

饲料中添加水飞蓟素对吉富罗非鱼生长性能、 肝脏脂肪代谢酶和抗氧化能力的影响

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摘要: 为评估饲料中添加水飞蓟素对吉富罗非鱼生长性能、肝脏脂肪代谢酶和抗氧化能力的影响, 分别在5组等氮等能的吉富罗非鱼饲料中添加不同水平的水飞蓟素[0(对照组)、100、200、400和800 mg/kg], 饲喂初始体质量为(8.17±0.31) g的吉富罗非鱼幼鱼9周, 测定实验鱼的生长性能、血清生化指标、肝脏脂肪代谢酶活性及抗氧化酶活性。结果显示, 随饲料中水飞蓟素添加水平增加, 吉富罗非鱼终末体质量、增重率、特定生长率和肥满度均呈先增加后降低的趋势, 各指标均在100 mg/kg实验组达到最大, 100 mg/kg实验组的饲料系数显著低于对照组。饲料中添加水飞蓟素显著降低了实验鱼肝体比和脏体比。100 mg/kg实验组全鱼、肌肉和肝脏的粗脂肪含量显著低于对照组。血清谷草转氨酶和谷丙转氨酶活性随水飞蓟素水平增加呈先降低后升高的趋势, 分别在200和100 mg/kg实验组达到最低, 两组之间无显著性差异。饲料中添加水飞蓟素后, 血清中的甘油三酯含量显著低于对照组; 肝脏中的脂蛋白脂酶和总酯酶活性显著高于对照组; 肝脏脂肪酶活性呈先上升后降低趋势, 在200 mg/kg实验组最高, 且与对照组有显著性差异。饲料中添加水飞蓟素后, 肝脏谷胱甘肽过氧化物酶活性显著高于对照组, 肝脏过氧化氢酶活性显著低于对照组; 肝脏丙二醛含量显著低于对照组。100 mg/kg实验组罗非鱼的肝细胞界限清晰, 无核偏移, 空泡较少。研究表明, 饲料中添加适量水飞蓟素提高了吉富罗非鱼生长性能, 调节了肝脏脂肪代谢酶活性和抗氧化能力, 对防治肝脏细胞损伤有积极作用。在本实验条件下, 推荐吉富罗非鱼幼鱼饲料中水飞蓟素添加水平为100 mg/kg。

关键词: 吉富罗非鱼; 水飞蓟素; 生长性能; 肝脏代谢酶; 抗氧化

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吉富罗非鱼(GIFT *Oreochromis niloticus*)是我国罗非鱼养殖的主要品系之一。近年来, 由于其市场价格较低, 罗非鱼配合饲料中多选用菜粕、棉粕、豆粕等植物蛋白原料以及一些非常规蛋白原料(血粉、蚕蛹粉、羽毛粉等)替代鱼粉^[1-3]。植物蛋白原料替代鱼粉常因饲料氨基酸不平衡和存在抗营养因子^[4]、适口性差、消化率

低等因素对鱼类生长和生理功能产生一定负面影响。有报道指出, 单一植物蛋白源或混合植物蛋白源完全替代鱼粉(100%)会降低罗非鱼^[1, 5]、大菱鲂^[6](*Scophthalmus maximus*)特定生长率和蛋白质效率, 增大饲料系数; 增加了罗非鱼^[5]鱼体脂肪含量。同时, 在当前高密度集约化养殖模式下, 更易导致罗非鱼肝脏代谢负荷增

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大和抗应激能力降低。因此有必要开发出具有保肝促生长的罗非鱼饲料添加剂。中草药或其提取物由于毒副作用小,易降解,对鱼体和环境无害^[7-8],已经在水产动物饲料中得到广泛研究与应用^[9-13]。

水飞蓟素(Silymarin)是从菊科植物水飞蓟果实及种子中萃取后得到的天然黄酮类化合物,其主要成分为水飞蓟宾(Silybin)、异水飞蓟宾(Isosilybin)、水飞蓟宁(Silydianin)、水飞蓟亭(Silychristin)等,成品为淡黄色粉末^[14-15]。水飞蓟素具有清除自由基^[16]、抗脂质过氧化^[17]、稳定肝细胞膜^[18]、减轻肝细胞损伤^[19]、保护肝细胞酶系统、提高肝脏解毒功能^[20]和抗炎^[21]等作用。水飞蓟素对于由四氯化碳^[22]、半乳糖胺、醇类和其他肝毒素引起的肝损害具有保护作用^[23],常在临床医学上用于预防和治疗慢性肝病、酒精性肝病^[24]、肝硬化^[25]等疾病。当前已经证实水飞蓟素对异育银鲫(*Carassius auratus gibelio*)^[26]、鲫(*Carassius auratus*)^[27]和团头鲂(*Megalobrama amblycephala*)^[28]有保护肝脏或促进生长的作用。因此,本实验在饲料中添加不同水平的水飞蓟素,考察其对吉富罗非鱼生长、肝脏脂肪代谢和抗氧化能力的影响,为其在罗非鱼饲料中合理使用提供科学依据。

1 材料与方法

1.1 实验饲料

以玉米蛋白粉、豆粕、棉粕和菜粕为蛋白源,混合油脂(玉米油、大豆油质量比为1:1)为脂肪源,面粉为糖源配制5组饲料,其基础饲料配方和营养成分见表1。实验饲料中水飞蓟素(饲料级,有效含量80%)的添加水平为0(对照组)、100、200、400和800 mg/kg(水飞蓟素添加水平的增减用微晶纤维素来调平)。饲料原料粉碎过60目筛,称重后混匀,少量的成分采用逐级扩大法混合。用绞肉机挤压成长条状,自然风干后,用破碎机制成直径为2 mm、长约3 mm的圆柱形颗粒,选取颗粒大小适宜的饲料用胶袋密封,置于冰柜中冷藏备用。

1.2 实验鱼及日常管理

实验用鱼来源于广西罗非鱼国家级育种实验场。正式实验前挑选健康活泼、规格一致的

表1 基础饲料的原料组成及营养成分

Tab. 1 Ingredients and proximate chemical composition of the basic experimental diet %

| 项目 items | 含量 content |
|--|------------|
| 原料 ingredients | |
| 玉米蛋白粉 corn gluten meal | 10.00 |
| 豆粕 soybean meal | 22.00 |
| 棉粕 cottonseed meal | 8.00 |
| 菜粕 rapeseed meal | 28.00 |
| 面粉 flour | 20.00 |
| 玉米油 corn oil | 1.50 |
| 大豆油 soybean oil | 1.50 |
| 胆碱 choline | 0.10 |
| 维生素预混料 ¹⁾ vitamin premix | 1.00 |
| 矿物质预混料 ²⁾ mineral premix | 4.00 |
| 蛋氨酸 methionine | 0.20 |
| 膨润土 bentonite | 2.00 |
| 羟甲基纤维素钠 carboxymethyl cellulose sodium | 1.00 |
| 微晶纤维素 microcrystalline cellulose | 0.70 |
| 营养成分 proximate composition | |
| 粗蛋白质 crude protein | 32.04 |
| 粗脂肪 crude lipid | 4.63 |
| 水分 moisture | 11.00 |
| 粗灰分 crude ash | 7.43 |

注: 1) 维生素预混料可为每千克饲料提供: 视黄醇醋酸酯 5000 IU, VD₃ 2000 IU, α -维生素E醋酸酯 60 mg, 盐酸硫胺素 5 mg, 核黄素 20 mg, VB₆ 10 mg, L-维生素C-2-磷酸镁 120 mg, VK₃ 5 mg, 肌醇 400 mg, 烟酸 120 mg, 泛酸钙 10 mg, 叶酸 1 mg, 生物素 0.1 mg; 2) 矿物质预混料可为每千克提供: 磷酸二氢钙 26 000 mg, 乳酸钙 6540 mg, 硫酸亚铁 42.5 mg, 硫酸镁 1340 mg, 磷酸二氢钠 1744 mg, 氯化钠 870 mg, 氯化铝 3 mg, 碘酸钾 2.5 mg, 氯化钾 1500 mg, 氯化铜 2 mg, 硫酸锰 16 mg, 氯化钴 20 mg, 硫酸锌 60 mg
Notes: 1) The vitamin premix provided the following per kg of diet: retinol acetate 5000 IU, cholecalciferol 2000 IU, tocopherol 60 mg, thiamin hydrochloride 5 mg, riboflavin 20 mg, pyridoxine hydrochloride 10 mg, L-ascorbyl-2-monophosphate-Mg 120 mg, menadione 5 mg, inositol 400 mg, nicotinic acid 120 mg, calcium pantothenate 10 mg, folic acid 1 mg, biotin 0.1 mg. 2) The mineral premix provided the following per kg of diet: Ca(H₂PO₄)₂ 26 000 mg, Ca(CH₃CHOHCOO)₂ 6540 mg, FeSO₄ 42.5 mg, MgSO₄ 1340 mg, NaH₂PO₄ 1744 mg, NaCl 870 mg, AlCl₃ 3 mg, KIO₃ 2.5 mg, KCl 1500 mg, CuCl₂ 2 mg, MnSO₄ 16 mg, CoCl₂ 20 mg, ZnSO₄ 60 mg

吉富罗非鱼暂养于长江水产研究所室内循环水养殖系统养殖桶(500 L)中。用对照组饲料驯化2周后,挑选规格均匀的吉富罗非鱼[平均初始体质量为(8.17±0.31) g]随机投放到15个养殖桶中,

每养殖桶放养40尾,随机分为5组,每组3个重复,分别投喂5组实验饲料,每天投喂3次(8:30~9:30, 12:30~1:30, 16:30~17:30),表观饱食投喂。每天早上8:00反冲1次,清除桶内粪便并加注约20%新水。每3周称取一次鱼体质量,每天记录水温、摄食情况和实验鱼死亡情况。饲养期间水温为24~30 °C,溶解氧>5 mg/L, pH: 6.5~7.0,总氨氮<0.2 mg/L,亚硝酸盐<0.05 mg/L,自然光照周期,养殖实验持续9周。

1.3 样品采集

养殖实验开始前,实验鱼均饥饿24 h,随机选取10尾鱼,使用MS-222(100 mg/L)将样品鱼麻醉后用于初始体成分分析。养殖实验结束后,将鱼体饥饿24 h,以桶为单位计数并称重。每桶随机取3尾麻醉的样品鱼测定全鱼常规营养成分。另外取3尾样品鱼用于测定生长性能、血清生化指标和组织常规营养成分、肝脏脂肪代谢酶、抗氧化酶活性及肝脏石蜡切片。具体操作如下:先测量体长体质量;再从尾部静脉采血,全血在4 °C静置4 h后,以3000 r/min离心10 min,制成血清;然后解剖取出内脏并称重,再分离出肝脏称重,取小块肝脏放置于10 mL离心管(管内盛有4%甲醛固定液)中用于制作石蜡切片,余下肝脏装袋保存;最后取肌肉(第一根背鳍至最后一根背鳍之间,侧线以上白肌)。将所有样品存放在-80 °C冰箱备用。

1.4 指标测定

生长性能指标测定 根据以下公式,计算增重率、特定生长率、饲料系数、成活率、肥满度、肝体比和脏体比。

增重率(weight gain rate, WGR, %)=($W_t - W_0$)/ $W_0 \times 100$

特定生长率(specific growth rate, SGR, %)=($\ln W_t - \ln W_0$)/ $t \times 100$

饲料系数(feed conversion rate, FCR, %)= $W_f / (W_t - W_0)$

成活率(survive rate, SR, %)= $N_t / N_0 \times 100$

肥满度(condition factor, CF, g/cm³)= $W_f / L^3 \times 100$

肝体比(hepatosomatic index, HSI, %)= $W_h / W \times 100$

脏体比(viscerosomatic index, VSI, %)= $W_v / W \times 100$

式中, W_t 代表鱼终末体质量(g), W_0 代表鱼初始体质量(g), t 代表实验天数, W_f 代表投喂饲料总质量(g), L 代表鱼体体长(cm), W 代表鱼体质量(g), N_t 代表终末尾数, N_0 代表初始尾数, W_h 代表鱼体肝脏质量(g), W_v 代表鱼体内脏质量(g)。

常规营养成分测定 饲料水分采用105 °C恒温干燥失重法测定(GB/T 5009.3);肌肉、肝脏和全鱼水分含量采用冷冻干燥法测定(使用CHRIST型冷冻干燥机冷冻干燥48 h);粗蛋白质采用凯氏定氮法(GB/T 5009.5);粗脂肪采用索氏抽提法(GB/T 5009.6);粗灰分采用马弗炉550 °C灼烧称重法(GB/T 5009.4)。

血清生化指标测定 总胆固醇(TCHO)、高密度脂蛋白胆固醇(HDL-C)、低密度脂蛋白胆固醇(LDL-C)、甘油三酯(TG)含量及谷草转氨酶(AST)、谷丙转氨酶(ALT)活性由Sysmex全自动生化分析仪(CHEMIX-800)测定,所用试剂均购自Sysmex公司。

肝脏脂肪代谢酶和抗氧化酶测定 脂蛋白脂酶(LPL)、肝脂酶(HL)、脂肪酶(LPS)活性、过氧化氢酶(CAT)、谷胱甘肽过氧化物酶(GSH-PX)活性、丙二醛(MDA)和溶菌酶(LZM)含量均采用南京建成生物技术研究所测试盒测定。

肝脏组织切片观察 肝脏样品在4%甲醛固定液中固定24 h后转入到70%酒精中,按照常规方法进行脱水、透明、包埋、切片和H. E染色,石蜡封片,在200倍光镜下观察其形态和结构的变化。

1.5 数据处理

实验结果均采用平均值±标准差(mean±SD)表示,采用SPSS 18.0软件进行单因素方差分析(One-Way ANOVA)和Duncan氏多重比较法对实验结果的差异显著性进行处理分析,以 $P < 0.05$ 为显著性水平。

2 结果

2.1 饲料中添加水飞蓟素对吉富罗非鱼幼鱼生长性能和饲料利用的影响

随水飞蓟素添加量的增加,FBW、WGR和SGR呈现先增加后降低的趋势,100 mg/kg实验组的FBW、WGR和SGR显著高于对照组($P < 0.05$) (表

2); 100 mg/kg实验组的FCR最低, 显著低于对照组($P<0.05$)。饲料中添加水飞蓟素显著降低了实验鱼的HSI和VSI($P<0.05$), 实验鱼CF随饲料中水

飞蓟素水平增加呈先上升后下降的趋势, 100 mg/kg组的CF显著高于对照组和其他实验组($P<0.05$)。各实验组间鱼体SR差异不显著($P>0.05$)。

表2 饲料中添加水飞蓟素对吉富罗非鱼幼鱼生长性能和饲料利用的影响

Tab. 2 Effects of dietary silymarin levels on growth performance and feed utilization of juvenile GIFT *O. niloticus*

| 项目 items | 水飞蓟素水平/(mg/kg) dietary silymarin levels | | | | |
|-----------------------------|---|---------------------------|---------------------------|----------------------------|--------------------------|
| | 0 | 100 | 200 | 400 | 800 |
| 初均体质量/g IBW | 8.18±0.33 | 8.15±0.44 | 8.19±0.24 | 8.16±0.35 | 8.18±0.45 |
| 末均体质量/g FBW | 73.99±1.32 ^a | 82.81±2.76 ^b | 80.22±2.04 ^b | 78.25±3.01 ^{ab} | 75.16±1.27 ^a |
| 增重率/% WGR | 805.90±39.15 ^a | 916.95±45.36 ^c | 879.19±3.88 ^{bc} | 859.10±31.02 ^{ab} | 818.46±3.60 ^a |
| 特定增长率/(%/d) SGR | 4.27±0.02 ^a | 4.38±0.04 ^b | 4.35±0.03 ^{ab} | 4.32±0.02 ^{ab} | 4.26±0.10 ^a |
| 饲料系数 FCR | 1.34±0.09 ^b | 1.17±0.04 ^a | 1.23±0.03 ^{ab} | 1.34±0.08 ^b | 1.29±0.06 ^b |
| 肝体比/% HSI | 2.76±0.20 ^d | 1.69±0.08 ^b | 1.42±0.18 ^a | 1.82±0.13 ^b | 2.18±0.13 ^c |
| 脏体比/% VSI | 9.41±0.30 ^b | 8.77±0.35 ^a | 8.81±0.38 ^a | 8.88±0.16 ^a | 8.87±0.38 ^a |
| 肥满度/(g/cm ³) CF | 3.98±0.09 ^c | 4.24±0.05 ^d | 3.21±0.10 ^b | 3.14±0.19 ^{ab} | 3.04±0.10 ^a |
| 存活率/% SR | 92.50±2.50 | 94.17±2.89 | 95.00±5.00 | 94.18±1.44 | 93.33±1.44 |

注: 同一行数据肩标字母不同表示差异显著($P<0.05$)。下同

Notes: Values with different letter superscripts in the same row mean significant differences ($P<0.05$). The same below

2.2 饲料中添加水飞蓟素对吉富罗非鱼幼鱼全鱼、肌肉和肝脏营养成分的影响

饲料中添加水飞蓟素对肝脏和肌肉组织中水分含量无显著影响($P>0.05$)(表3); 对全鱼和肌肉组织中粗蛋白质含量无显著影响($P>0.05$); 200和800 mg/kg实验组的肝脏粗蛋白质含量显著高于对照组($P<0.05$); 100 mg/kg实验组全鱼、肌肉和肝脏粗脂肪均显著低于对照组($P<0.05$); 此外, 饲料中添加水飞蓟素增加了全鱼粗灰分含量($P<0.05$)。

2.3 饲料中添加水飞蓟素对吉富罗非鱼血清生化指标的影响

400和800 mg/kg实验组血清TCHO含量显著低于对照组($P<0.05$), 100和200 mg/kg组与对照组无显著差异($P>0.05$)(表4); 各处理组实验鱼血清中HDL-C和LDL-C含量无显著差异($P>0.05$); 各水飞蓟素实验组血清TG含量显著低于对照组($P<0.05$); AST和ALT活性随水飞蓟素水平增加呈先下降后上升趋势, 分别在200和100 mg/kg实验组最低。

2.4 饲料中添加水飞蓟素对肝脏脂肪代谢酶的影响

罗非鱼饲料中添加水飞蓟素后, 肝脏中LPL和总酯酶(TL、LPL和HL的总和)活性显著高于对照组($P<0.05$)(表5); HL活性在100和200 mg/kg实验组显著高于对照组和800 mg/kg实验组; 肝脏LPS活性值变化同LPL和HL呈相似的趋势, 在200 mg/kg实验组达到最大值, 且显著高于对照组($P<0.05$), 800 mg/kg实验组显著低于对照组($P<0.05$)。

2.5 饲料中添加水飞蓟素对吉富罗非鱼肝脏抗氧化酶的影响

饲料中添加水飞蓟素显著降低了吉富罗非鱼肝脏CAT活力($P<0.05$)(表6); 显著提高了GSH-PX活性, 并随水飞蓟素添加量增加呈先上升后降低的趋势, 在100 mg/kg实验组达到最高。肝脏MDA含量较对照组显著降低($P<0.05$), 并随着水飞蓟素水平升高而逐渐降低。饲料中添加水飞蓟素对肝脏LZM含量无显著影响($P>0.05$)。

表3 饲料中水飞蓟素水平对吉富罗非鱼幼鱼全鱼、肌肉和肝脏营养成分的影响

Tab. 3 Effects of dietary silymarin levels on body, muscle and liver composition of juvenile GIFT *O. niloticus*

| 营养成分 nutrient component | 水飞蓟素水平/(mg/kg) dietary silymarin levels | | | | |
|-------------------------|---|--------------------------|-------------------------|--------------------------|--------------------------|
| | 0 | 100 | 200 | 400 | 800 |
| 全鱼 whole body | | | | | |
| 水分/% moisture | 73.20±1.11 ^b | 73.04±0.87 ^b | 70.71±0.98 ^a | 72.03±1.24 ^{ab} | 71.97±1.13 ^{ab} |
| 粗蛋白质/% crude protein | 15.05±0.36 | 14.98±0.30 | 15.90±0.98 | 15.15±1.01 | 15.74±0.92 |
| 粗脂肪/% crude lipid | 7.38±0.30 ^b | 6.55±0.77 ^a | 7.09±0.45 ^{ab} | 7.00±0.10 ^{ab} | 7.42±0.77 ^b |
| 粗灰分/% crude ash | 3.0±0.21 ^a | 3.8±0.13 ^b | 4.28±0.24 ^c | 4.21±0.29 ^c | 3.81±0.08 ^b |
| 肌肉 muscle | | | | | |
| 水分/% moisture | 77.24±0.87 | 77.68±0.70 | 77.74±0.71 | 78.08±1.88 | 76.81±0.84 |
| 粗蛋白质/% crude protein | 19.17±0.50 | 19.10±0.65 | 19.10±1.24 | 18.43±1.12 | 19.47±0.70 |
| 粗脂肪/% crude lipid | 1.27±0.13 ^c | 0.87±0.10 ^a | 1.18±0.03 ^{bc} | 1.02±0.21 ^{ab} | 1.14±0.20 ^{bc} |
| 肝脏 liver | | | | | |
| 水分/% moisture | 68.93±1.19 | 71.14±1.39 | 70.52±1.76 | 69.68±2.57 | 70.13±1.54 |
| 粗蛋白质/% crude protein | 10.32±0.78 ^a | 10.96±0.45 ^{ab} | 11.38±0.89 ^b | 10.66±0.35 ^{ab} | 11.35±0.35 ^b |
| 粗脂肪/% crude lipid | 4.34±0.20 ^c | 1.65±0.13 ^a | 3.58±0.53 ^b | 4.21±0.42 ^c | 3.49±0.11 ^b |

表4 饲料中添加水飞蓟素对吉富罗非鱼血清生化指标的影响

Tab. 4 Effects of dietary silymarin levels on serum biochemical indices of GIFT *O. niloticus*

| 项目 items | 水飞蓟素水平/(mg/kg) dietary silymarin levels | | | | |
|---------------------------|---|-------------------------|--------------------------|-------------------------|-------------------------|
| | 0 | 100 | 200 | 400 | 800 |
| 胆固醇/(mmol/mL) cholesterol | 2.66±0.31 ^b | 2.67±0.08 ^b | 2.69±0.08 ^b | 2.42±0.03 ^a | 2.46±0.04 ^a |
| 高密度脂蛋白胆固醇/(mmol/mL) HDL-C | 1.13±0.08 | 1.12±0.06 | 1.09±0.03 | 1.15±0.04 | 1.07±0.08 |
| 低密度脂蛋白胆固醇/(mmol/mL) LDL-C | 1.14±0.15 | 1.08±0.10 | 1.13±0.07 | 1.04±0.09 | 1.16±0.16 |
| 甘油三酯/(mmol/mL) TG | 2.73±0.06 ^d | 2.35±0.07 ^a | 2.52±0.15 ^b | 2.55±0.03 ^b | 2.64±0.13 ^c |
| 谷草转氨酶/(U/L) AST | 34.33±1.21 ^b | 24.50±0.83 ^a | 22.83±1.60 ^a | 23.33±1.97 ^a | 36.67±1.37 ^b |
| 谷丙转氨酶/(U/L) ALT | 27.83±2.31 ^b | 24.33±2.42 ^a | 29.33±1.21 ^{bc} | 31.67±2.50 ^c | 47.84±2.31 ^d |

表5 饲料中添加水飞蓟素对吉富罗非鱼肝脏脂肪代谢酶的影响

Tab. 5 Effects of dietary silymarin levels on liver lipid metabolism enzymes of GIFT *O. niloticus*

| 项目 items | 水飞蓟素水平/(mg/kg) dietary silymarin levels | | | | |
|-----------------------|---|--------------------------|--------------------------|--------------------------|-------------------------|
| | 0 | 100 | 200 | 400 | 800 |
| 脂蛋白酯酶/(U/mg prot) LPL | 1.46±0.08 ^a | 2.72±0.05 ^c | 2.60±0.30 ^c | 1.83±0.11 ^b | 1.91±0.13 ^b |
| 肝酯酶/(U/mg prot) HL | 1.78±0.09 ^a | 2.93±0.33 ^c | 2.54±0.27 ^{bc} | 2.20±0.27 ^{ab} | 1.90±0.19 ^a |
| 总酯酶/(U/mg prot) TL | 3.25±0.15 ^a | 5.55±0.31 ^c | 5.14±0.18 ^c | 4.03±0.20 ^b | 3.82±0.32 ^b |
| 脂肪酶/(U/g prot) LPS | 111.97±2.45 ^b | 110.04±6.73 ^b | 130.48±3.52 ^c | 107.97±8.16 ^b | 83.76±4.27 ^a |

表 6 饲料中添加水飞蓟素对吉富罗非鱼肝脏抗氧化酶活性的影响

Tab. 6 Effects of dietary silymarin levels on liver antioxidant activities of GIFT *O. niloticus*

| 项目 items | 水飞蓟含量/(mg/kg) dietary silymarin levels | | | | |
|------------------------------|--|-------------------------|-------------------------|-------------------------|-------------------------|
| | 0 | 100 | 200 | 400 | 800 |
| 过氧化氢酶/(U/mg prot) CAT | 7.59±0.33 ^c | 2.54±0.10 ^a | 4.10±0.14 ^b | 4.24±0.25 ^b | 4.19±0.18 ^b |
| 谷胱甘肽过氧化物酶/(U/mg prot) GSH-PX | 13.59±0.68 ^a | 32.94±2.32 ^c | 29.13±1.34 ^c | 25.51±0.76 ^b | 21.65±0.42 ^b |
| 丙二醛/(mol/mg prot) MDA | 1.63±0.11 ^d | 1.43±0.08 ^c | 1.35±0.05 ^{bc} | 1.30±0.13 ^b | 1.16±0.07 ^a |
| 溶菌酶/(g/mg prot) LZM | 0.14±0.02 | 0.17±0.01 | 0.15±0.03 | 0.16±0.01 | 0.16±0.02 |

2.6 饲料中添加水飞蓟素对吉富罗非鱼肝脏形态的影响

对照组肝细胞索尚清晰，但细胞核仁多偏向细胞一边，有些细胞核有裂解，一些肝细胞呈空泡(图1)；水飞蓟素添加水平为100 mg/kg

时，肝细胞界限清晰，核仁清晰可见，位于细胞中央，空泡较其他实验组少；添加水平为200和400 mg/kg时，空泡化较对照组严重；添加水平为800 mg/kg时肝细胞较对照组和其他实验组致密，肝细胞界限模糊，肝细胞索不成明显放射状，核仁偏移，部分裂解。

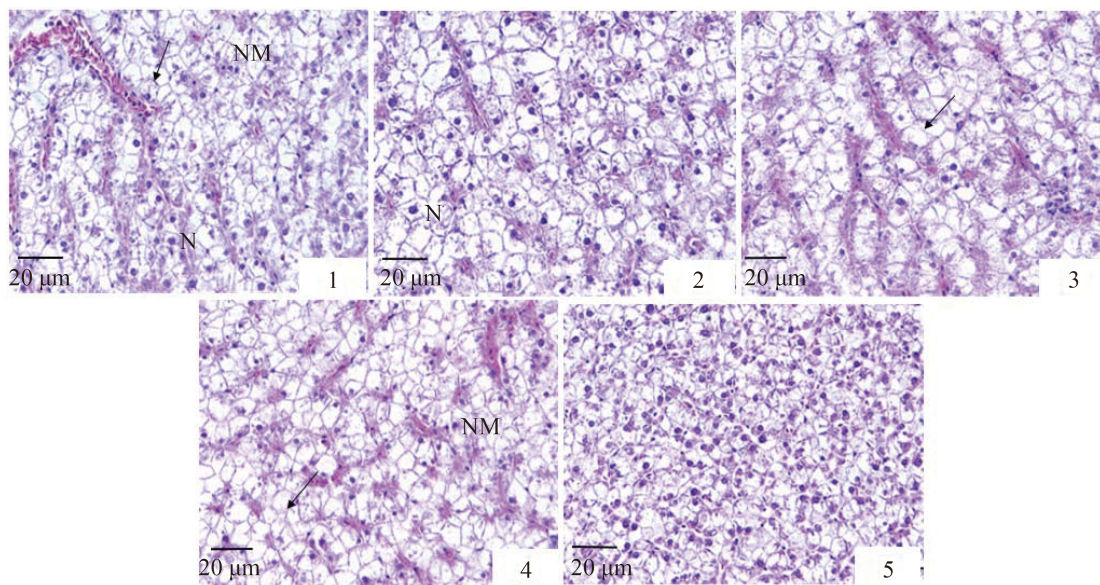


图 1 吉富罗非鱼肝脏肝细胞石蜡切片H.E染色

1-5. 饲料中水飞蓟素添加水平在0、100、200、400和800 mg/kg时肝脏细胞切片(200倍光镜下拍摄, N. 细胞核, NM. 细胞核偏移, 箭头细胞空泡化)

Fig. 1 Hepatocytes of tilapia observed in H.E staining paraffin sections

1-5. Liver histological structure of tilapia fed experimental diets supplemented with different concentrations of silymarin (×200; scale bar=20 μm; N nucleus; NM nuclear migration; arrow cellular vacuolization)

3 讨论

饲料中添加适量水飞蓟素能够促进动物生长。王英伟^[29]和刘为民^[30]在对猪和雏鸭的研究上表明：水飞蓟素能够促进猪和雏鸭的消化吸收，提高生长性能；王永庆^[28]也在团头鲂的研究中指出：饲料中添加水飞蓟素显著改善了团头

鲂生长，提高了饲料效率；李林明^[31]报道饲料中添加水飞蓟素对提高凡纳滨对虾(*Litopenaeus vannamei*)增重率、存活率和降低饵料系数有积极影响。在本实验中，饲料中添加100 mg/kg的水飞蓟素提高了罗非鱼增重率和特定生长率，降低了饵料系数，与以上的报道相符。这可能与水飞蓟素有保护肝脏的药理作用有关，肝脏

是机体物质和能量代谢的重要器官,添加适量水飞蓟素促进了肝脏物质能量代谢,提高了罗非鱼的生长性能。同时,添加100 mg/kg水飞蓟素显著降低了全鱼、肌肉和肝脏粗脂肪含量,减少肝脏脂肪沉积,表明了饲料中添加适量水飞蓟素能够改善吉富罗非鱼幼鱼肝脏脂肪代谢,提高了脂肪利用率,对促进鱼体生长有重要作用。本实验中,各添加组的肝体比和脏体比显著低于对照组,增大了鱼体可食用部分,表明添加水飞蓟素能有效降低肝脏和内脏脂肪,维持肝脏的正常形态。水飞蓟素添加水平超过200 mg/kg后,罗非鱼生长性能无明显改善,可能是因为过量水飞蓟素进入肠道后会刺激肠收缩,影响肠上皮吸收营养物质。但辜玲芳等^[26]报道饲料中添加100 mg/kg水飞蓟素对异育银鲫生长性能无显著影响,与本实验结果不同,可能与实验鱼养殖周期较短,尚未达到异育银鲫最佳剂量和最佳作用时间有关。

谷草转氨酶(AST)和谷丙转氨酶(ALT)主要存在于肝细胞内,肝细胞受损时,细胞膜通透性增强,细胞内释放AST和ALT,从而提高了血清中AST和ALT的活性,临床上常以血清中转氨酶活性来衡量肝脏是否受损^[32-33]。本实验中,100、200和400 mg/kg组AST活性显著低于对照组和800 mg/kg组,ALT在100 mg/kg时也显著低于其他组,说明添加适量的水飞蓟素可以降低血清中AST和ALT活性,这可能与水飞蓟素能清除自由基、促进肝细胞的修复和再生、维持肝脏健康作用有关^[28]。Shaker等^[22]报道100 mg/kg水飞蓟素显著降低了大鼠血清的AST和ALT活性。刘兴霞等^[34]报道添加水飞蓟宾磷脂胆碱复合物能降低患脓毒症幼鼠血清中AST和ALT活性,缓解肝损伤。本实验中当添加水平超过400 mg/kg后反而会增加肝脏转氨酶活性,表明添加高浓度水飞蓟素对肝脏修复和再生能力会起反作用,可能是高浓度的水飞蓟素在清除鱼体自由基的同时对肝细胞有应激作用,从而引起肝细胞通透性增大,释放到血清中的转氨酶活性升高^[35]。

血脂的主要成分是TCHO和TG,血脂含量可以反映机体脂类代谢情况。本实验中,饲料中添加水飞蓟素降低了罗非鱼血清TG含量,TCHO含量在400和800 mg/kg显著低于对照组,HDL-C和LDL-C与对照组无显著性差异,表明适量水飞蓟素能提高罗非鱼的血脂代谢能力。肝

脏是鱼类进行脂肪酸 β 氧化和调节脂肪累积的重要器官,LPL、HL和TL为重要的脂肪分解酶,其活性直接影响肝胰腺的脂肪代谢。Wong等^[36]和Mukherjee^[37]研究指出,LPL主要催化血浆中乳糜微粒和极低密度脂蛋白(VLDL),将TG水解成甘油和脂肪酸。HL存在于肝内皮细胞表面,对低密度脂蛋白(LDL)和高密度脂蛋白(HDL)代谢发挥重要作用^[38],LPS对鱼类消化食物中脂肪起重要作用。本实验中,饲料添加适量水飞蓟素提高了肝脏中LPL、HL、TL、LPS酶活性,表明水飞蓟素促进了脂肪降解与吸收,提高了脂肪利用效率。血清甘油三酯下降,肝脂和体脂含量下降,脂肪沉积量减少有利于维护肝脏正常形态。关于水飞蓟素调节脂肪代谢的报道较少,其机制仍需进一步研究。

动物机体抗氧化酶如CAT和GSH-PX主要存在于肝脏和红细胞中^[39],CAT参与活性氧代谢过程,在清除 O_2^- 、 H_2O_2 、过氧化物以及减少羟基自由基形成等方面发挥重要作用^[40],在动物体内主要具有抗氧化和抗炎症等作用^[41],可以在一定程度上提高机体免疫力,改善机体健康状态。GSH-PX是机体内清除过氧化氢和有机过氧化物的主要酶,可以在CAT活性较低或 H_2O_2 含量较少的组织中替代CAT清除 H_2O_2 ^[40],同时具有解毒作用^[42]。本实验结果显示:饲料中添加水飞蓟素显著降低了肝脏CAT活性,这与水飞蓟素具有提高被损伤肝脏CAT活性^[27, 43],改善抗氧化能力的观点不同,造成这种差异的原因可能与实验对象和实验周期有关。亦有研究者发现基因工程改造后的CAT缺失的小鼠依然表现正常,表明CAT只是在特定条件下才对动物是必不可少的^[40]。添加水飞蓟素提高了罗非鱼肝脏GSH-PX活性,GSH-PX活性增加是对组织中脂质过氧化作用的反应,是组织通过酶性通路防止过氧化物造成氧化损伤。同时GSH-PX活性增大亦补偿了CAT活性较低时氧自由基对鱼体的损害。MDA是脂质过氧化的最终产物,水飞蓟素实验组肝脏MDA含量显著低于对照组,这可能与水飞蓟素具有抗脂质过氧化作用有关。Bindoli等^[44]也指出,水飞蓟素可以抑制脂质过氧化产物MDA生成;用100 mg/kg水飞蓟素治疗四氯化碳引起的肝损伤时,肝脏MDA含量会显著降低^[22];此外,在异育银鲫^[26]上的类似报道也证实水飞蓟素具有缓解脂质过氧化的作用。

肝细胞损伤常见状态为肝细胞脂肪变性、

水样变性、羽毛性变性、嗜酸性变性和肝细胞增多甚至肝坏死^[45]。水飞蓟素可以抑制肝血窦扩张和炎性细胞浸润引起的肝细胞坏死^[16]。本实验中, 对照组肝细胞出现核偏移和空泡化现象, 可能是因为基础饲料为混合植物蛋白源, 氨基酸不平衡, 消化酶活力降低, 影响了鱼体对营养物质的代谢吸收, 对肠道和肝脏造成了一定损伤。赵贵萍等^[46]研究表明, 饲料中豆粕替代45%鱼粉, 会使肝血窦增大, 肝脏空泡化, 同时因脂肪滴积累会使肝细胞核偏移。100 mg/kg水飞蓟素实验组肝脏细胞空泡数量较其他组减少, 核仁清晰, 位于细胞中央, 细胞形态正常, 表明适量水飞蓟素能改善肝脏损伤、病变。这与水飞蓟素具有保护肝脏的药效有关, 其保肝机理与清除自由基、抗脂质过氧化能力有关^[23]。机体内CAT、GSH-PX以及SOD常通过各自作用途径维持着机体内自由基产生和清除的动态平衡。同时, 100 mg/kg实验组体脂、肝脏脂肪降低, 血清AST、ALT活性降低, TG含量降低, 肝脏CAT活性降低, GSH-PX活性增加, MDA含量降低等结果均表明: 饲料添加适量水飞蓟素对改善肝脏损伤有积极作用, 这与组织切片结果亦相互印证。添加过量水飞蓟素会降低生长、对肝脏形态产生一系列负面影响, 可能与其药物剂量过大产生毒性有关, 其具体机理仍需进一步深入研究。

4 小结

本实验研究表明: 饲料中添加100 mg/kg水飞蓟素提高了吉富罗非鱼生长性能, 提高了脂肪代谢酶活性, 降低了鱼体和肝脏脂肪沉积, 同时提高了罗非鱼的抗氧化能力。但饲料中添加过量的水飞蓟素, 亦会引起罗非鱼肝脏损伤, 不利于罗非鱼生长。

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Effects of dietary silymarin on growth performance, enzymes of hepatic lipid metabolism and antioxidant ability in GIFT *Oreochromis niloticus*

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Abstract: Silymarin was extracted from *Silybum marianum* (St. Mary's thistle, milk thistle), and has been used to treat liver, spleen and gallbladder disorders due to functions in anti-radical, antioxidant, anti-lipid peroxidation, anti-inflammatory and to protect the stability of plasma membrane. However, it remains unknown whether silymarin has the same functions in juvenile GIFT *Oreochromis niloticus*. A 9-week feeding experiment was performed to investigate the effects of dietary silymarin levels on growth performance, hepatic lipid metabolism enzymes activities and antioxidant ability in juvenile GIFT [initial body weight (8.17±0.31) g]. Five practical diets were formulated with incorporation of silymarin at the levels of 0 (control), 100, 200, 400 and 800 mg/kg. At the end of the feeding trial, growth performance, body composition, serum biochemical indices, hepatic lipid metabolism enzymes activities and antioxidant enzymes activities were measured. The results were as follows: ① Compared with those in the other groups, the highest weight gain rate (WGR), specific growth rate (SGR) and condition factor (CF) were observed in fish fed the diets containing 100 mg/kg silymarin. By contrast, feed conversion rate (FCR) of the fish fed the diets containing 100 mg/kg silymarin was the lowest and significantly decreased compared with control group. Dietary silymarin supplementation significantly decreased hepatosomatic index (HSI) and viscerosomatic index (VSI). There was no significant difference of survival rate among all the groups. ② The lipid content in whole body, muscle and liver was the lowest at the group of 100 mg/kg diet and significantly lower than the control group. Dietary silymarin supplementation significantly decreased triglyceride in serum. The activities of serum aspartate transaminase (AST) and alanine aminotransferase (ALT) were both decreased initially and then increased as silymarin level increased, and the lowest activity of AST and ALT were observed in the group fed 200 and 100 mg/kg diet, respectively. ③ For lipid metabolism enzymes, dietary silymarin supplementation significantly elevated the activities of lipoprotein lipase (LPL) and total lipase (TL) in liver, and the highest activity of lipase (LPS) was in the group of 200 mg/kg diet, while it was significantly lower than the control group. ④ Feed added silymarin significantly increased glutathione peroxidase (GSH-PX) activity and decreased catalase (CAT) activity in liver, and significantly reduced malondialdehyde (MDA) content in liver. ⑤ Liver slices of GIFT treated with dietary silymarin addition at 100 mg/kg showed few hepatocytes with empty vacuoles and less nuclear migration. In conclusion, appropriate dietary silymarin supplementation could promote growth performance, decrease muscle, whole body and hepatopancreas lipid contents, and regulate lipid metabolism enzymes and antioxidant ability in liver. On the basis of the above results, the proper dose of silymarin in juvenile GIFT diet was recommended to be 100 mg/kg.

Key words: GIFT *Oreochromis niloticus*; silymarin; growth performance; lipid metabolism enzymes; antioxidant ability

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