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饲料n-3 HUFA水平对珍珠龙胆石斑鱼幼鱼生长、免疫力及相关基因表达和抗病力的影响

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摘要: 本实验旨在研究饲料中不同n-3高不饱和脂肪酸(HUFA)水平对珍珠龙胆石斑鱼幼鱼生长性能、非特异性免疫、免疫相关基因表达及对抵抗哈维氏弧菌能力的影响。配制n-3 HUFA水平分别为0.65%(对照组)、1.00%、1.35%、1.70%、2.05%和2.40%的6种等氮等脂的实验饲料, 投喂初始体质量为(12.06±0.01) g的珍珠龙胆石斑鱼幼鱼8周。结果显示: ①饲料n-3 HUFA水平对饲料系数(FCR)、存活率(SR)、肝体比(HSI)和肥满度(CF)均无显著性影响; 1.35%组增重率(WGR)和特定生长率(SGR)显著高于2.40%组。②1.00%组的体粗脂肪含量显著低于1.70%和2.40%组。③攻毒前, 1.35%和1.70%组血清超氧化物歧化酶(SOD)、过氧化氢酶(CAT)的活性和补体C3的浓度显著高于对照组。攻毒后, 血清CAT、谷胱甘肽过氧化物酶(GSH-Px)、溶菌酶(LZM)活性和C3含量急剧上升, SOD活性显著下降。攻毒前, 2.40%组肠道*TLR22*和*MyD88* mRNA的表达量显著增加, 2.05%组*TNF-α*和*IL-1β*的表达量显著高于1.00%和1.35%组; 此外, 1.70%组肾脏*TLR22*和*IL-1β*的表达量显著低于对照组。攻毒后, 1.35%组肠道*MyD88* mRNA的表达量显著高于1.00%和1.70%~2.40%组, 1.70%组*TNF-α*和*IL-1β*的表达量有最小值; 1.70%组肾脏*IL-10*的表达量显著高于其他各组, 而*IL-1β*的表达量呈相反趋势, 显著低于2.40%组。研究表明, 适宜的饲料n-3 HUFA(1.47%~1.70%)可以提高珍珠龙胆石斑鱼的生长性能和非特异性免疫力, 并抑制促炎基因的表达。

关键词: 珍珠龙胆石斑鱼; n-3高不饱和脂肪酸; 非特异性免疫; 炎症相关基因
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海水鱼将亚麻酸(LNA, C18:3n-3)转化为n-3高不饱和脂肪酸(n-3 HUFA)——二十碳五烯酸(EPA, C20:5n-3)和二十二碳六烯酸(DHA, C22:6n-3)的能力很低, 因此EPA、DHA是海水鱼的必需脂肪酸[1-4]。n-3 HUFA是细胞膜磷脂的重要组成部分, 在水生动物细胞膜流动性、脂质性能、生殖发育、脂质代谢、免疫功能等方面发挥着重要作用[1-2, 5-6]。因此, 为了满足海水养殖鱼类必需脂肪酸的需求, 需要从饲料中添加。n-3 HUFA主要由鱼油和一些来自单细胞藻类、远洋生物和底栖无脊椎动物的替代油源供应[7]。另外, 过量的n-3 HUFA会导致鱼类生长[1, 8]和免疫力下降[2, 9-10], 增加氧化应激和损伤细胞组织[11]。因此, 确定海水鱼类对n-3 HUFA的需要量, 可以提高鱼油资源的利用率。

当前, 高密度养殖业、饲喂频率的增加和

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植物油的替代已导致许多鱼类疾病的发生，给水产养殖业造成了巨大的损失[12-13]。Li等[14]报道随着棕榈油替代鱼油比例的增加，*TLR*2、*TLR*3、*TLR*9、*TLR*22、*MyD*88 mRNA和n-p65对t-p65蛋白的表达显著上调，从而诱导大黄鱼(*Larimichthys crocea*)的炎症反应，激活TLR-NF-κB信号通路。Toll样受体(TLRs)是非特异性免疫系统的重要组成部分，可以识别保守的病原体相关分子模式[15]，而髓样分化因子88(MyD88)是一种结合蛋白，接受TLR并激活NF-κB[16-17]；当外部病原体入侵时，TLRs通过依赖MyD88或不依赖MyD88途径激活NF-κB信号通路，诱导免疫反应并清除病原体[18-19]。营养免疫学是利用饲料营养素预防水生动物疾病的一种新策略，它可以避免耐药性和残留，克服疫苗预防操作中的困难。以往的研究表明，n-3 HUFA可以作为一种重要的营养素，对水产动物的机体免疫功能进行调节，以应对疾病的侵袭[20-22]。

珍珠龙胆石斑鱼(*Epinephelus fuscoguttatus* ♀ × *E. lanceolatu* ♂)是我国及东南亚地区新培育的一种杂交石斑鱼，具有较好的生长性能和抗病力，市场前景广阔[23]。近年来，珍珠龙胆石斑鱼的脂质营养研究主要集中在对脂肪需要量[24]和脂肪源替代[25]，但对n-3 HUFA需要量及其营养免疫方面的研究尚无报道。因此，本实验旨在研究n-3 HUFA对珍珠龙胆石斑鱼生长、组织脂肪酸组成及免疫力、抗病力和免疫相关基因表达的影响。

1 材料与方法

1.1 实验设计

通过调整鱼油和玉米油添加量，配制成6种含n-3 HUFA水平分别为0.65%(对照组)、1.00%、1.35%、1.7%、2.05%和2.40%的等氮等脂的实验饲料。实验饲料组成和营养成分见表1，脂肪酸组成见表2。将所有原料粉碎过60目筛，按饲料配方比例准确称取，混合均匀后，压制成直径分别为2.0 mm和2.5 mm的颗粒饲料，最后将饲料风干至含水12%左右，分装于−20 ℃冰箱保存。

1.2 实验管理及养殖条件

珍珠龙胆石斑鱼购于湛江东海岛商业孵化场，购回后在水泥池(2.0 m×4.0 m×2.0 m)驯化2周。养殖实验在广东海洋大学海洋生物研究基地室内海水养殖系统中进行。鱼苗禁食24 h后分组，根据实验设计，挑选720尾体格健壮、大小均匀、平均体质量为(12.06±0.01) g的鱼苗随机分为6组，每组3个重复，每重复1个1 m^3的玻璃钢桶，每桶放40尾鱼，每天表观饱食投喂2次(08:00和17:00)，根据天气和摄食情况调整投喂量，养殖期8周。养殖期间不间断充气，每天换水1次，换水量50%。养殖期间水温23~26 ℃，溶解氧5~6 mg/L，盐度21~24。

1.3 样品采集及分析方法

样品采集　养殖实验结束禁食24 h后，对每个桶实验鱼称重、计数。从每桶中随机取3尾鱼，−20 ℃冰箱保存，备测体成分。每桶随机取6尾鱼采血，血液合并于1.5 mL离心管中，于4 ℃冰箱静置过夜后4 000 r/min离心15 min，取上清液置于−80 ℃冰箱，备测生化指标。每桶取3尾鱼，剥离肝脏和肌肉备测脂肪酸组成；取其后肠和肾混合放置于装有RNA later的离心管中，最后转移至−80 ℃冰箱保存，备测炎症相关基因表达量。养殖实验结束后，从每个桶随机抽取10尾鱼进行攻毒实验。根据Liu等[26]的方法，实验鱼腹腔注射6.7×10^7 CFU的哈维氏弧菌(*Vibrio harveyi*)，感染后24 h取血清、后肠和肾脏备测。

饲料、全鱼常规营养成分的测定　饲料、全鱼常规营养成分的测定采用AOAC方法进行。水分采用105 ℃烘干恒重法测定；粗蛋白质含量采用凯氏定氮法测定；粗脂肪含量采用索氏提取法测定(抽提剂为石油醚)；粗灰分含量采用550 ℃马弗炉煅烧法测定。

脂肪酸测定　饲料、肝脏和肌肉的脂肪酸谱根据Qiu等[27]的方法测定。将冷冻干燥后的样品放入10 mL玻璃试管中，加入3 mL KOH-甲醇溶液(1 mol/L)，于72 ℃水浴锅中加热20 min，冷却至室温。再加入3 mL HCl-甲醇溶液(2 mol/L)(现用现配)，振荡混合后于72 ℃水浴锅中加热20 min，冷却至室温，加入1 mL正己烷，剧烈振荡1 min进行萃取，静置分层时加1 mL水帮助分层。用1 mL注射器吸取上清液置于1 mL样品瓶中，并通过GC-MS(Agilent technologies 7890B - 5977A，USA)进行测定。结果以总脂肪酸的百分比表示。

抗氧化指标和免疫参数的分析　超氧化物歧化酶(SOD)、过氧化氢酶(CAT)、谷胱甘肽

表 1　实验饲料配方(% 干物质)

Tab. 1　Composition and nutrient levels of experimental diets (% dry matter)

原料　ingredients	饲料n-3 HUFAs水平　dietary n-3 HUFAs level					
	0.65	1.00	1.35	1.70	2.05	2.40
白鱼粉　white fish meal	30.00	30.00	30.00	30.00	30.00	30.00
酪蛋白　casein	20.00	20.00	20.00	20.00	20.00	20.00
豆粕　soybean meal	5.00	5.00	5.00	5.00	5.00	5.00
小麦谷朊粉　wheat gluten	8.00	8.00	8.00	8.00	8.00	8.00
玉米蛋白粉　corn gluten	5.00	5.00	5.00	5.00	5.00	5.00
面粉　wheat flour	18.00	18.00	18.00	18.00	18.00	18.00
鱼油　fish oil [1]	0.00	1.00	1.84	2.75	3.68	4.60
玉米油　corn oil	8.00	7.00	6.16	5.25	4.32	3.40
大豆卵磷脂　soybean lecithin	1.00	1.00	1.00	1.00	1.00	1.00
维生素预混料　vitamin premix [2]	0.20	0.20	0.20	0.20	0.20	0.20
矿物质预混料　mineral premix [3]	0.50	0.50	0.50	0.50	0.50	0.50
磷酸二氢钙　$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50	1.50
抗氧化剂　ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
诱食剂　attractant	0.10	0.10	0.10	0.10	0.10	0.10
氯化胆碱　choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
微晶纤维素　microcrystalline cellulose	1.15	1.15	1.15	1.15	1.15	1.15
羧甲基纤维素钠　CMC	1.00	1.00	1.00	1.00	1.00	1.00
总计　total	100	100	100	100	100	100
水分　moisture	12.36	13.17	12.87	13.09	13.10	12.66
粗蛋白　crude protein	50.67	50.57	50.91	50.92	51.45	50.55
粗脂肪　crude lipid	11.04	11.48	12.08	11.15	10.50	11.54
粗灰分　crude ash	8.65	8.46	8.46	8.33	8.39	8.40

注：1. 鱼油脂肪酸组成(占总脂肪酸的百分比)为C20:5n-3, 22.50；C22:6n-3, 15.56。2. 每千克维生素预混料中含有维生素A 10.00 g，维生素D_3 50.00 g，维生素E 99.00 g，维生素K_3 5.00 g，维生素B_1 25.50 g，维生素B_2 25.00 g，维生素B_6 50.00 g，维生素B_{12} 0.10 g，泛酸钙61.00 g，烟酸101.00 g，生物素2.50 g，肌醇153.06 g，叶酸6.25 g，纤维素411.59 g。3. 每千克矿物质预混料中含有KIO_4 0.03 g，$CoCl_2·6\ H_2O$ 4.07 g，$CuSO_4·5\ H_2O$ 19.84 g，$FeC_6H_5O_7$ 13.71 g，$ZnSO_4·7\ H_2O$ 28.28 g，$MnSO_4·7H_2O$ 0.12 g，NaH_2PO_4 80.00 g，$MgSO_4·H_2O$ 12.43 g，KCl 15.33 g，Na_2SeO_3 2.00 g，沸石粉824.19 g

Notes: 1. fatty acid composition of fish oil (percentage of total fatty acids): C20:5n-3, 22.50; C22:6n-3, 15.56. 2. Contained the following per kg of vitamin premix: vitamin A 10.00 g, vitamin D_3 50.00 g, vitamin E 99.00 g, vitamin K_3 5.00 g, vitamin B_1 25.50 g, vitamin B_2 25.00 g, vitamin B_6 50.00 g, vitamin B_{12} 0.10 g, calcium pantothenate 61.00 g, nicotinic acid 101.00 g, biotin 2.50 g, inositol 153.06 g, folic acid 6.25 g, cellulose 411.59 g. 3. Contained the following per kg of mineral premix: KIO_4 0.03 g, $CoCl_2·6\ H_2O$ 4.07 g, $CuSO_4·5\ H_2O$ 19.84 g, $FeC_6H_5O_7$ 13.71 g, $ZnSO_4$•7 H_2O 28.28 g, $MnSO_4·7H_2O$ 0.12 g, NaH_2PO_4 80.00 g, $MgSO_4·H_2O$ 12.43 g, KCl 15.33 g, Na_2SeO_3 2.00 g, zeolite power 824.19 g

过氧化物酶(GSH-Px)、溶菌酶(LZM)、补体C3(C3)采用ELISA酶联试剂盒(上海江莱公司)测定，测定方法参照试剂盒说明书进行。

RNA提取、cDNA合成和实时定量聚合酶链反应(q-PCR)　使用Trizol试剂(TaKaRa，日本)提取石斑鱼幼鱼的后肠和肾脏总RNA。总RNA的质量及浓度通过分光光度法(Nanodrop 2000，Thermo Fisher Scientific，美国)测定分析，使用1.2%的琼脂糖凝胶电泳对提取的RNA进行质量检验。cDNA的合成使用反转录试剂盒Prime Script™ RT Reagent Kit with g DNA Eraser(Perfect Real Time)(TaKaRa，日本)。实时PCR分析在定量热循环仪(Bio-Rad CFX96；Bio-Rad Labs，Hercules，CA，USA)中进行，反应体积为10 μL，包含5 μL

表 2 实验饲料脂肪酸组成

Tab. 2 Fatty acids composition of experimental diets %

脂肪酸 fatty acids	饲料n-3 HUFAs水平 dietary n-3 HUFAs level					
	0.65	1.00	1.35	1.70	2.05	2.40
C14:0	0.69	0.70	0.73	0.92	1.02	1.04
C16:0	12.87	12.74	13.12	13.20	13.21	13.24
C18:0	2.24	2.36	2.67	3.00	3.30	3.64
C20:0	0.37	0.36	0.38	0.39	0.41	0.43
ΣSFA[1]	16.17	16.16	16.90	17.51	17.94	18.35
C16:1	1.04	1.17	1.39	1.71	1.99	2.31
C18:1	25.47	25.38	25.47	25.33	25.15	24.86
ΣMUFA[2]	13.26	13.27	13.43	13.52	13.57	13.59
C18:2n-6	49.52	46.96	43.03	37.85	33.77	29.03
C20:4n-6	0.18	0.31	0.45	0.66	0.83	1.03
Σn-6 PUFA[3]	49.70	47.27	43.48	38.51	34.60	30.06
C18:3n-3	1.29	1.35	1.43	1.54	1.65	1.80
C20:5n-3	2.12	3.26	4.49	6.57	8.02	9.91
C22:6n-3	2.26	3.07	3.94	5.35	6.55	7.95
Σn-3 PUFA[4]	5.67	7.68	9.87	13.46	16.22	19.65
n-3/n-6 PUFA	0.11	0.16	0.23	0.35	0.47	0.65
n-3 HUFA[5]	4.38	6.33	8.43	11.92	14.57	17.86
DHA/EPA[6]	1.07	0.94	0.88	0.82	0.82	0.80

注：1. 饱和脂肪酸总和；2. 单不饱和脂肪酸总和；3. n-6 多不饱和脂肪酸总和；4. n-3 多不饱和脂肪酸总和；5. n-3 高不饱和脂肪酸 (DHA + EPA)；6. 22:6n-3/20:5n-3. 表6和表7同

Notes: 1. sum of saturated fatty acids; 2. sum of mono-unsaturated fatty acids; 3. sum of n-6 poly- unsaturated fatty acids; 4. sum of n-3 poly- unsaturated fatty acids; 5. n-3 high unsaturated fatty acids(DHA + EPA); 6. 22:6n-3/20:5n-3. Tab.6 and Tab.7 were the same

SYBR ® Green实时PCR混合试剂(Bio-Rad Labs)、1 μL cDNA、上下游引物各0.8 μL和3.2 μL DEPC水。引物序列设见表3。每6 s采集1次数据，热程序设计为95 ℃ 30 s下1个循环，95 ℃ 5 s，60 ℃ 34 s下40个循环，然后95 ℃ 5 s，60 ℃ 60 s下1个循环，最后50 ℃ 30 s下1个循环。在本实验中，以投喂0.65% n-3 HUFA组作为基因相对表达中的对照组，采用$2^{-\Delta\Delta Ct}$方法计算基因相对表达水平[28]。

1.4 生长性能指标计算方式

增重率(WGR，%)=(末均重−初均重)/初均重×100%

特定生长率(SGR，%/d)=(ln末均重−ln初均重)/实验天数×100%

成活率(SR，%)=实验结束时鱼尾数/实验开始时鱼尾数×100%

饲料系数(FCR)=摄食干饲料总重/(终末体质量−初始体质量)

1.5 数据处理

实验结果用平均值±标准差(mean±SD)表示，数据采用 SPSS 24.0 软件进行单因素方差分析(One-Way ANOVA)，组间若有显著性差异，再进行 Turkey氏多重比较检验，$P<0.05$表示差异显著。

2 结果

2.1 饲料n-3 HUFA水平对珍珠龙胆石斑鱼生长性能的影响

饲料n-3 HUFA水平FCR、SR均无显著影响($P>0.05$)。随着饲料中n-3 HUFA水平的增加，

表 3　实时定量(q-PCR)引物设计序列

Tab. 3　Primers pair sequences for real-time PCR

基因 gene	核苷酸序列 (5'-3') nucleotide sequence (5'-3')	登录号 GenBank accession no.
TLR22 [1]	F: CGAGCCAGGTAAACCCATCA	JQ965995.1
	R: CTCATCAAACAGGCGGAAGC	
MyD88 [2]	F: TCCTCTCGTCGCCCTGAAT	HQ197956.1
	R: CCGCTTTGGTGGGGTTTAC	
TNF-α [3]	F: GTGGCCTACACGACTGCACC	FJ491411.1
	R:TACAAAGGGCCACAGTGAGA	
IL-1β [4]	F: CGACATGGTGCGGTTTC	EF582837.1
	R: TCTGTAGCGGCTGGTGG	
IL-10 [5]	F: ACACAGCGCTGCTAGACGAG	KJ741852.1
	R: GGGCAGCACCGTGTTCAGAT	
TGF-β1 [6]	F: CGATGTCACTGACGCCCTGC	GQ205390.1
	R: AGCCGCGGTCATCACTTATC	
Nrf2 [7]	F: GAAGGAGCGTCTGTTGAGTGA	KU892416.1
	R: GAAGATGCTGCCGTTAGTTGA	
β-Actin	F: GGCTACTCCTTCACCACCACA	AY510710.2
	R: TCTGGGCAACGGAACCTCT	

注：1. toll样受体 22，2. 髓样分化因子 88，3. 肿瘤坏死因子，4. 白细胞介素-1β，5. 白细胞介素-10，6. 转化生长因子 β1，7. 核因子E2相关因子2

Notes: 1. toll-like receptor 22, 2. myeloid differentiation factor 88, 3. tumour necrosis factor α, 4. interleukin 1β, 5. interleukin 10, 6. transforming growth factor β1, 7. nuclear factor erythroid 2-related factor 2-like 2

WGR呈现先升高后降低的趋势，且在1.35%组显着高于对照组和2.40%组(P<0.05)；SGR与WGR呈相同的趋势，1.35%组明显高于2.40%组，而与其他各组无显著性差异(P>0.05)(表4)。以增重率为判断依据，构建二次回归曲线模型，得出分析方程$y = -51.844\ x^2+152.91\ x+282.16$ ($R^2 = 0.719\ 3$)；饲料干物质1.47% n-3 HUFA有最佳的WGR(图1)。

2.2　饲料n-3 HUFA水平对珍珠龙胆石斑鱼体成分指标的影响

全鱼水分和粗蛋白含量在各处理之间无显著差异(P>0.05)。1.00%组的体粗脂肪含量显著低于1.70%和2.40%组(P<0.05)，而与其他各组无显著性差异(P>0.05)(表5)。随着饲料中n-3 HUFA水平的增加，粗灰分含量逐渐降低，且在2.40%组达到最低值，显著低于0.65%~1.35%组(P<0.05)。

2.3　饲料n-3 HUFA水平对珍珠龙胆石斑鱼肝脏和肌肉脂肪酸组成的影响

肝脏和肌肉的脂肪酸谱反映了饲料脂肪酸组成情况。饲料n-3 HUFA水平对肝脏ΣSFA和ΣMUFA无显著性影响(P>0.05)。随着饲料中n-3 HUFA水平的增加，肝脏中18：2n-6和Σn-6 PUFA含量显著降低(P<0.05)。而与之相反的是，肝脏C20：4n-6、C20：5n-3、C22：6n-3、Σn-3 PUFA、n-3/n-6 PUFA和n-3 HUFA的含量显著增加(P<0.05)(表6)。在肌肉组织中也发现了类似的结果。然而，饲料n-3 HUFA显著增加了肌肉C18：3n-3的含量(P<0.05)，但对肝脏C18：3n-3无显著性影响(P>0.05)。此外，对照组肝脏和肌肉的DHA/EPA比值显著高于其他各组(P<0.05)(表7)。

2.4　饲料n-3 HUFA水平对珍珠龙胆石斑鱼血清抗氧化能力和免疫指标的影响

饲料n-3 HUFA水平显著影响攻毒前血清SOD、CAT、GSH-Px、LZM活性和C3含量(P<0.05)，但对攻毒后LZM无显著影响(P>0.05)(图2)。1.00%~1.70%组攻毒前血清SOD活性显著高于对照组(P<0.05)；与对照组相比，攻毒后血清SOD活性在1.00%~1.70%组显著增加(P<0.05)(图2-a)。随着饲料n-3 HUFA水平的增加，攻毒前CAT的活性先上升后下降，1.00%~1.70%组CAT活性显著高于其他各组(P<0.05)；攻毒后，各处理组的CAT活性显著高于对照组(P<0.05)(图2-b)。攻毒前1.35%组血清GSH-Px活性显著高于对照组(P<0.05)，而攻毒后两组间无显著性差异(P>0.05)(图2-c)。1.35%组攻毒前LZM活性显着高于2.05%和2.40%组(P<0.05)，但与其他各组之间无显著性差异(P>0.05)(图2-d)。随着n-3 HUFA水平的增加，攻毒前C3的含量先增加后下降，1.35%和1.70%组的C3含量显著高于其他各组(P<0.05)；然而，攻毒后1.00%和1.35%组C3的含量显著低于对照组(P<0.05)(图2-e)。攻毒后，血清CAT、GSH-Px、LZM和C3较攻毒前均明显增加，而SOD则呈现相反趋势。

2.5　饲料n-3 HUFA水平对珍珠龙胆石斑鱼肠道炎症相关基因表达的影响

攻毒前，2.40%组*TLR22*和*MyD88* mRNA表达量达到最大值，显著高于0.65%~1.70%组(P<0.05)；然而，*Nrf2* mRNA的表达量在1.35%组达

表 4 饲料不同水平n-3 HUFA对珍珠龙胆石斑鱼生长性能的影响

Tab. 4 Effect of dietary n-3 HUFAs on growth performance of *E. fuscoguttatus*×*E. lanceolatu*

项目 item	饲料n-3HUFAs水平 dietary n-3HUFAs level						*P*-值 *P*-value
	0.65	1.00	1.35	1.70	2.05	2.40	
初体质量/g IBW	12.05±0.01	12.06±0.02	12.06±0.01	12.06±0.02	12.05±0.00	12.05±0.00	0.556
末体质量/g FBW	55.85±2.01^{a}	56.52±0.76^{ab}	61.68±1.23^{b}	59.14±1.37^{ab}	56.58±2.99^{ab}	54.77±2.87^{a}	0.014
增重率/% WGR	363.39±16.42^{a}	368.71±5.61^{ab}	411.34±9.86^{b}	390.29±10.74^{ab}	369.42±24.87^{ab}	354.41±23.70^{a}	0.014
特定生长率/(%/d) SGR	3.07±0.07^{ab}	3.09±0.02^{ab}	3.26±0.04^{b}	3.18±0.04^{ab}	3.09±0.11^{ab}	3.03±0.11^{a}	0.018
饲料系数 FCR	0.81±0.02	0.80±0.01	0.79±0.02	0.80±0.01	0.79±0.01	0.82±0.01	0.083
存活率/% SR	99.38±1.25	100.00±0.00	100.00±0.00	98.75±1.44	100.00±0.00	100±0.00	0.164

注：同行肩标不同小写字母表示差异显著 (P<0.05)，下同

Notes: in the same row, values with different letter superscripts mean significant different (P<0.05), the same below

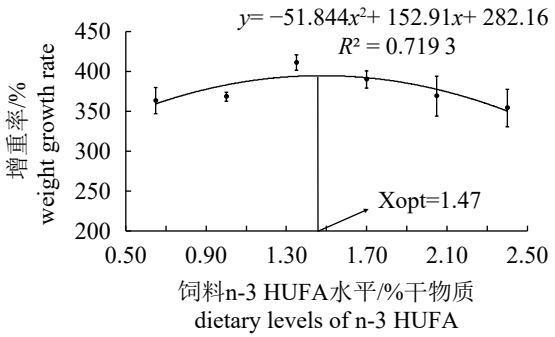

图 1 饲料n-3 HUFA水平与珍珠龙胆石斑鱼增重率之间的关系

Fig. 1 Relationship between dietary n-3HUFA and WGR of *E. fuscoguttatus*×*E. lanceolatu*

到最大值，并且显著高于1.00%组(P<0.05)(图3-a)。*IL*-10和*MyD*88 mRNA的表达呈现相同的趋势，而*TGF-β*1在各处理组间无显著差异(P>0.05)；2.05%组*TNF-α*和*IL*-1*β* mRNA表达量获得最大值，且显著高于1.00%和1.35%组(P<0.05)(图3-b)。攻毒后，各组之间*TLR*22和*Nrf*2 mRNA的表达无显著性差异(P>0.05)，而1.70%组*MyD*88 mRNA的表达量达到最大值，且显著高于1.00%、1.70%、2.05%及2.40%组(P<0.05)(图3-c)。与对照组相比，各组*IL*-1*β* mRNA的表达量均显著下降，1.70%组*IL*-1*β* mRNA的表达量有最小值(P<0.05)。同样，1.70%组*TNF-α* mRNA的表达量最低且显著低于2.05%和2.40%组(P<0.05)(图3-d)。

2.6 饲料n-3 HUFA水平对珍珠龙胆石斑鱼肾脏炎症相关基因表达的影响

攻毒前，1.70%组*TLR*22 mRNA表达有最低值，且显著低于对照组和2.40%组(P<0.05)；*MyD*88 mRNA的表达与*TLR*22具有相同的趋势。1.70%~2.40%组*Nrf*2 mRNA的表达量显著低于1.35%组(P<0.05)(图4-a)。与对照组相比，*IL*-10 mRNA的表达量在2.05%和2.40%组显著下降(P<0.05)。各处理组*TGF-β*1 mRNA的表达水平均显著低于对照组(P<0.05)。1.70%组*IL*-1*β* mRNA的表达量显著低于对照组和1.35%组(P<0.05)(图4-b)。攻毒后，*TLR*22和*MyD*88 mRNA的表达量分别在1.35%和1.70%组达到最大值，显著高于2.40%组(P<0.05)且与对照组无显著差异(P>0.05)。*Nrf*2

表 5 饲料不同水平n-3 HUFA对珍珠龙胆石斑鱼体组成的影响

Tab. 5 Effect of dietary n-3 HUFAs on body composition of *E. fuscoguttatus*×*E. lanceolatu*

项目 item	饲料 n-3 HUFAs 水平 dietary n-3 HUFAs level						*P*-值 *P*-value
	0.65	1.00	1.35	1.70	2.05	2.40	
水分 moisture	71.17±0.50	71.21±0.25	70.67±0.18	70.96±0.52	70.94±0.38	70.96±0.61	0.554
粗蛋白 crude protein	60.04±0.47	59.87±0.66	59.24±0.60	59.22±0.86	59.25±0.41	58.95±0.70	0.298
粗脂肪 crude lipid	24.05±1.11^{ab}	23.69±0.24^{a}	25.10±1.28^{ab}	25.91±0.58^{b}	25.14±0.13^{ab}	26.01±0.65^{b}	0.017
粗灰分 crude ash	15.79±0.34^{c}	15.61±0.21^{bc}	15.44±0.08^{bc}	14.83±0.15^{ab}	14.90±0.36^{ab}	14.44±0.46^{a}	0.001

表 6　饲料不同水平n-3 HUFA对珍珠龙胆石斑鱼肝脏脂肪酸组成的影响

Tab. 6　Effect of dietary n-3 HUFAs on fatty acid composition in liver of *E. fuscoguttatus* × *E. lanceolatu*　%

脂肪酸 fatty acid	饲料 n-3 HUFAs 水平　dietary n-3 HUFAs level						*P*-值 *P*-value
	0.65	1.00	1.35	1.70	2.05	2.40	
C14:0	3.44±0.65	2.83±0.39	3.41±0.57	2.94±0.16	3.50±0.06	3.05±0.37	0.285
C16:0	23.70±4.67	20.71±2.53	25.09±2.41	22.41±1.34	25.25±0.93	23.49±2.61	0.363
C18:0	3.67±0.50^{a}	3.69±0.33^{a}	4.66±0.35^{b}	4.42±0.13^{a}	4.67±0.14^{b}	4.61±0.45^{ab}	0.006
C20:0	0.24±0.06	0.24±0.01	0.25±0.01	0.25±0.02	0.27±0.04	0.30±0.01	0.236
ΣSFA^{a}	31.06±5.75	27.46±3.23	33.42±3.06	30.01±1.53	33.68±0.89	31.44±3.39	0.287
C16:1	4.92±0.09^{a}	3.27±0.16^{b}	4.41±0.11^{c}	4.45±0.11^{c}	5.03±0.16^{a}	4.41±0.10^{c}	0.000
C18:1	26.22±0.51	26.89±0.07	27.17±0.70	26.76±0.59	27.43±0.67	27.35±0.54	0.155
ΣMUFA^{b}	31.14±0.43^{ab}	30.15±0.17^{a}	31.58±0.75^{ab}	31.21±0.55^{ab}	32.45±0.82^{b}	31.76±0.61^{ab}	0.011
C18:2n-6	29.27±4.51^{b}	29.24±2.70^{b}	21.01±2.72^{ac}	21.81±0.24^{a}	16.88±1.09^{c}	16.64±2.02^{c}	0.000
C20:4n-6	0.21±0.03^{a}	0.32±0.04^{ab}	0.41±0.03^{b}	0.61±0.07^{c}	0.64±0.09^{c}	0.82±0.06^{d}	0.000
Σn-6 PUFA^{c}	29.48±4.53^{b}	29.55±2.73^{b}	21.42±2.70^{a}	22.41±0.27^{ab}	17.51±1.18^{a}	17.46±2.06^{a}	0.000
C18:3n-3	0.72±0.25	0.77±0.10	0.63±0.11	0.81±0.06	0.72±0.11	0.86±0.09	0.394
C20:5n-3	0.83±0.03^{a}	1.54±0.04^{b}	1.66±0.14^{b}	2.61±0.10^{c}	2.82±0.31^{c}	3.39±0.18^{d}	0.000
C22:6n-3	2.47±0.63^{a}	3.06±0.46^{a}	3.49±0.14^{a}	4.88±0.24^{b}	5.16±0.40^{b}	6.65±0.72^{c}	0.000
Σn-3 PUFA^{d}	4.01±0.87^{a}	5.37±0.55^{a}	5.78±0.37^{a}	8.31±0.33^{b}	8.70±0.62^{b}	10.90±0.97^{c}	0.000
n-3/n-6 PUFA	0.14±0.02^{a}	0.18±0.01^{b}	0.27±0.02^{c}	0.37±0.01^{d}	0.50±0.01^{e}	0.63±0.03^{f}	0.000
n-3 HUFA^{e}	3.30±0.62^{a}	4.60±0.46^{ab}	5.15±0.27^{b}	7.50±0.34^{c}	7.98±0.50^{c}	10.04±0.89^{d}	0.000
DHA/EPA^{f}	2.57±0.21^{a}	2.16±0.04^{b}	2.11±0.12^{b}	1.87±0.02^{b}	1.93±0.22^{b}	1.96±0.12^{b}	0.001

mRNA的表达量在1.70%组达到最大值，显著高于对照组(P<0.05)，但与其他各组无显著差异(P>0.05)(图4-c)。1.70%组*IL*-10 mRNA的表达量显著高于其他各组(P<0.05)；而1.70%组*IL*-1β mRNA的表达水平显著低于2.40%组(P<0.05)，且与其他组无显著性差异(P>0.05)(图4-d)。

3　讨论

研究表明，满足海水鱼类生长和发育的必需脂肪酸需要量为0.5%~2.0%[29]。本实验结果表明，饲料n-3 HUFA(干物质)水平为1.47%时，珍珠龙胆石斑鱼有最佳的WGR(图1)。这与斜带石斑鱼(*E. coioides*)[30]、黑鲷(*Acanthopagrus schlegelii*)[1]和欧氏六线鱼(*Hexagrammos otakii*)[31]的研究结果一致。Zuo等[2]发现饲料n-3 HUFA (0.60%~0.98%)水平显著促进了大黄鱼的生长和免疫。舌齿鲈(*Dicentrarchus labrax*)摄食0.7% n-3 HUFA(干物质含量)时具有较高的生长速率[32]。牙鲆(*Paralichthys olivaceus*)[8]、黑鲷[33]对n-3 HUFA(干物质含量)需要量为0.7%~1.0%，低于本实验结果。差异的原因可能与品种、规格、DHA/EPA比例及饲料脂肪水平和饲养环境有关[2, 29, 34]。

脂质代谢过程是脂类合成和分解代谢的动态平衡。当合成代谢增加或分解代谢减少时，机体的平衡被打破并导致过多的脂质沉积[35]。本实验全鱼粗脂肪水平先下降后上升，1.00%组显著低于2.40%组，这与黑鲷[33]和草鱼(*Ctenopharyngodon idella*)[36]的研究结果相一致。这表明适宜的n-3 HUFA水平可以提高脂质分解代谢并减少脂肪过度沉积。n-3 HUFA通过抑制脂肪酸合成酶(FAS)和葡萄糖-6-磷酸脱氢酶(G6PD)活性，抑制了虹鳟(*Oncorhynchus mykiss*)的脂质合成[37]。然而，饲料n-3 HUFA水平对黑鲷[1]和舌齿鲈[32]全鱼粗脂肪含量无显著性影响，这可能源于品种不同或各品种脂质代谢的差异。已有研究表明，肝脏和肌肉脂肪酸组成反映了饲料脂肪酸的组

表 7 饲料不同水平n-3 HUFA 对珍珠龙胆石斑鱼肌肉脂肪酸组成的影响

Tab. 7 Effect of dietary n-3HUFAs on fatty acid composition in muscle of *E. fuscoguttatus*×*E. lanceolatu* %

脂肪酸 fatty acid	饲料 n-3 HUFAs 水平 dietary n-3 HUFAs level						*P*-值 *P*-value
	0.65	1.00	1.35	1.70	2.05	2.40	
C14:0	1.73±0.12	1.65±0.18	1.68±0.18	1.73±0.11	1.83±0.16	1.85±0.17	0.601
C16:0	17.70±0.92	17.06±0.77	17.54±0.38	17.32±1.00	17.95±0.53	17.63±0.67	0.758
C18:0	4.35±0.08^{a}	4.34±0.16^{a}	4.86±0.05^{b}	5.02±0.07^{bc}	5.02±0.12^{bc}	5.29±0.10^{c}	0.000
C20:0	0.38±0.02^{a}	0.38±0.02^{a}	0.39±0.01^{ab}	0.42±0.01^{abc}	0.43±0.01^{bc}	0.44±0.01^{c}	0.001
ΣSFA^{a}	24.15±1.11	23.43±0.96	24.48±0.59	24.48±1.16	25.23±0.63	25.21±0.77	0.206
C16:1	2.13±0.25^{a}	2.06±0.13^{a}	2.34±0.18^{ab}	2.45±0.28^{ab}	2.63±0.23^{ab}	2.82±0.21^{b}	0.008
C18:1	24.45±0.51	24.61±0.14	24.64±0.27	24.66±0.35	24.71±0.57	25.06±0.20	0.521
ΣMUFA^{b}	26.58±0.75	26.67±0.26	26.99±0.20	27.11±0.63	27.35±0.77	27.88±0.11	0.093
C18:2n-6	37.55±1.39^{d}	36.87±1.05^{d}	33.51±0.71^{c}	30.60±0.86^{b}	26.95±0.51^{a}	24.80±0.76^{a}	0.000
C20:4n-6	0.28±0.01^{a}	0.34±0.01^{b}	0.46±0.01^{c}	0.61±0.03^{d}	0.66±0.03^{e}	0.80±0.02^{f}	0.000
Σn-6 PUFA^{c}	37.83±1.38^{d}	37.21±1.04^{d}	33.97±0.70^{c}	31.21±0.89^{b}	27.62±0.53^{a}	25.60±0.78^{a}	0.000
C18:3n-3	1.15±0.03^{a}	1.24±0.11^{ab}	1.22±0.05^{ab}	1.32±0.04^{b}	1.34±0.05^{bc}	1.49±0.02^{c}	0.000
C20:5n-3	1.80±0.03^{a}	2.41±0.20^{b}	3.01±0.14^{c}	4.20±0.21^{d}	5.00±0.09^{e}	6.03±0.09^{f}	0.000
C22:6n-3	3.45±0.19^{a}	4.03±0.08^{a}	4.97±0.14^{b}	6.18±0.45^{c}	6.53±0.15^{c}	7.55±0.05^{d}	0.000
Σn-3 PUFA^{d}	6.39±0.25^{a}	7.67±0.36^{b}	9.20±0.28^{c}	11.70±0.69^{d}	12.87±0.21^{e}	15.07±0.11^{f}	0.000
n-3/n-6 PUFA	0.17±0.00^{a}	0.21±0.01^{b}	0.27±0.01^{c}	0.37±0.01^{d}	0.47±0.01^{e}	0.59±0.02^{f}	0.000
n-3 HUFA^{e}	5.25±0.22^{a}	6.43±0.28^{b}	7.98±0.24^{c}	10.38±0.65^{d}	11.53±0.22^{e}	13.58±0.11^{f}	0.000
DHA/EPA^{f}	1.91±0.09^{e}	1.68±0.11^{d}	1.65±0.07^{cd}	1.47±0.05^{bc}	1.31±0.02^{ab}	1.25±0.02^{a}	0.000

成[27, 38]。在本研究中，珍珠龙胆石斑鱼的肝脏和肌肉脂肪酸组成有类似的结果。肝脏和肌肉中含有高水平的C16∶0和C18∶1，与银黑鲷(*Sparidentex haster*)[39]、黑鲷[33]及大黄鱼的结果相一致[2]，这表明C16∶0和C18∶1的含量不仅是这些组织中的主要能量来源，更可能是合成磷脂的主要PUFA。此外，肝脏和肌肉中C18∶3n-3、C20∶5n-3、C22∶6n-3和n-3 HUFA的含量随着日粮中n-3 HUFA水平的增加而增加，这与银黑鲷[39]和金头鲷(*Sparus aurata*)[40]的研究一致。本实验发现肌肉中n-3 HUFA的含量显著高于肝脏，黑鲷[1]和大黄鱼[2]的研究中也有相同结果。这可能与n-3 HUFA在满足生长最低需求后沉积在肌肉组织中有关。

在正常生理条件下，动物不断产生自由基并不断清除，因此机体自由基的浓度始终处于动态平衡[41]。过量的多不饱和脂肪酸会产生大量的自由基和活性氧(ROS)，易导致鱼体内酶的失活，破坏细胞膜结构，进而损害其他重要的生理功能，SOD、CAT和GSH-Px是清除机体ROS的主要抗氧化酶[42-44]。本实验中，攻毒前1.70%组SOD和CAT活性显著高于对照组；高水平的n-3 HUFA(2.05%和2.40%组)显著低于对照组血清GSH-Px的活性；攻毒后，1.35%组CAT活性显著高于其他各组，这表明适宜的n-3 HUFA可以显著提高珍珠龙胆石斑鱼的抗氧化能力，而高水平的n-3 HUFA会导致氧化应激反应，与以往研究一致[1, 36]。随着饲料中n-3 HUFA水平从0.15%增加到0.60%，大黄鱼血清SOD活性也明显增加[2]。补体系统和溶菌酶(LZM)是生物体抵抗微生物病菌感染的重要组成部分，它们都具有裂解细菌细胞的功能[45-46]。有研究表明，n-3 HUFA可以调节鱼类的免疫反应，促进体外巨噬细胞的杀菌能力[47-48]。在本研究中，攻毒前1.35%组中LZM的活性显着高于2.05%和2.40%组；适宜n-3 HUFA(1.35%~1.70%)可增加补体C3的浓度。用

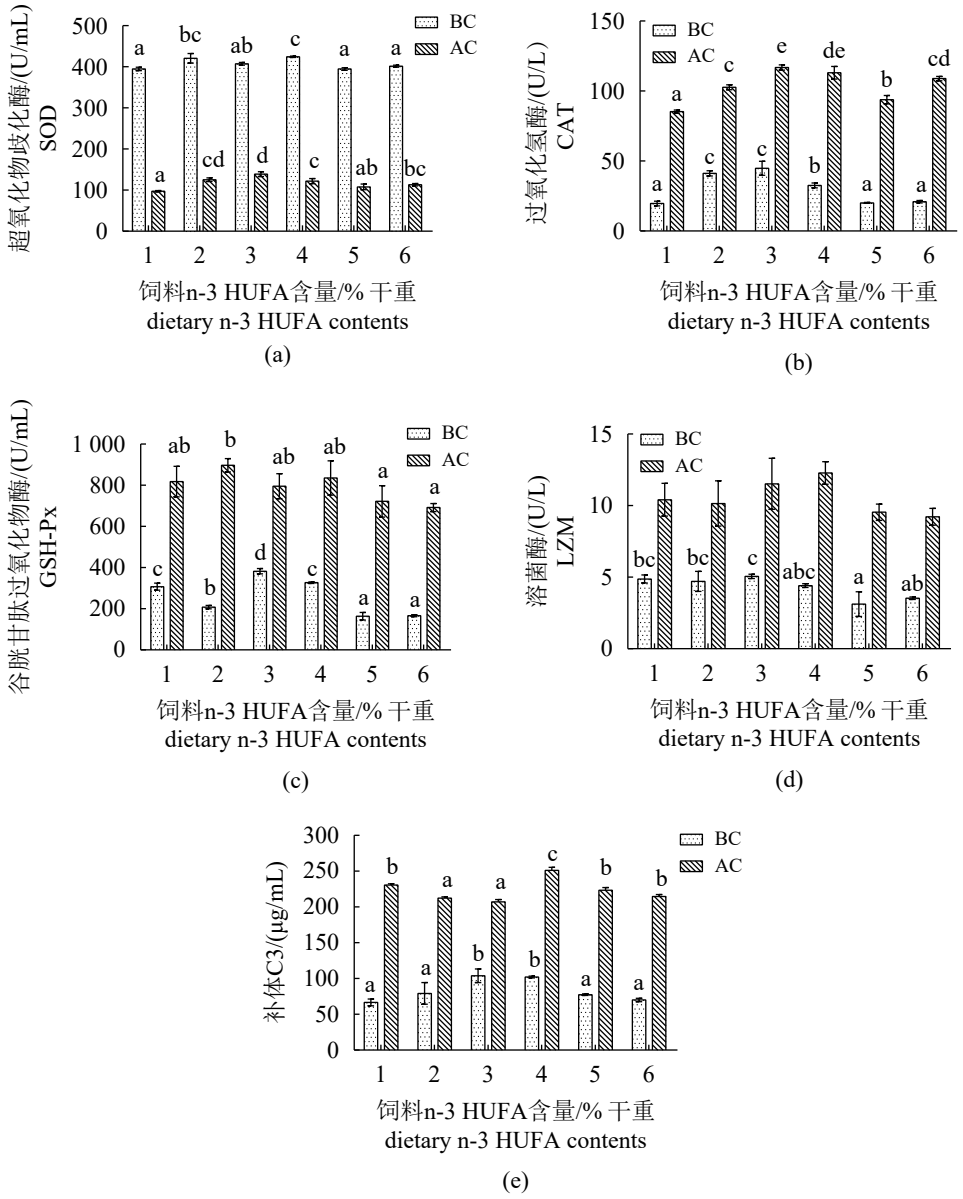

图 2　饲料不同水平对珍珠龙胆石斑鱼抗氧化能力和免疫指标的影响

BC.攻毒前；AC.攻毒后。同列肩标不同小写字母表示差异显著(P<0.05)，下同

Fig. 2　Effect of dietary n-3HUFAs on antioxidant and immunity ability of *E. fuscoguttatus*×*E. lanceolatu*

BC. before challenge, AC. after challenge. 1. 0.65%, 2. 1.00%, 3. 1.35%, 4. 1.70%, 5. 2.05%, 6. 2.40%. In the same row, values with different bar superscripts mean significant different(P<0.05), the same below

低或适宜水平的n-3 HUFA饲喂大黄鱼可以显著改善其免疫参数[2]；同样的，0.85%组仿刺参(*Apostichopus japonicus*)的LZM活性显著高于对照组，而在1.61%和1.95%组其活性急剧下降[22]。上述结果表明，适宜n-3 HUFAs可以提高免疫参数，而过量n-3 HUFA会导致免疫功能下降，这可能是由于过量n-3 HUFA会导致脂质过氧化，增加了氧化应激。然而，攻毒后SOD的活性明显低于攻毒前的活性，这可能因为哈维氏弧菌破坏了抗氧化系统的第一道防线。Sitjà-bobadilla等[49]发现，在寄生虫感染后，金头鲷血清SOD和LZM活性在开始时会增加，但由于免疫疲劳会很快耗尽。因此，本实验中高水平n-3 HUFA组表现出较差的生长性能，这可能是因为过量的n-3 HUFA可导致鱼体内脂质过氧化，产生大量氧自由基和ROS，导致体内酶失活，破坏细胞膜结构，进而影响了生长。饲料中高水平n-3 HUFA可降低吞噬细胞的吞噬能力和杀菌活性，从而

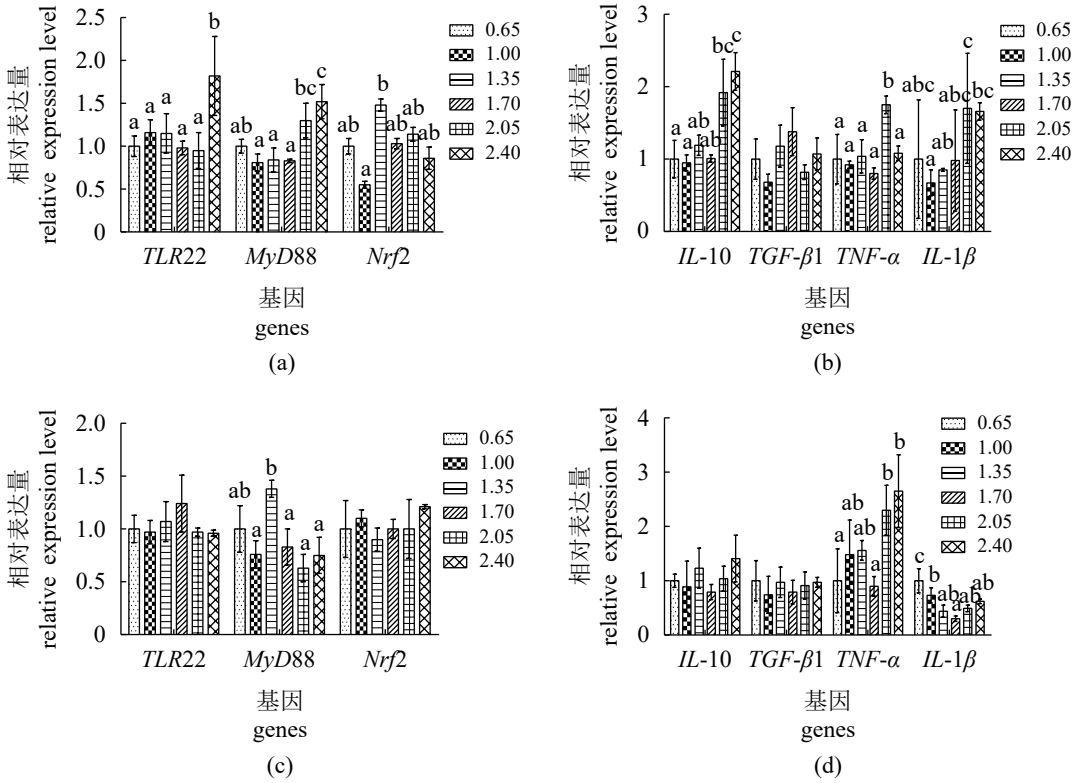

图 3 饲料不同水平n-3 HUFA对珍珠龙胆石斑鱼肠道攻毒前(a,b)和攻毒后(c,d)炎症相关基因表达的影响

Fig. 3 Effect of dietary n-3 HUFAs on expression of inflammatory related genes in intestine of *E. fuscoguttatus*×*E. lanceolatu* before (a,b) and after (c,d) challenge

降低斑点叉尾鮰(*Ictalurus punctatus*)的抗病力[50]。

核因子E2相关因子2(Nrf2)是一种有高度保守的碱性亮氨酸拉链结构的转录因子，对氧化还原反应敏感。在ARE抗氧化反应元件介导的II期解毒和抗氧化酶诱导中发挥中心作用[51]。本研究中，无论攻毒前后，1.35%组肾脏*Nrf2*表达量均达到最高值，而随着n-3 HUFA水平从2.05%增加到2.40%，*Nrf2*表达量则下降，这与大黄鱼[28]和花鲈(*L. japonicus*)[52]的结果一致，说明适宜的n-3 HUFA可以提高机体的抗氧化能力。本研究发现Nrf2在组织中的表达水平与血清中抗氧化酶的活性有一定的相关性。已有研究表明，细胞氧化应激产生的ROS和氮被认为是导致慢性炎症的主要因素之一[53]。TLRs通过MyD88相关激酶招募白细胞介素-受体1 (IL-1R)，随后诱导NF-κB和丝裂原活化蛋白激酶类[54]。在哺乳动物中，饱和脂肪酸和亚油酸能刺激TLR4/NF-κB通路，可能引发炎症[55-56]。在本研究中，缺乏或过量的n-3 HUFA均上调了攻毒前肠道和肾脏*TLR22*、*MyD88*和促炎基因(*TNF-α*和*IL-1β*) mRNA的表达，表明激活了TLR-NF-κB通路。随着棕榈油替代水平的提高，大黄鱼肝脏中*TLR2*、*TLR3*、*TLR9*、*TLR22*、*MyD88* mRNA及促炎基因的表达均增加[14]，这与本实验结果一致。本研究发现1.35%和1.70%组攻毒前肾脏和肠道*TLR22*、*MyD88*，促炎基因(*TNF-α*、*IL-1β*)和抗炎基因(*IL-10*、*TGF-β1*)的mRNA表达量相对较低，这可能是因为高水平的Nrf2表达抑制炎症的产生。以往的研究已经表明，DHA对炎症的保护作用是通过上调*Nrf2*介导*HO-1*表达，从而抑制了NF-κB信号通路的表达[57]。实验观察到，攻毒后适宜的n-3 HUFA明显上调了肠道和肾脏*TLR22*和*MyD88*的表达。Zuo等[2]同样发现，大黄鱼在感染刺激隐核虫(*Cryptocaryon irritans*)后期，0.6% n-3 HUFA明显上调了肝脏和肾脏*TLR22*的表达，这表明TLRs和MyD88在疾病识别和防御中发挥重要作用。在注射脂多糖(LPS)后，5% PUFA组小鼠血清*IL-10*表达量显著高于对照组(2.5%)[58]。低水平LC-PUFA组金头鲷感染植物性细菌后，肠道*TNF-α*和*IL-1β*表达显著增加[21]。本实验中，1.70%组肾脏*IL-10*的表达量显著高于其他各组，同样有最低的*IL-1β*表达量，显著低于2.40%组。

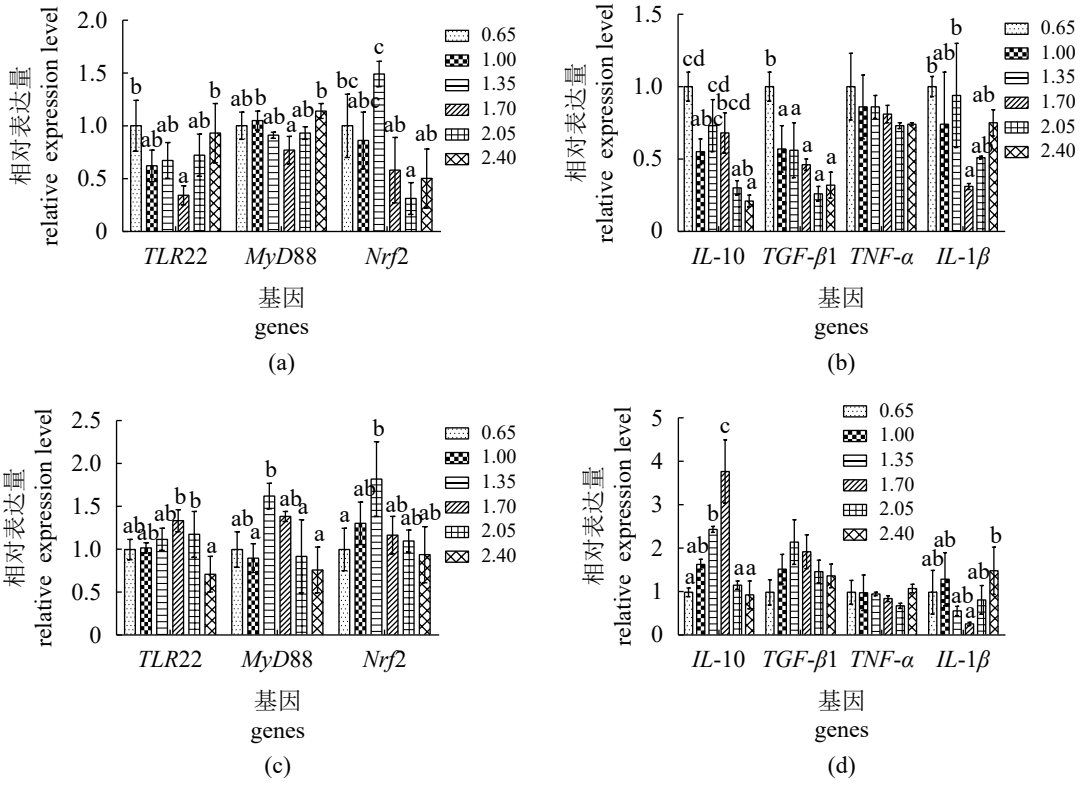

图 4　饲料不同水平n-3 HUFA对珍珠龙胆石斑鱼肾脏攻毒前(a,b)和攻毒后(c,d)炎症相关基因表达的影响

Fig. 4　Effect of dietary n-3 HUFAs on expression of inflammatory related genes in kidney of *E. fuscoguttatus*×*E. lanceolatu* before (a,b) and (c,d) after challenge

这与上述研究结果相一致，表明在感染哈维氏弧菌后，适宜的n-3 HUFA可以通过上调抗炎基因和下调促炎基因的表达量来提高珍珠龙胆石斑鱼的抗病性。

本研究表明，适宜的n-3 HUFA(1.47%~1.70%)可显著提高珍珠龙胆石斑鱼幼鱼的生长性能、非特异性免疫能力、抗病性和炎症抑制能力。

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Effects of dietary n-3 highly unsaturated fatty acids on growth, immunity and related gene expression and disease resistance of hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatu*)

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Abstract: This study was conducted to investigate the effects of dietary n-3 highly unsaturated fatty acids (n-3 HUFA) on growth performance, non-specific immunity, expression of some immune-related genes and resistance to *Vibrio harveyi* in juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀× *E. lanceolatu*♂). Six isoproteic and isolipidic experimental diets were formulated with graded levels of n-3 HUFA (0.65%, 1.00%, 1.35%, 1.70%, 2.05% and 2.40% of dry matter, respectively), and 0.65% group was used as control group. Each diet was randomly allocated to triplicate groups of fish in 1 000 L fiberglass tank, and each tank was stocked with 40 fish [initial weight (12.06 ± 0.01) g] for 8 weeks. Results showed that feed conversion ratio (FCR), survival rate (SR), hepatosomatic index (HSI) and condition factor (CF) were not significantly affected by dietary n-3 HUFA levels. Weight gain (WG) and specific growth rate (SGR) in 1.35% group were significantly higher than those in 2.40% group. Crude lipid of body in 1.00% group was significantly lower than those in 1.70% and 2.40% groups Liver and muscle fatty acid profiles reflected that of diets. Before challenge, the activity of serum superoxide dismutase (SOD), catalase (CAT) and content of complement 3 (C3) in 1.35% and 1.70% groups significantly higher than those of control group. After challenge, serum CAT, glutathione peroxidase (GSH-Px), lysozyme (LZM) and C3 all increased sharply, while SOD showed the opposite trend. Before challenge, the expression levels of intestine toll-like receptor 22 (TLR22) and myeloid differentiation factor 88 (MyD88) mRNA in 2.40% group were significantly increased, and the expression levels of tumour necrosis factor α (TNF-α) and interleukin 1β (IL-1β) mRNA in 2.05% group were significantly higher than those in 1.00% and 1.35% groups. In addition, the TLR22 and IL-1β mRNA levels in kidney of 1.70% group were significantly lower than those in control group. After challenge, the expression level of MyD88 mRNA in intestine of 1.35% group was significantly higher than that in 1.00% group and from 1.70% to 2.40% group, while TNF-α and IL-1β obtained minimum values in 1.70% group. In the kidney, the interleukin 10 (IL10) mRNA expression was significantly higher in 1.70% group than those in other groups, while the IL-1β expression in 1.70% group showed the opposite trend and significantly lower than that in 2.40% group. Results of this study suggested that moderate dietary n-3 HUFA (1.47%-1.70% HUFA) could improve the growth performance, non-specific immunity and inhibit the inflammatory response of hybrid grouper.

Key words: *Epinephelus fuscoguttatus*♀×*Epinephelus lanceolatu*♂; n-3 HUFA; non-specific immunity; inflammatory related gene

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