水生生物学报
ACTA HYDROBIOLOGICA SINICA

果胶高载引起的黄颡鱼绿肝症和肝纤维化与肠道菌群失调的关联

姚仕彬 任胜杰 蔡春芳 曹霞敏 吴萍 叶元土 姜光明 丁惠明 张铖

GREEN LIVER SYMPTOM AND LIVER FIBROSIS CAUSED BY PECTIN OVERLOAD IN *PELTEOBAGRUS FULVIDRACO* MAY BE RELATED TO THE INTESTINAL DYSBACTERIOSIS

YAO Shi-Bin, REN Sheng-Jie, CAI Chun-Fang, CAO Xia-Min, WU Ping, YE Yuan-Tu, JIANG Guang-Ming, DING Hui-Ming, ZHANG Cheng

在线阅读 View online: https://doi.org/10.7541/2022.2021.0257

---

doi: 10.7541/2022.2021.0257

果胶高载引起的黄颡鱼绿肝症和肝纤维化与肠道菌群失调的关联

姚仕彬[1,2] 任胜杰[1,2] 蔡春芳[1,2] 曹霞敏[1,2] 吴 萍[1,2] 叶元土[1,2]
姜光明[3] 丁惠明[4] 张 铖[4]

(1. 苏州大学基础医学与生物科学学院, 苏州 215123; 2. 江苏省水产动物营养重点实验室, 苏州 215123;
3. 苏州市吴中区农业农村局, 苏州 215104; 4. 苏州市阳澄湖国家现代农业示范区发展有限公司, 苏州 215138)

摘要: 日粮中黏性膳食纤维果胶较高时可引起黄颡鱼(*Pelteobagrus fulvidraco*)的绿肝症和肝纤维化, 研究旨在探讨肠道菌群在上述病理过程中的潜在作用。分别配制含30%糊精和果胶的对照日粮和高果胶日粮, 命名为CON和PEC日粮, 再在CON和PEC日粮中添加0.2%的抑菌剂甲硝唑, 命名为CONM和PECM日粮。用4种日粮饲养初始体质量为(21.5±3.6) g/尾的黄颡鱼, 分别于饲养7d和56d后采样分析。16S rRNA分析结果显示, 日粮中添加甲硝唑抑制了梭杆菌门(Fusobacteria)和厚壁菌门(Firmicutes)的细菌, 使变形菌门(Proteobacteria)成为第一优势菌, 表明甲硝唑发挥了抑菌作用。饲养7d后CON组、CONM组、PEC组和PECM组的绿肝率分别为0、12%、27%和88%, 饲养56d后各组鱼肝脏纤维化程度依次增强。甲硝唑显著降低了肠道内容物中胆盐水解酶活力。肝脏中参与胆汁酸合成、转运及调控的基因表达活性与绿肝率及血清中胆汁酸含量不一致。上述结果提示肠道菌群紊乱可能是引起绿肝症和肝纤维化的重要原因, 胆汁酸可能介导了此病理过程。

关键词: 果胶; 肠道菌群; 胆盐水解酶; 胆汁酸; 黄颡鱼
中图分类号: S963.7 **文献标识码:** A **文章编号:** 1000-3207(2022)11-1591-07

植物性食物或饲料中含有丰富的不能被单胃动物消化吸收的碳水化合物, 即膳食纤维, 包括非淀粉多糖(纤维素、半纤维素、果胶、树胶和β-葡聚糖等)、抗性淀粉、抗性低聚糖及木质素等[1]。膳食纤维虽然不能被单胃动物消化吸收, 但会影响肠道环境, 同时膳食纤维也是肠道细菌的能源, 能够促进肠道细菌的增殖, 提高其活力[1—3]。

肠道细菌在维持宿主代谢稳态中起着关键作用[4, 5], 其结构和功能的改变可能导致宿主代谢失调, 进而发展成各种疾病[3, 6]。适度提高日粮膳食纤维含量可改善肠道菌群的结构和功能, 因此, 医学领域正尝试用某些种类膳食纤维操控肠道菌群结构与功能, 以达到改善健康的目的[7, 8]。

植物性蛋白源中膳食纤维含量往往达30%以上, 例如, 豆粕中膳食纤维含量达36.7%[9], 豆渣中膳食纤维更是高达63%[10]。随着低鱼粉、无鱼粉饲料的开发和应用, 养殖鱼类往往处于膳食纤维高载状态, 这可能会引起肠道菌群功能紊乱, 进而发展出各种疾病。Singh等[11]已发现, 膳食纤维高载可导致小鼠胆汁淤积, 并诱发肝癌。我们在前期研究中也注意到膳食纤维短期高载(7d)可导致黄颡鱼(*Pelteobagrus fulvidraco*)绿肝症, 长期高载(56d)引起肝纤维化[12]。但肠道菌群是否参与了这一病理过程尚不清楚。本研究通过向黄颡鱼饲料中添加甲硝唑来抑制肠道厌氧菌, 探讨肠道菌群在膳食纤维高载致病过程中的作用, 为进一步解析膳食纤维的生理效应提供依据。

1 材料与方法

1.1 实验饲料制备

所用果胶(食品级>99%, 酯化度75%, 黏度1200 mPa)购自郑州明瑞化工有限公司, 用于抑制肠道厌菌群

收稿日期: 2021-10-03; **修订日期:** 2022-07-04
基金项目: 江苏省水产三新工程项目(Y2018-20); 江苏省教委自然科学基金重点项目(20KJA240001); 苏州市科技项目(SNG2020060)资助 [Supported by the Fishery Science and Technology Projects of Jiangsu Province (Y2018-20); the Major Project of the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (20KJA240001); the Science and Technology Project of Suzhou (SNG2020060)]
作者简介: 姚仕彬(1986—), 男, 博士研究生; 主要从事水产动物营养与饲料学研究。E-mail: 407241082@qq.com
通信作者: 蔡春芳, E-mail: caicf@suda.edu.cn

的甲硝唑(Metronidazole)购自国药集团化学试剂苏州有限公司(纯度≥99%)。其他饲料原料均由鑫裕饲料苏州有限公司提供。向饲料中分别添加30%的糊精和果胶, 命名为CON和PEC饲料。再向这两种饲料中分别添加0.2%的甲硝唑, 配制成名为CONM和PECM的饲料。饲料配方见表 1。

1.2 实验鱼饲养

养殖实验在苏州市阳澄湖国家现代农业示范区发展有限公司研究生工作站进行。黄颡鱼幼鱼购自浙江省湖州市南浔某育苗场。在室内水泥池驯化后选取规格一致的黄颡鱼随机分到24个容积为400 L的PC养殖缸中, 每缸放鱼20尾, 再用CON饲料驯养2周, 确保摄食状态良好。2周后称重[初始体质量(21.5±3.6) g], 并将各缸鱼初重差异调至*CV*<3%。随机分组, 分别投CON、PEC、CONM和PECM饲料, 每种饲料投喂6缸鱼。日常管理和环境条件同Ren 等[16]。

表 1 实验饲料组成及营养水平(风干基础)

Tab. 1 Composition and nutrient levels of experimental diets (air-dry basis)

成分Ingredient	饲料Diet			
	CON	PEC	CONM	PECM
白鱼粉White fish meal	300	300	300	300
乌贼膏Squid paste	20	20	20	20
螺旋藻粉Spirulina powder	30	30	30	30
玉米蛋白粉Corn protein powder	80	80	80	80
糊精Dextrin	300	0	300	0
果胶Soluble pectin	0	300	0	300
甲硝唑Metronidazole	0	0	2	2
混合植物粕Mixed plant meal	200	200	200	200
预混料Vitamin and mineral premix	10	10	10	10
酵母提取物Yeast extract	0.5	0.5	0.5	0.5
磷酸二氢钙Calcium dihydrogen phosphate	5	5	5	5
沸石粉Zeolite power	24.5	24.5	22.5	22.5
鱼油Fish oil	10	10	10	10
大豆油Soybean oil	10	10	10	10
大豆磷脂Soybean phospholipid	10	10	10	10
总量Total (g/kg)	1000	1000	1000	1000
概略组分Proximate composition				
水分Moisture	10.4	9.7	10.2	10.1
粗蛋白Crude protein	35.0	35.7	35.1	35.2
粗脂肪Crude lipid	4.4	4.7	4.1	4.5
粗灰分Ash	8.0	12.4	8.7	12.1

注: 所用饲料原料及概略养分测定方法同Cai等[12]

Note: The feed ingredients used in this experiment and the methods used to determine the proximate nutrition of diet are the same as in Cai, *et al*.[12]

1.3 采样及样品分析

于饲喂7d和56d后采样, 采样于禁食12h时进行。试验鱼先用200 mg/L的MS-222麻醉, 随机选取6尾鱼用于采血, 制备血清, 用于血清总胆汁酸(TBA)浓度测定(宁波美康生物科技股份有限公司生产的血清总胆汁酸测定试剂盒)。每箱3尾鱼于无菌状态下取肠道内容物(指肠道中未被吸收的消化物), 采用试剂盒(上海酶联生物科技有限公司, 中国)测定胆盐水解酶(BSH)活性; PEC组和PECM组后肠内容物也用于肠道菌群结构分析, 以验证饲料中甲硝唑的有效性。肠道菌群采用16S rRNA 法分析, 先用E.Z.N.A Soil DNA试剂盒(Omega, 美国)按其说明书抽提DNA, 将提取的 DNA 样品送往苏州金维智生物科技股份有限公司进行建库和扩增, V3+V4区高通量测序。饲养56d后的鱼每箱取3尾, 解剖取其肝脏, 甲醛固定后制作石蜡切片, 用masson染色观察肝脏纤维化程度。其余的鱼解剖, 取肝组织液氮速冻后–80℃冰箱保存, 用于*FXR*、*CYP7A1*、*CYP27A1*、*ABCB11*和*NTCP*等参与胆汁酸调控、合成和转运的相关基因的表达分析, 方法同Ren等[13]。所有的鱼均用于绿肝率的统计, 方法同Cai等[12], 即由3名视觉正常的实验者观察后一致认为肝脏局部或全部有肉眼可见的胆汁样绿色时, 将其记录为绿肝鱼, 绿肝率(%)=绿肝鱼的数量/每缸鱼数×100。

1.4 数据分析

数据以平均值mean±SD表示。各处理间差异经方差齐性检验后进行Duncan多重比较, 统计软件为SPSS 19.0, 显著水平设为$P<0.05$。

2 结果

2.1 甲硝唑有效性验证

对PEC组和PECM组黄颡鱼肠道内容物行16S rRNA V3+V4区高通量测序分析, 在黄颡鱼肠道中共发现20个门的细菌(图 1)。PEC组在饲养7d后丰度较高的为变形菌门(Proteobacteria)、梭杆菌门(Fusobacteria)和厚壁菌门(Firmicutes), 分别约占32%、42%和17%, 而PECM组变形菌门丰度最高, 约占87%。饲养56d后, PEC组梭杆菌门占比升至85%, PECM组仍以变形菌门为第一优势菌门, 丰度约占92%。上述结果表明饲料中添加的甲硝唑使肠道菌群结构发生了明显的改变, 厌氧菌大幅度减少。

2.2 绿肝率

由图 2可知, CON组黄颡鱼在7d和56d均无绿肝出现。饲喂7d时CONM组绿肝率达12%, 显著高于CON组($P<0.05$)。PEC组和PECM组均出现绿肝症状, 但后者的绿肝率(88%)约为前者(27%)的

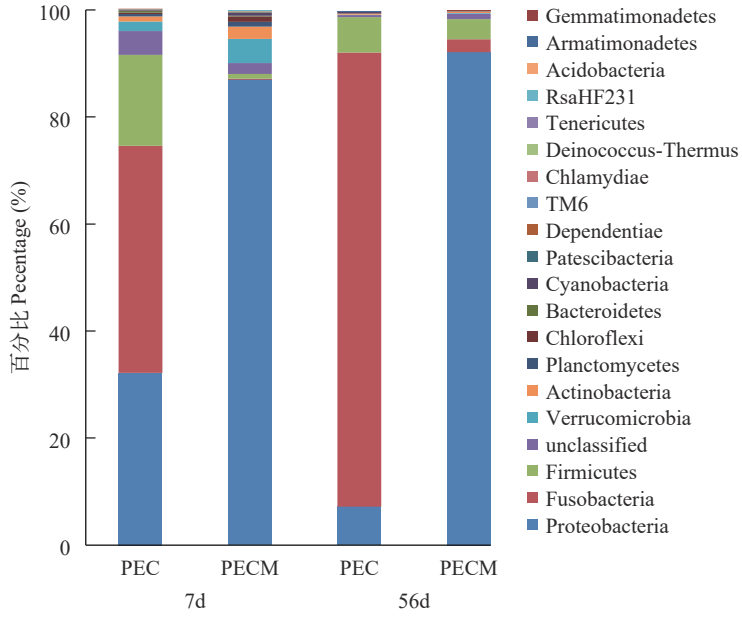

图 1　甲硝唑对肠道菌群结构的影响

Fig. 1　Metronidazole on the composition of intestinal flora of yellow catfish, *Pelteobagrus fulvidraco*

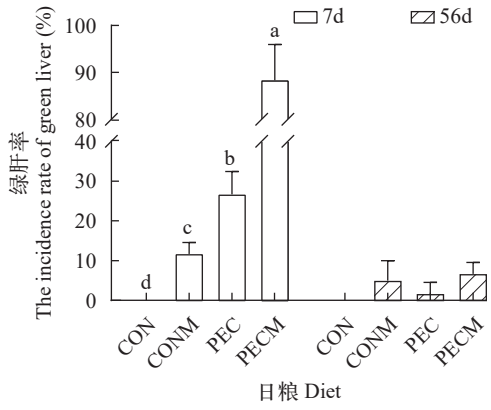

图 2　不同日粮饲喂黄颡鱼7d和56d后绿肝率

Fig. 2　The incidence rate of green liver in yellow catfish, *Pelteobagrus fulvidraco*, fed the diets for 7d and 56d respectively

同一系列柱上的小写字母不同表示组间具有统计学差异(P<0.05, n=3); 下同

Different letters above the bars of the same series mean significantly different (P<0.05, n=3). The same applies below

3倍。饲喂56d后绿肝率均较低, 且各组间无显著差异(P>0.05)。

2.3　肝组织学

饲养56d后, 肝组织切片masson染色结果显示, CON组纤维化(蓝色信号)不明显而PEC组纤维化明显。总体上, 纤维化程度由高到低依次为PECM组>PEC组>CONM组> CON组(图 3)。

2.4　胆盐水解酶活性和血清总胆汁酸浓度

由图 4可知, 7d时, CONM和PEC组肠道内容物BSH活性均显著低于CON组(P<0.05); PECM组显著低于PEC组(P<0.05)。56d时, CONM组和PEC组BSH活性均显著低于CON组(P<0.05), PECM组低于PEC组但差异不显著(P>0.05)。

7d时PEC组血清TBA浓度显著高于CON组(P<0.05), 而CONM和PECM组显著低于CON组(P<0.05)。56d时, CONM组、PEC组和PECM组血清TBA浓度显著高于CON组(P<0.05), 且各组间差异显著, 由高到低为CONM组>PEC组>PECM组>CON组(P<0.05)。

2.5　肝脏胆汁酸合成、转运及调控基因表达

由图 5可见, 同CON组相比, PEC组7d后肝脏中*fxr*、*cyp7a1*和*ntcp*基因表达显著升高(P<0.05), *cyp27a1*和*abcb11*基因表达显著降低(P<0.05)。CONM组*fxr*、*cyp7a1*、*cyp27a1*、*abcb11*和*ntcp*基因表达均显著高于CON组(P<0.05)。同PEC组相比, PECM组*fxr*基因表达显著降低(P<0.05), *cyp27a1*和*ntcp*基因表达显著升高(P<0.05)。56d时PEC组*fxr*、*cyp7a1*、*cyp27a1*和*abcb11*基因表达显著低于CON组(P<0.05), *ntcp*无显著变化。CONM组*fxr*和*cyp27a1*表达也显著低于CON组(P<0.05), *cyp7a1*、*abcb11*和*ntcp*表达则显著高于CON组(P<0.05)。同PEC组相比, PECM组*fxr*和*ntcp*表达无显著差异(P>0.05), *cyp7a1*、*cyp27a1*和*abcb11*基因表达显著升高(P<0.05)。

3　讨论

3.1　甲硝唑的抑菌作用

由PEC组和PECM组肠道菌群结构分析结果可

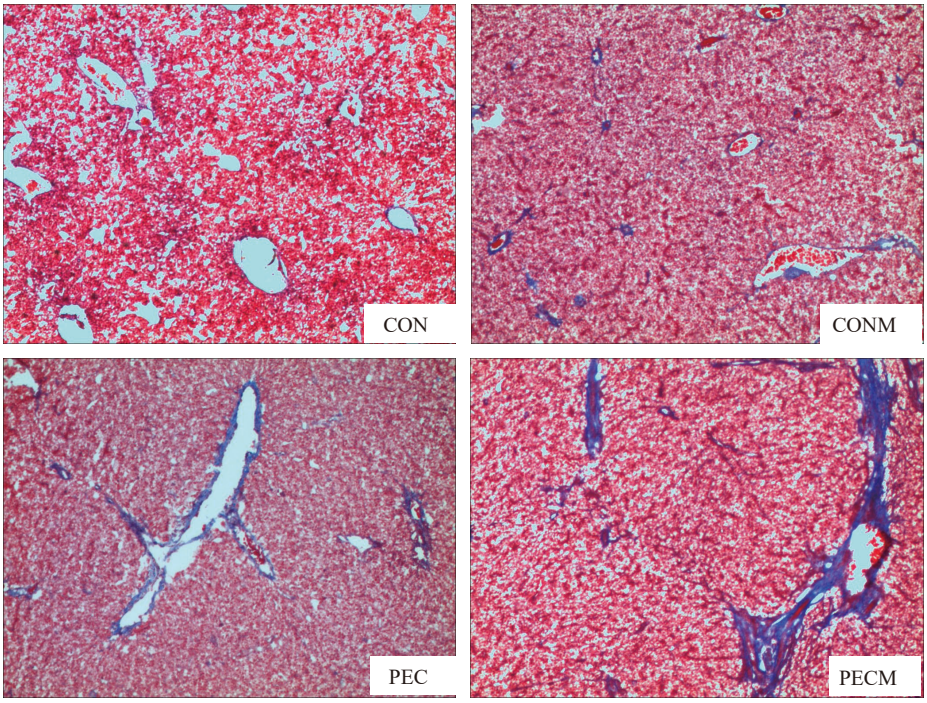

图 3 摄食不同饲料56d后黄颡鱼肝组织masson染色切片照

Fig. 3 Masson stained sections of liver tissue of yellow catfish, *Pelteobagrus fulvidraco*, fed the diets for 56d

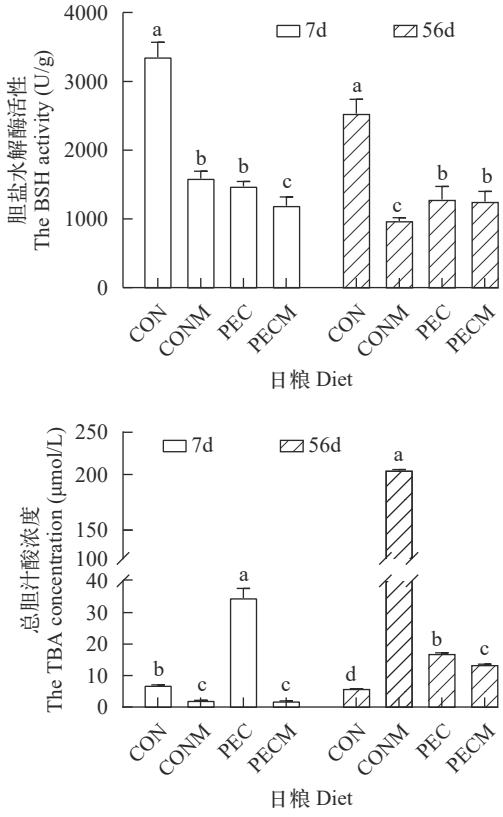

图 4 不同日粮饲喂黄颡鱼7d和56d后肠道内容物中胆盐水解酶活性和血清总胆汁酸浓度

Fig. 4 The digesta bile salt hydrolase (BSH) activity and serum total bile acid concentration of yellow catfish, *Pelteobagrus fulvidraco*, fed the diets for 7d and 56d respectively

见, 添加甲硝唑后变形菌门丰度提高, 而厚壁菌门和梭杆菌门的丰度下降。这一结果与甲硝唑对斑马鱼肠道菌群结构的影响一致[14], 表明本研究中添加的甲硝唑没有在饲料制备过程中被完全破坏, 发挥了抑制肠道厌氧菌的作用。

3.2 肠道菌群结构与绿肝症和肝纤维化的关联性

本研究结果显示, 7d时CON组没有绿肝症出现, 但CONM组有12%的鱼出现了绿肝症, PECM组绿肝率则是PEC组的3倍。上述结果暗示肠道菌群失调可能是绿肝症的重要原因。绿肝症是肝汁淤积的表现[12, 15]。秦艳等[16]和李红等[17]分析了肝内胆汁淤积症患儿肠道菌群, 发现患儿肠道菌群紊乱, 与本实验结果一致。

7d 时PECM组变形菌门丰度达到了87%, 远高于PEC组(图 1)。导致这一结果的原因应该与果胶自身对肠道菌群的影响有关: 研究表明高果胶饮食也会提高肠道变形菌门的相对丰度[18], 从而与甲硝唑的作用相叠加, 最终导致PECM组变形菌门成为绝对优势菌(图 1)。PECM组绿肝率较PEC组高3倍, 这是否与变形菌门丰度提高有关值得关注。饲养56d时各组鱼绿肝率均较低(图 2), 这一结果与我们的前期研究一致[12], 可能与体内胆固醇[12]、牛磺酸[19, 20]等胆汁成分及其合成原料的下降有关。

长期胆汁淤积可损伤肝细胞, 继而发展为肝纤维化[15]。我们在前期研究中发现, 与CON组相比, PEC组黄颡鱼的肝组织发生了明显的纤维化[12], 这

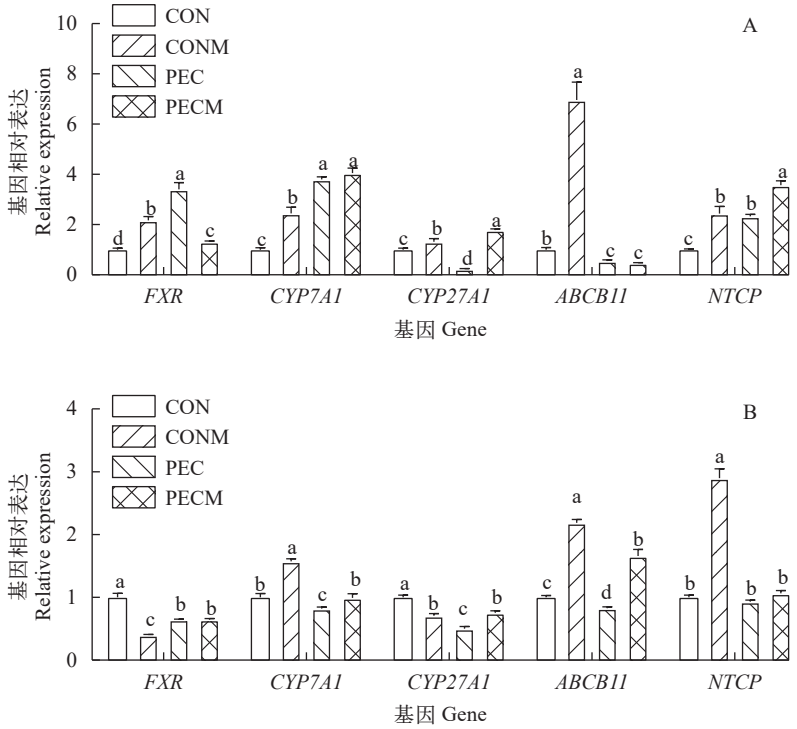

图 5　不同日粮饲喂黄颡鱼7d (A)和56d (B)后肝脏胆汁酸合成、转运及调控相关基因mRNA相对表达

Fig. 5　Relative mRNA expression of genes related to liver bile acid synthesis, transport and regulation in the liver of yellow catfish, *Pelteobagrus fulvidraco*, fed the diets for 7d (A) and 56d (B)

一现象在本实验中得到重现(图 4)。结合各组绿肝率的差异, 表明黄颡鱼肝纤维化也与胆汁淤积有关, 而纤维化后肝脏功能障碍则可能是56d时绿肝率反而较低的另一原因。

3.3　肠道菌群引起绿肝症和肝纤维化的潜在分子机制

肠道菌群在胆汁酸代谢中发挥着重要作用, 它借助BSH和类固醇脱氢酶等通过脱氢、脱羟基和脱硫等作用改变胆汁酸池的组成[21—23], 本研究结果显示, 添加甲硝唑后BSH活性显著下降, PEC组BSH活性显著低于CON组, 表明其胆汁酸的生物转化效能下降。胆汁酸是法尼醇X受体(FXR)的配体, 不同胆汁酸对FXR的激活作用不同甚至相反[24], FXR又影响着胆汁酸的合成与转运[25]。胆固醇-7α-羟化酶(CYP7A1)是胆汁酸合成经典途径的限速酶, 甾醇27α-羟化酶(CYP27A1)则是替代途径的限速酶。由本研究结果可见, 7d时CONM组、PEC组和PECM组的*fxr*和*CYP7A1*表达均高于CON组, 此外, CONM组和PECM组的*cyp27a1*也显著高于CON组(P<0.05, 图 5), 表明*fxr*高表达未能有效抑制胆汁酸合成。Na^+/牛磺胆酸协同转运体 (NTCP) 是一种表达于肝细胞基侧膜的转运蛋白, 将肝门静脉内的牛磺胆酸转运至肝细胞内。NTCP缺陷病的共同临床特点是高胆汁酸血症[26]。本研究中7d 时PEC组血清TBA浓度较高, 56d时CONM组TBA含量较高, 然而, 其对应的*ntcp*表达也均较高, 因此不能用它解释血清TBA的变化。*abcb11*编码胆盐输出泵(BSEP), BSEP是肝脏从血液中清除胆汁酸盐的限速步骤[27]。当BSEP蛋白功能异常或表达减少时, 阻碍胆汁酸外排至胆管, 从而造成肝内胆汁淤积[28]。从本研究结果看, CONM组在56d时*abcb11*表达量最高(图 5), 但其血清TBA含量也最高(图 4)。上述结果表明与CON组相比, CONM、PEC和PECM组的鱼均存在胆汁酸代谢紊乱的情况。大量研究表明肠道菌群失调会引起胆汁酸代谢紊乱[21, 22], 与本实验结果相支持。肠道内的胆汁酸除了乳化脂肪促进其吸收外, 还具有信号传导功能, 通过FXR、G蛋白偶联胆汁酸受体等调节胆汁酸的合成、转运及炎性反应[29, 30], 肠道菌群失调后胆汁酸代谢紊乱, 这可能是果胶高载引起绿肝症和肝组织纤维化的重要途径。

4　结论

本研究再次证明果胶短期高载可致绿肝症, 长期高载则会引起肝组织纤维化。饲料中添加甲硝唑提高了绿肝率和肝脏纤维化程度, 提示肠道菌群紊乱可能是绿肝症和肝纤维化的重要原因, 这一病理过程可能由胆汁酸介导。

参考文献:

[1] Stephen A M, Champ M M J, Cloran S J, *et al*. Dietary fibre in Europe: Current state of knowledge on definitions, sources, recommendations, intakes and relationships to health [J]. *Nutrition Research Reviews*, 2017, **30**(2): 149-190.

[2] Gidenne T. Dietary fibres in the nutrition of the growing rabbit and recommendations to preserve digestive health: a review [J]. *Animal*, 2015, **9**(2): 227-242.

[3] Makki K, Deehan E C, Walter J, *et al*. The impact of dietary fiber on gut microbiota in host health and disease [J]. *Cell Host & Microbe*, 2018, **23**(6): 705-715.

[4] Song Z, Cai Y, Lao X, *et al*. Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome [J]. *Microbiome*, 2019, **7**(1): 9.

[5] Vaiserman A M, Koliada A K, Marotta F. Gut microbiota: a player in aging and a target for anti-aging intervention [J]. *Ageing Research Reviews*, 2017(35): 36-45.

[6] Rehman T. Role of the gut microbiota in age-related chronic inflammation [J]. *Endocrine, Metabolic & Immune Disorders Drug Targets*, 2012, **12**(4): 361-367.

[7] Cheng J, Jiang X, Li J, *et al*. Xyloglucan affects gut-liver circulating bile acid metabolism to improve liver damage in mice fed with high-fat diet [J]. *Journal of Functional Foods*, 2020(64): 103651.

[8] Morrison K E, Jašarević E, Howard C D, *et al*. It's the fiber, not the fat: significant effects of dietary challenge on the gut microbiome [J]. *Microbiome*, 2020, **8**(1): 15.

[9] Miao J Z, Lü Z Q, Cao Z H, *et al*. Study on enzyme extraction of dietary fiber from soybean residue [J]. *China Food Additives*, 2009(5): 120-124. [苗敬芝, 吕兆启, 曹泽虹, 等. 酶法提取豆粕中总膳食纤维的研究 [J]. 中国食品添加剂, 2009(5): 120-124.]

[10] Lou H W, Chi Y J. Effects of extrusion-cooking on soluble dietary fiber in soybean residue [J]. *Journal of the Chinese Cereals and Oils Association*, 2009, **24**(6): 31-35. [娄海伟, 迟玉杰. 挤压蒸煮对豆渣中可溶性膳食纤维含量的影响 [J]. 中国粮油学报, 2009, **24**(6): 31-35.]

[11] Singh V, Yeoh B S, Chassaing B, *et al*. Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer [J]. *Cell*, 2018, **175**(3): 679-694.

[12] Cai C, Ren S, Cui G, *et al*. Short-term stress due to dietary pectin induces cholestasis, and chronic stress induces hepatic steatosis and fibrosis in yellow catfish, *Pelteobagrus fulvidraco* [J]. *Aquaculture*, 2020(516): 734607.

[13] Ren S, Cai C, Cui G, *et al*. High dosages of pectin and cellulose cause different degrees of damage to the livers and intestines of *Pelteobagrus fulvidraco* [J]. *Aquaculture*, 2020(514): 734445.

[14] Zha J W. Effect of competition and synergism between gut bacteria on *Aeromonas hydrophia* infection in zebrafish [D]. YangLing: Northwest A & F University, 2019: 10-12. [查继伟. 斑马鱼肠道菌群协同竞争关系对嗜水气单胞菌感染的影响 [D]. 杨凌: 西北农林科技大学, 2019: 10-12.]

[15] Machiko C, Hiroyuki N, Kaoru H, *et al*. Occurrence of the myxosporean emaciation disease caused by enteromyxum leei in cultured malabar grouper epinephelus malabaricus [J]. *Fish Pathology*, 2013, **48**(3): 88-96.

[16] Qin Y, Qin H R, Du L F. Analysis on physical development of infants with intrahepatic cholestasis and intestinal flora [J]. *Maternal and Child Health Care of China*, 2021, **36**(13): 3003-3005. [秦艳, 秦海荣, 杜丽芳. 肝内胆汁淤积症患儿体格发育和肠道菌群情况分析 [J]. 中国妇幼保健, 2021, **36**(13): 3003-3005.]

[17] Li H, Jiang Z X, Tang J Q, *et al*. Characteristics of gut microbiota in children with infantile cholestatic hepatopathy [J]. *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, 2021, **31**(2): 141-143. [李红, 江治霞, 汤建桥, 等. 婴儿胆汁淤积性肝病肠道菌群的特征性分析 [J]. 中西医结合肝病杂志, 2021, **31**(2): 141-143.]

[18] Xu R, Lu Y, Wang J, *et al*. Effects of the different dietary fibers on luminal microbiota composition and mucosal gene expression in pig colons [J]. *Journal of Functional Foods*, 2019(59): 71-79.

[19] Takagi S, Murata H, Goto T, *et al*. The green liver syndrome is caused by taurine deficiency in yellowtail, *Seriola quinqueradiata* fed diets without fishmeal [J]. *Aquaculture Science*, 2005, **53**(3): 279-290.

[20] Cao X, Ren S, Cai C, *et al*. Dietary pectin caused great changes in bile acid profiles of *Pelteobagrus fulvidraco* [J]. *Fish Physiology and Biochemtry*, 2021, **47**(6): 2015-2025.

[21] Long S L, Gahan C G, Joyce S A. Interactions between gut bacteria and bile in health and disease [J]. *Molecular Aspects of Medicine*, 2017(56): 54-65.

[22] Zeng Z H, Liu R R, Tang L, *et al*. Interaction between gut microbiota and bile acid metabolism [J]. *Chinese Journal of Microecology*, 2021, **33**(7): 849-856. [曾忠花, 刘容容, 汤俐, 等. 肠道菌群与胆汁酸代谢的互相作用 [J]. 中国微生态学杂志, 2021, **33**(7): 849-856.]

[23] Ridlon J M, Kang D J, Hylemon P B. Bile salt biotransformations by human intestinal bacteria [J]. *Journal of Lipid Research*, 2006, **47**(2): 241-259.

[24] Vaquero J, Monte M J, Dominguez M, *et al*. Differential activation of the human farnesoid X receptor depends on the pattern of expressed isoforms and the bile acid pool composition [J]. *Biochemical Pharmacology*, 2013, **86**(7): 926-939.

[25] Cariello M, Piccinin E, Garcia-Irigoyen O, *et al*. Nuclear receptor FXR, bile acids and liver damage: introducing the progressive familial intrahepatic cholestasis with FXR mutations [J]. *Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease*, 2018, **1864**(4): 1308-1318.

[26] Song Y Z. Research advances in the pathogenesis, clinical manifestations, and diagnosis/treatment of sodium-

taurocholate cotransporting polypeptide deficiency [J]. *Journal of Clinical Hepatology*, 2019, **35**(8): 1690-1692. [宋元宗. 钠牛磺胆酸共转运多肽缺陷病的发病机制、临床表现及诊疗进展 [J]. 临床肝胆病杂志, 2019, **35**(8): 1690-1692.]

[27] Stindt J, Kluge S, Dröge C, *et al*. Bile salt export pump-reactive antibodies form a polyclonal, multi-inhibitory response in antibody-induced bile salt export pump deficiency [J]. *Hepatology*, 2016, **63**(2): 524-537.

[28] Feng H, Wang X. Research progress on the relationship between bile salt output pump and cholestasis [J]. *Chinese Journal of Clinical Rational Drug Use*, 2021, **14**(24): 179-181. [冯红, 王翔. 胆盐输出泵与胆汁淤积关系的研究进展 [J]. 临床合理用药杂志, 2021, **14**(24): 179-181.]

[29] Xie G, Jiang R, Wang X, *et al*. Conjugated secondary 12 alpha-hydroxylated bile acids promote liver fibrogenesis [J]. *Ebiomedicine*, 2021, **66**(5): 103290.

[30] Cai S Y, Boyer J L. The role of bile acids in cholestatic liver injury [J]. *Annals of Translational Medicine*, 2021, **9**(8): 737.

GREEN LIVER SYMPTOM AND LIVER FIBROSIS CAUSED BY PECTIN OVERLOAD IN *PELTEOBAGRUS FULVIDRACO* MAY BE RELATED TO THE INTESTINAL DYSBACTERIOSIS

YAO Shi-Bin[1, 2], REN Sheng-Jie[1, 2], CAI Chun-Fang[1, 2], CAO Xia-Min[1, 2], WU Ping[1, 2], YE Yuan-Tu[1, 2], JIANG Guang-Ming[3], DING Hui-Ming[4] and ZHANG Cheng[4]

(1. *School of Basic Medicine and Biological Science*, *Soochow University*, *Suzhou* 215123, *China*; 2. *Key Laboratory of Aquatic Animals Nutrition of Jiangsu Province*, *Suzhou* 215123, *China*; 3. *Agricultural and Rural Bureau of Wuzhong District*, *Suzhou* 215104, *China*; 4. *Suzhou Yangchenghu National Modern Agriculture Demonstration Zone Development Co.*, *Ltd*, *Suzhou* 215138, *China*)

Abstract: The content of dietary fibers in commonly used plant feedstuffs is often more than 30%, which is the fermentation substrate for intestinal flora. With the extensive use of low fish meal feed, cultured fish species are in a state of high dietary fiber loading. However, the nutritional stress induced by dietary fiber as well as the mechanism has not received enough attention. In the previous study, we found that high viscous dietary fiber pectin induced green liver symptom and liver fibrosis in yellow catfish, *Pelteobagrus fulvidraco*. The purpose of this study was to explore the role of intestinal flora in the above pathological process. The diets containing 30% dextrin and pectin were named CON and PEC diets respectively. Metronidazole was then added to CON and PEC diets in a dosage of 0.2% and the obtained diets were named CONM and PECM. The four diets were fed yellow catfish with an initial weight of (21.5±3.6) g, and sampling was conducted at 7d and 56d respectively. 16s rRNA analysis showed that metronidazole inhibited bacteria of Fusobacteria and Firmicutes, resulted in Proteobacteria became the first dominant bacteria. After 7 days of feeding, the green liver rate of fish fed diet CON, CONM, PEC, PECM was 0, 12%, 27% and 88% respectively. The green liver rate of all groups was low after 56d. The degree of liver fibrosis after 56d was the highest in fish fed diet PECM, followed by PEC, and the lightest in CON. Metronidazole significantly reduced the activity of bile salt hydrolase in digesta. The serum TBA concentration in fish fed diet PEC was significantly higher than those in CON, while that in fish fed diet CONM and PECM was significantly lower than those in CON. At 56d, the serum TBA concentration in fish fed diet CONM, PEC and PECM was significantly higher than that fed CON diet. The gene expression activity involved in bile acid synthesis, transport and regulation in liver was inconsistent with the green liver rate and the serum bile acid concentration. These results indicated that dysbacteriosis was an important cause of green liver symptom and liver fibrosis induced by pectin, which might be mediated by bile acid. In view of the extensive physiological effects of intestinal flora as well as bile acids, attention should be paid to the effect of dietary fiber in aquatic feed preparation.

Key words: Pectin; Intestinal flora; Bile salt hydrolase; Bile acid; *Pelteobagrus fulvidraco*