

双低菜粕替代鱼粉对克氏原螯虾幼虾生长性能、抗氧化能力和消化系统组织形态的影响

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REPLACING FISH MEAL WITH RAPESEED MEAL ON GROWTH PERFORMANCE, ANTIOXIDANT CAPACITY AND DIGESTIVE SYSTEM MORPHOLOGY OF JUVENILE *PROCAMBARUS CLARKII*

HUANG Ying, LOU Ge-Ge, LIU Xuan-Yu, MAN Zhou, JIANG Neng-Zuo, GUO Ya-Zhe, ZHU Xiao-Ming, GE Ru-Xiang, LIU Hao-Kun, CHEN Xin-Hua, TONG Meng-Qi

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双低菜粕替代鱼粉对克氏原螯虾幼虾生长性能、抗氧化能力和消化系统组织形态的影响

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摘要: 以双低菜粕替代饲料中不同比例[0(R0)、25%(R25)、50%(R50)、75%(R75)和100%(R100)]的鱼粉蛋白, 配制成双低菜粕含量分别为0、21.25%、42.50%、63.75%和85%的5种等氮等能饲料, 饲喂平均初始体重为(2.49±0.01) g的克氏原螯虾(*Procambarus clarkii*)幼虾42d, 探讨其对克氏原螯虾幼虾生长性能、饲料利用率、消化能力及抗氧化能力的影响。结果显示: (1)R0、R25和R50组之间终末体重(FBW)、特定生长率(SGR)、饲料效率(FE)和蛋白质效率(PER)无显著差异($P>0.05$), R75和R100组的FBW、SGR、FE和PER显著低于R0组($P<0.05$); 与R0组相比, R50、R75和R100组克氏原螯虾幼虾粗蛋白和粗脂肪含量显著降低($P<0.05$); R75和R100组克氏原螯虾幼虾肝胰腺蛋白酶活性、肝胰腺脂肪酶活性及肠道胰蛋白酶活性均显著低于R0组($P<0.05$), 各组间淀粉酶活性无显著差异($P>0.05$); R75和R100组肝胰腺和肠道NPY和TRY基因表达量显著低于R0组($P<0.05$), MSNP基因表达量显著高于R0组($P<0.05$)。 (2)与R0组相比, R100组肝胰腺碱性磷酸酶(AKP)、谷丙转氨酶(GPT)和谷草转氨酶(GOT)活性显著升高($P<0.05$); 随着菜粕水平的升高, R100组丙二醛(MDA)含量显著高于R0组($P<0.05$), 肝胰腺过氧化氢酶(CAT)、超氧化物歧化酶(SOD)、谷胱甘肽过氧化物酶(GPX)和谷胱甘肽S转移酶(GST)活性呈下降趋势且R75和R100组的以上4种的酶活性显著低于R0组($P<0.05$); R50、R75和R100组肝胰腺和肠道AKP基因表达量显著上升($P<0.05$), CAT、SOD和GPX基因表达量显著下降($P<0.05$)。 (3)R25组克氏原螯虾幼虾肝胰腺和肠道组织结构与R0组无显著差异, 随着替代比例的升高, 克氏原螯虾幼虾肝胰腺上皮细胞空泡化逐渐加重, R细胞萎缩程度加深, 肝小管管腔畸形并变大; 肠道上皮细胞脱落, 肠绒毛明显变短。 综上结果, 双低菜粕在克氏原螯虾幼虾饲料中的添加比例以不超过21.25%为宜。

关键词: 菜粕; 生长; 抗氧化能力; 基因表达; 克氏原螯虾幼虾

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鱼粉是一种昂贵的优质蛋白源, 为鱼类和甲壳类动物饲料中蛋白质的主要来源^[1]。然而, 由于野生渔业种群减少, 鱼粉产量下降, 导致供应短缺和价格上涨^[2]。在过去的几十年里, 随着水产饲料工业的迅速发展, 寻找优质且低成本的鱼粉替代蛋白源得到了广泛的研究。为了降低饲料成本, 促进水产养殖业的可持续发展, 水产商业饲料中植物蛋白

源的添加比例逐渐增加。

菜粕在全球油粕产量中仅次于豆粕^[3]。因其价格低廉、资源丰富和加工方便而被广泛用于水产饲料^[4]。菜粕的蛋白质含量高, 且拥有较高水平的必需氨基酸, 如蛋氨酸和半胱氨酸^[5]。在众多的植物蛋白源中, 菜粕具有较好的氨基酸平衡模式^[6]。已有研究表明, 菜粕可以一定比例添加到甲壳动物

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饲料中而不会降低其生长性能,如红螯螯虾(*Cherax quadricarinatus*)^[7]、中华绒螯蟹(*Eriocheir sinensis*)^[8]和南美蓝对虾(*Litopenaeus stylirostris*)^[9]等。然而,与其他植物蛋白源一样,菜粕含有许多抗营养因子,包括低聚糖、酚类化合物、单宁、植酸、硫代葡萄糖苷及其衍生物等,这些抗营养因子会对动物的生长性能和健康状况产生负面影响,应限制其在饲料中的添加量^[10, 11]。高水平菜粕会导致水产动物饲料利用率低下^[12-14],消化酶活性降低^[15],肝肠组织结构受损^[16, 17],代谢抑制^[18],免疫功能减退^[19, 20]。双低菜粕是双低油菜籽产油后的副产物,其常规营养成分与普通菜粕无显著差异^[21],但其芥酸和硫代葡萄糖苷含量大幅降低且赖氨酸、蛋氨酸及精氨酸的含量较高^[22, 23]。双低菜粕作为优质饲料蛋白源,目前已应用于水产养殖方面^[24]。

克氏原螯虾(*Procambarus clarkii*)的环境适应性好,繁殖能力强^[25],是我国当前养殖产量最大的淡水甲壳类动物。该虾因口感美味,营养价值高,深受消费者欢迎。目前,在克氏原螯虾的养殖过程中人工配合饲料应用较为广泛,但由于没有统一的营养需求标准,克氏原螯虾饲料市场良莠不齐^[26, 27]。克氏原螯虾为杂食性动物,多种蛋白源均可应用于其饲料中^[28],包括鱼粉、肉粉、虾粉、豆粕、菜粕和昆虫粉等^[29],目前鲜有关于双低菜粕在其饲料中适宜添加比例的研究。本试验研究不同双低菜粕替代比例对克氏原螯虾生长性能、体成分、消化酶活性、抗氧化能力、抗氧化和摄食相关基因表达等方面的影响,旨在为该虾配合饲料中添加适宜比例的双低菜粕提供理论依据。

1 材料与方法

1.1 试验饲料与试验分组

试验饲料的配方和常规化学成分如表1所示,氨基酸组成如表2所示。用双低菜粕替代基础饲料中0(R0)、25%(R25)、50%(R50)、75%(R75)和100%(R100)的鱼粉蛋白,配置成双低菜粕含量分别为0、21.25%、42.50%、63.75%和85%的5种等氮等能饲料。饲料原料由福建大昌生物科技实业有限公司提供。将所有饲料原料粉碎过后过40目筛,按照配方比例从小到大的顺序逐次添加并混合均匀,之后使用饲料机(SLP-45,上海华夏渔业机械仪器工贸公司)制作成直径1 mm大小的颗粒,60℃烘干后密封,放置在-20℃冰箱备用。

1.2 试验动物与养殖管理

克氏原螯虾虾苗选自湖北省莱克集团小龙虾良种选育中心。在正式试验开始前,克氏原螯虾虾

表1 试验基础饲料配方及化学组成(%干物质)

Tab. 1 Diet formulation and chemical composition of experimental diets (% in dry matter)

原料Ingredient	替代水平Replacing level (%)				
	0 (R0)	25 (R25)	50 (R50)	75 (R75)	100 (R100)
白鱼粉White fish meal	50.00	37.50	25.00	12.50	0.00
双低菜粕Rapeseed meal	0.00	21.25	42.50	63.75	85.00
鱼油Fish oil	3.00	3.89	4.78	5.67	6.55
玉米淀粉Corn starch	39.95	30.20	20.50	10.70	1.00
纤维素Cellulose	0.05	0.16	0.22	0.38	0.45
多维多矿预混物Vitamin and Mineral premix	1.00	1.00	1.00	1.00	1.00
氯化胆碱Choline chloride	0.50	0.50	0.50	0.50	0.50
磷酸二氢钙CaH ₂ PO ₄	0.50	0.50	0.50	0.50	0.50
胆固醇Cholesterol	0.50	0.50	0.50	0.50	0.50
卵磷脂Lecithin	1.00	1.00	1.00	1.00	1.00
海藻酸钠Sodium alginate	1.00	1.00	1.00	1.00	1.00
羧甲基纤维素Carboxymethyl cellulose	1.70	1.70	1.70	1.70	1.70
鱿鱼粉Squid Power	0.80	0.80	0.80	0.80	0.80
饲料化学成分Chemical composition (% in dry matter)					
粗蛋白Crude protein	35.82	35.24	35.62	35.88	35.43
粗脂肪Crude lipid	8.61	8.58	8.35	8.65	8.32
能量Gross energy (kJ/g in dry matter)	16.53	16.63	16.08	16.91	16.89

注: 饲料原料由福建大昌生物科技实业有限公司提供

Note: Feed ingredients are provided by Fujian Dachang Biotechnology Industry CO., LTD

表2 试验饲料氨基酸组成

Tab. 2 Amino acid composition of experimental diets (% in dry matter)

氨基酸 Amino acid	替代水平Replacing level (%)				
	0(R0)	25(R25)	50(R50)	75(R75)	100(R100)
天冬氨酸Asp	3.03	2.9	2.76	2.52	2.28
苏氨酸Thr	1.43	1.46	1.49	1.48	1.45
丝氨酸Ser	1.51	1.51	1.50	1.43	1.37
谷氨酸Glu	4.57	4.88	5.35	5.63	5.88
甘氨酸Gly	2.52	2.44	2.24	1.99	1.67
丙氨酸Ala	2.01	1.93	1.83	1.68	1.46
胱氨酸Cys	0.23	0.33	0.40	0.46	0.51
缬氨酸Val	1.64	1.65	1.71	1.66	1.69
蛋氨酸Met	0.71	0.60	0.49	0.4	0.32
异亮氨酸Ile	1.33	1.33	1.36	1.32	1.28
亮氨酸Leu	2.35	2.36	2.39	2.28	2.16
酪氨酸Tyr	1.00	1.01	1.00	0.92	0.84
苯丙氨酸Phe	1.29	1.30	1.34	1.29	1.26
赖氨酸Lys	2.37	2.27	2.23	2.11	1.88
组氨酸His	0.64	0.70	0.77	0.79	0.83
精氨酸Arg	2.07	2.10	2.10	2.08	1.92
脯氨酸Pro	1.46	1.60	1.76	1.85	1.90
合计Total	30.16	30.37	30.72	29.89	28.70

苗放到室内养殖系统中暂养3周, 对其进行驯化, 暂养期间投喂5组饲料构成的混合饲料。试验开始时, 对克氏原螯虾进行24h的饥饿处理, 挑取体质健康、体格相近的克氏原螯虾幼虾[平均体重(2.49 ± 0.01) g]随机放入15个养殖水箱, 每箱16尾。共设置5个试验组, 每组3个重复。在试验期间, 每天定时投喂饲料3次(9:00、17:00和22:00), 投喂结束后清除缸内残饵和排泄物, 换水量为1/3。发现死虾后捞出, 每天记录摄食量及死虾数量, 一共持续42d。

克氏原螯虾采用静水养殖的方式, 试验养殖系统由15个矩形塑料养殖箱(100 cm×50 cm×50 cm)组成, 养殖箱水深保持在15 cm。每天测两次(8:30和16:00)水温和室温, 每周测一次水体DO值和氨氮水平。在整个养殖过程, 养殖水体维持以下参数: 水温(21 ± 2)℃, DO值 ≥ 6 mg/L, 氨氮含量 < 0.4 mg/L。光照周期为12L:12D (8:30到20:30)。

1.3 试验取样

在养殖试验结束禁食24h后, 对每缸虾进行计数、称重并记录。每个缸中随机选3尾虾称取体重, 解剖后称取肝胰腺重量, 用于计算肝体比, 并测量去壳后虾肉的重量计算出肉率。此3尾虾取肝胰腺和中肠, 用中性甲醛固定后进行组织切片制作。另取3尾虾在冰盘上解剖后取肝胰腺和肠道, 肝脏和肠道分别混合后部分用作酶活性的测定, 剩余部分用于基因表达的分析, 均迅速放入冻存管中经液氮急冻, 稍后转移到-80℃冰箱中保存。再取3尾虾用于体成分分析。

1.4 样品的测定

参照文献[30]的方法对本试验饲料及样品虾进行水分、粗蛋白、粗脂肪和灰分的含量测定。样品在105℃下烘干至恒重, 通过失重法测定水分。粗蛋白含量使用凯氏定氮仪(Kjeltec8400, FOSS)测定。粗脂肪含量使用索氏抽提仪(ST243, FOSS)进行测定。样品在马福炉中550℃燃烧3h, 以失重法测定灰分。

肝胰腺中碱性磷酸酶(AKP)、谷丙转氨酶(GPT)、谷草转氨酶(GOT)、过氧化氢酶(CAT)、超氧化物歧化酶(SOD)、谷胱甘肽过氧化物酶(GPX)、谷胱甘肽S转移酶(GST)活性及丙二醛(MDA)含量均采用南京建成生物工程研究所的试剂盒测定, 测定方法按说明书进行。

从各组虾的肝胰腺组织中分离出总RNA。分离出的RNA浓度由超微量核酸蛋白测定仪(Scandrop 100, Analytik Jena, Germany)按照说明书进行确认, 按 A_{260}/A_{280} 的比例计算。基因的引物(正向和反向)序列、解链温度、产物大小见表3。使用逆转

录试剂盒进行逆转录操作, 引物的扩增效率在有效范围(0, 1)之内。将cDNA保存在-80℃下, 直至进行qPCR分析。所有qPCR反应均在analytickjenaq-tower 2.2荧光定量PCR仪(德国)中进行。qPCR系统由cDNA模板9 μL, 10 μL SYBR Green Realtime PCR Master Mix, 10 μmol/L正/反定量引物各0.5 μL组成。qPCR反应条件如下: 95℃ 15s, 60℃ 1min, 40个循环。根据上述qPCR反应系统配置所有组分, 并在PCR平板离心机中以4℃和6000 r/min的速度离心30s。然后将其置于定量PCR仪中, 根据该程序进行扩增。分析熔融曲线以确认这些反应中存在单一产物。为了使基因表达数据正常化, 使用18S基因作为管家基因。采用 $2^{-\Delta\Delta C_t}$ 法计算各组的基因表达水平。

1.5 数据处理

计算公式: 存活率(SR, %)= $100 \times N_t/N_0$; 特定生长率(SGR, %/d)= $100 \times (\ln W_t - \ln W_0)/t$; 饲料效率(FE, %)= $100 \times (W_t - W_0)/F$; 蛋白质效率比(PER)= $100 \times (W_t - W_0)/F \times P$; 肝体比(HSI)= $100 \times (W_z/W)$; 出肉率(FY, %)= $100 \times (W_f/W)$ 。式中, N_0 为试验开始时试验

表3 本试验所用引物

Tab. 3 Primer pairs used for RT-qPCR analysis of related genes in this study

基因名称 Gene name	引物序列 Primer sequence (5'—3')	溶解温度 T_m (°C)	登录号 GenBank number
18S	GCAATAACAGGTCTG TGATGCC AGGGACGTAATCAGC GCAA	60	X90672.1
AKP	CTACAGTGCCCCACA CTACG GGGTATGAAGGCGTT CTCGT	60	KC007367.1
CAT	GGAGAAGGCCGACGA CTTAG GTGCTTCTCAGCCTCT TCGT	60	KM068092.1
SOD	CGTAGGTACGGTGTA TGGGC TGCTACGCCTTGGTAG TTGG	60	KC333177.1
GPX	CCGCTCTTCACCTTCT TG GCGAGTGTATGGCTT ACC	60	JN835259.1
NPY	TCCGAGACAGAGAGG CGTAT AGGTGGGTGAGAGGT GACAT	60	AB036713.1
TRY	CCTGCATGAGGACTT CGACT GGACCGACGTTCTCG TTGAA	60	AY596942.1
MSNP	TCGAAGCAACAGGCT CTCTC GTTGGTTGAGGCAGA TGGGA	60	AB294248.1

虾的尾数, N_t 为试验结束后试验虾的存活尾数, W_0 为平均初始体重(g), W_t 为平均终末体重(g), t 为试验时长(d), F 为饲料摄入干重(g), P 为饲料中粗蛋白质含量(%), W 为虾体质量(g), W_z 为肝胰脏质量(g), W_f 为去壳虾肉质量(g)。试验数据以平均值 $\pm$ 标准误(mean $\pm$ SE)表示, 采用SPSS 20.0软件进行统计分析。试验结果经一元方差(One-way ANOVA)分析后, 用Duncan's 进行多重比较, 当 $P<0.05$ 时, 为差异显著。

2 结果

2.1 双低菜粕替代鱼粉对克氏原螯虾幼虾生长性能的影响

如表4所示, 当双低菜粕替代比例 $\geq 75\%$ 时, 克氏原螯虾幼虾平均终末体重、特定生长率、饲料效率和蛋白质效率与R0组相比显著降低($P<0.05$)。当双低菜粕替代比例 $\geq 50\%$ 时, 克氏原螯虾幼虾存活率显著低于R0组($P<0.05$)。与R0组相比, R50和R100组克氏原螯虾幼虾肝体比显著增高($P<0.05$)。当双低菜粕替代比例 $\geq 75\%$ 时, 显著降低了克氏原螯虾幼虾出肉率($P<0.05$)。

2.2 双低菜粕替代鱼粉对克氏原螯虾幼虾体成分的影响

如表5所示, 与R0组相比, 当双低菜粕替代比

例 $\geq 75\%$ 时, 克氏原螯虾幼虾的水分显著提高($P<0.05$)。当双低菜粕替代比例 $\geq 50\%$ 时, 克氏原螯虾幼虾的粗蛋白和粗脂肪均显著低于R0组($P<0.05$)。当双低菜粕替代比例 $\geq 75\%$ 时, 克氏原螯虾幼虾的灰分显著下降($P<0.05$)。

2.3 双低菜粕替代鱼粉对克氏原螯虾幼虾消化酶活性的影响

如表6所示, 与R0组相比, 当双低菜粕替代比例 $\geq 75\%$ 时, 克氏原螯虾幼虾肝胰腺胰蛋白酶、肝胰腺脂肪酶和肠道胰蛋白酶活性显著降低($P<0.05$)。当双低菜粕替代比例为100%时, 显著降低了克氏原螯虾幼虾肠道脂肪酶活性($P<0.05$)。饲料中双低菜粕的添加对克氏原螯虾幼虾肝胰腺淀粉酶和肠道淀粉酶活性无显著影响($P>0.05$)。

2.4 双低菜粕替代鱼粉对克氏原螯虾幼虾抗氧化指标的影响

如表7所示, 与R0组相比, 当双低菜粕替代比例 $\geq 75\%$ 时, 克氏原螯虾幼虾肝胰腺AKP活性显著升高($P<0.05$)。R100组克氏原螯虾幼虾肝胰腺GPT活性显著上升($P<0.05$)。当双低菜粕替代比例 $\geq 25\%$ 时, 克氏原螯虾幼虾肝胰腺GOT活性显著上升($P<0.05$), CAT和GST活性显著下降($P<0.05$)。当双低菜粕替代比例 $\geq 75\%$ 时, SOD和GPX活性显著下降($P<0.05$)。克氏原螯虾幼虾肝胰腺MDA含量随

表4 双低菜粕替代鱼粉对克氏原螯虾幼虾生长性能的影响(平均值 $\pm$ 标准误)

Tab. 4 Effects of replacing fish meal with rapeseed meal on growth performance of juvenile *P. clarkii* (mean $\pm$ SE)

指标Index	替代水平Replacing level (%)				
	0	25	50	75	100
初始体重IBW (g)	2.49 $\pm$ 0.01	2.49 $\pm$ 0.02	2.49 $\pm$ 0.02	2.47 $\pm$ 0.03	2.49 $\pm$ 0.04
终末体重FBW (g)	8.57 $\pm$ 0.15 ^a	8.50 $\pm$ 0.15 ^a	8.43 $\pm$ 0.35 ^a	7.80 $\pm$ 0.12 ^b	7.70 $\pm$ 0.06 ^b
存活率SR (%)	82.05 $\pm$ 2.57 ^a	76.92 $\pm$ 4.44 ^a	64.10 $\pm$ 2.56 ^b	56.41 $\pm$ 2.56 ^{bc}	51.28 $\pm$ 2.57 ^c
特定生长率SGR (%/d)	2.21 $\pm$ 0.03 ^a	2.20 $\pm$ 0.03 ^a	2.18 $\pm$ 0.06 ^{ab}	2.05 $\pm$ 0.04 ^{bc}	2.01 $\pm$ 0.03 ^c
饲料效率FE (%)	66.70 $\pm$ 1.71 ^a	64.00 $\pm$ 1.25 ^{ab}	63.66 $\pm$ 2.77 ^{ab}	60.91 $\pm$ 0.15 ^b	59.80 $\pm$ 0.29 ^b
蛋白质效率比PER	1.86 $\pm$ 0.05 ^a	1.82 $\pm$ 0.04 ^{ab}	1.79 $\pm$ 0.08 ^{ab}	1.70 $\pm$ 0.00 ^b	1.69 $\pm$ 0.01 ^b
肝体比HSI (%)	4.68 $\pm$ 0.31 ^b	4.59 $\pm$ 0.12 ^b	5.48 $\pm$ 0.14 ^a	5.09 $\pm$ 0.25 ^{ab}	5.62 $\pm$ 0.22 ^a
出肉率FY (%)	12.24 $\pm$ 0.60 ^a	13.17 $\pm$ 0.42 ^a	12.20 $\pm$ 0.36 ^a	10.60 $\pm$ 0.14 ^b	9.90 $\pm$ 0.29 ^b

注: 同列数据后面英文字母不同者表示各组之间差异显著 ($P<0.05$); 下同

Note: Different superscript letters within each column represent significant differences ($P<0.05$). The same applies below

表5 双低菜粕替代鱼粉对克氏原螯虾幼虾水分、粗蛋白、粗脂肪和灰分的影响(平均值 $\pm$ 标准误)

Tab. 5 Effects of replacing fish meal with rapeseed meal on moisture, crude protein, crude lipid and ash of juvenile *P. clarkii* (mean $\pm$ SE)

指标Index	替代水平Replacing level (%)				
	0	25	50	75	100
水分Moisture	73.95 $\pm$ 0.75 ^a	74.20 $\pm$ 1.60 ^a	74.75 $\pm$ 0.95 ^a	78.75 $\pm$ 0.55 ^b	79.15 $\pm$ 0.55 ^b
粗蛋白Crude protein	13.39 $\pm$ 0.16 ^a	12.94 $\pm$ 0.30 ^{ab}	12.41 $\pm$ 0.07 ^{bc}	11.92 $\pm$ 0.05 ^{cd}	11.73 $\pm$ 0.23 ^d
粗脂肪Crude lipid	2.71 $\pm$ 0.09 ^a	2.54 $\pm$ 0.03 ^a	2.23 $\pm$ 0.08 ^b	1.64 $\pm$ 0.13 ^c	1.22 $\pm$ 0.10 ^d
灰分Ash	9.18 $\pm$ 0.36 ^a	8.52 $\pm$ 0.52 ^{ab}	9.24 $\pm$ 0.22 ^a	7.13 $\pm$ 0.75 ^b	6.98 $\pm$ 0.33 ^b

着饲料中双低菜粕替代比例的增高而上升,当双低菜粕替代比例为100%时显著高于R0组($P<0.05$)。

2.5 双低菜粕替代鱼粉对克氏原螯虾幼虾肝胰腺和肠道组织学的影响

如图1所示, R0组克氏原螯虾幼虾的肝胰腺组织结构完整, 肝小管排列紧密, 界限明显清晰, 管腔呈星形, 细胞分布均匀饱满, 形态正常。R25组和R0组相比肝胰腺组织结构无显著差异, 无明显病变。R50组肝小管排列松散, 部分肝小管管腔扩张, 上皮细胞肿胀、空泡化, 部分基膜溶解。R75组肝小管和管腔扩张更加明显, 部分上皮细胞坏死解体、空泡化, B细胞肿大, 数量增多, R细胞萎缩变小, 数量明显减少; 肝小管退化, 基膜界限不清晰, 部分基膜破裂分离。R100组肝小管完全退化, 结构紊乱, 受损严重, 上皮细胞大量坏死, R细胞进一步萎缩消失, 无完整细胞结构, 部分破裂组织落入管腔和肝小管间隙。

如图2所示, R0组正常克氏原螯虾幼虾的肠道组织结构完整, 上皮细胞排列紧密, 呈规则栅栏状排列, 固有层紧密。R25组和R0组的肠道组织结构相比并无显著差异, 无明显病变。R50组固有层萎缩, 出现肠细胞脱落和空泡化现象。R75组上皮层紊乱, 上皮细胞形状不规则, 呈异常排列。R100组上皮细胞与结缔组织分离, 上皮细胞严重受损, 肌

层细胞坏死, 肠道结构不完整, 肠绒毛明显变短。

2.6 双低菜粕替代鱼粉对克氏原螯虾幼虾肝胰腺和肠道相关基因表达的影响

如图3所示, 所有双低菜粕替代组肝胰腺的AKP基因表达量均显著高于R0组($P<0.05$)。随着双低菜粕替代比例的提高, 克氏原螯虾幼虾肝胰腺CAT基因表达量逐渐降低, 均显著低于R0组($P<0.05$)。当双低菜粕替代比例 $\geq 50\%$ 时, 与R0组相比, 克氏原螯虾幼虾肝胰腺SOD和GPX基因表达量显著降低($P<0.05$)。当双低菜粕替代比例 $\geq 75\%$ 时, 克氏原螯虾幼虾肝胰腺NPY和TRY基因表达量显著低于R0组($P<0.05$)。R100组克氏原螯虾幼虾肝胰腺MS-NP基因表达量显著高于R0组($P<0.05$)。

如图4所示, 当双低菜粕替代比例 $\geq 50\%$ 时, 与R0组相比, 克氏原螯虾幼虾肠道AKP基因表达量显著升高($P<0.05$)。所有双低菜粕替代组肠道的CAT基因表达量均显著低于R0组($P<0.05$)。当双低菜粕替代比例 $\geq 50\%$ 时, 克氏原螯虾幼虾肠道SOD基因表达量显著低于R0组($P<0.05$)。当双低菜粕替代比例 $\geq 75\%$ 时, 与R0组相比, 克氏原螯虾幼虾肠道GPX基因表达量显著降低($P<0.05$)。克氏原螯虾幼虾肠道NPY基因表达量随着双低菜粕替代比例的提高而降低, 当双低菜粕替代比例 $\geq 50\%$ 时显著低于R0组($P<0.05$)。当双低菜粕替代比例 $\geq 75\%$

表6 双低菜粕替代鱼粉对克氏原螯虾幼虾消化酶活性的影响(平均值 $\pm$ 标准误)

Tab. 6 Effects of replacing fish meal with rapeseed meal on digestive enzyme in juvenile *P. clarkii* (mean $\pm$ SE)

指标Index	替代水平Replacing level (%)				
	0	25	50	75	100
肝胰腺胰蛋白酶Hepatopancreatic trypsin (U/mg)	9.64 $\pm$ 0.36 ^a	9.22 $\pm$ 0.39 ^a	9.11 $\pm$ 0.08 ^a	6.69 $\pm$ 0.70 ^b	6.78 $\pm$ 0.24 ^b
肝胰腺脂肪酶Hepatopancreatic lipase (U/g prot)	16.44 $\pm$ 0.55 ^a	16.82 $\pm$ 1.72 ^a	15.37 $\pm$ 1.54 ^{ab}	11.14 $\pm$ 1.03 ^c	12.19 $\pm$ 1.19 ^{bc}
肝胰腺淀粉酶Hepatopancreatic amylase (U/mg prot)	1.07 $\pm$ 0.07	1.25 $\pm$ 0.09	1.20 $\pm$ 0.04	1.26 $\pm$ 0.21	1.12 $\pm$ 0.23
肠道胰蛋白酶Intestinal trypsin (U/mg prot)	13.55 $\pm$ 0.92 ^a	13.81 $\pm$ 1.36 ^a	12.52 $\pm$ 1.52 ^{ab}	9.62 $\pm$ 0.40 ^b	9.34 $\pm$ 0.37 ^b
肠道脂肪酶Intestinal lipase (U/g prot)	22.20 $\pm$ 1.74 ^a	22.57 $\pm$ 1.86 ^a	20.12 $\pm$ 1.18 ^{ab}	19.04 $\pm$ 1.62 ^{ab}	16.47 $\pm$ 1.36 ^b
肠道淀粉酶Intestinal amylase (U/mg prot)	1.50 $\pm$ 0.13	1.52 $\pm$ 0.15	1.59 $\pm$ 0.17	1.36 $\pm$ 0.04	1.44 $\pm$ 0.16

表7 双低菜粕替代鱼粉对克氏原螯虾幼虾肝胰腺生化指标的影响(平均值 $\pm$ 标准误)

Tab. 7 Effects of replacing fish meal with rapeseed meal on biochemical indexes in hepatopancreas of juvenile *P. clarkii* (mean $\pm$ SE)

指标Index	替代水平Replacing level (%)				
	0	25	50	75	100
碱性磷酸酶AKP (金氏单位/ mg prot)	18.86 $\pm$ 1.26 ^b	18.15 $\pm$ 1.31 ^b	18.14 $\pm$ 0.42 ^b	23.91 $\pm$ 0.72 ^a	24.86 $\pm$ 0.72 ^a
谷丙转氨酶GPT (U/g prot)	28.91 $\pm$ 1.26 ^b	29.66 $\pm$ 2.22 ^b	32.73 $\pm$ 0.87 ^b	30.55 $\pm$ 1.49 ^b	49.97 $\pm$ 2.35 ^a
谷草转氨酶GOT (U/g prot)	19.21 $\pm$ 1.37 ^b	29.75 $\pm$ 3.73 ^a	28.34 $\pm$ 1.65 ^a	27.80 $\pm$ 0.78 ^a	34.13 $\pm$ 0.43 ^a
过氧化氢酶CAT (U/mg prot)	12.71 $\pm$ 0.43 ^a	11.17 $\pm$ 0.10 ^b	9.95 $\pm$ 0.17 ^c	9.81 $\pm$ 0.46 ^c	9.73 $\pm$ 0.34 ^c
超氧化物歧化酶SOD (U/mg prot)	94.14 $\pm$ 3.18 ^a	88.50 $\pm$ 5.67 ^a	80.77 $\pm$ 2.62 ^{ab}	68.13 $\pm$ 4.15 ^b	60.42 $\pm$ 5.43 ^c
谷胱甘肽过氧化物酶GPX (U/mg prot)	108.98 $\pm$ 1.09 ^a	105.39 $\pm$ 4.21 ^a	106.72 $\pm$ 2.71 ^a	88.54 $\pm$ 2.77 ^b	82.18 $\pm$ 8.03 ^b
谷胱甘肽S转移酶GST (U/mg prot)	58.15 $\pm$ 1.13 ^a	54.16 $\pm$ 0.78 ^b	52.11 $\pm$ 0.78 ^b	51.05 $\pm$ 0.73 ^b	51.15 $\pm$ 1.42 ^b
丙二醛MDA (nmol/mg prot)	5.55 $\pm$ 0.17 ^b	5.75 $\pm$ 0.26 ^{ab}	5.99 $\pm$ 0.17 ^{ab}	6.14 $\pm$ 0.13 ^{ab}	6.24 $\pm$ 0.17 ^a

时, 克氏原螯虾幼虾肠道 TRY 基因表达量显著低于 $R0$ 组($P<0.05$)。当双低菜粕替代比例 $\geq 50\%$ 时, 克氏原螯虾幼虾肠道 $MSNP$ 基因表达量显著高于 $R0$ 组($P<0.05$)。

3 讨论

3.1 双低菜粕替代鱼粉对克氏原螯虾幼虾生长性能和体成分的影响

菜粕通常被作为重要的植物蛋白源添加到部分水产动物的饲料中^[18]。与本试验结果相同, 饲料中高水平的菜粕可显著降低日本鲈(*Lateolabrax japonicus*)^[31]和凡纳滨对虾(*Litopenaeus vannamei*)^[32]的存活率。存活率的降低可能和菜粕中的有毒物

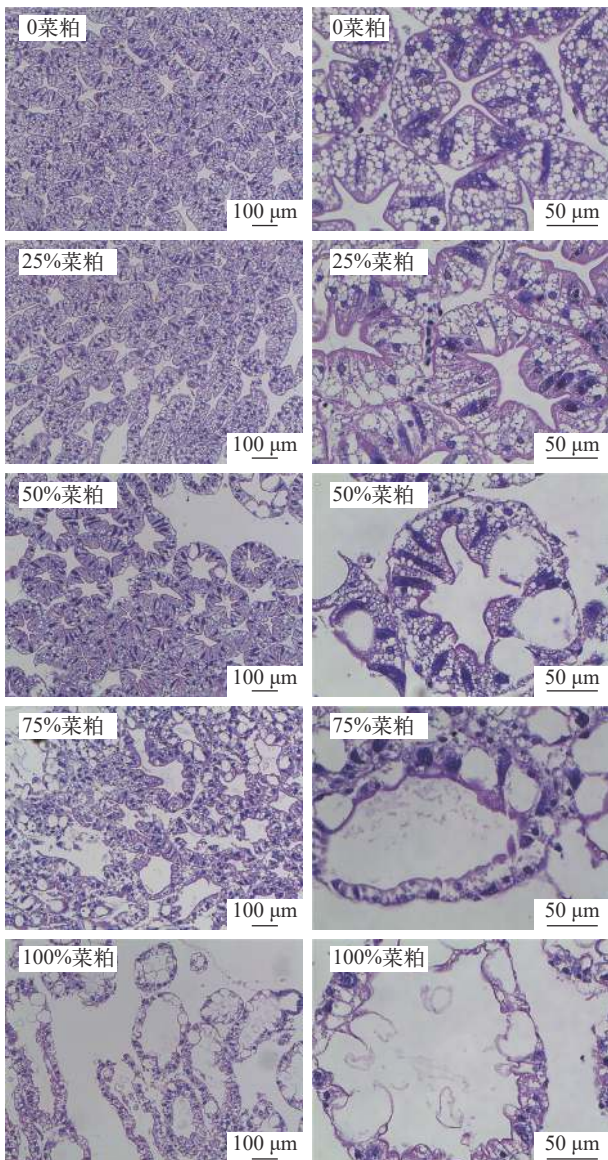


图1 双低菜粕替代鱼粉对克氏原螯虾幼虾肝胰腺组织学的影响
Fig. 1 Effects of replacing fish meal with rapeseed meal on hepatopancreas histology of juvenile *P. clarkii*

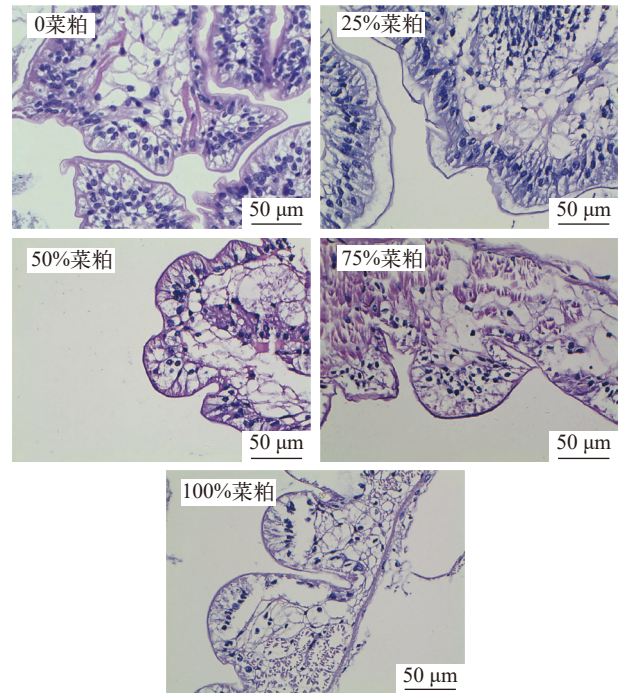


图2 双低菜粕替代鱼粉对克氏原螯虾幼虾中肠组织学的影响
Fig. 2 Effects of replacing fish meal with rapeseed meal on midgut histology of juvenile *P. clarkii*

质有关, 如: 单宁被认为是动物中毒致死的原因^[33]。另有研究表明, 菜粕替代鱼粉比例达到60%时, 显著降低了大黄鱼(*Larimichthys crocea*)的终末体重和特定生长率^[34]。当菜粕替代饲料中40%鱼粉的蛋白质时, 显著降低了日本鲈的饲料效率^[31]。当饲料中双低菜粕含量达到8.2%时, 显著提高了日本沼虾的饲料系数^[35]。在本试验中, 饲料中双低菜粕替代鱼粉比例 $\geq 75\%$ 时, 对克氏原螯虾幼虾终末体重和特定生长率产生了不良影响, 显著降低了饲料效率, 与以上结论相似。生长性能的降低是由于菜粕中含有抗营养物质, 如硫代葡萄糖苷、植酸和单宁等^[36], 这些抗营养因子的高摄入量会导致代谢抑制^[37]。硫代葡萄糖苷和其分解产物如: 异硫氰酸盐(ITC)、恶唑烷硫酮(OZT)、硫氰酸酯(TC)和腈(RCN), 可通过刺激消化道黏膜而影响饲料利用, 严重危害动物的健康^[15, 38]。此外, 菜粕中氨基酸不平衡和必需氨基酸的缺乏^[39], 以及菜粕含有高水平粗纤维而影响饲料的消化率, 从而导致水产动物的生长性能不佳^[40]。当菜粕替代豆粕比例达到60%时, 可显著降低奥尼罗非鱼(*Oreochromis niloticus*×*Oreochromis aureus*)的蛋白质效率^[41]。本试验结果与上述试验相同, 克氏原螯虾幼虾饲料中双低菜粕替代鱼粉比例 $\geq 75\%$ 时蛋白质效率显著降低, 可能是菜粕中的植酸会与蛋白质结合形成不溶性复合物, 大大降低蛋白质的生物效力和消化率^[38]。

肝体比是评价生物体健康、营养状况、能量储备的指标^[42]。有毒物质可导致肝脏肿大从而影

响肝体比^[43]。出肉率作为衡量水产养殖动物肌肉品质、经济性状和生产性能的重要指标,反映了水

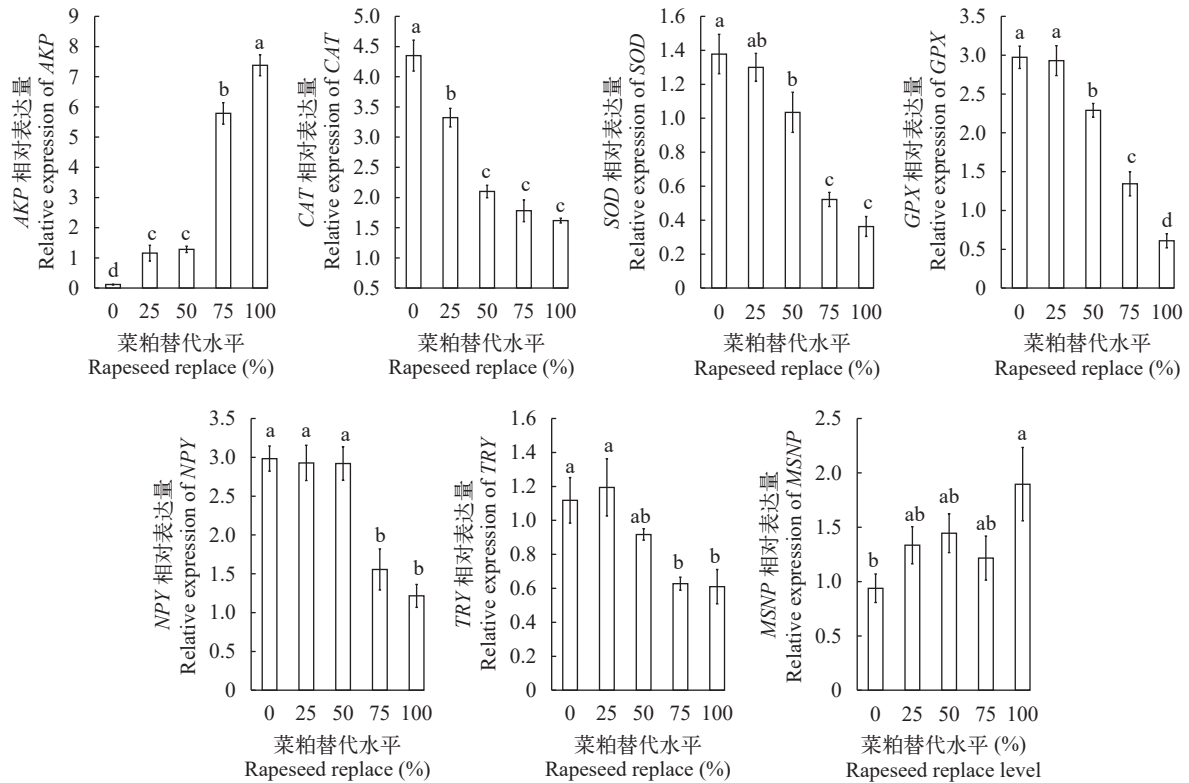


图3 双低菜粕替代鱼粉对克氏原螯虾幼虾肝胰腺基因表达的影响

Fig. 3 Effects of replacing fish meal with rapeseed meal on gene expression in hepatopancreas of juvenile *P. clarkii*

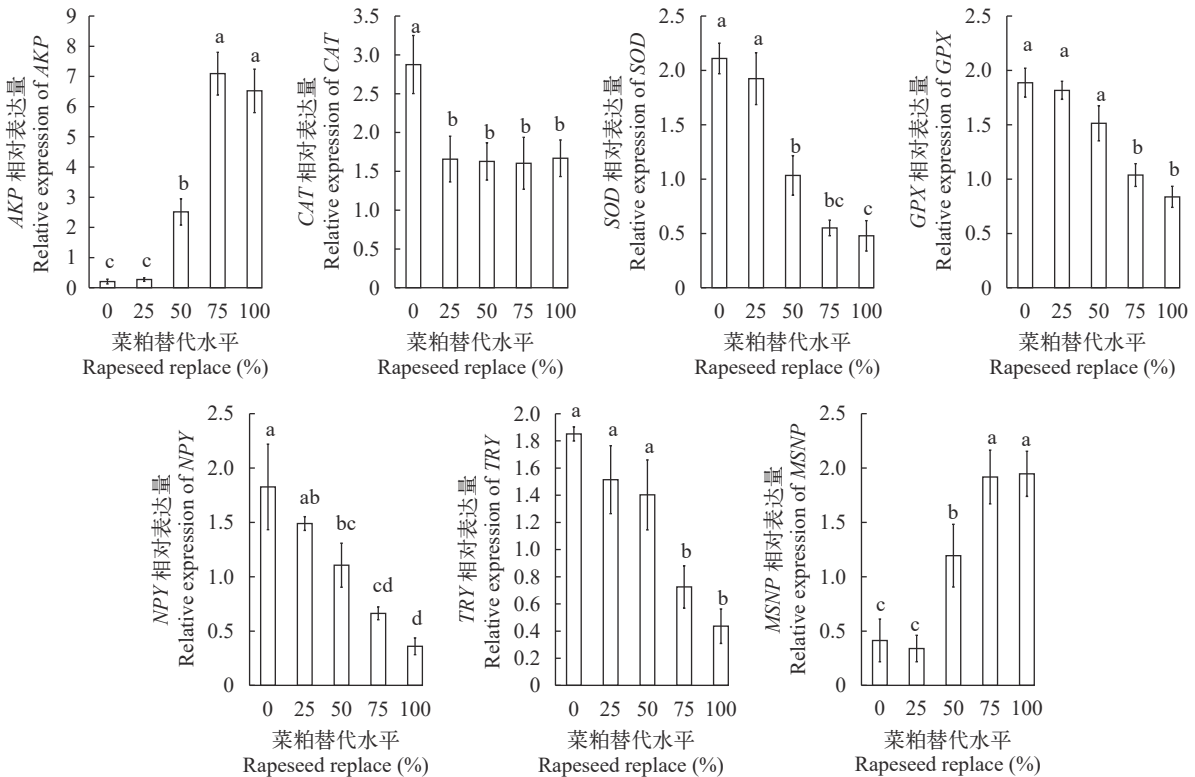


图4 双低菜粕替代鱼粉对克氏原螯虾幼虾肠道基因表达的影响

Fig. 4 Effects of replacing fish meal with rapeseed meal on gene expression in intestines of juvenile *P. clarkii*

生生物的生长情况,是评价甲壳动物食用价值的重要指标^[44,45]。与本试验结果相同,高菜粕替代水平(34.8%、45%和100%)可显著提高吉富罗非鱼(*Oreochromis niloticus*, GIFT)^[46]、奥尼罗非鱼和尼罗罗非鱼(*Oreochromis niloticus*)^[41,47]的肝体比。可能是植物性饲料中赖氨酸的缺乏,限制了蛋白质的合成,导致未用于蛋白质合成的氨基酸部分转化为脂质或糖原并沉积在肝脏中,从而增加动物的内脏质量,提高了肝体比^[48]。混合植物蛋白(玉米蛋白粉、小麦面筋、挤压豌豆、菜粕和挤压全麦)高水平替代饲料中鱼粉后,显著降低金头鲷(*Sparus aurata*)的出肉率^[49]。在本试验中,饲料中双低菜粕的添加显著降低了克氏原螯虾幼虾的出肉率,这可能是因为饲料中缺乏蛋氨酸和赖氨酸对肌肉细胞的生长和增殖产生了不利影响^[14]。

菜粕可显著提高鳊(*Siniperca chuatsi*)的鱼体水分,降低粗脂肪含量^[18]。凡纳滨对虾饲料中双低菜粕替代鱼粉后,全虾脂肪有下降的趋势^[50]。当饲料中菜粕添加比例达到32%时,显著降低了虹鳟(*Oncorhynchus mykiss*)的鱼体粗蛋白含量,不同菜粕替代水平对虹鳟鱼体粗脂肪含量无显著影响^[51]。张明明等^[52]以吉富罗非鱼为研究对象,在饲料中添加60%的双低菜粕,可显著降低鱼体粗脂肪含量。在本试验中,当双低菜粕替代鱼粉比例 $\geq 50\%$,克氏原螯虾幼虾的全虾粗蛋白和粗脂肪均显著低于R0组,表明蛋白质和脂肪合成受到抑制^[16]。菜粕中的单宁和植酸可抑制动物对营养物质的吸收,减少克氏原螯虾幼虾体蛋白质和脂肪的沉积^[53]。

3.2 双低菜粕替代鱼粉对克氏原螯虾幼虾摄食和消化的影响

水生动物对营养素的消化和利用在很大程度上取决于肠道中消化酶活性的水平,消化酶活性可受外源和内源因素的影响,饵料是主要的外源因素之一^[54]。使用菜粕替代饲料中50%的鱼粉时,显著降低了红螯螯虾肝胰腺胰蛋白酶和脂肪酶活性^[7]。饲料中菜粕含量对黄颡鱼(*Pseudobagrus ussuriensis*)肠道内 α -淀粉酶和脂肪酶活性没有显著影响,当替代鱼粉水平为 $\geq 20\%$ 时,黄颡鱼胃蛋白酶活性显著降低^[20]。日本鲈的蛋白酶、脂肪酶和淀粉酶活性随着饲料中菜粕水平的增加而显著降低^[31]。在本试验中,饲料中双低菜粕替代比例达到75%时,可显著降低克氏原螯虾幼虾肝胰腺胰蛋白酶、脂肪酶和肠道胰蛋白酶活性。消化酶活性的降低和菜粕中的抗营养因子相关^[7],例如植酸在动物体内会影响一系列的消化酶活性,抑制消化和营养吸收^[55],而单宁可以和消化酶结合从而降低消化酶的活性^[53]。

神经肽Y(NPY)是中枢系统中强大的食欲刺激剂,是食欲调控网络中主要的基因,主要起促进机体摄食的作用^[56,57]。在同一饲料蛋白水平下以植物蛋白(豆粕、菜粕和棉粕)为蛋白源的试验组斑点叉尾鲷(*Ictalurus punctatus*)大脑中 npv 基因表达量显著低于以鱼粉为蛋白源的试验组^[58]。在杂交石斑鱼(*Epinephelus fuscoguttatus*♀ $\times$ *Epinephelus lanceolatus*♂)幼鱼饲料中,以大豆蛋白替代部分鱼粉显著降低了其 npv 基因表达量^[59]。胰蛋白酶(TRY)是一种重要的消化酶,TRY基因表达的下调会影响消化功能,从而影响养殖动物的生长^[60]。与鱼粉组相比,高菜粕组显著降低了草鱼肝胰腺 try 基因表达量^[61]。Myosuppressin属于一组神经肽,仅在昆虫或甲壳动物中发现^[62],具有抑制肠道收缩^[63]、拒食特性^[64]和抑制神经肽分泌^[65]等多种特性。在本试验中,双低菜粕替代组肝胰腺和肠道NPY和TRY基因表达量下降,MSNP基因表达量上升,表明饲料内双低菜粕的添加会抑制克氏原螯虾幼虾的食欲,导致食物摄入量减少,并降低其肠道消化能力。这可能是菜粕中的单宁、芥子碱及硫代葡萄糖苷水解产生的异硫氰酸酯等都会降低饲料的适口性,进而影响养殖动物的摄食。另有研究表明,NPY基因表达量下降可能是因为菜粕中赖氨酸的缺乏,赖氨酸可通过味觉受体T1R1增加钙离子的释放调节NPY^[66]。

3.3 双低菜粕替代鱼粉对克氏原螯虾幼虾抗氧化性能的影响

AKP是溶酶体系统的主要组成部分,可消除和水解微生物^[67],参与细胞的吞噬作用和细胞内解毒^[68,69]。在本试验中,双低菜粕高比例替代鱼粉显著提高了克氏原螯虾幼虾肝胰腺AKP活性,有研究表明,当有毒物质进入肝胰脏时,AKP活性升高发挥其解毒功能^[70]。GPT和GOT是重要的氨基酸转氨酶,在蛋白质代谢中起重要作用,其活性直接反映了蛋白质代谢的效率,也是反映肝细胞生理状态的主要敏感指标^[71,72]。与本试验结果相同,饲料中添加菜粕显著提高了亚洲红尾鲢肝脏GPT和GOT活性,激活了蛋白质的降解^[15]。CAT、SOD、GPX和GST作为主要的抗氧化酶在体液免疫系统中起着重要作用^[73,74]。CAT能够保护细胞免受抗氧化系统中 H_2O_2 引起的损伤^[75]。甲壳类动物的SOD是主要的抗氧化指标,它可以清除生物体中过量的自由基和防御其他氧化损伤^[76]。GPX可以催化 H_2O_2 转化为 H_2O 或将过氧化脂质还原为正常状态,以防止细胞膜和其他生物组织的破坏^[77]。GST是一种参与宿主细胞解毒的关键酶^[78]。MDA是脂质过氧化的产物,其含量间接反映了体内活性氧自由基的

含量和组织细胞脂质过氧化程度^[19]。饲料中菜粕和棉粕的添加量增加20%可显著降低草鱼(*Ctenopharyngodon idellus*)血清中SOD和GPX的活性^[4]。黄颡鱼血清中SOD和CAT活性随着饲料中菜粕水平的提高而降低,血清MDA含量则随着饲料中菜粕水平的提高而提高^[20]。芥酸是菜粕中天然存在的主要抗营养因子之一,随着草鱼饲料中芥酸含量的增加,其肠道GPX、GST、CAT、CuZnSOD和MnSOD活性逐渐降低,MDA含量逐渐上升^[79]。使用植物蛋白替代鱼粉,会显著降低水产动物的抗氧化能力^[80,81]。本试验结果与上述相同,双低菜粕替代75%的鱼粉时可显著降低克氏原螯虾幼虾肝胰腺GPX、GST、CAT和SOD活性,提高幼虾MDA含量,表明高比例的双低菜粕替代可降低克氏原螯虾幼虾的抗氧化能力。

在本试验中,克氏原螯虾幼虾肝胰腺和肠道AKP基因表达量随着双低菜粕添加比例的增多而升高,可能是为了维持体内环境稳态而采取的主动防御措施,机体试图通过免疫反应来适应不利情况,同时这也是一种被动的病理显示^[82,83]。高水平棉粕和菜粕饲料饲喂的草鱼肠道*cuznsod*、*mnsod*、*cat*基因表达量均显著低于鱼粉组^[19]。投喂高水平豆粕饲料的大菱鲆(*Psetta maxima* L.) *sod*和*gpx*基因表达量显著低于鱼粉组^[84]。饲料中植物蛋白(豆粕、花生粕和菜粕)水平从50%提高到70%时,显著降低中华绒螯蟹(*Eriocheir sinensis*)肝胰腺*cytmnsod*和*mtmnsod*基因表达量^[85]。在本试验中,克氏原螯虾幼虾肝胰腺和肠道CAT、SOD和GPX基因表达量均随着双低菜粕替代比例的增高而降低,双低菜粕可能通过下调CAT、SOD和GPX基因表达量来降低肠道和肝胰腺抗氧化酶的活性,从而降低克氏原螯虾幼虾机体抗氧化能力。这可能是由于菜粕所含的抗营养因子和有毒物质的负面影响。

3.4 双低菜粕替代鱼粉对克氏原螯虾幼虾肝脏和肠道组织学的影响

肝胰腺是甲壳动物消化、吸收、储存、排泄和代谢中心^[86,87],也是主要的解毒器官^[78]。随着饲料中双低菜粕替代水平的提高,大黄鱼肝脏细胞空泡化程度逐渐加深,细胞核浓缩变小,菜粕替代比例为100%时,肝脏细胞几乎完全空泡化,细胞核消失^[88]。使用双零菜粕替代尼罗罗非鱼饲料中37.5%以上的鱼粉时,其肝细胞被严重破坏,排列紊乱^[5]。在本试验中,随着饲料中双低菜粕替代比例的提高,克氏原螯虾幼虾肝胰腺受损程度逐步加深,R75和R100组肝小管完全退化,结构紊乱,受损严重,R细胞萎缩和消失。R细胞是营养物质储存的主要场

所,可用于监测甲壳动物的营养状况,R细胞的萎缩和消失可能意味着摄入的饲料不能满足克氏原螯虾幼虾营养需求^[89]。高比例的双低菜粕替代可引起克氏原螯虾幼虾B细胞肿大,这可能是由氧化应激造成的,相似的结果在低鱼粉饲料对南美白对虾的研究中也有报道^[90]。肝胰腺病理损伤将会导致其功能的改变和对分泌、吸收、储存和解毒等基本过程的干扰^[91]。这些损伤推测是由菜粕中的抗营养因子导致的,如菜粕中的脲会对动物的肝脏产生巨大的毒性损害作用^[92]。

由于肠道是营养吸收的主要场所,因此可以用肠道组织学来评估营养吸收的效率,而肠道结构的损坏会降低其吸收功能^[93]。投喂含41%菜粕的饲料会造成红螯螯虾的围食膜与黏膜皱褶完全分离^[7]。菜粕替代饲料中37.5%鱼粉显著降低了翘嘴鲌(*Siniperca chuatsi*)肠道的绒毛高度、绒毛宽度和绒毛密度,增加了肠壁厚度和杯状细胞数目,且造成肠绒毛大量脱落^[94]。用菜粕替代鱼粉和豆粕对尼罗罗非鱼的肠道形态有显著影响,75%替代组肠绒毛黏膜皱襞严重空泡化;100%替代组肠道出现死亡细胞数量增多、炎症及组织壁和绒毛完全变性等现象^[17]。在本试验中,随着饲料中双低菜粕替代比例的提高,克氏原螯虾幼虾肠道受损程度逐步加深。肠道组织学的变化可能与菜粕中所含抗营养因子的水解产物(如异硫氰酸酯、植酸-蛋白质复合物)^[17]和不可利用的碳水化合物(包括非淀粉多糖、抗性淀粉和某些低聚糖)^[13]相关。

4 结论

本研究结果表明,饲料中双低菜粕替代克氏原螯虾幼虾饲料中鱼粉蛋白的比例 $\geq 50\%$ 时,会对克氏原螯虾的消化酶活力和抗氧化能力,损坏其肝胰腺和肠道组织结构,对其摄食、消化和抗氧化相关基因的表达产生负面影响。克氏原螯虾幼虾饲料中双低菜粕含量以不超过21.25%为宜。

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REPLACING FISH MEAL WITH RAPESEED MEAL ON GROWTH PERFORMANCE, ANTIOXIDANT CAPACITY AND DIGESTIVE SYSTEM MORPHOLOGY OF JUVENILE *PROCAMBARUS CLARKII*

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Abstract: To elucidate the effects of rapeseed meal replacing fish meal on growth performance, feed utilization, digestive activity and antioxidant capacity of juvenile *Procambarus clarkii*, five isonitrogenous and isocaloric diets were formulated to feed juvenile *Procambarus clarkii* with the average initial body weight of (2.49±0.01) g for 42d. The inclusion levels of rapeseed meal were 0, 21.25%, 42.50%, 63.75% and 85% by using rapeseed meal instead of fish meal in different proportions [0 (R0), 25% (R25), 50% (R0), 75% (R0) and 100% (R0)], respectively. The results showed that: (1) no significant differences in FBW, SGR, FE, and PER were observed among the R0, R25 and R50 groups ($P>0.05$), however, FBW, SGR, FE, and PER in R75 and R100 groups were significantly lower than those in R0 group ($P<0.05$). Compared with the R0 group, the crude protein and crude lipid contents of juvenile *Procambarus clarkii* in the R50, R75 and R100 groups were significantly reduced ($P<0.05$). The activities of hepatopancreatic trypsin, hepatopancreatic lipase and intestinal trypsin of juvenile *Procambarus clarkii* in the R75 and R100 groups were significantly lower than those in the R0 group ($P<0.05$), while there was no significant difference in amylase activity among all the groups ($P>0.05$). *NPY* and *TRY* genes expressions levels in hepatopancreas and intestine of R75 and R100 groups significantly decreased than those of R0 group ($P<0.05$), while *MSNP* gene expression level significantly increased ($P<0.05$). (2) Hepatopancreatic alkaline phosphatase (AKP), alanine aminotransferase (GPT), and aspartate aminotransferase (GOT) activities in the R100 group significantly increased compared with the R0 group ($P<0.05$). With the increase of rapeseed meal level, the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione S-transferase (GST) in hepatopancreas showed a decreasing tendency, which in the R75 and R100 groups were significantly lower than those in the R0 group ($P<0.05$). The expression levels of *AKP* gene in hepatopancreas and intestine of the R50, R75 and R100 groups significantly increased compared with the R0 group ($P<0.05$), while the expression levels of *CAT*, *SOD* and *GPX* genes significantly decreased ($P<0.05$). (3) The structures of hepatopancreas and intestine in the R25 group were not significantly different from those in the R0 group. With the increase of replacement ratio, the vacuolation of hepatopancreas epithelial cells in the juvenile *Procambarus clarkii* gradually increased, R cells atrophy by degrees, and the hepatopancreas tubules lumens were misshapened and had become enlarged. The intestinal cells fall off and the intestinal villi become significantly shorter. In summary, the appropriate content of rapeseed meal in the feed for juvenile *Procambarus clarkii* should not be more than 21.25%.

Key words: Rapeseed meal; Growth; Antioxidant capacity; Gene expression; Juvenile *Procambarus clarkii*