文章编号: 1000-0615(2019)11-2405-08 DOI: 10.11964/jfc.20180311205

茶多酚对大菱鲆生长、抗氧化能力及脂肪代谢相关基因表达的影响

李宜聪[1], 廖 凯[2], 姬仁磊[1], 方 炜[1],
徐 丹[1], 麦康森[1], 艾庆辉[1*]

(1. 中国海洋大学水产学院, 农业农村部水产动物营养与饲料重点实验室,
海水养殖教育部重点实验室, 山东 青岛 266003;
2. 宁波大学海洋学院, 浙江 宁波 315211)

摘要: 为了研究饲料中添加茶多酚对大菱鲆生长、抗氧化能力及脂肪代谢相关基因表达的影响, 以初始体质量为(13.51+0.31) g的大菱鲆幼鱼为实验对象, 设计4组添加不同梯度茶多酚(0%、0.01%、0.02%和0.05%, 干重添加量分别为0、100、200和500 mg/kg)的等氮等脂实验饲料, 进行为期70 d的摄食生长实验。结果显示: ①与对照组相比, 饲料中添加0.01%~0.02%茶多酚显著提高了大菱鲆幼鱼增重率(WGR); 饲料效率(FE)随饲料中茶多酚添加水平升高而升高, 但各组间差异不显著; 随饲料中茶多酚添加水平升高, 大菱鲆肝体比(HSI)呈降低趋势, 且显著低于对照组; ②鱼体组成分析表明, 投喂添加茶多酚饲料组大菱鲆鱼体和肝脏粗脂肪含量呈下降趋势, 且在茶多酚添加水平为0.02%~0.05%时达到最低值, 显著低于对照组; ③与对照组相比, 投喂添加茶多酚饲料组大菱鲆血清总抗氧化能力(T-AOC)及谷胱甘肽过氧化物酶(GSH-Px)活性显著升高, 且各添加组间差异不显著; 超氧化物歧化酶(SOD)活性随茶多酚的添加水平升高呈先升高后降低趋势, 且在添加水平为0.02%时显著高于对照组; 血清丙二醛(MDA)含量随茶多酚添加水平升高而降低, 且在添加水平为0.02%~0.05%时显著低于对照组; ④大菱鲆肝脏固醇调节元件结合蛋白 1(SREBP-1)表达量随着饲料中茶多酚添加水平升高而降低, 且在添加水平为0.02%~0.05%时达到最低值, 显著低于对照组; 脂肪酸合成酶(FAS)表达量随茶多酚添加水平的升高呈先降低后升高趋势, 且在添加水平为0.02%时显著低于对照组; 过氧化物酶体增殖物激活受体α(PPAR α)表达量变化趋势与FAS相反, 且在添加水平为0.02%时达到最高值。肉毒碱棕榈酰转移酶1(CPT1)表达量随饲料中茶多酚添加水平升高而升高, 显著高于对照组, 且各添加组间差异不显著。研究表明, 高脂饲料中添加茶多酚能促进大菱鲆生长、降低肝脏脂肪过度沉积并提高血清抗氧化能力, 高脂饲料中添加0.02%茶多酚是大菱鲆幼鱼生长的最适添加量。

关键词: 大菱鲆; 茶多酚; 生长; 体组成; 抗氧化能力; 脂肪代谢
中图分类号: S 963.73 **文献标志码:** A

高脂饲料具有蛋白质节约效应, 能促进鱼类生长并有效减少有机物和氮元素的损失, 具有保护环境、节约成本的优点[1-2]。然而, 高脂饲料在鱼类集约化养殖过程中的广泛应用也带来了诸多问题, 例如引起肝脏脂肪异常沉积和肝脏损伤[3]、降低疾病抵抗力和鱼肉品质等[4-5]。

收稿日期: 2018-03-13 **修回日期:** 2018-04-04
资助项目: 国家鲆鲽类产业技术体系建设专项(CARS-50-G08); 鳌山人才计划(2015ASTP); 国家重点研发计划(2018YFD0900402)
通信作者: 艾庆辉, E-mail: qhai@ouc.edu.cn

大菱鲆(*Scophthalmus maximus*)是我国北方地区最重要的养殖经济鱼类之一。目前大菱鲆的养殖主要使用人工配合饲料，其生长所需的最适脂肪水平为12%~15%，而实际养殖过程中一般使用脂肪水平16%以上的高脂饲料，易造成养殖大菱鲆脂肪摄入过剩引起病变。大菱鲆的脂代谢机理与其他一些鱼类类似，所以大菱鲆是一种适合研究脂代谢机理的海水鱼模型动物[6]。

茶多酚是茶叶中所含的一种多酚类化学物质，其主要的活性物质为表没食子儿茶素没食子酸酯(EGCG)，化学式为$C_{22}H_{18}O_{11}$。茶多酚作为一种天然免疫增强剂，已经被研究证实在哺乳动物中具有提高抗氧化能力[7]、抗菌抗病毒[8-9]、减少血浆甘油三酯(TG)[10]及总胆固醇(TC)含量[11]、抑制机体及肝脏脂肪过度沉积[10, 12]等诸多生物学作用。另外，在水生动物中关于茶多酚的研究还相对较少，仅在牙鲆(*Paralichthys olivaceus*)[13]、虹鳟(*Oncorhynchus mykiss*)[14]、异育银鲫(*Carassius auratus gibelio*)[15] 和尼罗罗非鱼(*Oreochromis niloticus*)[16]中发现茶多酚能促进抗氧化和抗炎能力，而关于茶多酚对鱼类脂代谢的影响目前尚未见报道。因此，本研究旨在探讨高脂饲料中添加茶多酚对大菱鲆生长和抗氧化能力的影响，并通过测定脂肪代谢相关基因表达量的变化初步探究茶多酚对大菱鲆脂代谢的调控机理，力求寻找一种高效可行的降脂免疫增强剂，为大菱鲆养殖业提供基础支持。

1 材料与方法

1.1 实验饲料制备

以白鱼[大西洋鳕(*Gadus morhua*)]粉、豆粕为主要蛋白源，鱼油、豆油及卵磷脂为主要脂肪源，高筋小麦粉为主要糖原配制成4种等氮等脂的实验饲料。其中蛋白质水平为50%左右，脂肪水平为18%左右，茶多酚(Sigma，纯度98%以上，有效成分儿茶素含量90%以上)添加水平为0%、0.01%、0.02%和0.05%(干重添加量分别为0、100、200和500 mg/kg)。饲料中茶多酚添加水平参考Tian等[17]、龙萌等[18]及刘振兴等[19]实验结果确定。实验饲料组成及营养成分分析见表1。

所有原料粉碎后过60 μm筛网，采用逐级混匀的方式将原料混匀，然后加入油源及水揉匀，经双螺杆饲料挤条机加工成硬质颗粒饲料(2 mm×2 mm)，并用55 °C烘箱烘干10 h以上，密封包装储存于–20 °C冰箱备用。

1.2 实验设计和养殖管理

实验用大菱鲆幼鱼为山东海阳黄海水产有限公司养殖场人工孵化的同一批幼鱼。在正式养殖实验前，将大菱鲆幼鱼放于室内流水养殖系统(2.0 m×2.0 m×1.5 m)中暂养2周，并以1号商业饲料(青岛七好生物科技有限公司，规格1 mm×1 mm，粗蛋白含量50%，粗脂肪含量12%)每日2次饱食投喂，使之适应养殖系统及配合饲料。

正式实验开始前, 对实验鱼饥饿24 h，挑选身体健康、大小一致的大菱鲆幼鱼[初始体质量为(13.51±0.31) g] 360尾，随机分配至12个塑料桶中(0.6 m×0.6 m×0.9 m)，每个桶中放养30尾。每种饲料随机分为3个重复，每天饱食投喂2次(8:00和18:00)。实验期间海水水温为18~24 °C，pH为7.8~8.0，盐度为29~33，溶解氧含量在5.0 mg/L以上，养殖周期为70 d。

1.3 样品采集

养殖实验持续10周，实验结束前将实验鱼饥饿24 h，将每个水箱所有存活的实验鱼计数并称重。每个处理组取9尾鱼，称重计数后用2 mL无菌注射器于尾静脉取血，4 °C离心，取上清液保存于–80 °C冰箱待测。解剖得到肝脏和内脏分别称重，用于计算肝脏指数和内脏指数。取3尾鱼解剖得到肝脏，并存放于1.5 mL无RNA酶离心管中，保存于–80 °C冰箱，用于RNA提取及实时荧光定量实验。最后取5尾鱼放于–20 °C冰箱中保存，用于鱼体常规成分分析。

1.4 实验分析

饲料原料及鱼体粗脂肪、粗蛋白和水分的测定参照AOAC方法[21]进行；肝脏及肌肉脂肪含量测定采用氯仿—甲醇法。

血清超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GSH-Px)活性，总抗氧化能力(T-AOC)及丙二醛(MDA)含量采用南京建成生物研究所试剂盒进行测定，所有实验操作严格遵循说明书进行。

肝脏总RNA参照Trizol试剂盒(TaKaRa，日本)提取，根据PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa，日本)说明书通过引物[20]进行反转录(表2)。

表 1　饲料配方及常规营养成分(干重)

Tab. 1　Formulation and proximate composition of the experimental diets　%

原料成分 ingredients	饲料茶多酚添加水平　dietary tea polyphenols level			
	0%	0.01%	0.02%	0.05%
鱼粉　fish meal	30.00	30.00	30.00	30.00
小麦谷朊粉　wheat gluten meal	12.00	12.00	12.00	12.00
酪蛋白　casein	13.00	13.00	13.00	13.00
小麦粉　wheat meal	7.65	7.64	7.63	7.60
豆粕　soybean meal	16.00	16.00	16.00	16.00
豆油　soybean oil	14.00	14.00	14.00	14.00
磷脂　phospholipid	2.00	2.00	2.00	2.00
矿物质预混料　mineral premix[1]	1.00	1.00	1.00	1.00
维生素预混料　vitamin premix[1]	0.50	0.50	0.50	0.50
氯化胆碱　choline chloride	0.10	0.10	0.10	0.10
磷酸二氢钙　monocalcium phosphate	1.00	1.00	1.00	1.00
丙酸钙　calcium propionic acid	0.10	0.10	0.10	0.10
防霉剂　mold inhibitor[2]	0.10	0.10	0.10	0.10
诱食剂　attractant mixture[3]	1.00	1.00	1.00	1.00
乙氧基喹啉　ethoxyquin	0.05	0.05	0.05	0.05
茶多酚　tea polyphenol	0.00	0.01	0.02	0.05
海藻酸钠　sodium alginate	1.50	1.50	1.50	1.50
合计　total	100	100	100	100
营养成分　proximate composition[4]				
干物质　dry matter	92.11	92.07	91.80	91.79
粗蛋白　crude protein	50.23	50.70	50.31	50.91
粗脂肪　crude lipid	18.36	18.88	18.21	18.22

注：1.维生素和矿物质预混料参考[20]；2.防霉剂，富马酸与丙酸钙按1∶1混合而成；3.诱食剂，甘氨酸与甜菜碱按1∶3混合而成；4.营养成分为实测值

Notes: 1. vitamin premix and mineral premix according to [20]; 2. mold inhibitor, fumaric acid and calcium are mixed by 1/1; 3. attractant, glycine and betaine are mixed by 1/3; 4. nutrient composition was actual measurement values

1.5　计算及统计方法

存活率(survival rate, SR, %)=$FN/IN\times100\%$

增重率(weight gain rate, WGR, %)=$(W_t-W_0)/W_0\times100\%$

特定生长率(specific growth rate, SGR, %/d)=$(\ln W_t-\ln W_0)/t\times100\%$

肝体比(hepato-somatic index, HSI, %)=$(W_l/W_t)\times100\%$

脏体比(viscera-somatic index, VSI, %)=$(W_v/W_t)\times100\%$

饲料效率(feed efficiency, FE)=$(W_t-W_0)/W_f$

式中，FN为存活数量(尾)，IN为初始数量(尾)，W_t为终末体质量(g)，W_0为初始体质量(g)，W_l为肝脏湿重(g)，W_v为内脏团湿重(g)，W_f为摄食干重(g)，t为养殖时间(d)。

实时荧光定量结果采用$2^{-\Delta\Delta C_t}$法进行计算。数据采用SPSS 18.0进行单因素方差分析(One-Way ANOVA)，当差异显著时($P<0.05$)，采用Tukey氏检验进行多重比较，实验数据以平均值±标准误(mean±SE)表示。

表 2 荧光定量PCR所用引物序列

Tab. 2 Primer pair sequences for Real-time PCR in the present study

目的基因 target genes	正向引物 forward primer(5′-3′)	反向引物 reverse primer(5′-3′)
CPT 1	GCCTTTCAGTTCACCATCACA	ATGCGGCTGACTCGTTTCTT
PPAR α	CGATCAGGTGACCCTGTTAA	TGGAACTTGGGCTCCATCd
SREBP-1	CGATCCGCACTCCAAGT	CCGCACTGCCCTGAAT
FAS	GGCAACAACACGGATGGATAC	CTCGCTTTGATTGACAGAACAC
β-actin	GTAGGTGATGAAGCCCAGAGCA	GTAGGTGATGAAGCCCAGAGCA

注：*CPT* 1. 肉碱棕榈酸酰基转移酶 1；*PPAR α*. 过氧化物增殖物活化受体 α；*SREBP*-1. 固醇调节元件结合蛋白；*FAS*. 脂肪酸合酶

Notes: *CPT* 1. carnitine palmitoyltransferase 1; *PPAR α*. peroxisome proliferator-activated receptor α; *SREBP*-1. sterol regulatory element-binding protein 1; *FAS*. fatty acid synthase

2 结果

2.1 茶多酚添加水平对大菱鲆生长性能的影响

大菱鲆SR和FE随饲料中茶多酚添加水平升高而升高，但各组间差异不显著(P>0.05)。大菱鲆VSI随饲料中茶多酚添加水平升高而降低，但各组间差异不显著(P>0.05)。大菱鲆FW、WGR和SGR随饲料中茶多酚添加水平升高呈先升高后降低趋势，且在添加水平为0.01%~0.02%时达到最高值，显著高于对照组(P<0.05)，而0.05%添加组与对照组差异不显著(P>0.05)。与对照组相比，大菱鲆HSI随饲料中茶多酚添加水平升高而显著降低(P<0.05)，各添加组间差异不显著(P>0.05)(表3)。

2.2 茶多酚添加水平对大菱鲆鱼体组成变化的影响

与对照组相比，饲料中添加茶多酚对大菱鲆幼鱼鱼体水分和粗蛋白含量影响不显著(P>0.05)。大菱鲆肌肉脂肪含量随饲料中茶多酚添加水平升高而降低，但各组间差异不显著(P>0.05)。大菱鲆鱼体及肝脏脂肪含量随饲料中茶多酚添加水平升高而降低，各添加水平显著低于对照组，且在添加水平为0.02%~0.05%时显著低于其他各组(P<0. 05)(表4)。

2.3 茶多酚添加水平对大菱鲆血清抗氧化能力的影响

与对照组相比，投喂添加茶多酚饲料组大菱鲆血清T-AOC及GSH-Px活性显著升高(P <0.05)，且各添加组间差异不显著。血清SOD活性随茶多酚添加水平升高呈先升高后降低趋势，各添加组显著高于对照组，且在添加水平为0.02%时达到最高值(P <0.05)。血清MDA含量随饲料中茶多酚添加水平升高而降低，且在添加水平为0.02%~0.05%时显著低于对照组(P <0. 05)；0.01%添加组

表 3 饲料茶多酚添加水平对大菱鲆生长性能的影响(湿重)

Tab. 3 Effects of graded levels of tea polyphenol on growth of *S. maximus*(wet basis)

指标 indicators	饲料茶多酚添加水平/% dietary tea polyphenols level			
	0%	0.01%	0.02%	0.05%
终末体质量/g W_t	46.21±2.30^b	50.16±1.07^a	51.37±1.50^a	48.33±1.66^{ab}
存活率/% SR	97.78±1.11	100	100	100
增重率/% WGR	242.04±10.94^b	270.97±11.81^a	278. 27±15.32^a	266.88±9.42^{ab}
特定生长率/(%/d) SGR	1.75±0.06^b	1.87±0.05^a	1.90±0.08^a	1.84±0.04^{ab}
饲料效率 FE	1.11±0.03	1.13±0.04	1.14±0.08	1.15±0.07
肝体比/% HSI	1.63±0.20^a	1.32±0.22^b	1.37±0.26^b	1.29±0.15^b
脏体比/% VSI	5.45±0.33	5.46±0.12	5.43±0.38	5.42±0.22

注：表中同一行中不同上标字母表示差异显著(P<0.05)，下同

Notes: the data with the different superscripts in the same row are significantly different (P<0.05), the same below

表 4　饲料茶多酚添加水平对大菱鲆体组成的影响(湿重)

Tab. 4　Effects of graded levels of tea polyphenol on body composition of *S. maximus*　%

指标 indicators	饲料茶多酚添加水平/% dietary tea polyphenols level			
	0%	0.01%	0.02%	0.05%
全鱼 whole fish				
水分 moisture	78.70±0.32	76.59±0.73	76.75±0.15	77.32±0.62
粗蛋白质 crude protein	15.92±0.05	16.02±0.06	15.99±0.03	15.92±0.04
粗脂肪 crude lipid	4.28±0.04^{a}	4.10±0.01^{b}	3.77±0.03^{c}	3.78±0.01^{c}
肝脏及肌肉指标 liver and muscle indicators				
肝脏粗脂肪 liver crude lipid	9.80±0.05^{a}	9.03±0.09^{b}	8.27±0.05^{c}	8.12±0.02^{c}
肌肉粗脂肪 muscle crude lipid	0.98±0.04	0.94±0.01	0.92±0.01	0.90±0.01

与对照组差异不显著(表5)。

2.4　茶多酚添加水平对大菱鲆肝脏*SREBP*-1、*FAS*、*CPT* 1及*PPAR α*表达量的影响

大菱鲆肝脏*SREBP*-1 mRNA表达量随饲料中茶多酚添加水平升高而降低，且在添加水平为0.02%~0.05%时达到最低值，显著低于对照组(P<0.05)；0.01%添加组与对照组差异不显著(P>0.05)。肝脏*FAS* mRNA表达量随饲料中茶多酚添加水平升高呈先降低后升高趋势，且在添加水平为0.02%时达到最低值，显著低于对照组(P < 0.05)；其余添加组与对照组差异不显著。与对照组相比，饲料中添加茶多酚显著提高了肝脏*CPT*-1 mRNA表达量(P <0. 05)，各添加组间差异不显著。肝脏*PPAR α* mRNA表达量随饲料中茶多酚添加水平升高呈先升高后降低趋势，且在添加水平为0.02%时达到最高值，显著高于对照组(P <0.05)；其余添加组差异不显著(P>0.05)(表6)。

表 5　饲料茶多酚添加水平对大菱鲆血清抗氧化酶活性影响

Tab. 5　Effects of graded levels of tea polyphenol on antioxidant enzyme activities of *S. maximus*

指标 indicators	饲料茶多酚添加水平/% dietary tea polyphenols level			
	0%	0.01%	0.02%	0.05%
SOD/(U/mL)	15.89±0.28^{c}	17.96±0.27^{b}	19.23±0.25^{a}	18.97±0.15^{ab}
T-AOC/(U/mL)	6.18±0.08^{b}	7.02±0.08^{a}	7.29±0.05^{a}	7.24±0.03^{a}
GSH-Px/(U/mg)	53.22±1.79^{b}	74.25±2.80^{a}	75.59±0.98^{a}	73.92±1.94^{a}
MDA/(mmol/mL)	7.79±0.17^{a}	6.92±0.19^{ab}	6.70±0.16^{b}	6.71±0.24^{b}

表 6　饲料茶多酚添加水平对大菱鲆肝脏脂肪代谢相关基因表达量的影响

Tab. 6　Effects of graded levels of tea polyphenol on expressions of lipid metabolism related genes of *S. maximus*

指标 indicators	饲料茶多酚添加水平/% dietary tea polyphenols level			
	0%	0.01%	0.02%	0.05%
SREBP-1	1.00±0.06^{a}	0.88±0.02^{ab}	0.80±0.02^{b}	0.82±0.03^{b}
FAS	1.00±0.07^{a}	0.86±0.03^{ab}	0.81±0.02^{b}	0.84±0.02^{ab}
CPT 1	1.00±0.05^{b}	1.26±0.03^{a}	1.32±0.03^{a}	1.27±0.04^{a}
PPAR α	1.00±0.06^{b}	1.24±0.09^{ab}	1.28±0.09^{a}	1.23±0.05^{ab}

3　讨论

本实验结果显示，饲料中添加0.01%~0.02%茶多酚显著促进了大菱鲆的生长，这与在团头鲂[18](*Megalobrama amblycephala*)、尼罗罗非鱼[19]和虹鳟[22]中的研究结果相一致。说明适当添加茶多酚对鱼类的生长具有促进作用。而有关茶多酚的促生长作用机理尚未有确切报道，还需要进一步探究。茶多酚0.01%~0.02%添加组生长显著，但组间差异不显著，可能是因为2个添加水平都满足了鱼类生长需要；而0.05%添加水平导致生长下降可能是因为饲料中过高的茶多酚会导致其摄食和饲料转化率下降所致。

本实验中添加茶多酚显著降低了大菱鲆肝脏脂肪含量及肝体比，这与李金龙[23]的研究结果相似，其研究发现饲料中添加茶多酚能一定程度降低青鱼(*Mylopharyngodon piceus*)鱼体及肝脏脂肪沉积。关于茶多酚的降脂作用机制在鱼类

中未见报道，只在小鼠(*Mus musculus*)上发现茶多酚能通过调控脂质代谢基因的表达来发挥作用[24]。本实验中分析脂肪代谢基因的结果发现，饲料中添加0.02%茶多酚同时显著上调了*PPAR α*及*CPT* 1 mRNA表达量，*PPAR α*在脂肪分解代谢的调控过程中发挥重要的作用[25]。*PPAR α*被激活后能显著上调 *CPT* 1基因表达量，而后者是长链脂肪酸进入线粒体氧化分解的关键限速酶，所以*CPT* 1 mRNA表达量的上升可能促进脂肪酸的氧化分解，由此推测茶多酚可能是通过促进脂肪酸氧化分解来发挥降脂作用。另外，在本实验中，饲料中添加0.02%茶多酚可显著下调*SREBP*-1和*FAS* mRNA的表达量。*SREBP*-1在肝脏甘油三酯和脂肪酸合成过程中发挥重要作用，有研究发现，在肥胖小鼠肝脏中*SREBP*-1表达水平上升，且*SREBP*-1转基因小鼠肝脏脂肪酸合成明显加快，甘油三酯生成增多[26]。结合本实验结果，可以推测茶多酚能抑制*SREBP*-1活化、降低其目的基因*FAS*表达量，进而抑制脂肪酸的合成。

除降脂作用外，茶多酚在提高鱼体抗氧化能力方面也具有重要作用。SOD、GSH-Px等酶是鱼体抗氧化系统的关键组成部分[27]。当饲喂高脂饲料时，由于脂质过氧化导致机体抗氧化能力受到损伤并产生大量过氧化的有害产物(如MDA)，从而损伤机体健康，饲料中添加茶多酚则能减少这种损伤[28]。在本实验高脂饲料中添加茶多酚，显著提高了大菱鲆血清抗氧化能力，缓解了因饲喂高脂饲料所引起的机体抗氧化能力下降导致的危害，这与李金龙[23]的研究结果一致。这可能因为茶多酚能通过提供电子氢，清除机体内多余的自由基或过氧化物，且能与发生氧化反应所必备的多种金属离子络合在一起，从而降低氧化反应速率，起到抗氧化的作用。

综上所述, 饲料中添加茶多酚能促进大菱鲆生长、降低肝脏脂肪沉积和提高抗氧化能力，其降脂作用机理可能为促进脂肪氧化分解并抑制脂肪酸合成。另外，在本实验条件下结合生长、体成分分析、抗氧化酶活性变化及成本效果问题分析得出，0.01%~0.02%茶多酚是适宜添加量。

参考文献：

[1] Yigit M, Tsuda K, Suzuki Y, *et al*. The protein sparing effects of high lipid levels in diets for rainbow trout (*Oncorhynchus mykiss*, W. 1792) with special reference to reduction of total nitrogen excretion[J]. The Israeli Journal of Aquaculture, 2002, 54(2): 79-88.

[2] Boujard T, Gelineau A, Coves D, *et al*. Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (*Dicentrarchus labrax*) fed high fat diets[J]. Aquaculture, 2004, 231(1-4): 529-545.

[3] Boujard T, Laurent L, Aupérin B. Feeding behaviour, energy expenditure and growth of rainbow trout in relation to stocking density and food accessibility[J]. Aquaculture Research, 2002, 33(15): 1233-1242.

[4] Ai Q H, Mai K S, Zhang C, *et al*. Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus*[J]. Aquaculture, 2004, 242(1): 489-500.

[5] Du Z Y, Clouet P, Huang L M, *et al*. Utilization of different dietary lipid sources at high level in herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic fatty acid oxidation[J]. Aquaculture Nutrition, 2008, 14(1): 77-92.

[6] Morzel M, Sohier D, Hvd V. Evaluation of slaughtering methods for turbot with respect to animal welfare and flesh quality[J]. Journal of the Science of Food & Agriculture, 2003, 83(1): 19-28.

[7] Benzie I F, Szeto Y T. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay[J]. Journal of Agricultural and Food Chemistry, 1999, 47(2): 633-636.

[8] Hattori M, Kusumoto I T, Namba T, *et al*. Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*[J]. Chemical & Pharmaceutical Bulletin, 1990, 38(3): 717-720.

[9] Nakayama M, Suzuki K, Toda M, *et al*. Inhibition of the infectivity of influenza virus by tea polyphenols[J]. Antiviral Research, 1993, 21(4): 289-299.

[10] Wang S, Huang Y, Xu H, *et al*. Oxidized tea polyphenols prevent lipid accumulation in liver and visceral white adipose tissue in rats[J]. European Journal of Nutrition, 2016: 1-12.

[11] Chan P T, Fong W P, Cheung Y L, *et al*. Jasmine green tea epicatechins are hypolipidemic in hamsters (*Mesocricetus auratus*) fed a high fat diet[J]. Journal of Nutrition, 1999, 129(6): 1094-1101.

[12] Chaudhari P N, Hatwalne V G. Effect of epicatechin on liver lipids of rats fed with choline deficient diet[J]. Indian Journal of Nutrition & Dietetics, 1977, 14(5): 136-139.

[13] Cho S H, Lee S M, Park B H, *et al*. Effect of dietary inclusion of various sources of green tea on growth, body composition and blood chemistry of the juvenile olive flounder, *Paralichthys olivaceus*[J]. Fish Physiology & Biochemistry, 2007, 33(1): 49-57.

[14] Xu Q Y, L I. Effects of tea polyphenols on growth performance, blood biochemical Indices and non-specific immune indices of rainbow trout (*Oncorhynchus mykiss*)[J]. Chinese Journal of Animal Nutrition, 2008.

[15] Gu L F, Hou Y Q, Ding B Y, *et al*. Effects of several plant extracts on growth performance and blood biochemical indices in *Carassius auratus gibelio*[J]. Freshwater Fisheries, 2008.

[16] Liu Z X, Hao K, Le H, *et al*. Effects of tea polyphenols on growth performance, antioxidant effect and non-specific immune indices of *Oreochromis niloticus*[J]. Guangdong Agricultural Sciences, 2012, 39(23): 113-115.

[17] Tian C, Ye X, Zhang R, *et al*. Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPARγ-adiponectin pathway[J]. Plos One, 2013, 8(1): 12-14.

[18] 龙萌, 侯杰, 苏玉晶, 等. 酵母硒和茶多酚对团头鲂幼鱼生长和生长轴基因表达、营养品质及抗病力的影响[J]. 水产学报, 2015, 39(1): 97-107.
Long M, Hou J, Su Y J, *et al*. Effects of selenium yeast and tea polyphenols on growth and related gene transcription in the hypothalamus-pituitary-growth axis,muscle composition and disease resistance of juvenile Wuchang bream (*Megalobrama amblycephala*)[J]. Journal of Fisheries of China, 2015, 39(1): 97-107(in Chinese).

[19] 刘振兴, 柯浩, 郝乐, 等. 茶多酚对罗非鱼生长性能、抗氧化功能和非特异免疫指标的影响[J]. 广东农业科学, 2012, 39(23): 113-115.
Liu Z X, Ke H, Hao L, *et al*. Effects of tea polyphenols on growth performance, antioxidant effect and non-specific immune indices of *Oreochromis niloticus*[J]. Guangdong Agricultural Sciences, 2012, 39(23): 113-115(in Chinese).

[20] 彭墨, 徐玮, 麦康森, 等. 亚麻籽油替代鱼油对大菱鲆幼鱼生长、脂肪酸组成及脂肪沉积的影响[J]. 水产学报, 2014, 38(8): 1131-1139.
Peng M, Xu W, Mai K S, *et al*. Growth performance,fatty acids composition and lipid deposition injuvenile turbot (*Scophthalmus maximus*) fed diets with various fish oil substitution levels by linseed oil[J]. Journal of Fisheries of China, 2014, 38(8): 1131-1139(in Chinese).

[21] Association of Official Analytical Chemists. Official methods of analysis of AOAC international[M]. 16th ed. Arlington: AOAC International, 1995.

[22] 徐奇友, 李婵, 许红, 等. 茶多酚对虹鳟生长性能、生化指标和非特异性免疫指标的影响[J]. 动物营养学报, 2008, 20(5): 547-553.
Xu Q Y, Li C, Xu H, *et al*. Effects of tea polyphenols on growth performance, blood biochemical indices and non-specific immune indices of rainbow trout (*Oncorhynchus mykiss*)[J]. Chinese Journal of Animal Nutrition, 2008, 20(5): 547-553(in Chinese).

[23] 李金龙. 茶多酚对青鱼幼鱼生长、免疫及脂肪代谢的影响[D]. 长沙: 湖南农业大学, 2013: 15-16.
Li J L. Effects of tea polyphenols on growth,immunity and lipid metabolisn in juvenile *Mylopharyngodon piceus*[D]. Changsha: Hunan Agricultural University, 2013: 15-16(in Chinese).

[24] Ikeda I, Tsuda K, Suzuki Y, *et al*. Tea catechins with a galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats[J]. Journal of Nutrition, 2005, 135(2): 155.

[25] Bose M, Lambert J D, Ju J, *et al*. The major green tea polyphenol, (−)− epigallocatechin -3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice[J]. Journal of Nutrition, 2008, 138(9): 1677.

[26] 宁丽军. 尼罗罗非鱼过氧化物体增殖激活受体(PPARα)激活及其降脂机制研究[D].上海: 华东师范大学, 2016: 26-27.
Ning L J. The activition and the related regulatory mechanism of PPARα in lipid metabolism of Nile tilapia, *Oreochromis niloticus*[D]. Shanghai: East China Normal University, 2016: 26-27(in Chinese).

[27] Shearer K D. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids[J]. Aquaculture, 1994, 119(1): 63-88.

[28] 张克烽, 张子平, 陈芸, 等. 动物抗氧化系统中主要抗氧化酶基因的研究进展[J]. 动物学杂志, 2007, 42(2): 153-160.
Zhang K F, Zhang Z P, Chen Y, *et al*. Antioxidant defense system in animals[J]. Chinese Journal of Zoology, 2007, 42(2): 153-160(in Chinese).

Effects of tea polyphenols on growth, antioxidant capacity and lipid metabolism related genes expression of turbot (*Scophthalmus maximus*)

LI Yicong [1], LIAO Kai [2], JI Renlei [1], FANG Wei [1], XU Dan [1], MAI Kangsen [1], AI Qinghui [1*]

(1. *Key Laboratory of Aquaculture Nutrition and Feed*, *Ministry of Agriculture and Rural Affairs*, *Key Laboratory of Mariculture*, *Ministry of Education*, *Ocean University of China*, *Qingdao* 266003, *China*;
2. *School of Marine Sciences*, *Ningbo University*, *Ningbo* 315211, *China*)

Abstract: In aquaculture, high fat diets are widely used for its protein sparing effect which can improve protein utilization efficiency, promote growth and protect environment pollution. Hoevever, it can also lead to excessive lipid deposition of liver and lower disease resistance. In order to solve this problem, additives should be explored. Tea polyphenols are natural plant extracts which have been proved to have the effects of of promoting growth, lipid reduction and immunity improving in many animals. Therefore, the purpose of the experiment is to investigate the effects of dietary tea polyphenols (TP) level on growth, antioxidant capacity and lipid metabolism of turbot. Four diets with graded levels of TP (0.00%, 0.01%, 0.02% and 0.05%. dry weight 0, 100, 200 and 500 mg/kg) was formulated in this experiment. Fish were cultured in indoor aquaculture system, and each diet was randomly assigned to three replicate groups of juvenile fish [initial average weight of (13.51+0.31) g] and fed twice daily (8:00, 18:00).The feeding trial lasted 70 d. The results showed as follows: ① Compared with the control group, fish fed the diet with 0.01%–0.02% TP had significantly higher weight gain rate (WGR). Feed efficiency (FE) was increased with the increasing TP level and no significant differences were detected among dietary treatments. Compared with the control group, Hepato-somatic index (HSI) was significantly increased by the supplement with TP. ② The lipid contents of whole fish and liver were decreased with increasing TP level and significantly lower in 0.02%–0.05% group compared with the control group. ③ Compared with the control group, the activities of total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-Px) were significantly higher in fish fed the diet with TP, and no significant differences were detected among fish fed diets with TP. The activity of superoxide dismutase (SOD) first increased and then decreased with the increasing TP level, and 0.02% supplement level was the highest. The content of malondialdehyde (MDA) was decreased with increasing TP level, and significantly higher in 0.02% group compared with the control group. ④ The expression of sterol regulatory element binding protein 1(SREBP-1) was decreased with increasing TP level and significantly higher in 0.02%–0.05% groups compared with the control group. The expression of fatty acid synthetase (FAS) was first decreased and then increased with increasing TP level and significantly lower in 0.02% group compared with the control group. The expression of peroxisome proliferator-activated receptor α (PPAR α) first increased and then decreased with increasing TP level and significantly higher in 0.02% group compared with the control group. The expression of carnitine palmitoyl transferase 1(CPT 1) increased with increasing TP level and significantly higher compared with the control group. This research indicated that high fat diets supplemented with TP could promote growth, reduce liver fat deposition, and increase antioxidant activity. The optimum supplemental level of TP in diets for turbot is approximatly 0.02%.

Key words: *Scophthalmus maximus*; tea polyphenols; growth; body composition; antioxidant ability; lipid metabolism

Corresponding author: AI Qinghui. E-mail: qhai@ouc.edu.cn

Funding projects: China Agriculture Research System (CARS-50-G08); Aoshan Talents Program (2015ASTP); National Key R & D Program of China (2018YFD0900402)