; Deng et al., 2010)。也有报道表明，在基于植物蛋白源的饲料中叉尾鮰(Twibell and Wilson, 2004)，牙鲆(Chen, 2006; Deng et al.,2010)的生长，能由饲料胆固醇补充提高。但是，大西洋鲑(Bjerkeng et al., 1999)，杂交条纹鲈(Sealey et al., 2001)和牙鲆(Deng et al., 2010)在鱼粉饲料上补充胆固醇抑制了生长。因此，胆固醇补充可能是在植物蛋白基础上有需要。另外，在本实验中，胆固醇好牛磺酸的组合比T组和C组或对照组提高了大菱鲆幼鱼的生长。这样，观察到的胆固醇和牛磺酸系统促生长效果可以被解释为使含SBM和WGM的饲料（缺乏牛磺酸和胆固醇）又达到营养平衡。

bot compared to fish fed other diets containing either T-1.0% ($P<0.05$) or

C-1.0% ($P>0.05$) or the control ($P<0.05$) (Table 2). Thus, the observed synergistic growth-promotion effect of combined cholesterol and taurine can be explained by a re-equilibration of the diet containing SBM and WGM, which was short of taurine and cholesterol.

In the present study, the whole-body lipid content in fish fed C-1.0% diet was significantly higher than the control diet while that in fish fed T-1.0% diet was significantly lower than the control diet. Dietary addition of 1.0% cholesterol significantly resulted in higher app f
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involved in the whole-body lipid storage of fish fed plant-based diets with taurine supplementation needs further elucidation.

在本实验汇总，C组的总体脂肪含量显著高于对照组，但T组显著低于对照组。在SBM饲料中添加1%胆固醇显著导致了更高的粗脂肪表观消化系数和牙鲆的肝脏脂肪酶活性（Chen，2006）在本研究中，高植物蛋白饲料含1.25%胆固醇可以提高饲料脂肪的利用，增加了总体脂肪的贮存，与之相比，Gaylord et al. (2006)报道在植物饲料中添加牛磺酸（0.5-1.5%）鱼的总体脂肪含量没有影响。在植物饲料中添加牛磺酸对总体脂肪储存的及理论需要更深入的研究。

Taurine may interfere with lipid metabolism of fish, which had been shown in other animals where taurine exhibits hypolipidemic effects (Militante and Lombardini, 2004). Furthermore, the plasma cholesterol-lowering effects of taurine supplementation have been well dem
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bile acids by improved activity of CYP7A1 because of unchanged bile acid concentration and CYP7A1 activity (Table 4, Fig. 1).

牛磺酸可能会干涉到鱼脂肪的代谢，这在其他动物中有所表现，牛磺酸有降脂肪的效应(Militante and Lombardini, 2004)。另外，牛磺酸补充对血浆胆固醇的降低效应已经在大鼠(Murakami et al., 1996; Yokogoshi et al., 1999)，小鼠(Chen et al., 2005; Murakami et al., 1999)，仓鼠(Murakami et al., 2002)，人类(Mizushima et al., 1996)中有所证明。在大鼠，小鼠和仓鼠中，牛磺酸增加了胆汁酸的合成，同时增加了CYP7A1mRNA的表达活性，这是在肝胆汁酸合成中的限速酶(Murakami et al., 1996,1999, 2002; Yokogoshi et al., 1999)。表明通过刺激CYP7A1增加了胆固醇向胆汁酸的转化，这可能是牛磺酸降胆固醇效应的主要机理。在本实验中，与C组相比，TC组总血浆胆固醇显著下降，这不能归因于通过增加了CYP7A1的活性来增加了胆固醇向胆汁酸的转化，因为胆汁酸水平和CYP7A1活性都没变。

The liver cholesterol-lowering effects of taurine supplementation have been well established in rats or mice fed high cholesterol diet (Chen et al., 2005; Gandhi et al., 1992; Park and Lee, 1998; Yan et al., 1993). The cholesterol metabolism is affected by *de novo* cholesterol synthesis in liver of fish based on fish fed high cholesterol diet. A possible mechanism is that taurine supplementation led to a prior storage of cholesterol in liver of fish fed high plant protein based on high cholesterol diet, and then caused plasma cholesterol-lowering effects.

补充牛磺酸降低肝胆固醇的效果已经在饲喂高胆固醇饲料的大鼠和小鼠中证明了(Chen et al., 2005; Gandhi et al., 1992; Park and Lee, 1998; Yanet al., 1993)。胆固醇代谢受到肝内源胆固醇合成的影响。胆固醇的生物合成由负反馈机制控制，关键酶是HMG-CoAr(Maita et al., 2006)。在本实验中，TC组鱼肝的HMG-CoAr mRNA的相对表达显著低于C组。但是，TC组与C组相比，TC，FC和胆固醇酯水平并没改变。因此，牛磺酸补充可能抑制了高胆固醇饲料中鱼肝的胆固醇合成。可能的机理是牛磺酸补充导致高胆固醇的高植物蛋白饲料的鱼肝脏优先储存胆固醇，然后引发了血浆胆固醇降低效果。

In conclusion, the results of the present study showed that supplementation of either cholesterol or taurine and their combination in di rbot
fe lant
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ef ased
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HMG-CoAr mRNA.

总之，本实验的结果表明补充胆固醇，牛磺酸或二者组合能够显著提高植物蛋白饲料的大菱鲆幼鱼的生长表现。而且，当饲喂含高胆固醇的高植物蛋白饲料时，补充牛磺酸的血浆胆固醇降低效应归因于降低了胆固醇的合成能力，由于减少了HMG-CoAr mRNA的相对表达。

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